MUCOSAL IMMUNITY: NEW STRATEGIES FOR PROTECTION AGAINST VIRAL AND BACTERIAL PATHOGENS

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Mucosal Immunity: New Strategies for Protection Against Viral and Bacterial Pathogens

Differentiation and Function of Organized Mucosal Lymphoid Tissues

J1-001 AN OVERVIEW OF INTERACTIONS BETWEEN EPITHELIAL AND LYMPHOID CELLS IN THE HUMAN MUCOSAL IMMUNE SYSTEM, Per Brandtzaeg, Inger Nina Farstad, Trond S. Halstensen, Frode Jahnsen, Ellen Nilsen, Dag E. Nilssen, and Helge Scott, Laboratory for Immunohistochemistry and Immunopathology, Institute of Pathology, University of Oslo, Rikshospitalet, N-0027 Oslo, Norway.

Lympho-epithelial interactions occur in inductive tissues such as Peyer's patches (PP) and at various mucosal effector sites, both in terms of cytokine signalling and cellular contact. The initial encounter between mucosal immune cells and luminal antigens is facilitated by antigen-sampling M cells. These specialized cells express less HLA class II molecules than the rest of the dome epithelium of PP and are therefore hardly involved in antigen presentation to $CD4^+$ T-helper cells. However, the M cells may by lysosomal activity degrade the antigens they transport. Their basal pockets contain relatively few $CD68^+$ macrophages but many T and B cells; the latter show a putative naive ($sIgD^+sIgM^+$) or primed ($sIgD^-sIgM^+/sIgA^+$) phenotype and apparently represent an extension of the underlying lymphoid follicle. The T cells at these sites are dominated by $CD4^+CD45R0^+$ memory cells (approx. 70%), thus contrasting ordinary intestinal intraepithelial lymphocytes (IEL) that normally are composed mainly of $TCR\alpha/\beta^+CD8^+$ cells (80-90%). Moreover, they commonly express the early activation marker CD69, perhaps reflecting diversified immune stimulation conducted by adjacent B cells that fastidiously take up partially processed antigens from the overlying M cells. Activated B and T cells rapidly home to mucosal effector sites where extravasation may largely be determined by their expression of the integrin $\alpha4\beta7$. Subsequent outward migration of $CD8^+$ cells apparently depends on further immune activation in the lamina propria, and their preferential retention within the epithelium is probably mediated by interactions between an epithelial ligand and the integrin $\alpha E\beta7$ which is chiefly expressed on $CD8^+$ cells. This may also be true for the small fraction of $TCR\gamma/\delta^+$ IEL. Both subsets are induced to proliferate within the epithelium in immunostimulatory conditions such as celiac disease, but their *in vivo* function remains obscure. An increased population of $TCR\alpha/\beta^+CD8^+$ IEL in patients with generalized B-cell deficiency might reflect a compensatory mucosal defense function. Interestingly, IgA-deficient subjects without infections have increased numbers of $TCR\gamma/\delta^+$ IEL and so have AIDS patients except those with a particularly short life expectancy. In the upper respiratory tract epithelium, the proportion of $CD4^+$ T cells is usually higher than in the gut; this fact, together with differences in the epithelial expression of accessory molecules such as ICAM-1, might render IEL in the airways more prone to overstimulation and could contribute to local hypersensitivity. In the gut immunological overstimulation appears in the main to be mediated via lamina propria $CD4^+$ T cells that by their cytokines affect local B-cell maturation, IEL activity, and epithelial function. This is evidenced in the gluten-sensitive celiac lesion where the activated ($CD25^+$) cell fraction is increased; mucosal restimulation *in vitro* has facilitated cloning of gluten-specific $CD4^+$ T cells and characterization of their cytokines, IFN-γ being the most prominent. This cytokine can efficiently upregulate important immunological molecules such as secretory component (SC or pIgR) and HLA-DR on epithelial cells. Local enhancement of secretory immunity may thus be explained by cytokine-mediated interactions between $CD4^+$ T cells, B cells, and the epithelium.

J1-002 *TRANSPORT OF ANTIGENS AND MICROORGANISMS BY M CELLS*, Marian R. Neutra, Paul J. Giannasca, Andreas Frey, Fan Zhou, and Karen T. Giannasca, Harvard Medical School and Children's Hospital, Boston.

Transepithelial transport of antigens by M cells in the epithelium associated with lymphoid follicles in the intestine and rectum delivers immunogens directly to organized mucosal lymphoid tissues, the inductive sites for mucosal immune responses. We are defining the factors that influence M cell transport of antigens and microorganisms, developing methods for targeting immunogens to these cells, and analyzing the protective roles of specific secretory IgA antibodies. For example, we have used a panel of lectins and antibodies to investigate the composition of complex carbohydrates associated with M cells in various regions of the intestinal tract of adult BALB/c mice. Subpopulations of M cells in Peyer's patches show differential binding of lectins with distinct fucose-specificities to their apical, basolateral, and intracellular membranes, while M cells overlying colonic and rectal lymphoid follicles are characterized by terminal galactose residues. Lectins selectively adhere to and are taken up by M cells *in vivo*, suggesting that M cell-specific glycoconjugates could serve as "receptors" for targeting of lectin-antigen conjugates to the mucosal immune system. We also tested whether cholera toxin (CT) B subunit can adhere preferentially to M cells when immobilized on the surfaces of particles of two disparate sizes: 10 nm and 1 μm. Our results suggest that the M cell glycocalyx is sufficient to block CTB-mediated binding of 1 μm microparticles to the apical plasma membrane while particles in the nanometer range are able to penetrate this barrier. Ferritin, a soluble model antigen, was used to test whether liposomes can provide an effective delivery vehicle for mucosal immunization via the rectum, and whether IgA can serve to target liposomes to M cells. The results showed that liposomes co-administered with CT are effective for mucosal immunization via the rectum, and that coating of liposomes with IgA can enhance the local secretory immune response to antigen, apparently by increasing liposome uptake via M cells. In a series of studies, we have shown that secretion of monoclonal IgA antibodies recognizing a single surface epitope on enteric pathogens can prevent interaction of pathogens with epithelial surfaces. IgAs against surface-exposed epitopes were protective when applied to epithelial cell monolayers *in vitro* , fed orally to suckling mice, and secreted *in vivo* using a "backback" tumor method. Current efforts are focused on enhancing M cell uptake of gp120-containing vaccines administered orally or rectally, induction of anti-gp120 IgA antibodies on rectal and female genital mucosal surfaces, and testing the protective potential of specific IgAs. This basic information may contribute to design of mucosal vaccines that elicit anti-gp120 secretory IgA (sIgA) antibodies in rectal and cervical secretions of women, and that reduce the risk of sexual or vertical transmission of AIDS.

J1-003 EXPERIMENTAL APPROACHES TO INVESTIGATE THE M CELL PHENOTYPE. Eric Pringault[1-2], Sophie Kerneis[2], Anna Bogdanova[2],Ochine Karapetian[2], Jean-Pierre Kraehenbuhl[2], [1]Molecular Biology Department, Pasteur Institute, 28 rue du Dr. Roux 75015 Paris, France. [2] Cell Biology Department, Swiss Institute for Experimental Research on Cancer, CH-1066 Epalinges-Lausanne, Switzerland.

M cells from the follicle-associated epithelium (FAE) can be distinguished by electron microscopy from neighbouring enterocytes by the absence of a brush-border and a huge invagination of the basoleral membrane, forming a pocket containing lymphocytes and/or macrophages. Since TEM analysis only gives a "snapshot" of a section of a Peyer's patch, it is impossible to know if these lymphoid cells are permanent residents of this pocket or whether the interactions are transient . The FAE renewal is dependant on the proliferation of crypt cells surrounding the follicle. FAE and M cell formation seems to require a paracrine or cell contact-mediated cross-talk with the lymphoid compartment of the Peyer's patch. M cells could arise either from fully differentiated enterocytes present in this FAE (transdifferentiation) or directly from immature crypt cells (independant cell sublineage). In the first case, the M cell phenotype corresponds to the aspect of an enterocyte having acquired a new specialized function of lumen-to-follicle transcytosis, concommitent to interaction with one of several lymphocytes. To perform this function, the enterocyte disassembles its brush-border, reorganizes its cytoskeleton network and changes its columnar shape. Whether these modifications are reversible or not has to be considered. In the first hypothesis, M cell as a specific entity is unlikely. In the second case, a cross-talk with lymphoid cells triggers a M cell differentiation program in immature crypt cells in a process close to that postulated for other mucosal cell types such as goblet cells or enteroendocrine cells. Committed M cells never acquire absorptive enterocyte characteristics (for instance they never assemble a brush-border) but instead acquire their specific functions. New experimental approaches have been developed to investigate the mechanism of FAE and M cell formation. We have investigated the expression of villin, an actin-associated brush-border protein, which is a marker of the differentiation of epithelial cells from the endodermic lineage. Villin expression was observed in all cells of the FAE from the mouse intestinal Peyer's patches. In particular, immature precursors located in the surrounding crypts express villin before the brush-border assembly occurs. Although this protein is concentrated at the level of the brush-border in enterocytes of the FAE, a subpopulation of epithelial cells in this FAE displayed a cytoplasmic distribution of villin. This subpopulation included typical M cells. The cytoplasmic distribution of this protein indicates that the microfilament network is strongly modified in M cells compared to the adjacent enterocytes and provides new criteria to identify M cells by immunocytochemistry and optical microscopy. Villin expression in mouse M cells is a strong argument of their epithelial intestinal origin. Villus effacement and appearence of epithelial domes, associated with changes in the enterocyte phenotype, occur upon injection of Peyer' patches cells isolated from a donor Balb/c mouse between the muscularis and the intestine mucosa of a recipient mouse, in a duodenal segment devoided of Peyer's patches . 9-11 days after injection, we have observed a *de novo* formation of Peyer's patch-like structures, displaying various degrees of maturity. This implies that FAE-like structures could be induced from crypts from which normally arises villus-lining epithelium and emphasizes the inductive role of lymphoid compartment. In addition, attempts to induce FAE and M cell formation in cell culture have been initiated, using previously established intestinal cell lines or FAE cell lines trans-immortalized by the SV40 large T antigen under the control of the villin promoter, co-cultured with Peyer's patch lymphocytes.

Roles of Epithelial Cells in Induction of Mucosal Immune Responses

J1-004 THE ROLE OF THE THYMUS IN INTESTINAL INTRAEPITHELIAL T LYMPHOCYTE DEVELOPMENT. Leo Lefrançois, Sara Olson and Lynn Puddington. University of Connecticut Health Center, Division of Rheumatic Diseases, Farmington, CT 06030.

Recent evidence has indicated that the intestinal epithelium may be a major extrathymic site of T cell production. However, which of the multiple intestinal intraepithelial lymphocyte (IEL) subsets are extrathymic in origin has been controversial. Our results indicate that the thymus is an integral component of IEL maturation and is required for a novel two-stage process of T cell production. In neonatally thymectomized mice T cell receptor (Tcr) $\gamma\delta$ IEL were depleted and some Tcr$\alpha\beta$ IEL were of an immature phenotype. Thymus grafting experiments revealed that all Tcr$\gamma\delta$ and Tcr$\alpha\beta$ IEL subsets could be thymus-derived, including Tcr$\alpha\beta$ cells lacking Thy1 and CD8β. *In utero* anti-Tcr$\gamma\delta$ monoclonal antibody (mAb) treatments resulted in depletion of $\gamma\delta$ IEL without subsequent reemergence of this subset in adulthood whereas anti-Tcr$\alpha\beta$ mAb treatment only marginally reduced the $\alpha\beta$ IEL subset. These findings suggest that Tcr$\alpha\beta$ and Tcr$\gamma\delta$ IEL arose at distinct developmental stages. Overall, the results indicate that some IEL precursors are thymus-derived but require further thymic influence to mature in the periphery. Our current efforts are focussed on determining the mechanism by which the thymus influences IEL devlopment and whether Tcr rearrangement and T lymphocyte selection occurs in the intestine.

J1-005 ANTIGEN PRESENTATION BY ENTEROCYTES. Lloyd Mayer, Xian Y. Yio, Asit Panja, and Yin Li. Mount Sinai Medical Center, New York, NY 10029.

Differences in antigenic exposure and anatomy logically dictate that the requirements for regulating immune responses in the intestine would be quite distinct from those of the systemic immune system. Our laboratory has postulated that these differences relate to a unique system of antigen presentation which includes an active role for intestinal epithelial cells. These cells constitutively express class II antigens, are capable of taking up soluble proteins by fluid phase endocytosis and present processed peptides to immunocompetent T cells. However, in contrast to conventional APCs which express class II molecules, normal intestinal epithelial cells appear to selectively activate CD8+ suppressor T cells. This activation is not inhibited by antibodies to conventional restriction elements (Class I and Class II) but is inhibited by antibodies to CD8, suggesting that the CD8 molecule itself is involved in this process. In fact, co-culture of allogeneic epithelial cells with peripheral blood T cells results in the activation of CD8 associated p56lck. Activation of this kinase is necessary but not sufficient to drive the proliferation of CD8+ T cells in this system. We have therefore turned our attention towards identifying a molecule which might serve as a novel ligand for CD8, activating p56lck and inducing CD8+ T cell proliferation. A series of anti-epithelial cell mAbs were generated and screened for their ability to inhibit in this system. Two distinct mAbs were identified, B9 and L12, which were capable of inhibiting CD8+ T cell proliferation in IEC:PB T cell co-cultures but displayed different tissue staining characteristics and ability to inhibit the activation of CD8 associated p56lck. Both mAbs appear to recognize a 180 kDa molecule from intestinal epithelial cell lines and freshly isolated epithelial cells. This 180kDa molecule is heavily N-glycosylated (protein core = 76kd) and exists in two forms on the cell surface, a GPI anchored form on the apical surface and a transmembrane form on the basolateral surface. However, B9 stains intestinal epithelial cells from the duodenum to the rectum in an apical pattern (villous>crypt) as well as thymic epithelial and syncytiotrophoblast cells. L12 stains intestinal epithelium in a similar pattern but also stains airway epithelium (which activate CD4+ T cells). Furthermore, mAb B9 but not L12 inhibits the activation of p56lck in IEC:PB T cell co-cultures and mAb B9 affinity purified gp180 can bind specifically to T cells and, when aggregated, will activate CD8 associated p56lck. L12 and B9 appear to recognize distinct carbohydrate side chains on the same protein backbone, probably accounting for these differences. Differential glycosylation of the protein core may therefore dictate distinct functional activities. Thus it appears that B9 and L12 mAbs may identify either a novel restriction element or adhesion molecule which serves as a unique regulatory element in mucosal immune responses.

J1-006 ROLE OF EPITHELIAL CELLS IN THE MUCOSAL IMMUNE RESPONSE TO BACTERIA, Catharina Svanborg, William Agace, Hugh Connell, Spencer Hedges, Maria Hedlund, Majlis Svensson and Maria Wagner, Department of Medical Microbiology, Lund University, S-223 62 Lund.

Epithelial cells play an active role in the mucosal immune system. They sense the microbial components in the environment, respond to their presence with the production of mediators, and interact with other cells in the mucosal compartment. We have shown that epithelial cells produce cytokines in response to bacterial stimulation. The spectrum of cytokines produced and the magnitude of the epithelial cytokine response is influenced by bacterial virulence factors (P- and Type 1 fimbriae, LPS, invasion etc). The epithelial response to *E.coli* includes interleukin 6 (IL-6) an endogenous pyrogen, enhancer of the acute phase response and a differentiation factor for mucosal B lymphocytes. The secretion of interleukin 8 (IL-8) a neutrophil chemoattractant causes a granulocyte influx to the site of infection, and facilitates bacterial clearance. Neutrophil transmigration across the epithelial layer involves the interaction of CD11b/CD18 on the granulocyte with ICAM-1 on the epithelial cells. Bacteria upregulate ICAM-expression on the epithelial cells.

The epithelial cell can also respond to cytokines produced by other types of cells in the mucosal compartment. Such cytokines include interleukin 1α (IL-1α), tumor necrosis factor α (TNF-α) γ-interferon (γ-IFN) and interleukin 4 (IL-4). Exogenous cytokines modify the epithelial response to bacteria (IL-1 and IL-4 are enhancers while γ-interferon can act as an inhibitor). In this way a mucosal network is created and cells like intra-epithelial lymphocytes, mast cells and T cells can modify the epithelial response to bacteria.

The mucosal inflammatory response is important for the resistence to infection. Animals with genetic or pharmacologic deficiencies in this response have lost the ability to clear mucosal Gram negative infection. In contrast, mice with immuno deficiencies have intact antimicrobial defenses. This emphasizes the importance of the epithelial-microbial interactions, as an early regulatory event in mucosal immunity.

Induction of Immune Responses in Mucosal Lymphoid Follicles

J1-007 CYTOKINES REGULATE IgA PRODUCTION AND PROVIDE EARLY SIGNALS FOR THE ACTIVATION OF IMMUNE AND INFLAMMATORY RESPONSES IN THE MUCOSAL ENVIRONMENT, Martin F. Kagnoff, Lars Eckmann, George Huang, Pyeung-Hyeun Kim, Hyun Jung, Joshua Fierer, and Ewa Morzycka-Wroblewska, Laboratory of Mucosal Immunology, University of California, San Diego, La Jolla.

Cytokines regulate Ig isotype expression at mucosal surfaces at a cellular and molecular level by controlling heavy chain switching, gene transcription, and mRNA stability. We have characterized some of the molecular mechanisms that underlie cytokine regulated IgA production by murine B cells and B cell lines. TGFß1 stimulates isotype switching from IgM to IgA. In contrast, other cytokines, including IL-2, IL-5, or IL-4 and IL-5 in combination, predominantly upregulate IgA secretion. For the latter, this involves at least two different mechanisms. In the early period after agonist stimulation, increased cellular mRNA levels for the secreted form of α heavy chain (α_S) and κ light chain reflect increased transcription of those genes. At later time points, the coordinate stabilization of α_S and κ light chain mRNA appears to be the predominant mechanism that underlies increased IgA secretion. This dual mechanism for regulating α_S and κ light chain mRNA levels meets the host's need for a rapid and transient increase in IgA production in response to short lived stimuli and a delayed, but prolonged, response to persistent stimuli such as may be seen during ongoing microbial infection. Together, these two mechanisms are synergistic and are likely essential for attaining high level IgA secretion. In addition to regulating the IgA response, cytokines produced by mucosal tissues play an essential role in delivering signals which govern the activation, growth, differentiation and migration of cells that are crucial to both the host's natural and acquired immune response. Epithelial cells that line the mucosal surface are particularly important in this regard as they can provide the earliest signals for initiation and amplification of mucosal immune and inflammatory responses which have important consequences for how the host responds to pathogenic microbes. Supported by NIH grants DK35108 and DK47739, and a Pediatric AIDS Foundation grant.

J1-008 **The neutralizing antibody response against vesicular stomatitis virus**

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The in vitro neutralising and in vivo protective antibody response against vesicular stomatitis virus (VSV), Indiana (IND) is specific for one major epitope on the glycoprotein (G) and has the following characteristics: The IgM response is largely T help independent and reaches peak titers early by day 4 after infection; the subsequent switch to IgG after day 6 is strictly T help dependent.

Affinity maturation of IgG antibodies is generally thought to play an important role in the adaptive immune response to improve effector functions of IgG within two weeks to several months of antigen encounter. Since this concept has been based on IgG responses against chemically defined haptens, we validated it in a viral system and analysed neutralising IgG antibody responses against VSV, (a close relative of rabies virus), early on d6 or on d12 and late after hyperimmunization on d150. The results indicate that primary neutralising IgG antibodies recognize a single major epitope with high affinities of 10^8 to 10^{10} l/mol and with rapid on-rates already on day 6 of a primary response and with no evidence for further antigen dose- and time-dependent affinity maturation.

We have analysed B cell and T helper cell tolerance in mice expressing VSV-G as transgene under various promotors in a membrane associated form. Autoantibodies to VSV-G could not be induced by VSV-G in adjuvant or by recombinant vaccinia virus expressing VSV-G , but were triggered by with wild-type VSV. The data show that helper T cell tolerance is crucial in maintenance of B-cell non-reactivity and that cognate T-B recognition is necessary to break apparent unresponsiveness of self-reactive B cells. The influence of antigen epitope density and order on B cell induction or antibody production was assessed with VSV-G (IND); it can be found in a highly repetitive form in the envelope of VSV and in a poorly organized form on the surface of infected cells. In transgenic mice, expressing VSV-G under the K^b-promotor also in the bone-marrow, B cells were unresponsive to the randomly distributed VSV-G (IND) present as self antigen but responded promptly to the same antigen presented in the highly organized form. Thus, antigen organization influences B cell tolerance. These results may help to understand rules for optimal induction of antibodies and mechanisms of virus-induced autoimmunity.

Cellular Traffic in the Mucosal Immune System

J1-009 TISSUE SPECIFIC LYMPHOCYTE ADHESION AT THE EPITHELIUM MEDIATED BY CADHERINS AND INTEGRINS, Karyn L. Cepek[1], Sunil K. Shaw[1], Christina M. Parker[1], Gary J. Russell[1], Jon S. Morrow[2], David L. Rimm[2], and Michael B. Brenner[1], [1]Lymphocyte Biology Section, Department of Rheumatology and Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, [2]Department of Pathology, Yale University, New Haven, CT 06510

In contrast to sessile cell types, lymphocytes traffic through the vasculature and migrate to be found diffusely distributed in tissues or organized in lymphoid structures. A complex array of adhesion molecules including selectins, integrins, and their counter-receptors mediate lymphocyte homing and migration into tissues and may be constitutively expressed or induced. However, the molecules mediating the retention of lymphocytes in a tissue specific manner within the parenchyma have not been defined.

Positioned along the epithelium at the basolateral surface of enterocytes, intestinal intraepithelial lymphocytes are T cells of the mucosal immune system which serve as a model for understanding the tissue specific compartmentalization of lymphocytes. We hypothesized that the localization of IEL is mediated by specific interactions between adhesion molecules expressed selectively on this subpopulation of T cells and tissue restricted adhesion molecules on epithelial cells. We demonstrate that heterotypic adhesion interactions between epithelial cells and intraepithelial lymphocytes in vitro are mediated by the tissue specific adhesion molecule, E-cadherin, and the T lymphocyte specific adhesion molecule, integrin $\alpha^E\beta_7$.

J1-010 ROLES OF INDUCTIVE SITES IN REGION-SPECIFIC SECRETORY IMMUNE RESPONSES OF DIVERSE MUCOSAL TISSUES, Jiri Mestecky[1], Zina Moldoveanu[1], Michael W. Russell[1], Miroslav Novak[2], Hong-Yin Wu[1], G. Hajishengallis[1], Alan C. Menge[3], and Suzanne M. Michalek[1], [1]Departments of Microbiology and Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, [2]Secretech Corporation, Birmingham, AL 35205, and [3]Department of OB/GYN, University of Michigan Medical Center, Ann Arbor, MI 48109

Most vaccines currently considered for further improvement or development are required to induce, in addition to systemic responses, protective immunity against microorganisms that either infect or enter the host through the enormous surface areas of mucosal membranes of the gastrointestinal, respiratory, and genitourinary tracts, and the conjunctiva. Numerous experiments performed in humans and animals have revealed that stimulation of inductive sites such as Peyer's patches results in parallel immune responses manifested by the appearance of S-IgA antibodies in the external secretions of remote glands. However, recent experiments suggest that inductive sites associated with the upper respiratory tract, rectum, and perhaps genital tract may also function as sources of IgA-committed cells that populate, with some selectivity, certain remote effector sites. Furthermore, the combination of various immunization routes and the use of suitable antigen-delivery systems may accomplish an important task – the induction of mucosal immune responses at a location relevant to the site of entry of a given pathogen. Despite pronounced species differences (humans, monkeys, rats, and mice), it is apparent that antigen-specific IgA antibodies can be induced in certain secretions (e.g., female genital tract) not only by immunization in the vicinity of corresponding mucosal tissues (e.g., vagina and rectum) but also by oral and especially intranasal immunization. On the other hand, intra-rectal immunization or genital infection may result in some cases in the appearance of antibodies in salivary secretions. Furthermore, the induction of antibodies at a desired mucosal site can be accentuated with the use of a suitable antigen-delivery system including a relevant microbial vector, incorporation of antigens in biodegradable microspheres or liposomes, and linkage or co-administration of antigens with cholera toxin B subunit. Collectively, these studies have demonstrated a certain degree of compartmentalization as well as complementarity of various inductive sites in the mucosal immune system. As many pathogens display distinct mucosal tissue tropism, further exploitation of immunization routes and efficient antigen-delivery systems will ultimately direct the desired immune response to the relevant site where an infection can be effectively countered. Supported by USPHS grants AI 28147, DE 06746, DE 08182, and DE 09081.

Exploitation of Mucosal Inductive Sites By Microbial Pathogens

J1-011 NEONATAL INFECTION OF PEYER'S PATCH LYMPHOCYTES BY MOUSE MAMMARY TUMOR VIRUS (MMTV).

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MMTV expresses a superantigen after infection of B cells. Superantigen expressing B cells can activate a large proportion of the T cell repertoire since amongst the polymorphic parts of the T cell receptor only the Vβ region of the T cell receptor is of major importance for superantigen activation. The superantigen response is important for the survival of the virus in the host. This retrovirus is transmitted from mother to offspring via milk during the first two weeks after birth. To analyze the route of infection and the ontogeny of the superantigen response we analyzed the lymph nodes draining the intestine of mice continuously drinking MMTV infected milk. Amongst the lymphoid organs we could detect early infection by PCR on day four after birth exclusively in Peyer's patches. Later on viral DNA was detectable in all lymphoid organs and finally in the mammary gland. The neonatal immune system was capable of mounting a superantigen response and reorganization of the B cell compartment very early in the Peyer's patches. This is in contrast to the response in the peripheral lymphoid organs where a superantigen response became detectable only two weeks after birth.

J1-012 THE DEVELOPMENT AND MAINTENANCE OF THE 'NORMAL' STEADY STATE OF GALT IN NEONATES AND FORMERLY GERM-FREE MICE MAY BE DRIVEN DIFFERENTIALLY BY VARIOUS COMMENSAL ENTERIC BACTERIA AND ENTERIC VIRUSES, John J. Cebra[1], Nico A. Bos[2], D. Craig Hooper[1], David R. Kramer[1], Fan Lee, and Khushroo E. Shroff[1], [1]Department of Biology, University of Pennsylvania, Philadelphia, PA 19104 and [2]Department of Histology and Cell Biology, University of Groningen, Groningen NL-9713 EZ, The Netherlands

A comparison of the histology and cellularity of lymphoid tissues of the GALT and of the functional activity and differentiation into subsets of the cellular elements of these tissues from antigen-free (AF), germ-free (GF), and conventionally reared (CNV) mice suggests that chronic exposure of the gut mucosa to environmental antigens (Ags) - such as commensal bacteria - and/or acute oral infection with enteric viruses can account for the 'physiologically normal' state of the GALT. For instance: [1] both oral reovirus infection and enteric colonization with a gram-negative bacteria (Morganella morganii) of GF mice result in transient germinal center (GC) reactions (12-14 day maximum) in Peyer's patches (PP), which generate specific IgA plasmablasts and IgA memory cells. Although infectious reovirus is cleared and the M. morganii persist at high density in the intestinal lumen, the GC reaction wanes in both cases, although IgA memory cells persist for long periods. We suggest that a successful secretory IgA response can attenuate chronic stimulation of GC reactions in PP, even though the bacteria persist; [2] although the intraepithelial leukocyte compartment (IELs) typically contain a preponderant population of CD8 $(\alpha/\beta)^+$, Thy-1$^+$, α/β TCR$^+$ T cells that are constitutive 'killers' by re-directed cytotoxic assays, AF and GF mice have quiescent IELs that are mainly CD8$^+$ (α/α), Thy-1$^-$ and γ/δ TCR$^+$. Neither acute enteric reovirus infection nor chronic gut colonization with M. morganii perturb the IELs of GF mice towards the phenotype of CNV mice. Segmented filamentous bacteria -- obligate, gram-positive anaerobes -- have been shown to convert the lamina propria of GF mice into 'normal' IgA production upon colonization (Klassen et al., I & I 61:303, 1993). We find that such colonization also perturbs the IEL compartment towards the phenotype of CNV mice; [3] by contrasting CNV F_1 immunocompetent neonates that result from reciprocal crosses of immunocompetent and severe-combined immunodeficient (SCID) parents, and by swapping litters to vary birth vs. nurse mothers, we have found that [a] neonates at 10 d. post-partum are fully competent to mount a preferential, specific IgA response in GALT; [b] having a SCID mother results in a 'premature' development of 'natural' IgA antibody (Ab) responses in GALT; [c] reovirus infection of neonates born of immunocompetent mothers can accelerate the development of 'natural' IgA responses; [d] specific anti-reovirus IgA Ab from the nurse mothers can both inhibit the development of specific anti-reovirus Ab in challenged neonates as well as the accompanying development of 'natural' IgA Abs; [4] a comparison of CD4$^+$ T cells from PP of AF, GF, and CNV mice, using antigen-presenting cells (APC) from each group in turn shows that the 'spontaneous' prolif-eration of PP T and APC cells normally observed (compared with splenic cells) is related to environmental Ag exposure.

J1-013 FROM MECHANISMS OF INTESTINAL CELLS AND TISSUE INVASION BY *Shigella* TO IgA-MEDIATED MUCOSAL PROTECTION AGAINST SHIGELLOSIS, Philippe J. Sansonetti, Unité de Pathogénie Microbienne Moléculaire, INSERM U389, Institut Pasteur, 28 rue du Docteur Roux, F - 75724 PARIS Cédex 15, FRANCE.

Shigellosis, an invasive disease of the human colon, is particularly prevalent in developing countries where children are the principal victims. It is caused by *Shigella*, a gram-negative bacteria which has the capacity to invade epithelial cells. There is a need for a vaccine against shigellosis. Unfortunately, most of the attempts at protecting against this invasive infection, either parenterally or orally, have been rather unsuccessful. Empiric approaches have therefore reached their limits in this case and two major domains need to be developped in order to achieve rational vaccine development : an in-depth molecular and cellular analysis of the interactions between the bacterial pathogen and host cells and tissues ; a fundamental study of the bases of mucosal defences against pathogens, particularly at the level of the intestine.

Molecular and cellular analysis of shigellosis has shown that bacteria have the capacity to induce their own entry into epithelial cells *via* a macropinocytic process involving the triggering of major cytoskeletal rearrangement that induce membrane ruffles. Three proteins called Ipa (IpaB, 62 kDa, IpaC, 42 kDa, and IpaD, 37 kDa) are secreted by the pathogen upon contact with cells and work is underway to elucidate the signalling pathways that they activate to induce cytoskeletal rearrangements. Once the bacterium has entered the cell, the Ipa proteins achieve lysis of the phagocytic vacuole and the bacterium induces polymerization of actin at one of its poles, thus generating a motility process caused by IcsA, a 120 kDa bacterial surface protein.

Bacteria then reach the intermediate junction of the polarized epithelial layer and, with the assistance of cadherins, are transported from cell to cell. This global process allows efficient intracellular colonization of the epithelium. Actually, the process starts *in vivo* at the level of lymphoid follicles associated with the mucosa. Bacteria induce an inflammation which destabilizes the epithelium and allows further invasion.

Recent evidence indicates that monoclonal IgA antibodies directed against the *Shigella* LPS, more precisely again serotype-specific epitopes, have the capacity to inhibit the invasive process.

It is hoped that the convergence of these two approaches will allow to establish efficient vaccination strategies against this pathogen.

Production and Function of Secretory Iga in Protection of Mucosal Surfaces

J1-014 REGULATION OF EXPRESSION OF THE POLYMERIC IMMUNOGLOBULIN RECEPTOR, Charlotte S. Kaetzel[1], Janet F. Piskurich[2], Kenneth R. Youngman[2], Carol M. Tamer[2], and May H. Blanchard[2], [1]University of Kentucky, Lexington, KY 40536 and [2]Case Western Reserve University, Cleveland, OH 44106.

Transport of secretory IgA into external fluids is mediated by the polymeric immunoglobulin receptor (pIgR) on the surface of mucosal epithelial cells. The pIgR is expressed constitutively in a variety of mucosal epithelia, and has been shown to be up-regulated by the recombinant cytokines IFN-γ, TNF-α and IL-4 in HT-29 human colon carcinoma cells. We have demonstrated that activation of freshly isolated human intestinal lamina propria mononuclear cells (LPMC) induces production of natural cytokines, and these act synergistically as potent stimulators of pIgR expression in HT-29 cells. LPMC from normal colonic mucosal were stimulated with PMA and calcium ionophore. The resulting supernatants consistently induced dose-dependent increases in pIgR expression by HT-29 cells, up to 65-fold. Ab-mediated neutralization of IFN-γ, TNF-α and IL-4 suggested that the central regulator of pIgR expression in these supernatants was IFN-γ. Our data thus demonstrate that natural cytokines, predominantly IFN-γ, produced by stimulated human intestinal lymphocytes and macrophages have the capacity to up-regulate dramatically pIgR expression in an intestinal epithelial cell line, suggesting that their action in vivo leads to enhancement of local defense functions mediated by IgA. For use as tools for studying the molecular mechanisms by which cytokines regulate pIgR expression, we isolated both cDNA and genomic DNA encoding human and mouse pIgR. Comparisons of the amino acid sequences of human, mouse, rat, bovine and rabbit pIgR revealed that regions of the molecule implicated in Ig-binding, signal transduction and transcytosis have been preferentially conserved during evolution. Northern analysis demonstrated that the tissue specificity of pIgR mRNA expression is similar in humans and mice, with the exception that pIgR mRNA is expressed in mouse, but not human liver. Using a sensitive ribonuclease protection assay, we demonstrated that induction of pIgR by IFN-γ in HT-29 cells is mediated by accumulation of pIgR mRNA. Cycloheximide treatment abolished the IFN-γ induced increase in pIgR mRNA, indicating that up-regulation of pIgR mRNA by IFN-γ requires *de novo* protein synthesis. The newly synthesized protein(s) could be transcription factor(s) that interact with IFN-γ response elements in the pIgR gene or, alternatively, factor(s) that increase the stability of pIgR mRNA. Analysis of the 5'-flanking region of the human pIgR gene revealed sequence homology with elements from MHC class II, Fcγ1 receptor and cytokeratin genes. Preliminary results from experiments involving transfection of chimeric reporter genes into HT-29 cells suggest that *cis*-acting element(s) within the promoter-proximal region of the human pIgR gene may contribute to up-regulation of transcription of this gene by IFN-γ.

J1-015 MOLECULAR EVENTS IN PRODUCTION AND TRANSPORT OF SECRETORY IgA: A BASIS FOR DESIGN OF REAGENTS FOR PASSIVE IMMUNE PROTECTION, Jean-Pierre Kraehenbuhl[1], Sophie Kerneis[1], José Berdoz[1], Blaise Corthésy[2], Lorenz Rindisbacher[2], Irène Corthésy-Theulaz[3], Armelle Phalipon[4], Marian R. Neutra[5], and Eric Pringault[1,6], [1]Swiss Institute for Experimental Cancer Research and Institute of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland, [2]Institute of Animal Biology, University of Lausanne,1015 Lausanne-Dorigny, [3]Division of Gastroenterology, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland, [4]Department of Molecular Pathogenicity, Institut Pasteur, Paris, France, [5]Childrens Hospital and Harvard Medical School, Boston, Massachussets, USA, [1,6]Unité des Membranes, Institut Pasteur, Paris, France

The major class of antibody produced by mucosal tissues lining the digestive, respiratory and urogenital tracts is secretory IgA (sIgA), made of two IgA monomers, one joining (J) chain and one or two molecules of secretory component (SC). Two cells cooperate to produce sIgA antibodies: the local plasma cells that secrete dimeric IgA and the epithelial cells that express the polymeric immunoglobulin (poly-Ig) receptor mediating transepithelial transport of the dimeric IgA. During transport, the receptor is cleaved and secretory component (SC) which corresponds to the five receptor's Ig-like domains remains covalently bound to the IgA dimer providing resistance to proteolysis. In order to identify the mechanisms whereby sIgA antibodies protect mucosal surfaces, we have developed an *in vitro* system that mimics the *in situ* situation. Human or mouse intestinal epithelial cells that express the poly-Ig receptor are co-cultivated with hybridoma or myeloma cells that produce dimeric IgA antibodies. The monolayers are subsequently exposed to different pathogenic microorganisms, protective antibodies are identified and the mechanisms of protection can be analyzed. To facilitate the generation of monoclonal IgA antibodies, a novel strategy has been designed for the isolation of the rearranged genomic variable heavy and light chain genes (Fv) of mouse immunoglobulin from hybridoma cell DNA.The amplified fragments are inserted into expression vectors containing mouse constant α heavy and light chain DNA. The recombinant immunoglobulin DNA is then expressed in CHO or myeloma cells. In CHO cells IgA dimerization is obtained by J chain DNA co-transfection. Antibodies specific for *Clostridium difficile* toxin A and *Helicobacter pylori* urease have been generated by this procedure, *Shigella flexneri* liposaccharide were obtained by screening hydridoma cells for IgA producers. Our data indicate that IgA antibodies prevent microbial adhesion and/or invasion and that immune exclusion by pathogen crosslinking is not the unique mechanisms that operates at mucosal cell surfaces. The system also allows to analyze at which cell surface the antibodies exert protection and whether the IgA present in intracellular compartments play a role.

J1-016 HOST DEFENSE FUNCTIONS OF IGA AT MUCOSAL SURFACES, Michael E. Lamm[1], Mary B. Mazanec[1], John G. Nedrud[1], and Charlotte S. Kaetzel[2], [1]Case Western Reserve University, Cleveland, OH 44106 and [2]University of Kentucky, Lexington, KY 40536.

The long accepted host defense function of mucosal IgA is to act as a lumenal exclusion barrier to prevent antigens and microbes from adhering to or penetrating the lining of a mucous membrane in locations like the respiratory and intestinal tracts. This function is illustrated by the ability of passively administered specific IgA monoclonal antibodies to inhibit infection in immunologically naive mice when Sendai virus is instilled into the upper respiratory tract. A second mucosal location where IgA antibody can operate is within the lining epithelial cells in the case of intracellular pathogens like viruses. This situation follows from the natural route by which mucosal IgA reaches the lumenal secretions, namely by receptor-mediated endocytosis and transport through the interior of the lining epithelial cells rather than by diffusion between epithelial cells. This function of IgA has been demonstrated with Sendai and influenza viruses, initially in polarized epithelial monolayers and more recently by experiments in vivo in which IgA antibodies were more effective than IgG antibodies. Such intracellular virus neutralization can aid in recovery from infection. A third defense function of mucosal IgA antibodies is to complex antigens in the lamina propria and excrete them through the lining epithelium via the same route taken by free IgA, thereby minimizing the load of immune complexes reaching the general circulation. This function has been demonstrated both with polarized epithelial monolayers and in vivo after oral immunization.

J1-017 IgA, A HETEROGENEOUS IMMUNOGLOBULIN WITH VERSATILE FUNCTIONS, Michael W. Russell[1], Elena B. Nikolova[1], Lonette S. Phipps[1], Pamela L. White[1], Jiri Mestecky[1], and Mogens Kilian[2], [1]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, [2]Department of Medical Microbiology, University of Århus, Århus, Denmark.

IgA is the most heterogeneous of human immunoglobulins, occurring in monomeric, polymeric, and secretory (S-) molecular forms, and two subclasses, IgA1 and IgA2, which are differentially distributed in various physiological compartments. Circulating IgA, produced largely in the bone marrow, is predominantly monomeric, and mainly of the IgA1 subclass; S-IgA, comprising similar amounts of both subclasses, is the product of the mucosal immune system and is polymeric, containing J chain and secretory component (SC). Despite conflicting reports, the bulk of the evidence demonstrates that IgA antibodies are essentially non-inflammatory and probably have anti-inflammatory properties. Intact human IgA antibodies of all molecular forms fail to activate complement by either major pathway, and interfere with complement activation by IgG antibodies, thereby diminishing some of the consequent inflammatory reactions, as well as inhibiting C3b-dependent interactions with phagocytes. S-IgA antibodies at mucosal surfaces are well-known for their potential to inhibit adherence of microorganisms, which is considered to be an essential first step in pathogenesis. If the epithelium is breached, the abundant IgA in the mucosal environment may act to limit the inflammatory consequences of exposing the submucosal tissues to the antigenic load. However, neutrophils are rapidly recruited from the circulation to sites of bacterial invasion by chemotactic factors such as IL-8, which is produced by epithelial cells upon bacterial challenge, and this also increases surface expression of the IgA receptor, FcαR, on neutrophils. Such stimulated neutrophils show enhanced ability to phagocytose particles opsonized with IgA antibodies and to mount the oxidative metabolic response, thereby contributing to the elimination of invading microorganisms but without involving complement. Numerous significant mucosal pathogens have exploited the properties of IgA and the unique hinge-region of human IgA1, which they cleave by means of specific IgA1 proteases to yield Fabα and Fcα fragments. The Fabα fragments retain antigen-binding activity, and have the same ability as intact IgA1 to inhibit complement activation, but lack the protective properties associated with the Fcα.SC part of intact S-IgA. Organisms whose antigenic surfaces are blocked by Fabα antibody fragments are thus shielded from other specific immune defense mechanisms of the host. As the host responds by generating antibodies that inhibit IgA1 proteases, a determinant of protective immunity against these organisms may be the ratio: [IgA1-protease-inhibiting antibody] / [IgA1 antibody to surface antigens]. If this is correct, inclusion of IgA1 proteases in vaccines could add an important component to immunization strategies against these infections. Supported by US-PHS grants DE-09691 and DE-08228.

New Directions in Mucosal Vaccine Strategies I (Live Bacterial and Viral Vaccines and Vectors: An Update)

J1-018 BCG: A MUCOSAL VACCINE VECTOR, Solomon Langermann*, Susan Palaszynski*, Ariadna Sadziene‡, David McMurray¶, David Saunders*, and Scott Koenig*. MedImmune, Inc. Gaithersburg, MD*, University of Texas Health Science Center, San Antonio, TX‡, and Texas A&M University, College Station, TX¶

Intranasal (IN) vaccination of mice with live Bacille Calmette-Guerin (BCG) expressing the outer surface protein A (OspA) antigen from *Borrelia burgdorferi*, results in a prolonged and protective systemic immune response as well as a secretory IgA response against OspA. The secretory immune response is disseminated throughout the mucosal immune system including the upper respiratory, gastrointestinal, and urogenital tracts. Intranasal immunization also induces local, cellular immune responses to mycobacterial antigens within the lower respiratory tract of mice. In addition, immunization via the intranasal route induces highly organized, marked lymphocytic accumulations in bronchial, nasopharyngeal, and gut-associated lymphoid tissues. The appearance of lymphoid aggregates four to six weeks after immunization correlates with the emergence of a local immune response, and the persistence of such lymphoid aggregates may be associated with the sustained IgA response. Aside from the observed responses in mice, intranasal immunization of guinea pigs with recombinant BCG (rBCG) OspA also results in a systemic immune response to OspA as well as a local, cellular response to mycobacterial antigens. Furthermore, intranasal immunization with either recombinant or non-rBCG protects guinea pigs from aerosol challenge with virulent *M. tuberculosis*. Taken together, these results demonstrate that a single intranasal immunization with rBCG is a powerful method for inducing long-lasting secretory, systemic, and local immune responses. Thus, rBCG (or, in the case of TB, non-rBCG as well) may be a valuable vaccine vehicle for inducing primary protection against respiratory, gastrointestinal, and sexually transmitted organisms which gain entry via mucosal surfaces, while at the same time providing a long-lasting systemic IgG response.

J1-019 OVERVIEW OF MUCOSAL IMMUNIZATION WITH ATTENUATED *Vibrio cholerae, Shigella, Escherichia coli* and *Salmonella typhi*, Myron M. Levine[1], [1]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore MD 21201.

Engineered strains of *V. cholerae* (O1 & O139), *E. coli, Shigella*, and *S. typhi* can serve as well-tolerated, immunogenic and protective live oral vaccines to prevent, respectively, cholera, enterotoxigenic *E. coli* (ETEC) diarrhea, shigellosis and typhoid fever. The pathogenesis of each of these infections involves progressively greater invasion relative to the intestinal mucosa: vibrios and ETEC adhere via fimbriae but do not invade; *Shigella* invade colonocytes multiplying therein and in the lamina propria; *S. typhi* translocate the intestinal mucosa and ultimately reach the organ of the reticuloendothelial system (spleen, liver, bone marrow, etc.). A single oral dose of *V. cholerae* O1 strain CVD 103-HgR confers 100% protection against severe ($\geq$ 5.0 liter purge) and moderate ($\geq$ 3.0 l purge) cholera caused by either O1 biotype or serotype in the volunteer experimental challenge model. Protection commences as early as 8 days and continues undiminished for at least 6 months post-vaccination (longest interval tested so far). The key to broad protection against ETEC is to stimulate SigA-mediated mucosal immunity against the common antigenic types of ETEC fimbriae. Oral immunization of guinea pigs with an $_{\Delta}$*aroA*,$_{\Delta}$*virG* mutant of *S. flexneri* 2a elicted IgA O antibody in tears and protected against conjunctival sac challenge with wild type *S. flexneri* 2a. A recently modified volunteer model that results in high attack rates (ca 90%) of typical shigellosis following ingestion of low inocula (10^3 organisms) allows pertinent studies of human immunity to *Shigella*. Quantitation of trafficking gut-derived IgA antibody secreting cells (ASCs) in peripheral blood is emerging as a correlate of protection for oral *Shigella* vaccines. A single oral dose of well-tolerated $_{\Delta}$*aroC*,$_{\Delta}$*aroD S. typhi* strain CVD 908 is impressively immunogenic, eliciting vigorous serum antibodies and mucosal and cell-mediated immune responses. These oral vaccines are also being utilized as live vectors to stimulate relevant serum, mucosal or cell-mediated immune responses against expressed foreign antigens derived from unrelated pathogens.

Viruses

J1-020 RECOMBINANT POLIOVIRUS EXPRESSING HIV PROTEINS AS MUCOSAL VACCINES, Casey D. Morrow, Donna C. Porter, Marie J. Anderson, Patricia N. Fultz and Zina Moldoveanu, University of Alabama at Birmingham, Birmingham Alabama 35294.

In order for a vaccine to stimulate the mucosal immune system, it must be capable of delivering the desired antigens to immunoreactive sites such as the gut associated lymphoid tissue. Poliovirus is attractive for the development of such a vector because the natural transmission of the virus is via a fecal oral route and is thus stable in the harsh environment of the gastrointestinal tract. The attenuated strains of poliovirus have been used to produce an effective vaccine against poliomyelitis which has resulted in the virtual eradication of the disease in developed countries. The vaccine is relatively safe and is routinely given orally to infants resulting in the generation of both systemic and mucosal immunity.

To develop poliovirus as a vector, previous studies from this laboratory have demonstrated that gene segments of HIV *gag*, *pol* or *env* could be substituted for the capsid gene of poliovirus. Following *in vitro* transcription, the transfection of RNA's into tissue culture cells resulted in the expression of HIV proteins. In recent studies, we have constructed poliovirus genomes which contain the complete full length HIV-1 *gag* or SIV *gag* gene. Transfection of these RNA's into cells resulted in the expression of the Pr55^{gag} protein which was released from the cells in the form of a virus particle. We have also generated poliovirus genomes which contain the full length HIV-1 *env* (gp160) as well as replicons which encode HIV and SIV gp120. Transfection of these RNA's into cells results in the expression of the appropriate HIV or SIV envelope protein. Since the HIV/SIV genes were substituted for the P1 capsid gene, the *in vitro* transcribed RNA's were transfected into cells previously infected with the recombinant vaccinia virus (VV-P1), which expresses the poliovirus capsid precursor protein P1, to encapsidate the genomes. Reinfection of cells with the encapsidated genomes resulted in the expression of the HIV/SIV proteins demonstrating that the genomes were encapsidated. Serial passage of the encapsidated HIV/SIV-poliovirus genomes in the presence of VV-P1 resulted in sufficient quantities for immunization. Mice susceptible to poliovirus immunized with the encapsidated replicons produced antibodies specific for HIV or SIV proteins. In a related study, we have demonstrated that coinfection of cells with stocks of the encapsidated replicons and either type 1 or type 2 attenuated poliovirus results in the retention of the replicon genomes in virus stocks after serial passage. Administration of these virus stocks into mice (oral) or monkeys (rectal) resulted in the generation of both anti-poliovirus and anti-HIV specific antibodies in the serum and secretions. Studies are ongoing to formulate a cocktail vaccine containing encapsidated replicons expressing the *gag* and *env* proteins from different HIV strains which can be used for oral immunization. The results of these studies then establish the use of encapsidated poliovirus replicons is a new vaccine vector system for the delivery of antigens to the mucosal immune system.

J1-021 GENETIC ENGINEERING OF INFLUENZA VIRUS FOR USE IN VACCINATION, Peter Palese and Adolfo García-Sastre, Department of Microbiology, Mount Sinai School of Medicine, New York, NY 10029.

Only recently has it become possible to introduce site-specific mutations into the genome of influenza virus, a negative strand RNA virus. Ribonucleoprotein complexes consisting of synthetic RNAs and purified polymerase complex can be rescued into infectious influenza virus. This technology gives us a rational approach to the attenuation of influenza viruses for use as live virus vaccines. The introduction of changes in the noncoding region (promoter) of the neuraminidase gene has allowed the construction of viruses which are attenuated in the mouse model. Alternatively, changes in the coding regions of specific genes (the neuraminidase, the hemagglutinin or the NP gene) also resulted in the attenuation of influenza viruses. The successful development of live influenza A and B virus vaccines will depend on the stability and efficacy of such genetically engineered strains.

Influenza virus has the ability to induce in its host high level B and T cell immune responses--as well as specific mucosal immunity. Thus, influenza virus recombinants may be useful in eliciting protecting immune responses against different pathogens. Specific foreign epitopes derived from different viruses or parasites (plasmodia) have been introduced into the hemagglutinins and neuraminidases of influenza viruses, resulting in chimeric viruses capable of inducing a systemic as well as a mucosal immune response in mice. For example, novel influenza virus recombinants expressing epitopes of the HIV env protein were shown to induce in mice a neutralizing humoral and mucosal immune response against the virus. CD8+ T cell-specific responses were generated by chimeric influenza viruses expressing specific epitopes of the circumsporozoite (CS) protein of *P. yoelii* and *P. falciparum*. In addition to expressing foreign epitopes, it is now possible to express entire proteins derived from foreign pathogens by constructing chimeric influenza viruses which code for polyproteins and/or bicistronic genes.

These data support the concepts (1) that genetically engineered influenza viruses can serve as effective live virus vaccine candidates and (2) that influenza virus chimerae expressing foreign epitopes/proteins may allow simultaneous vaccination against multiple pathogens.

Mucosal Immunity: New Strategies for Protection Against Viral and Bacterial Pathogens

New Directions in Mucosal Vaccine Strategies II (Delivery Systems and Adjuvants for Immunization with Proteins, Peptides and DNA)

J1-022 DNA VACCINES, A NEW APPROACH TO IMMUNIZATION, H.L. Robinson[1], S. Lu[1], D. Feltquate[1], R.G. Webster[2], and J.R. Haynes[3], [1]Department of Pathology, University of Massachusetts Medical Center, Worcester, MA 01655;[2]Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN 38101-0318, [3]Agracetus Inc. Middleton, WI 53562

Direct DNA inoculations are being developed as a method of subunit vaccination. In this method, plasmid DNAs are constructed that are eukaryotic expression vectors for the proteins to be used as immunogens. The constructed DNAs are directly administered to the animal to be vaccinated. The take up and expression of the vaccine DNA by host cells initiate the immune response. Early studies using DNA vaccines have demonstrated the raising of long lasting humoral, cell mediated, and protective responses.

DNA vaccine trials in my laboratory have used influenza and immunodeficiency virus models. Vaccinated animals have included chickens, mice, ferrets, rabbits, and monkeys. Vaccine DNAs have been administered by injections in saline as well as gene gun delivery of DNA-coated gold beads. These trials have demonstrated immune responses following both parenteral and mucosal administrations of DNA. They have also demonstrated that gene gun delivery of DNA is much more efficient at raising responses than direct injections in saline.

Unsolved Problems in Mucosal Vaccine Strategies Against Specific Mucosal Pathogens: Roundtable

J1-023 **PROPHYLACTIC AND THERAPEUTIC IMMUNIZATION AGAINST *H. FELIS* INFECTION WITH *H. PYLORI* UREASE SUBUNITS,** P. Michetti, I. Corthésy-Theulaz, A.-C. Vaney*, R. Haas, J.-P. Kraehenbühl**, E. Saraga*, A.L. Blum. Division of Gastroenterology, CHUV, Lausanne; *Pathology Institute, Lausanne University; **Biochemistry Institute and Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland.

Infection of the gastric mucosa by *Helicobacter pylori* is an essential factor in the development of peptic ulcer disease and in gastric malignancies such as gastric carcinomas and lymphomas. We previously showed that immunization with *H. pylori* urease was protective against *H. felis* infection in mice. Since urease is toxic, we examined whether the enzymatically inactive recombinant subunits A (UreA) and/or B (UreB) of *H.pylori* urease were as effective as the holoenzyme. UreA and UreB were expressed in *E.coli*. Female BALB/c mice recieved four weekly doses of UreA or UreB or UreA+UreB. UreA and UreB were bound to hydroxyapatite (HAP). Cholera toxin was used as an adjuvant (CT). Control mice received CT and HAP alone. Animals were infected 7 days later with 3 doses of 1-2x10^8 *H.felis* at 2 day intervals. Infection was assessed 12 and 70 days after the last challenge by rapid urease test and microscopy. Twelve days post-challenge, all UreA- and UreB-immunized mice as well as all control mice were infected, but a decrease in urease test readings was observed in UreB-immunized mice (0.18±0.23 [mean±SD], versus 0.47±0.15 in controls, p=0.014; Mann Whitney U-test). Seventy days post-challenge, 10/17* UreA- and 16/20** UreB-immunized mice were completely free of infection, while 12/12 control were infected (*p=0.019; **p=0.00002; Fisher's Exact vs controls). We then sought to test whether such an immunization could be therapeutically effective when given to an infected host. Mice were first infected with *H. felis*, and after 3 weeks latency, were vaccinated as above. Gastric biopsies were assessed by urease test and by microscopy 3-4 or 8 weeks after vaccination. At 8 weeks a group of mice were tested non-invasively by ^{14}C-urea breath test (BT) to monitor disappearence of the infection. Fourteen of out sixteen cured mice were subsequently challenged twice with infectious *H. felis* to assess secondary immunity.

a) Cure of *H. felis* infection (infected/total)

H. felis	3-4 wks	8 wks	BT
Control	0/16	0/8	10/10
UreB	8/18*	5/9**	16/28†

Fisher Exact: *p=0.0019 **p=0.04 †p=0.002

b) Secondary immunity to *H. felis* (infected/total)

2x challenged	2.5 wks
4 naive mice	4/4
14/16	5/14

These results show that i) prophylactic vaccination with UreA and UreB are protective against *H. felis* infection but do not prevent a transient colonization of the gastric mucosa, ii) *H. pylori* UreB given orally to *H. felis*-infected mice induces clearance of the infection, iii) therapeutic immunization protects against reinfection.

Conclusion: 1) Recombinant enzymatically-inactive UreA and UreB induce protection against *H. felis* infection. 2) The lack of natural immunity against *Helicobacter* infection can be overcome. 3) Vaccination might be used both in the prevention and in the treatment of *Helicobacter* infections. Supported by SNF 32-36349.92 and by OraVax, Inc., Cambridge, MA

J1-024 PROTECTIVE IMMUNITY TO *BORDETELLA PERTUSSIS* INFECTION,
Roberta Shahin, Mary Leef and Jerko Barbic, Center for Biologics Evaluation and Research, FDA, Bethesda, Md.

We are using a murine model of *Bordetella pertussis* respiratory infection to analyze mechanisms of protective immunity at a mucosal site. Intranasal immunization with purified *B. pertussis* antigens encapsulated in either poly(DL-lactide-co-glycolide) microspheres or in multilamellar liposomes elicited high levels of specific serum IgG as well as IgG and IgA in mucosal secretions; this immunization also resulted in a decrease in bacterial recovery from the lungs and tracheas of animals experimentally infected with an aerosol of *B. pertussis* by 2-3 $\log_{10}$ cfu in comparison to infected controls. In contrast, intranasal immunization with formalin-fixed whole cell *B. pertussis* prior to infection resulted in almost complete clearance of the bacteria from the lungs and tracheas of immunized mice (a decrease of >6 $\log_{10}$ cfu), with little or no detectable specific antibody to known pertussis antigens in the serum or secretions. LPS resistant C3H/HeJ mice immunized with formalin-fixed *B. pertussis* cleared the infection as efficiently as LPS responsive C3H/HeN mice immunized with formalin-fixed *B. pertussis* suggesting that the observed clearance is not due to inflammation stimulated by endotoxin. Intranasal immunization with formalin-fixed *E. coli* did not protect against respiratory *B. pertussis* infection. Mice that were intranasally administered formalin fixed modulated *B. pertussis*, which do not express environmentally regulated virulence determinants under the control of the *bvg* locus, also cleared a *B. pertussis* challenge, but to a lesser extent than those immunized with formalin fixed virulent *B. pertussis*. Nude mice administered formalin-fixed *B. pertussis* intranasally had no decrease in bacterial recovery from the respiratory tract in comparison to unimmunized controls. Populations of T cells that proliferate specifically in response to formalin fixed virulent *B. pertussis* and formalin fixed modulated *B. pertussis* are generated in the draining lymph nodes of the lungs of mice immunized intranasally with formalin fixed *B. pertussis*. Upon antigen stimulation in vitro, these T cells produce γ-interferon, but not IL-4. These data suggest that the protection observed with formalin fixed *B. pertussis* administered intranasally is antigen specific, requires T cells, and may be due to both *bvg* regulated and *bvg* repressed and/or *bvg* independent gene products.

Roundtable I: Mechanisms of HIV and SIV Entry Via Mucosal Surfaces

J1-025 IN VITRO EVIDENCE THAT TRANSMISSION OF HIV INVOLVES MUCOSAL INFECTION, David M. Phillips, Rachael Pearce-Pratt, Xin Tan, and Vanaja R. Zacharopoulos., The Population Council, 1230 York Avenue, New York, New York 10021

We have established an in vitro model to examine if HIV could directly infect epithelia at the portal of entry. To accomplish this we grew established cell lines derived from the gut and cervix, which are the potential sites of HIV entry during sexual transmission. Initially, we attempted to infect these epithelial cell lines with HIV from culture supernatant of HIV-infected transformed T-lymphocytes but we had limited success. However, when we added chronically infected T-cell or monocyte-cell lines to the epithelia, we were able to detect infection with relatively insensitive methods, including immunocytochemistry, in situ hybridization, and p24 ELISA. This finding is especially relevant to AIDS as numerous studies have shown that the principal target cells of HIV, lymphocytes and monocyte/macrophages, are present in blood, semen and the vagina, and that HIV positive individuals contain infectious mononuclear cells in genital tract secretions. We asked why HIV-infected cells were so much more efficient in infecting epithelial cells than free virus. The first clue was that the mononuclear cells remained adherent to the epithelia even after the cultures were washed fairly vigorously. As early as 15 minutes after addition of the infected cells, we observed HIV budding from the mononuclear cell at the point of contact with the epithelium; but not at other regions of the HIV-infected cell. With the transmission electron microscope we observed HIV budding from the mononuclear cell at the point of contact with the epithelium, but not at other regions of the HIV-infected cell. At later times after addition of the HIV-infected mononuclear cells to the epithelium, we observed numerous virions apparently trapped in the space between adherent mononuclear and epithelial cells. Depending on the type of epithelium, virus appeared to be taken up by one or more of three different mechanisms. In an epithelium derived from a carcinoma of the human cervix, virions entered the epithelium via large smooth vesicles similar to those observed in nonspecific phagocytosis. In an intestine-derived epithelial cell line, we saw virions taken up by both coated pits and large smooth vesicles as well as by direct fusion with the cell membrane. We cloned a cell line of the cervix-derived epithelium which produces so much virus, that budding of HIV can be easily appreciated in the electron microscope. Virus is secreted both apically and basally. Based on these observations of transformed cell lines, we have proposed that transmission of HIV may involve a cell-to-cell entry mechanism involving HIV-infected immune system cells, that occur in body fluids of HIV-positive individuals, and intact CD4-negative epithelial cells at the site of entry. We suggest that this process may be initiated by adherence of HIV-infected cells to epithelia of noninfected individuals. Adherence could result in secretion of virus into a space between the infected mononuclear cell and the epithelium. Virions would enter the epithelial cell, causing productive infection, and new virus would be produced and secreted. New virions would then infect mononuclear cells in the connective tissue and, if it were a stratified epithelium, infect the epithelial cell below it to spread the infection.

Mucosal Immunity and HIV/SIV: Prospects for A Mucosal Vaccine

J1-026 GENERATION OF DIVERSITY IN T CELL EPITOPE HIERARCHY BY MUCOSAL IMMUNIZATION, AND PROTECTIVE IMMUNITY AGAINST RECTAL INFECTION BY SIV IN MACAQUES. Thomas Lehner[1], Roger Brookes[1], Lesley A. Bergmeier[1], Linda S. Klavinskis[1], Elaine Mitchell[1], Louisa Tao[1], Martin P. Cranage[2], Graham Hall[2], Michael Dennis[2], Nicola Cook[2], K. Doyle[3] and Ian Jones[3]. [1]Department of Immunology, UMDS at Guy's Hospital, London SE1 9RT and [2]PHLS, CAMR, Porton Down, Salisbury, Wilts SP4 OJG, [3]NERC Institute of Virology, Mansfield Road, Oxford OX1 3SR.

Sexual transmission of HIV occurs through the rectal or genital mucosa. A comparative investigation has been carried out in rhesus macaques immunized with SIV p27 expressed as hybrid Ty virus-like particles (p27:Ty-VLP) by the rectal or genital route augmented by oral immunization. These were studied in relation to intramuscular and a novel 'targeted lymph node' (TLN) immunization. Three levels of immunity were established: (1) secretory IgA antibodies at the rectal or genito-urinary mucosa, (2) T and B cell immune responses in the draining lymph nodes and (3) splenic and circulating T cell responses and serum IgG and IgA antibodies. Short term cell lines (STCL) of mononuclear cells were then generated from peripheral blood mononuclear cells (PBMC), spleen and the internal iliac lymph nodes by stimulation with SIV p27:Ty-VLP, followed by restimulation of the STCL with 22 overlapping synthetic peptides (20^{mers}), derived from the sequence of SIV p27 molecule. A hierarchy of 4 T cell epitopes was established which differed significantly with the 5 routes of immunization. Intramuscular immunization induced a significantly higher frequency of circulating or splenic T cell proliferation with peptides 121-140, 41-60 or 61-80 than the corresponding cells from macaques immunized by the genital routes ($p<0.05$-$p<0.01$). However, iliac lymph node cells showed significantly higher T cell proliferation with peptide 121-140 or 61-80 after genital than intramuscular immunizations ($p<0.05$).

In order to test the protective potential of the sub-cutaneous TLN route of immunization, 4 male rhesus macaques were injected with SIV gp120 and p27 antigen in Alum. Controls consisted of 7 macaques of which 4 were unimmunized and 3 were injected with Alum and an unrelated antigen. Serum, rectal, urethral, urinary and seminal fluid IgA and IgG antibodies to gp120 and p27 were elicited in the 4 immunized but not in the 7 control macaques. T cell proliferative ($CD4^+$ cell) responses to gp120 and p27 were found in the 4 immunized but not the control macaques. Rectal mucosal challenge with a cell-free molecular clone of SIVmac J5, infected all 7 control macaques, but of the 4 immunized macaques 2 yielded no detectable virus or provirus DNA and 2 showed a significant decrease in the viral load ($p<0.001$). The T cell epitope diversity expressed by the two macaques demonstrating sterilising immunity appears to differ from the two macaques which showed a decrease in the viral load. As the sIgA and IgG antibody titres of rectal and serum antibodies did not differ between the macaques with total and partial immunity against rectal infection with SIV, the hypothesis will be pursued that the T cell epitope hierarchy might be one of the major factors determining the level of immunity.

J1-027 MUCOSAL IMMUNE RESPONSES OF MACAQUES TO SIV INFECTION AND HIV/SIV VACCINES, Christopher J. Miller[1], Michael McChesney[1], Barbara Lohman[1], Yichen Lu[2], Raul Andino[3], Mark Feinberg[3]. [1]California Regional Primate Research Center, University of California-Davis, Davis, CA 95616, Virus Research Institute[2], Cambridge MA and [3]Gladstone Institute of Virology and Immunology, University of California , San Francisco.

In order to protect against the sexual transmission of HIV, an ideal vaccine would prevent or minimize the spread of the virus from the genital mucosa to the systemic lymphoid tissues. To accomplish this, a vaccine must elicit a protective genital mucosal immune response as well as elicit protective immunity in the systemic lymphoid tissue. Because little is known about the immune response of the female genital tract to infection or immunization, we have undertaken studies to characterize the local immune response to intravaginal infection with SIV and the mucosal immune response to SIV and HIV antigens delivered using novel immunization strategies. First we sought to determine if SIV-specific cytotoxic T lymphocytes (CTL) could be detected in the vaginal mucosa of four chronically, and 2 acutely, SIV-infected female rhesus macaques. We have previously demonstrated that large numbers of CD8+ lymphocytes are present in the vaginal epithelium and superficial submucosa of rhesus macaques. Using immunomagnetic beads, CD8+ T cells were isolated from the vaginal epithelium and submucosa and assayed for their ability to lyse autologous target cells expressing SIV antigens (gag/env) in a limiting dilution format chromium release assay. SIV-specific CTL to at least one SIV antigen were detected in the CD8+ vaginal intraepithelial lymphocytes all of the SIV-infected animals, but not in an uninfected control. The precursor frequency of SIV-specific CTL in the vaginal epithelium was similar to what has been reported in systemic lymphoid tissues. This is the first direct demonstration of viral specific CTL activity in the vaginal epithelium. Next we sought to elicit specific mucosal immune responses in immunized rhesus macaques. We immunized cynomologous macaques intrarectally with a recombinant poliovirus containing the HIV nef gene. By 2 weeks post-immunization, HIV nef-specific IgG and IgA were detected in the sera and HIV nef-specific IgA was detected in the rectal washes of one animal. The immune responses of the animals to additional booster immunizations are currently being characterized. In addition, 4 female rhesus macaques have been immunized perivaginally with naked DNA encoding the HIVgag, env and nef genes. The DNA used to immunize 2 of the animals was mixed a polyphosphacene adjuvant in PBS while the DNA used to immunize the other 2 animals was suspended in PBS alone. The systemic and mucosal immune responses of the animals to this vaccination protocol is currently being assessed.

Mucosal Immunity: New Strategies for Protection Against Viral and Bacterial Pathogens

Late Abstract

TRAFFICKING OF MUCOSAL IMMUNOCYTES: CELLULAR AND MOLECULAR ASPECTS, Cecil Czerkinsky, I.N.S.E.R.M Unit 80, Lyon, France and Department of Medical Microbiology and Immunology, University of Göteborg, Sweden. The concept of a common mucosal immune system that provides immune reactivity not only at the site of antigen deposition but also at certain remote mucosal sites may be explained by the utilization of organ-specific recognition molecules by circulating precursors of mucosal B and T cell immunoblasts and by the production of certain maturation factors (e.g. hormones, cytokines) produced in certain organs or microcompartments. This notion may explain the unification of immune responses in diverse mucosal sites, the preferential redistribution of mucosal immunocytes in certain tissues, and the physiological segregation of mucosal from systemic immune mechanisms.

Novel methods have been developed to enable studies of antigen-specific B and T cell responses in various mucosal and extramucosal tissues in primates and in rodents, using cholera toxin and its B subunit as prototype immunogens and carrier-delivery systems. The tissue localization and homing potential of antibody-forming cells and their circulating precursors have also been examined after oral, nasal, intratonsilar, rectal and/or genital immunization(s). The anatomical distribution of T- and accessory-cell-derived cytokines has also been examined by means of a novel technique. These tools are being employed to evaluate optimal mucosal immunization regimens against several pathogens including HIV-1. The results of these studies have obvious implications for the design of vaccine formulations against pathogens encountered in different mucosal sites.

Mucosal Immunity: New Strategies for Protection Against Viral and Bacterial Pathogens

Organization and Function of the Mucosal Immune System

J1-100 **INDUCTION OF SYSTEMIC AND MUCOSAL ANTIBODIES TO PNEUMOCOCCAL ANTIGENS BY MUCOSAL IMMUNIZATIONS.**
Ingeborg S. Aaberge, Else-Carin Groeng, Inger Lise Haugen, Rolf Dalseg, Martinus Løvik and Bjørn Haneberg, Vaccine Department, National Institute of Public Health, N-0462 Oslo, Norway.

To study the local mucosal and serum antibody responses to polysaccharide encapsulated bacteria, suspensions of heat inactivated *Streptococcus pneumoniae* type 4 were administered at various mucosal sites to adult BALB/c mice. At weekly intervals, four groups of six mice each were immunized four times, by either nasal, oral, gastric or colonic-rectal deliveries of $2x10^8$ bacteria, with 5µg cholera toxin as an adjuvant. A group of six untreated mice served as controls. Samples of serum, saliva, pulmonary lavage fluid and feces were collected one week after the last dose. Specific IgA and IgG antibodies to whole bacteria and IgA, IgG and IgM antibodies to type 4 polysaccharide, as well as total IgA and IgG, were determined by ELISA.

Nasal and oral immunizations induced significant IgA antibody responses to whole bacteria in serum, saliva and pulmonary secretions. In addition, nasal immunizations induced significant IgA antibody responses in extracts of feces. Gastric immunizations did not induce any response, and colonic-rectal immunizations gave IgA responses only in serum and feces. Significant IgG antibody responses to whole pneumococci were detected only after nasal immunizations, and only in serum and pulmonary secretions, which generally had high concentrations of total IgG. Nasal and colonic-rectal immunizations induced IgA antibody responses to type 4 polysaccharide in feces. Immunization by all mucosal routes induced serum IgA as well as IgM antibodies to the polysaccharide. However, no IgG antibody response to polysaccharide was detected neither in serum, nor in samples representing respiratory and intestinal secretions.

With the use of whole pneumococci, nasal immunizations were thus superior to other mucosal immunization sites which were tested for induction of both systemic and mucosal antibody responses. The lack of an IgA antibody response in feces to oral and gastric immunizations, while nasal immunizations induced strong fecal responses, is suggestive of a cellular link between the nasal inductor and the intestinal effector sites for mucosal antibody secretion. The results may indicate that the antibody responses to whole bacteria represent activity against different pneumococcal antigens.

J1-101 ENHANCED HOST DEFENSE MECHANISMS SUPPORTED BY DELIVERY OF 1,25 DIHYDROXYVITAMIN D3 (1,25 VITAMIN D3) AND DEHYDROEPIANDROSTERONE (DHEA) AS IMMUNOMODULATORS IN MURINE RESPIRATORY INFECTIONS. Barbara Araneo, Julie Zhu, Xia Chao, and Tad Dowell. Paradigm Biosciences Inc., 2401 Foothill Blvd. and the University of Utah School of Medicine, Salt Lake City, Utah 84132.

The objectives of the present study were to establish whether a prechallenge immunization of adult mice with a vaccine preparation that contains specific immunomodulators would enhance protection from a mucosal challenge dose of the homologous pathogen. The experimental immunomodulators were designed as vaccine supplements. These supplements were comprised of either 1,25 dihydroxyvitamin D3 (1,25 vitamin D3) alone or in conjunction with dehydroepiandrosterone (DHEA). Groups of adult Balb/c mice were immunized with a trivalent Influenza vaccine containing a Type A H3N2 strain, for which cross protection was elicited in mice by intranasal challenge with another H3N2 strain. Immunization groups, made up of 10 animals each, were 1) nonimmune, 2) vaccine with the vehicle, 3) vaccine with 1,25 vitamin D3 and 4) vaccine with 1,25 vitamin D3 and DHEA. Vaccine compositions were controlled for the volume of antigen, alum and ethanol vehicle used to dissolve the modulators. Either 1,25 vitamin D3 or DHEA plus 1,25 vitamin D3 in conjunction with the vaccine promoted higher titers of neutralizing serum antibody responses against Type A influenza. Only the groups given a prechallenge immunization with the 1,25 vitamin D3 or the combined modulators were able to produce detectable levels of virus neutralizing antibody in nasal wash material three days post challenge. These results indicate that immunomodulators can be effective in promoting host defense mechanisms against pathogens that colonize and cause infection through mucosal epithelia.

J1-102 THE PEYER'S PATCH HEV LYMPHOCYTE-ENDOTHELIAL CELL ADHESION CASCADE, Robert F. Bargatze$^{+\Delta}$, Mark A. Jutila^{+}, David P. Andrew$^{\Delta}$, and Eugene C. Butcher$^{\Delta+}$, $^{+}$Veterinary Molecular Biology, Montana State University, Bozeman, MT 59717; the $^{\Delta}$Laboratory of Immunology and Vascular Biology, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305 and the Center for Molecular Biology in Medicine, Veterans Administration Medical Center, Palo Alto, CA 94304. During lymphocyte recirculation the attachment of lymphocytes to high endothelial venules (HEV) of Peyer's patches (PP) has been shown to involve the participation of a number of adhesion receptors and their counter ligands. These include the CD11/18 (LFA-1) and $\alpha4/\beta7$ integrins, and L-selectin on the leukocyte, as well as ICAM and MAdCAM-1 on the endothelium. In addition, a pertussis toxin (Ptx) sensitive signaling step facilitates lymphocyte arrest from rolling by strengthening adhesion. It has been hypothesized that these receptors and signaling events participate in an integrated adhesion cascade, but this has never been directly shown. Using *in situ* video microscopy we found that 1) L-selectin can mediate normal lymphocyte-HEV rolling via MAdCAM-1 and 2) $\alpha4/\beta7$ can further act, after L-selectin initiation of rolling, to slow and 3) arrest the lymphocyte on the HEV by binding to an L-selectin independent epitope of MAdCAM-1. These events were found to be regulated by a Ptx sensitive pathway leading to functional activation of $\alpha4/\beta7$ integrin. Arrest via $\alpha4/\beta7$ can also be inhibited by treatment with a mAb directed against LFA-1. The studies suggest a possible role for LFA-1 in $\alpha4/\beta7$ activation. Examination of Mn++ or PMA preactivated-integrin-adhesion-participation in lymphocyte binding revealed an L-selectin-independent capacity of cells to rapidly arrest in PP HEV that was insensitive to anti-LFA-1 mAb or Ptx treatment. Anti-$\alpha4$ or anti-$\beta7$ mAb treatment of activated lymphocytes blocked the rapid binding/arrest behavior, showing that LFA-1 expression on activated cells is insufficient to facilitate arrest on PP HEV, even when cells were rolling via L-selectin. Thus, the arrest of lymphocytes from the blood onto PP HEV can occur through at least two distinct scenarios; 1) through a multi-step adhesion cascade initiated by L-selectin rolling on MAdCAM-1 followed by Ptx and anti-LFA-1 mAb sensitive-activation of $\alpha4/\beta7$ integrin binding to MAdCAM-1; or 2) through preactivated $\alpha4/\beta7$ integrin, that singularly arrests lymphocytes from the blood flow via MAdCAM-1. Funding for these studies was provided by GM37734, AI19957, and USDA grant number 93-37206-9348.

J1-103 ALTERED RECIRCULATION OF ACTIVATED B-CELLS IN IgA NEPHROPATHY, Marie C Béné, Anne Kennel, Edith Renoult, Michèle Kessler, Gilbert C Faure, Laboratoire d'Immunologie & Néphrologie, Faculté de Médecine & CHU de Nancy, 54500, Vandoeuvre les Nancy, France

IgA nephropathy is associated with recurrent infections of the upper respiratory tract, and anomalies of the partition of IgA and IgG-producing cells have been demonstrated in the tonsils of patients suffering from this renal disease. This suggests that the physiological regulation of cell trafficking between mucosal areas could also be altered in such patients. Indirect evidence supporting this hypothesis was obtained by studying frozen-cut sections of tonsils from IgAN patients and controls. In the former, the number of HEV-like structures, stained by FITC anti-fibronectin antiserum appeared clearly increased, and adhesion molecules (CD54, CD31) were expressed with strong intensity on endothelial cells. We further investigated the consequences of this hyperactivation of HEV by evaluating the numbers of IgA-containing activated B-cells in the peripheral blood of 30 IgAN patients, 18 normal controls and 34 patients suffering from other renal diseases than IgA nephropathy. Activated B-cells were assessed by the ELISAspot method and in direct immunofluorescence on cytospins. Both tests were performed after separation of peripheral lymphocytes by density gradient centrifugation. Significantly lower numbers of IgA spots (766 ± 105) were noted in IgAN patients, compared to both normal controls (1178 ± 141) and other renal patients (1123 ± 192). A strong correlation was noted in all groups between the numbers of circulating immunoblasts evidenced in immunofluorescence and the number of spots, suggesting that spot-forming cells could indeed represent a subset of activated B-cells on their way between induction sites and MALT areas. These data support the physiopathological hypothesis of a dysregulation of the mucosal immune system specific to IgAN among other nephropathies. The increased expression of adhesion molecules on larger and more numerous tonsillar HEV could be responsible for a faster relocation of circulating activated B-cells originated from inductive sites such as Peyer's Patches. These larger number of cells would then achieve their terminal differenciation in plasma cells and produce increased levels of IgA, some of which would eventually reach the peripheral blood and, ultimately, the renal mesangium.

J1-104 THE MAJORITY OF LYMPHOCYTES MIGRATING TO THE LAMINA PROPRIA OF THE INTESTINAL MUCOSA IN PIGS ARE T CELLS

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Lymphocyte migration studies in the gut immune system have often concentrated on IgA lymphoblasts and little is known to what extent small T and B lymphocytes migrate to the lamina propria (LP) of the mucosa. Therefore the migration pattern of gut derived lymphocytes was studied in 7 adult Göttingen minipigs in which the mesenteric lymph nodes had been excised three months before the main experiments. The gut lymphocytes were obtained by cannulating the intestinal lymph duct and sampling the lymph over three days. Prior to venous retransfusion of $\approx 5*10^8$ lymphocytes the cells were stained with fluorescein isothiocyanate (FITC). The pigs were killed 20 hours later and samples of jejunum and ileum were taken. The epithelial lymphocytes were isolated by EDTA incubation, the LP lymphocytes by collagenase incubation.

Using flow cytometry the percentages of FITC labelled cells were determined in the intraepithelial and LP cell fraction. Among the intraepithelial lymphocytes very few $FITC^+$ cells ($\leq 0.003\%$) were found, showing the low cell immigration during the observation period. In the LP $0.12 \pm 0.07\%$ of the lymphocytes were $FITC^+$. Calculations based on the total number of gut lymphocytes showed that in the LP $\approx 4\%$ of the injected cells were recovered. By indirect immunfluorescence the subset distributions of these $FITC^+$ cells were determined ($CD2^+$ 75.1 ± 16.5%, $CD4^+$ 32.7 ± 30.0%, $CD8^+$ 45.4 ± 30.8%, IgA^+ 7.4 ± 5.2% and IgM^+ 7.2 ±5.6%). A 4.6-fold enrichment of IgA^+ cells was observed in the LP in comparison to the injected cell population (IgA^+ 1.6 ± 0.9%). The IgM positive population remained stable (rate in gut lymph: 7.8%), and the CD2 positive population increased slightly.

In summary, a marked population of immigrated B cells was observed in the LP. This indicates their preferential migration to this tissue compartment. However, most of the immigrated cells were small T lymphocytes. It is so far not known whether these cells have a specific homing pattern for the intestinal mucosa.

J1-105 **MONOCLONAL IgA DERIVED FROM PERITONEAL B CELLS IS ENCODED BY SOMATICALLY MUTATED V_H GENES AND IS REACTIVE WITH COMMENSAL ANAEROBIC BACTERIA,** N.A. Bos, J.C.A.M. Bun, S.H. Popma, E.R. Cebra, G.J. Deenen, M.J.F. van der Cammen, F.G.M. Kroese and J.J. Cebra, Dept of Histology and Cell Biology, Immunology Section, University of Groningen, Oostersingel 69/I, NL-9713 EZ Groningen, The Netherlands.

We have transferred peritoneal cells from BALB/c mice into CB17-SCID mice. Six to eight months after injection only cells with the B1 phenotype were retained in these mice. In the lamina propria of the intestine many peritoneal donor-derived IgA-producing cells were present. The mesenteric lymph nodes of these mice were found to be a major site of proliferation and generation of IgA plasmablasts. We have established IgA-producing hybridomas from the mesenteric lymph nodes of such mice. All eight obtained IgA monoclonal antibodies reacted with different, but partially overlapping, faecal bacterial populations. Cloning and sequencing of the V_H genes of five hybridomas showed somatic mutations, especially in the CDR regions, suggestive of an antigen driven selection process.

J1-106 T LYMPHOCYTES TRAFFIC BETWEEN PERIPHERAL BLOOD AND LUNG: WHAT CAN WE LEARN FROM T CELL RECEPTOR REPERTOIRE STUDIES. SE Burastero, G Casorati, M Vavassori, B Borgonovo, D Gaffi, E Crimi and GA Rossi, from the DIBIT, San Raffaele Scientific Institute, 20132 Milan, and the University of Genoa, 16131 Genoa, Italy.

It has been shown that organs where type I hypersensitivity reactions take place contain allergen specific T cells, likely involved not only in driving B cells to switch to IgE production but also in modulating the underlying inflammation. It is not established whether and to what extent T cells at disease sites are locally expanded and/or recruited from peripheral blood. We addressed this issue by studying the clonal composition and the kinetic of expansion of lung T lymphocytes. At this purpose, 12 asthmatic subjects underwent fiberoptic bronchoscopy and bronchoalveolar lavage (BAL) in one or two segments of the same or controlateral lung, at baseline (6) and 6 or 24 hr following controlled allergen inhalation (6). Allergen specific T cell lines were derived from peripheral blood from these individuals using the same antigen used for bronchial challenge. PCR and heteroduplex analysis were combined to compare the T cell receptor (TcR) variable (V) gene usage and the T cell clonal heterogeneity in BAL T cells and allergen specific T cell lines derived from the peripheral blood. Polyclonality (no emerging bands, continuous smear) was found in 5 out of 6 BAL derived T cells from asthmatics in basal conditions, while oligoclonality (emerging bands), indicating local expansion of T cell clones was observed in one asthmatic individual who suffered a recent exposure to allergen and had an high degree of airway inflammation. At difference, oligoclonality of lung T cells was detected in 6 out of 6 allergen challenged subjects both 6 and 24 hr after an allergen challenge that induced a positive bronchial reaction (increase of airways resistance). The heteroduplex analysis also showed that, among expanded clones, some gave a similar pattern in the BAL and in the corresponding peripheral blood allergen specific T cell lines. The identity of clonotypic determinants of TcR V chains in these two compartments was confirmed by direct sequencing. In addition, the pattern of oligoclonality was virtually identical comparing BAL T cells taken from two different sites of the same or controlateral lung. Our results indicate that: 1) T lymphocytes are clonally expanded in the lower respiratory tract of asthmatic subjects as early as 6 hr after exposure to allergen; 2) these cells include allergen specific T lymphocytes; 3) the lung T cell repertoire contains circulating T cells, that likely have peculiar homing characteristics.

J1-107 THE HINGE DELETION ALLELIC VARIANT OF PORCINE IgA RESULTS FROM A MUTATION AT THE SPLICE ACCEPTOR SITE IN THE FIRST Cα INTRON, J. E. Butler, W.R. Brown, I. Kacskovics, B. Amendt and R. Shinde, Department of Microbiology, University of Iowa, Iowa City, IA 52242

Recently published genomic and cDNA sequences for porcine IgA suggested that the splice acceptor site for the Cα1-Cα2 intron was AA rather than AG dinucleotide. However, the use of riboprobes and an *in vitro* HeLa cell splicing system indicated that splicing occurred at a cryptic AG site 12 nucleotides into the Cα2 domain. The possibility that swine B-cells could use either site was tested by preparing the cDNAs from 13 different samples representing nine animals and amplifying the segment from the first Cα1 nucleotide to nucleotide 532 in Cα2 (genomic DNA numbering system). Analysis on a 6% polyacrylamide sequencing gel revealed two polynucleotide products in most samples which differed by the expected 12 nucleotides suggesting that swine could use both splice sites. Sequence analysis confirmed that the shorter form was spliced at the downstream site and the larger form at the apparent upstream AA site. However, when the genomic DNA from an animal expressing only the longer polynucleotide was cloned and sequenced, the upstream splice acceptor site was rather AG. Hence, the data suggest that porcine IgA occurs in two allelic forms designated IgA^a and IgA^b that differ by an apparent G to A mutation in the last nucleotide of intron 1 resulting in a short-hinge (2 amino acids, IgA^b) variant in which the downsteam cryptic splice site is used as well as a "normal-hinged" (6 amino acids, IgA^a) variant. Evidence that both transcripts are functional was confirmed by showing that serum IgA levels were similar in animals homozygous for each variant.

J1-108 EVIDENCE THAT RESISTANCE TO *STREPTOCOCCUS ZOOEPIDEMICUS* INFECTION AT THE EQUINE ENDOMETRIAL MUCOSAL SURFACE IS INDEPENDENT OF KILLING IN PERIPHERAL BLOOD. Robert C. Causey[1], Dale L. Paccamonti[2], William J.Todd[1,3]. [1]Veterinary Science, LAES, [2]Veterinary Clinical Sciences, [3] Veterinary Microbiology and Parasitology, SVM, LOUISIANA STATE UNIVERSITY, BATON ROUGE, LA 70803. Infection of the mucosal lining of the equine uterus by *S.zooepidemicus* is a common cause of infertility in the broodmare.Mares are classified as susceptible or resistant based on their ability to effectively clear an intrauterine inoculum of *S.zooepidemicus* from the uterus. *Streptococcus zooepidemicus* has been shown to resist the bactericidal properties of fresh blood in a manner similar to M + group A streptococci, based on the requirement for type-specific antibody to mediate successful phagocytosis in blood. To test the role of type-specific antibody in resistance to endometrial infection, 4 mares were repeatedly intrauterinely inoculated with uterine isolates of *S.zooepidemicus*.Response to infection was compared between isolates to which mares did and did not have circulating type-specific antibody.Presence or absence of type-specific antibody in the blood made no detectable difference to the ability of mares to eliminate the organism from the uterus. Therefore local resistance appeared to protect the mare from infection at the uterine mucosal surface. However, in 5 of 5 clinical cases of endometritis, in which local resistance had apparently been breached, high levels of type-specific antibody were present in peripheral blood. The equine uterus appears to posses local defenses against streptococcal infection that are independent of circulating humoral responses. The horse is a convenient model to investigate uterine mucosal immunity to bacterial infections.

J1-109 ANTIGEN PROCESSING PATHWAY IN CACO-2 CELLS. Benjamin M. Chain, Elaine Hughson , Quentin Anstee and David R. Katz. Antigen processing and presentation by epithelial cells of the gastro-intestinal tract may play an important role in regulation of mucosal immunity. As the first step in developing an in vitro model of this event, we have studied the expression of MHC class II molecules in the human intestinal cell line CacCo-2. Exposure of the baso-lateral surface of differentiated, but not undifferentiated CaCo-2 cells to γ-interferon results in the co-ordinate induction of expression of class II MHC (HLA-DR), and the invariant chain in this cell line. Confocal microscopy has been used to localise expression to a population of intra-cellular vesicles in the apical part of the cell, and to the baso-lateral cell surface. These vesicles are largely distinct from both the apical and baso-lateral fluid phase endocytosis compartment, although there is some limited overlap with the basolateral early endosomal (transferrin receptor positive) compartment. In contrast to MHC class II, invariant chain localises to intracellular vesicles, but is absent from the baso-lateral membrane, suggesting that it is processed and removed from the MHC dimer in a normal way. These results demonstrate that activation of CaCo-2 cells leads to a pattern of MHC expression which is consistent with a role in antigen presentation, and will form the basis for further functional studies of antigen processing by this cell line.

J1-110 IN VITRO ASSEMBLY OF SECRETORY IMMUNOGLOBULIN A (sIGA) FROM IGA AND RECOMBINANT SECRETORY COMPONENT, Blaise Corthésy and Lorenz Rindisbacher, Institut de Biologie animale, Université de Lausanne, CH-1015 Lausanne, Switzerland

Secretory IgA (sIgA), the principal immunoglobulin in mucous membrane secretions, consists of one dimeric IgA unit and two additional polypeptide chains, J chain and secretory component (SC). The heavy and light chains and the J chain are synthesized and assembled in plasma cells, while SC is made in the epithelial cells of mucous membranes and exocrine glands. Laboratory production of complete sIgA is therefore complicated since constituent polypetide chains come from two distinct types of cells.
In the frame of a project oriented toward the production of secretory IgA of defined specificity for passive oral protection, in vitro assembly of polypeptides from mouse and human origin has been performed to examine the degree and nature of SC-IgA complex formation. Dimeric IgA were obtained from mouse and human hybridoma, while murine and human *recombinant* SC was produced in *mammalian* and *insect* cells. We report here spontaneous covalent reassociation between purified components. Comparison to protease sensitivity of reconstituted sIgA versus IgA is also presented. Our data clearly establish in vitro reassociation as a source of sufficient amount of sIgA to unravel the function of SC both in vitro and in vivo.

J1-111 **Conversion of orally induced suppression of the mucosal immune response to ovalbumin into stimulation by conjugating ovalbumin to cholera toxin or its B subunit**, Bernard de Geus, Wil stok, Philip van der Heijden and André Bianchi. *Dept. of Immunology, ID-DLO, P.O.Box 65, 8200 AB Lelystad, The Netherlands.*

Oral pretreatment of mice with ovalbumin (OVA) not only supressed a subsequenly induced systemic immune response (oral tolerance) but also suppressed, even more effectively, a subsequent induced intestinal IgA response. In contrast, pretreatment with OVA conjugated to Cholera Toxin (CT) or its B subunit (CTB) resulted in a stimulative effect. The stimulative effect was enhanced when unconjugated OVA and polymerized OVA were removed from the conjugate mixtures by affinity chromatography. Thus, the effect of oral pretreatment depends on the balance between tolerizing and stimulating componets in the conjugate mixture. As OVA-CTB conjugates were at least as effective as OVA-CT conjugates in stimulation of the intestinal immune response, we concluded that the ability of the OVA conjugates to bind to the mucosal mucosa is a prerequisite in inducing the stimulative effect. These observations further demonstrate that conjugation of a protein to an appropriate carrier can convert the nature of the immunization from suppresive into stimulative.

J1-112 **T CELL RECEPTOR EXPRESSION IN IL-2 EXPANDED LYMPHOCYTES FROM ILEAL MUCOSA IN PATIENTS WITH SPONDYLARTHROPATHY.**
Filip De Keyser, Dirk Elewaut, Ilse Hoffman, Eric M. Veys. Dept. of Rheumatology, Ghent University Hospital, Belgium
Spondylarthropathy (SPA) is a chronic inflammatory disease affecting the axial skeleton and peripheral joints. The bowel, more precisely the terminal ileum, is an important extra-articular site of inflammation in this disease. TCR expression in peripheral blood lymphocytes (PBL) and in vitro IL-2 expanded lymphocytes from ileal mucosa from SPA patients was analysed in flow cytometry with a set of monoclonal anti-TCR Vß antibodies (anti-Vß5A, -Vß5B, -Vß5C, -Vß6, -Vß8, -Vß12; T Cell Diagnostics, Cambridge, MA).
Ileocolonoscopy with mucosal biopsy was performed on 7 patients meeting the ESSG classification criteria for SPA and on 4 control patients. TCR expression in PBL was similar in SPA, rheumatoid arthritis and controls. IL-2 expanded lymphocytes from ileal mucosa in patients with SPA and controls remained in general polyclonal after a 20 days' culture period. No significant changes in TCR expression were observed in ileal lymphocytes from control patients during the culture period. In 2 SPA patients an increased representation of Vß8 positive cells was observed (19% and 51% of CD3 lymphocytes in expanded ileal lymphocytes respectively versus 3 to 5% in PBL); one SPA patient showed an increase of Vß12 positive cells as well (21%). There was a gradual increase of Vß8 T lymphocytes with the passage of time. These data suggest an overrepresentation of in vivo pre-activated Vß8 T cells (which preferentially respond to IL-2 stimulation in vitro) in the terminal ileum of some selected patients with SPA and correlate with the previously reported overexpression of Vß8 in patients with inflammatory bowel disease (Crohn's disease).

J1-113 ADJUVANT EFFECT OF NON-TOXIC MUTANTS OF LT FOLLOWING INTRANASAL AND INTRAVAGINAL IMMUNISATION, M. Teresa De Magistris, Annalisa Di Tommaso, Mariagrazia Pizza, Rino Rappuoli, *Gordon Dougan and *Gill Douce, *IRIS, Immunobiology Research Institute Siena*, Siena, Italy and **Dept. of Biochemistry, Imperial College of Science, Technology and Medicine*, London, UK.
We tested the immunogenicity and the adjuvant effect on mucosal immunisation of wild type *E.coli* heat-labile toxin (LT) and of a non-toxic mutant containing a single mutation in the active site of LT (LTK63). Female Balb/c mice received three immunisations either intranasally or intravaginally with a mixture of LT or LTK63 and Ovalbumin. Specific antibodies were measured by ELISA in serum and in nasal, gut and vaginal washes. After two immunisations mice in all groups developed high anti-toxin serum Ig titers. Following intravaginal immunisation, anti-toxin IgA appeared in vaginal and gut but not in nasal washes, wherease after intranasal immunisation anti-toxin IgA were found in serum, nasal and vaginal washes. Both LT and LTK63 acted as adjuvants intranasally, inducing significant antibody titers in the serum and anti-Ovalbumin IgA in nasal washes. Their adjuvant effect following intravaginal immunisation is under evaluation. These results demonstrate that a) the adjuvant activity of LT can be dissociated from the enzymatic activity and b) non-toxic LT mutants induce an immune response in vagina.

J1-114 EXPRESSION OF RAT INTESTINAL DEFENSINS IN HEMORRHAGIC SHOCK, Gill Diamond, Michael D'Alessio and Michael Condon[1], Department of Anatomy, Cell Biology and Injury Sciences, UMDNJ, Newark, NJ 07103, and [1]Surgical Services, VA Medical Center, E. Orange, NJ 07019.
Intestinal mucosal barrier failure and subsequent translocation of bacteria and endotoxin has been proposed to contribute to the development of sepsis following hemorrhage. Evidence has accumulated indicating intestinal dysfunction to occur via alterations of its mechanical, humoral, cellular, immunologic and nonimmunologic defense mechanisms, following blood loss and injury. Among the host defense mechanisms identified in the small intestine is that of defensins. These antimicrobial peptides, found in phagocytic cells of numerous species, have also been shown to be expressed in human and mouse small intestinal Paneth cells. The expression of a gene encoding defensin-like peptides in the tracheal mucosa of the cow has been shown to be upregulated in response to bacteria. We therefore examined rats subjected to hemorrhagic shock for the presence of intestinal defensin peptides, as well as changes in mRNA levels encoding these peptides.
Sprague Dawley rats were bled to a mean arterial pressure of 30mm Hg, and maintained at this pressure until 60% of their shed blood was returned. Control rats were cannulated and not shocked. Rats were sacrificed either immediately or 2h post shock and their intestines removed, cut into 10 cm segments, and total RNA isolated. Segments were also homogenized for protein isolation.
Due to the high levels of sequence homology in the 5' region of defensin mRNA sequences, 3' RACE analysis was used with an upstream oligonucleotide primer designed from the 5' homologous region of mouse defensins. PCR of cDNA from oligo dT-primed transcripts using this primer yielded amplified products with high sequence identity to both human and mouse small intestinal defensins. Predicted amino acid sequences from these molecules indicated that these putative defensins were more similar to mouse intestinal defensins than to rat neutrophil defensins. Northern blot analysis of rat intestine mRNA using these sequences as probes indicated that steady-state levels of defensin mRNA in the ileum increased 6-fold after hemorrhagic shock.

J1-115 A DISTINCT ARRAY OF PROINFLAMMATORY CYTOKINES IS EXPRESSED IN HUMAN COLON EPITHELIAL CELLS IN RESPONSE TO BACTERIAL INVASION, Lars Eckmann, Hyun Jung, Suk-Kyun Yang, Joshua Fierer, Ewa Morzycka-Wroblewska and Martin F. Kagnoff, Department of Medicine, Laboratory of Mucosal Immunology, University of California, San Diego School of Medicine, La Jolla, CA 92093
Pathogenic bacteria that penetrate the intestinal epithelial barrier stimulate an inflammatory response in the adjacent intestinal mucosa. The present studies asked whether colon epithelial cells can provide signals that are important for the initiation and amplification of an acute mucosal inflammatory response. Infection of monolayers of human colon epithelial cell lines (T84, HT29, Caco-2) with invasive strains of bacteria (*S. dublin, S. dysenteriae, Y. enterocolitica, L. monocytogenes*, enteroinvasive *E. coli*) resulted in the coordinate expression and upregulation of a specific array of four proinflammatory cytokines, IL-8, MCP-1, GM-CSF and TNFα, as assessed by mRNA levels and cytokine secretion. Expression of the same cytokines was upregulated following TNFα or IL-1 stimulation of these cells. In contrast, cytokine gene expression was not altered following infection of colon epithelial cells with noninvasive bacteria or the noninvasive protozoan parasite, *G. lamblia*. Notably, none of the cell lines expressed mRNA for IL-2, IL-4, IL-5, IL-6, IL-12p40, IFN-γ or significant levels of IL-1 or IL-10 in response to the identical stimuli. The coordinate expression of IL-8, MCP-1, GM-CSF and TNFα appears to be a general property of human colon epithelial cells since an identical array of cytokines, as well as IL-6, also was expressed by freshly isolated human colon epithelial cells. Since the cytokines expressed in response to bacterial invasion or other proinflammatory agonists have a well documented role in chemotaxis and activation of inflammatory cells, colon epithelial cells appear to be programmed to provide a set of signals for the activation of the mucosal inflammatory response in the earliest phases following microbial invasion. Supported by NIH grants DK35108 and DK47739, a Pediatric AIDS Foundation grant, and fellowship support from the CCFA (L.E.).

J1-116 HUMAN SERUM IgA AND TRIGGERING OF Fc-alpha RECEPTOR INDUCE INTERLEUKIN-1 RECEPTOR ANTAGONIST AND MODULATE INFLAMMATORY CYTOKINE RELEASE IN HUMAN MONOCYTES, Martha M. Eibl[+], Hermann M. Wolf[+], Aysen Samstag[°], Michael B. Fischer[+] and Rafi U. Ahmad[+], [+]Institute of Immunology, University of Vienna, and [°]Immuno AG, Vienna, Austria

A deregulated expression and/or release of inflammatory cytokines accounts for the majority of pathophysiological events in a variety of systemic inflammatory diseases. Human serum IgA induces significant levels of IL-1ra release in human monocytes. IL-1ra release could also be induced by a monoclonal antibody (mAb) specific for the ligand binding site of the phagocyte Fc-alpha receptor (Fc-αR), indicating that IgA induces IL-1ra production through triggering of the FcαR. Comparably to human serum IgA, FcαR mAb also down-regulated TNF-alpha and interleukin-6 release in human monocytes activated with *Haemophilus influenzae* type b (Hib), while Hib-induced release of granulocyte-macrophage colony-stimulating factor (GM-CSF) was unaffected. By the induction of IL-1ra and down-regulation of TNF-alpha and IL-6 release in human monocytes, interaction of IgA with its receptor may actively contribute to the regulation of the inflammatory response at the level of the mucosal surfaces, thus preventing the development of local systemic pathology.

J1-117 **GUT INFLAMMATION IN PSORIATIC ARTHRITIS: A PROSPECTIVE ILEOCOLONOSCOPIC STUDY.**

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Psoriatic arthritis has been recently subdivided into 4 groups: patients with a symmetrical polyarticular (POLY) involvement, patients with an asymmetrical oligoarthritis (OLIGO), patients with predominantly axial involvement (AXIAL) and patients with a mutilating arthritis. The AXIAL and probably the OLIGO group are part of the spondylarthropathy concept. In previous ileocolonoscopic studies on patients with spondylarthropathies (in which the subgroup of psoriatic arthritis was excluded) microscopical inflammatory gut lesions were found in 60% of the cases.

Ileocolonoscopy was performed on 64 patients with psoriatic arthritis (37 male, 27 female). Inflammatory gut lesions were found in 10 of the 64 patients (15%). Three of the 15 patients (20%) with oligoarthritis and 7 of the 23 patients (30%) with predominantly axial involvement showed gut inflammation, whereas in none of the 26 patients with polyarthritis inflammatory gut lesions were discovered. HLA-B27 was absent in the polyarticular group, and was found in 60% of the oligoarticular group and in 65% of the axial group.

We can conlude that the gut inflammation is only present in the subgroups of psoriatic arthritis who belong to the spondylarthropathy concept suggesting that pathogenesis of locomotor inflammation in psoriatic arthritis is different in the various subgroups.

Prevalence of gut inflammation in psoriatic spondylartropathy is significantly lower compared to uncomplicated spondylarthropathy suggesting that not only the gut but also the skin can be a portal of entry for causative antigens in psoriatic arthritis.

J1-118 COMPARISON OF MUCOSAL AND SYSTEMIC IMMUNE RESPONSES IN MICE INFECTED WITH HETEROLOGOUS AND HOMOLOGOUS ROTAVIRUSES AND THE ROLE OF LOCAL ANTIBODY IN PROTECTION, N. Feng, J. Burns, L. Bracy and H. Greenberg, Dept of Medicine & Microbiology, Stanford University, Stanford, CA 94305

Rotaviruses are the single most important cause of severe dehydrating diarrhea in young children in the world. The basis of immunity to rotavirus infection is not fully understood. We have carried out a series experiments in a mouse model system to compare the immune response to heterologous and homologous rotavirus infection and to determine the specific role of IgA antibody in protection and resolution. Suckling mice were orally inoculated with either a virulent or attenuated "homologous" murine rotavirus (EHP), or a "heterologous" simian rotavirus (RRV) and the mice were challenged with a virulent murine rotavirus (EC_W) six weeks later. In separate studies, mice were transplanted with IgA producing hydridomas directed at selected rotavirus proteins. The absence of viral antigen shedding in feces after wild type challenge or the clearance of virus in chronically infected mice was defined as protection. Homologous rotaviruses, regardless of virulence, induced protection at very low dose during primary infection (10^1 PFU or 10^{-2} DD_{50}/mouse). Heterologous rotavirus required a much higher dose (10^7 PFU/mouse) to generate protection. We examined the mucosal and systemic humoral immune responses in these mice by measuring levels of rotavirus-specific fecal IgA and serum IgG as well as IgA and IgG secreting cells in GALT and spleens. Homologous rotaviruses were more efficient than heterologous rotavirus in inducing a humoral immune response, especially an intestinal IgA response which was directly correlated with protection. The role of helper T cells in the regulation of mucosal and systemic immune responses after heterologous or homologous rotavirus inoculation is currently being studied. We then confirmed the importance of local antibody in protection by demonstrating that IgA monoclonals were fully capable of preventing infection and of resolving chronic infection in SCID mice when transplanted into test mice. Of importance, IgA monoclonals without in vitro neutralizing activity were able to mediate protection and resolution in our model system. The mechanism of these novel antiviral effects will be discussed.

J1-119 THE CTLA-4 COUNTER-RECEPTOR IS REQUIRED FOR T CELL IL-4 PRODUCTION DURING THE PRIMARY BUT NOT CHALLENGE MUCOSAL IMMUNE RESPONSE TO A GASTROINTESTINAL PARASITE, William C. Gause*, Xia di Zhou*, S.-J. Chen*, Suzanne C. Morris#, Fred D. Finkelman#, Peter Linsley##, Joseph F. Urban**, and Pin Lu*. Departments of *Microbiology and #Medicine, USUHS, Bethesda, MD 20814, ##Department of Cellular Immunology, Bristol-Myers Squibb Pharmaceutical Research Institute, WA,Helminthic Diseases Laboratory, ARS, USDA, Beltsville, MD 20705-2350.

The costimulatory signal provided through CTLA-4 counter receptor interactions is required for T cell activation resulting in increased IL-2 production in vitro, but its role in IL-4 production is unclear and few in vivo studies have been performed to confirm results of in vitro experiments. We have examined the in vivo effects of blocking CTLA-4 ligands on the primary and challenge Th2-associated mucosal immune responses that follow oral infection of mice with the nematode parasite, *Heligmosomoides polygyrus*. Murine CTLA4-Ig administration on the day of immunization inhibited *H..-polygyrus*-induced increases in $CD4^+$,$TCR\alpha/\beta^+$ T cell IL-4 gene expression and IL-4 protein production as measured by RT-PCR and ELISPOT respectively at 8 days after primary inoculation. In addition, CTLA4-Ig partially blocked increased IL-3, IL-4, IL-5, and IL-9 cytokine gene expression in the Peyer's patch (PP), and completely blocked elevated serum IgE levels, but not blood eosinophils, at 14 days after inoculation. In contrast administration of CTLA4-Ig at days 3 and 4 after immunization did not affect either elevated cytokine production or IgE levels. In addition, CTLA4-Ig failed to suppress IL-4 gene expression by $CD4^+$,$TCR\alpha/\beta^+$ T cells from mice that were inoculated for a second time with *H. polygyrus*. These results suggest that stimulation of CD28 and/or CTLA-4 is required for T cell activation leading to IL-4 cytokine production, B cell activation, and IgE secretion during a Th2-like, primary mucosal immune response to a nematode parasite but that IL-4 production by primed T cells or memory T cells does not require CTLA-4 counter-receptor interactions.

J1-120 ALTERED IGA TRANSPORT IN J CHAIN DEFICIENT MICE, Barbara A. Hendrickson, David A. Conner, Daniel J. Ladd, Donna Kendall, Edward E. Max, Marian R. Neutra, Christine E. Seidman and Jonathan G. Seidman, Division of Infectious Diseases, Children's Hospital, and Department of Genetics, Harvard Medical School, Boston, MA 02115

We have studied J chain knockout mice to define the role of J chain in the polymerization and mucosal transport of IgA. J chain is covalently associated with dimeric IgA and IgM and is thought to play a critical role in the transport of polymeric immunoglobulins into mucosal secretions and bile. Most of the work investigating the function of J chain in this process has been done with purified preparations of immunoglobulin and J chain or with isolated cell lines. We sought to clarify the roles of J chain in an animal model by generating a mouse lacking J chain using the technique of gene targeting in murine embryonic stem (ES) cells. J chain deficient animals exhibited normal serum IgM levels but markedly elevated serum IgA. Although polymeric IgA was present in the mutant mice, a larger proportion of their serum IgA was monomeric than was found in wild-type mouse serum. Fecal and bile IgA levels were decreased in the J chain deficient mice, while breast milk and intestinal surface IgA levels were similar to normal mice. We conclude that J chain is not essential for IgA polymerization or transport by intestinal and mammary epithelial cells but is necessary for efficient hepatic transport of IgA and is involved in dimer stabilization.

J1-121 *IN SITU* IDENTIFICATION OF MURINE PEYER'S PATCH DENDRITIC CELLS: A UNIQUE POPULATION OF N418+ CELLS IS PRESENT IN THE SUBEPITHELIAL DOME, Brian L. Kelsall, and Warren Strober, Laboratory for Clinical Investigation, National Institutes of Health, Bethesda, MD 20892

While it is clear that antigens and microorganisms from the intestinal lumen are transported into the Peyer's patch (PP) by M cells present in the follicle associated epithelium (FAE), the details of antigen processing and presentation within the PP are not known. We identified PP dendritic cells (DC) and macrophages (MP) *in situ* by immunoperoxidase staining of frozen sections of PPs from B10.A mice using the avidin-biotin complex (ABC) technique. Cells typical of interdigitating DCs that stain with the monoclonal antibodies NLDC-145, M342, 2A1, and N418 (Anti-CD11c) were localized to the interfollicular T cell regions (IFR). MAC-1 (CD11b)^{hi} MPs were concentrated in the IFR, as well as the lamina propria of the overlying villi. Interestingly, we identified a population of N418+, MHC II+, MAC-1^{lo}, F4/80-, B220-, CD3-, CD4-, CD8- cells with dendritic morphology that is concentrated just beneath the FAE. We believe these are a population of DCs uniquely positioned to capture antigens transported by M cells. The lack of MAC-1^{hi}, F4/80+, or endogenous peroxidase positive cells beneath the FAE suggests there are few mature MPs at this site. In addition, the lack of staining for the intracellular DC antigen identified by M342 argues that the subepithelial DCs may be in an earlier state of differentiation than the DCs in the IFR. The identification of two populations of PP DCs has implications for studies of antigen handling in the PP, and the presence of N418+ cells in the subepithelial region suggests DCs play a major role in the processing of luminal antigens.

J1-122 CD1 MOLECULES EXPRESSED BY ENTEROCYTES PRESENT PEPTIDES DISTINCT FROM OTHER CLASS I ANTIGEN PRESENTING MOLECULES

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Department of Microbiology & Immunology, UCLA Los Angeles, CA 90024, Scripps Research Institute and R. W. Johnson Pharmaceutical Research Institute, La Jolla, CA 92037, and Ixsys Corp., La Jolla, CA 92037.

CD1 is a class I-like antigen presenting molecule expressed by intestinal epithelial cells in humans and rodents. We have expressed a soluble form of the mouse CD1 molecule in insect tissue culture cells. A potential motif for peptide binding antigen binding to CD1 molecules has been determined by using these soluble molecules to screen an epitope display library. The motif defined is distinct from those defined previously from other class I molecules, in that it is highly hydrophobic and contains two bulky tryptophan amino acids. Binding studies carried out with synthetic peptides containing the motif confirm that the two tryptophans are required for CD1 binding, and that peptides up to 22 amino acids long bind to CD1. With regard to peptide length, CD1 binding peptides therefore are more similar to those that bind class II than those that bind class I molecules. The immunologic relevance of this binding has been demonstrated. We have raised peptide-specific, CD1-restricted T cells that are CD8 positive. These cells are cytotoxic, and they synthesize γ interferon following antigen stimulation. The rules that govern in vitro binding to soluble CD1 molecules and stimulation of the CD1-restricted T cells are concordant: both tryptophans are required and T cells respond to long peptides without the need for further processing. In summary, the antigenic structures presented by CD1 molecules are quite distinct from those presented by classical class I molecules, suggesting that CD1 could play a distinct role in host defense in the mucosae.

J1-123 αβ CD8+ AND αα CD8+ MURINE INTESTINAL INTRAEPITHELIAL LYMPHOCYTES (iIEL) RESPOND DIFFERENTLY FROM LYMPH NODE CD8+ CELLS UPON STIMULATION VIA αβ T CELL RECEPTOR, Nan-Shih Liao, Yen-Jey Lai, Ray Dong, and Vasily Gelfanov, Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, Republic of China

Intestinal IEL locate between and beneath the epithelial cells covering the intestine lumen, and are probably the first group of lymphocytes that encounter antigens and pathogens passing through the intestine. The cellular composition of iIEL is more complicated than that of peripheral T cells, as shown by the presence of CD4+8+ and ααCD8+ cells. Among the ααCD8+ cells, one fourth to half are αβ TCR+ whereas the rest are γδ TCR+ cells. To understand activation requirements of iIEL subsets, we analyzed proliferation and cytokine production of purified αβ CD8+ and αα CD8+ iIEL subpopulations in comparison to LN CD8+ cells stimulated via αβ TCR by plate-bound H57.597 and an anti-CD28 mAb, 37.51. We found that anti-CD28 mAb enhances proliferation of αβ CD8+ iIEL, whereas αα CD8+ iIEL showed no proliferation in the absence of exogenous IL2. Interestingly, in the presence of exogenous IL2, αα CD8+ iIEL proliferate to the same level as αβ CD8+ iIEL. We also found that the kinetic of proliferative response of αβ CD8+ and αα CD8+ iIEL are much more delayed than that of CD8+ LN cells. The late kinetic of iIEL in comparison to that of LN CD8+ cells may explain previous reports on the unresponsiveness of αα CD8+ iIEL to TCR triggering.

J1-124 SIGNIFICANTLY DIFFERENT DEGREES OF ANTIGENIC HETEROGENEITY AMONG IgA1 PROTEASES SECRETED BY SPECIES OF *STREPTOCOCCUS, NEISSERIA* AND *HAEMOPHILUS*, Hans Lomholt, Knud Poulsen and Mogens Kilian, Department of Medical Microbiology, University of Aarhus, DK-8000, Denmark

Mucosal vaccines designed to induce a local response in the upper airways presumably will elicit antibodies primarily of the IgA1 subclass. A number of bacteria colonizing these mucosal surfaces secrete specific proteases that cleave the hinge region of human IgA1. The ensuing release of intact Fab and Fc fragments may result in coating of the bacterial surface by monomeric Fab fragments directed to vaccine antigens, however, devoid of effector functions residing in the Fc domain. If neutralization of the secreted IgA1 proteases is not accomplished, then the efficiency of mucosal vaccines against these organisms may be significantly reduced. We present results of enzyme neutralization studies employing rabbit antisera against selected IgA1 proteases. Extensive antigenic heterogeneity was found among IgA1 proteases of *Streptococcus pneumoniae* and these data will be compared to results on the population structure obtained by multilocus enzyme electrophoresis. In contrast, IgA1 proteases of *Neisseria gonorrhoeae*, which show a panmictic population structure, possessed highly cross-reactive neutralizing epitopes also shared by *Neisseria meningitidis* IgA1 proteases. Evidence based on DNA sequence data is presented that recombination has occurred *in vivo* among *N. meningitidis* IgA1 protease genes, as previously shown for IgA1 protease genes of *Haemophilus influenzae* and *N. gonorrhoeae*. Thus, horizontal genetic exchange constitutes a possible mechanism for modulation of the antigenic properties of these proteases. The significantly different degree of genetic and antigenic variation among IgA1 proteases secreted by different bacterial species that colonize and invade mucosal surfaces is reviewed.

J1-125 CYTOKINE GENE EXPRESSION DURING INTESTINAL MATURATION OF RATS, Elena Mengheri, Laura Ciapponi and Fabio Nobili, Istituto Nazionale della Nutrizione via Ardeatina 546, 00179 Roma, Italy

Several studies have demonstrated that the immune system can modulate epithelial cell renewal, differentiation and function in the gastrointestinal tract. We have analyzed the expression of some cytokines in lymphocytes of rat Peyer's patches during the weaning period to investigate whether these factors may play a role in the maturation of the mucosa. IL-2, IFNγ and IL-4 gene expression was analyzed by PCR assay at 16, 19, 21 and 30 days of age and did not show differences at the various ages. Since effects of other cytokines could not be excluded, we have studied whether treatment of rats with cyclosporinA (CsA), a potent immunosuppressor which selectively inhibits T cell activation, would affect the maturation of intestine. CsA induced a strong reduction in crypt cell proliferation as well as in villus and crypt length from 14 to 21 days of age. However, other indeces of intestinal maturation, such as lactase, sucrase and maltase activities were unaffected. To investigate the possibility that the effects of CsA were not due to an inhibition of mucosal T cells but rather to a direct effect of CsA on intestine, we have treated three different intestinal cell lines with CsA and measured ^{3}H-thymidine incorporation.The CsA induced a marked decrease in cell proliferation, suggesting that the effects found in vivo were not immunomediated.

J1-126 MUCOSAL IMMUNIZATION WITH CHLAMYDIAL ANTIGENS Miroslav Novak[1], Ryan McNamara[1], Jacqueline D. Duncan[1], Zina Moldoveanu[2], Richard W. Compans[3], Jiri Mestecky[2], [1]Secretech Inc., Birmingham, AL 35205; [2]Department of Microbiology and Medicine, University of Alabama at Birmingham, Birmingham, AL 35294; [3]Department of Microbiology and Immunology, Emory University, Atlanta, GA 30322.

Chlamydia trachomatis, mouse pneumonitis strain, was grown on McCoy cells and purified on discontinuous Renografin gradient. Major outer membrane protein (MOMP) was separated from purified elementary bodies (EB) by preparative SDS-PAGE. Mice were immunized s.c., orally, or rectally with EB in PBS solution (EB-sol.) or microencapsulated EB (EB-ms) or with MOMP in solution.

On day 28 after s.c. immunization with EB-sol. we observed 4-fold increase of serum IgG by ELISA over preimmune level (1:32000) and on week 8 and 16 this titer dropped to 1:16000, but using EB-ms we detected a 5-fold elevation of serum IgG and higher levels on week 8 and 16 - 1:64000. Purified MOMP was not immunogenic after s.c. immunization in ELISA, but in immunoblot we could detect anti-MOMP antibodies. Oral immunization with EB-sol. elevated secretory IgA in saliva modestly (1:20 - 1:40), but in vaginal wash we observed a 4 to 5 fold increase in specific IgA over preimmune titer (1:80 - 1:160). Oral immunization with EB-ms produced only modest increase in salivary and vaginal IgA (1:20 - 1:40). Rectal immunization with EB-sol. induced increase in serum IgG to 1:8000 - 1:32000 and secretory IgA in feces to high levels 1:40 - 1:160. Secretory IgA titer in the vaginal wash after this immunization was 1:20 - 1:40 and secretory antibodies were not detected in saliva. Supported by SBIR grant 1R43 AI34794-01.

J1-127 DEVELOPMENT AND SELECTION OF THE MURINE TCR GAMMA DELTA INTESTINAL EPITHELIAL LYMPHOCYTES, Chetan Panwala, Daniel Cruz, Daniel Magaña, Beate Sydora, Hilde Cheroutre, and Mitchell Kronenberg, Department of Microbiology and Immunology, University of California Los Angeles, Los Angeles, CA. 90024.

The intestinal epithelial lymphocytes (IEL) of the murine small intestine are comprised of both γδ and αβ TCR bearing T cells. The γδ IEL have a restricted repertoire of V gene segments, most express the Vγ5 gene segment. We sought to determine what mechanisms restrict Vγ gene usage in IEL. The two models that were tested were preferential rearrangement and/or antigenic selection. Using a PCR based strategy, we analyzed the productive and nonproductive rearrangements of both Vγ5 positive and Vγ5 negative IEL. If preferential rearrangement were responsible for predominant Vγ5 expression, then most Vγ5+ IEL also should have nonproductive Vγ5 rearrangements. Our findings include the following: 1. The γδ IEL repertoire is more diverse than previously reported with a significant proportion of γδ IEL expressing the Vγ1.1 and Vγ2 gene segment. 2. The repertoire is partly shaped by preferential rearrangement--only 3 of the 6 most common Vγ rearrangements occur including Vγ1.1-Jγ4, Vγ2-Jγ1, and Vγ5-Jγ1, but not Vγ3-Jγ1, Vγ4-Jγ1 and Vγ1.2-Jγ2. 3. Both the Vγ5 positive and Vγ5 negative populations have a similar set of rearrangements. IEL from mice with a rearranged Vγ3/Vδ1 transgene predominantly express Vγ3. These cells have a different phenotype with respect to surface markers such as CD5, CD8 and CD45, suggesting that there may be some sort of post rearrangement selection mechanism which only allows T cells with the appropriate receptors to mature. Where does this selection occur? There is a body of evidence which suggests that the IEL may develop through a thymus independent pathway. We have been able to detect RAG 2, and TdT mRNA in the gut along with Vγ5 excision products suggesting that development of IEL may be occurring in the intestine.

J1-128 B CELL REPERTOIRE IN INTESTINAL B CELLS.
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The B cell repertoire in intestinal mononuclear cells was studied by examining Ig heavy chain variable gene (VH) usage. Reverse transcription of INT mucosal RNA from normal or Crohn's disease (CD) intestine was followed by polymerase chain reaction with primers specific for each VH family. While all family members were expressed, mRNA transcripts for VH4 were present at higher levels than their genomic representation and were higher in CD than normal intestine. VH4 clones from RNA were isolated from intestinal biopsies of 5 normal and CD individuals. The PCR products were cloned and DNA sequencing performed. Analyses of sequences from normal individuals revealed all were members of VH4 family but there were no identical clones between individuals or from the same individual. In addition, 50% of the clones used the JH5 gene segment in the CDR3 (VDJ recombining region). In contrast, MNC from CD patients used predominantly JH4, as do splenic and lymph node B cells from normal individuals. In addition, in CD patients we found similar VH4 sequences in many of the clones. This was true with multiple clones from an individual patient and when sequences were compared with clones amplified from different patients. This suggests pauciclonality in the B cells in CD consistent with the hypothesis that there is a restricted immune response to a dominant antigen in this disease. There was marked divergence from germline sequences within the D region consistent with chronic antigenic exposure. Thus VH4-expressing intestinal B cells are quite different in individuals with CD and normals. In normals, VH4 B cells are polyclonal and express a JH gene segment rarely used in non-intestinal lymphocytes. In contrast CD VH4-B cells express a limited repertoire of variable region sequences associated with the most commonly used JH gene segment. These data suggest that normal mucosal B cells have a repertoire distinct from systemic B cells and that this distinction is abolished in CD.

J1-129 MUCOSAL IMMUNITY AGAINST SHIGELLOSIS: PROTECTIVE ROLE OF ANTI-LPS IgA ANTIBODIES.
Armelle Phalipon*, Muriel Kaufmann#, Pierre Michetti$, Jean-Marc Cavaillon§, Michel Huerre&, Philippe Sansonetti*, Jean-Pierre Kraehenbuhl#. *Unité de Pathogénie Microbienne Moléculaire, U389 Institut National de la Santé et de la Recherche Médicale, §Unité d'Immunoallergie, &Unité d'Anatomopathologie, Institut Pasteur, 25-28 rue du Dr Roux, 75015 Paris, France, #Institut Suisse de Recherches Expérimentales sur le Cancer et Institut de Biochimie, Université de Lausanne, CH-1066 Epalinges, Switzerland, $Division de Gastroentérologie, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland.
Shigella flexneri, a gram-negative bacillus, is the major etiological agent of the endemic form of shigellosis, a dysenteric syndrome causing high rate of mortality among infants, particularly in developing countries. It causes disease by invading the colonic mucosa, generating severe inflammation (for review Sansonetti). To determine the role of humoral mucosal immune response in protection against shigellosis, we have obtained a monoclonal dimeric IgA antibody specific for *Shigella flexneri* serotype 5a lipopolysaccharide (LPS) and used a murine pulmonary model which mimics the lesions occuring in natural intestinal infection. Adult BALB/c mice challenged with 10^7 *Shigella flexneri* organisms developed a rapid inflammatory response characterized by a polymorphonuclear cell infiltration around and within the bronchi with a concommittant systemic IL-6 response. Implantation of monoclonal IgA-secreting hybridoma cells in the back of mice resulted in the production of circulating anti-LPS monoclonal IgA antibodies that protected the animals against subsequent intranasal challenge with *S. flexneri* serotype 5a reflected by the absence of histopathological lesion and a significant decrease in IL-6 response. This protection was shown to be serotype-specific. The administration of the monoclonal antibody by the nasal route also protected mice against intranasal challenges. The protective dose was the same as that measured locally in the bronchoalveolar secretions using the "back pack" model. Our data indicate that an antibody directed against a bacterial surface antigen and present at the mucosal surface is sufficent to confer protection against *Shigella* infection.

Sansonetti P. J. Infectious Agent and Disease, 1994, 2, 201-206.

J1-130 PRIMARY STRUCTURE OF *Neisseria meningitidis* IgA1 PROTEASES IN RELATION TO CLEAVAGE SPECIFICITY,
Knud Poulsen, Hans Lomholt and Mogens Kilian, Department of Medical Microbiology, University of Aarhus, DK-8000, Denmark.
IgA1 is the predominant immunoglobulin subclass on mucosal membranes in the human upper respiratory tract. A number of bacterial pathogens including *N. meningitidis* secrete IgA1 proteases which specifically cleave the human IgA1 molecule in the hinge region and thereby eliminate the Fc mediated effector functions of IgA1. Thus, these proteases should be considered when developing oral vaccines exerting their function via specific IgA1 on mucosal surfaces. We present the cloning and sequencing of a *N. meningitidis* IgA1 protease gene which revealed overall homology to the corresponding gonococcal and *Haemophilus influenzae* proteases indicating that they share the same unique mechanism for secretion which involves autoproteolytic maturation of the preprotease during transport across the outer membrane. Apparently, the *N. meningitidis* IgA1 preprotease lacks a domain equivalent to the α-peptide which in gonococci is secreted concomitantly with the mature protease. Each of the IgA1 proteases cleaves one of several Pro-Ser (type 1 proteases) or Pro-Thr (type 2 proteases) peptide bonds in the hinge region of IgA1. For the three above mentioned species epidemic strains are associated with type 1 IgA1 protease production. The reason for this association remains unknown, though it may suggest that the type 1 IgA1 proteases have activities in addition to IgA1 cleavage. We have sequenced and compared a part of the IgA1 protease gene from a number of bacterial isolates and showed that a characteristic difference in sequence and length correlated with each of the different cleavage types.

J1-131 RESPIRATORY EPITHELIAL CELLS PRODUCE IL-8 IN RESPONSE TO PSEUDOMONAS AERUGINOSA ADHERENCE,
Alice Prince and Emily DiMango, Departments of Medicine and Pediatrics, College of Physicians and Surgeons, Columbia University, New York, NY 10032
P.aeruginosa has been recently shown to stimulate bronchial epithelial cells to produce IL-8, a major neutrophil chemoattractant. Piliated strains, PAO1, PAK, PA1244, induced an SV40 transformed bronchial epithelial cell line (1HAEo-) to produce 60-100% more IL-8 than did isogenic *pil* mutants. Non-adherent *rpoN* mutants were unable to stimulate IL-8 production. Purified pilin and flagellin were each found to trigger an IL-8 response while a PAO Fla$^-$ strain and a PAK *fliA* mutant were markedly diminished in their ability to stimulate IL-8. A direct correlation was found between adherence to the epithelial cells and IL-8 expression. The Fla mutants evoked less of an IL-8 response and were significantly less adherent than the *pil* mutants suggesting that flagellin has an important role in mediating attachment to epithelial cells. Attachment without invasion of the epithelium seemed sufficient for an IL-8 response as few ($<10^{-1}$ cfu/ml) bacteria were found within the epithelial cells and cytochalasin did not affect IL-8 production. Although the *P.aeruginosa* virulence factors LPS, elastase, exoenzyme S, and phospholipases did not effect an IL-8 response, incubation of the 1HAEo- cells with the *Pseudomonas* autoinducer, an N-acyl homoserine lactone compound, elicited dose dependent IL-8 expression which was not produced in reaction to other homoserine lactone analogues. *P.aeruginosa* contact with the respiratory epithelium as mediated by several discrete exoproducts appears to be sufficient to trigger inflammmation.

J1-132 **INTERACTIONS BETWEEN C-KIT AND STEM CELL FACTOR ARE REQUIRED FOR INTESTINAL IMMUNE SYSTEM HOMEOSTASIS, Lynn Puddington, Sara Olson, and Leo Lefrançois, University of Connecticut Health Center, Farmington, CT 06030**

Interactions between the protein tyrosine kinase receptor, c-kit, and its' ligand, stem cell factor (SCF) are known to be important for development of hematopoietic cells, melanocytes and germ cells. Within the hematopoietic lineage, development of T lymphocytes appears normal in mice bearing mutations in the c-kit (*W*) or SCF (*Sl*) genes. Our studies indicated that this was not the case for intraepithelial lymphocytes (IEL), a unique population of T lymphocytes that resides within the intestinal epithelium. In normal mice, IEL are composed of approximately equal numbers of cells bearing the $\alpha\beta$ or $\gamma\delta$ subtype of the T cell receptor for antigen (Tcr), and this relationship is maintained throughout life. In contrast, in mice encoding mutant c-kit (W/W^v) or SCF (Sl/Sl^d) age-dependent opposite effects were observed for IEL of each Tcr type. Although the normal IEL phenotype was observed in young mutant mice, beginning at 6-8 wk of age, the number of $\gamma\delta$ IEL decreased and the number $\alpha\beta$ IEL increased. In mutant mice 16 wk of age, IEL were composed of 96-98% $\alpha\beta$ and 2-4% $\gamma\delta$ cells. The increase in $\alpha\beta$ IEL was due largely, but not exclusively, to an increase in the $CD4^+8^+$ subset, suggesting that these cells may be developmental intermediates in the $\alpha\beta$ IEL lineage. Furthermore, mRNA encoding c-kit or SCF was present in IEL or intestinal epithelial cells, respectively, indicating a potential for direct interaction between c-kit and its' ligand during residence or development of IEL within the epithelium. These results suggest that interactions between c-kit and SCF are required for normal homeostasis of T lymphocytes within the intestinal epithelium.

J1-133 INTERLEUKIN-6 IS ESSENTIAL FOR DEVELOPMENT OF CONVENTIONAL MUCOSAL ANTIBODY RESPONSES IN VIVO, Alistair J Ramsay*, Alan J Husband+, Ian A Ramshaw*, Shisan Bao+ and Manfred Kopf#. *John Curtin School of Medical Research, Australian National University, Canberra, Australia, +Dept Veterinary Pathology, University of Sydney, Australia, #Max Planck Institute for Immunobiology, Freiburg, Germany.

IL-6 markedly and selectively enhances IgA production *in vitro* by isotype-committed B cells, although the *in vivo* relevance of these findings is unclear. The presence in mucosal tissues of T cells, macrophages and other cells capable of IL-6 production *in vitro* and the broad distribution of cells containing IL-6 mRNA in intestinal mucosa however, are consistent with the proposition that this factor is important in regulating the effector stage of IgA responses.

We have studied mucosal IgA antibody responses in mice rendered IL-6-deficient ($IL\text{-}6^-$) by targeted disruption of the IL-6 gene to obtain evidence for this hypothesis (Ramsay et al., *Science* **264**: 561, 1994). In these mice, greatly reduced numbers of IgA-producing cells were observed at mucosae and grossly deficient local antibody responses were recorded following mucosal challenge with either ovalbumin or recombinant vaccinia virus (rVV). The IgA response in lungs was completely restored after intranasal infection with rVV engineered to express IL-6. These findings demonstrate a critical role for IL-6 *in vivo* in the development of mucosal IgA antibody responses and illustrate the effectiveness of local vector-directed cytokine gene delivery.

The residual number of IgA plasma cells present in $IL\text{-}6^-$ mice may arise from a subset of IgA precursor cells which derive from the peritoneal cavity rather than MALT. Whereas a proportion of surface IgA^+ cells in the small intestines of normal mice express the CD5 marker, and are, therefore, presumably cells of peritoneal cavity origin, a higher level of CD5 expression occurs among surface IgA^+ cells in the gut of $IL\text{-}6^-$ mice. We conclude that there is an IL-6-independent subset of intestinal IgA plasma cells which accounts for the residual number of IgA plasma cells in $IL\text{-}6^-$ mice.

J1-134 SALIVARY ANTIBODIES TO CLONES OF ORAL STREPTOCOCCI STUDIED IN RELATION TO PERSISTENCE OF CLONES AND EPISODES OF INVASION, Jesper Reinholdt*, Jesper Hohwy*, and Mogens Kilian#, Institute of Odontology* and Institute of Medical Microbiology#, University of Aarhus, DK-8000, Aarhus, Denmark.

Streptococci belonging to *S.sanguis, S.oralis*, and *S.mitis* are predominant, lifelong members of the oral flora although specific antibodies are present in saliva and serum from early infancy. IgA1 protease activity, which is a property of most of these bacteria, may contribute to their apparent ability to evade immune control. However, also these enzymes are potential targets of inhibitory antibodies. In a recent study, we found considerable clonal diversity of the oral *S.mitis* biovar 1 population including differentiation of carbohydrate and protein antigens. Hence, potential immunological control might be reflected as clonal shifts. To evaluate the role of salivary IgA antibodies, we have sequentially sampled the oral flora and saliva of 6 subjects, including 2 infants, 4 times over a period of 313 days. By restriction enzyme analysis, the 4 bacterial samples from each subject were found to represent between 11 and 28 clones of *S.mitis* biovar 1 with a mean of 5 in each sample. In adults, but not in infants, some clones were detected at more than one occasion. No correlation of persistence to IgA1 protease production was found. Salivary IgA antibodies were monitored by SDS-PAGE and immunoblotting of extracted bacterial antigens and by ELISA. Essentially, no change in the specificity and titer of antibodies reacting with individual clones were detected irrespective of whether the clone persisted or disappeared. Antibodies reacting with individual clones amounted to approx. 0.5% and 1.5% of total IgA in infants and adults, respectively. These data do not support the hypothesis that the oral *S.mitis* biovar 1 population is regulated by salivary IgA antibodies. In another study, we analyzed serum and saliva from 4 subjects recovering from prolonged, invasive infection due to *S.sanguis* (1 subject), *S.oralis* (2 subjects), or *S.mitis* biovar 1 (1 subject) for antibodies to the causative bacteria and their potential IgA1 proteases. Whereas antibodies to bacterial cells and IgA1 protease were highly elevated in serum, S-IgA antibodies in saliva did not exceed the level in normal controls. The results of these studies may be of relevance to ongoing attempts to stimulate salivary S-IgA responses to indigenous, oral streptococci as a means of controlling dental caries.

J1-135 OVEREXPRESSION OF BIOLOGICALLY ACTIVE SECRETORY COMPONENT, Lorenz Rindisbacher and Blaise Corthésy, Institut de Biologie animale, Université de Lausanne, Switzerland

The biological role of secretory component (SC) in the secretory IgA complex is still largely unknown. Aiming to mass production of secretory IgA for passive protection of mucosal surfaces, we produced recombinant human SC using viral-based eukaryotic expression systems. An artificial translation stop codon was introduced into the cDNA for polymeric Ig receptor such that only the five extracellular domains corresponding to SC were expressed. In addition, sequences encoding six consecutive histidine residues at the carboxy terminus of the truncated protein were added to some constructs, which should allow simple purification of the product by metal-chelate affinity chromatography. The engineered SC genes were integrated into the genomes of baculovirus and vaccinia virus via homologous recombination. The resulting virus recombinants were used to infect Sf9 insect cells and a series of mammalian cell lines, respectively, which subsequently secreted SC into the serum-free culture medium at levels of 10 to 50 mg/liter. Apparent size differences of the products, depending on the host cell type, were shown to be due to variations in glycosylation patterns. Recombinant SC was purified by either Ni^{2+}-chelate- or ConA affinity chromatography. The proteins produced by both of the systems proved to retain their biological activity in terms of specific binding to dimeric IgA.

J1-136 GUT-HOMING CD4$^+$ MEMORY T CELLS IN THE MOUSE. Angelika Rudolphi and Jörg Reimann· Institute of Medical Microbiology and Immunology, Department of Bacteriology, University of Ulm, Ulm, FRG.

T lymphocyte subsets predominate in gut-associated lymphoid tissue (GALT) of immunocompetent mice. Murine CD4$^+$ T cells in the small and large intestine are the major recirculating T cell subset of mucosal T cell populations. No antigen receptor-expressing lymphoid cells were found in GALT of C.B-17 *scid/scid* (SCID) mice. The heterotopic transplantation of SCID mice with a full thickness gut wall graft from the ileum or colon of immunocompetent (congenic C.B-17+/+ or semi-syngeneic BALB/c^{dm2}) mice selectively reconstituted the CD3$^+$ TCR$\alpha\beta^+$ CD4$^+$ T cell subset in the gut. Donor-type CD4$^+$ T cells expressing the surface phenotype of mucosa-seeking memory T cells (CD44^{hi} LPAM-1/2$^+$ LECAM-1^{lo} CD2hi CD28^{hi}) were found in spleen, peritoneal cavity, mesenteric lymph nodes, the epithelial layer and the lamina propria of the small and large intestine of the immunodeficient SCID host, but not in peripheral lymph nodes. The mucosa-homing 'single-positive' (SP) CD4$^+$ CD8$^-$ effector T cells migrated into the epithelial layer of the small intestine. The transit of SP CD44$^+$ CD4$^+$ T cells from the lamina propria into the epithelial layer of the small intestine coincided with various changes in their surface phenotype. The cells coexpressed the CD8α-chain, *i.e.*, switched from the SP CD4$^+$ CD8$^-$ to the 'double-positive' (DP) CD4$^+$ CD8$\alpha\alpha^+$ phenotype. The cells downregulated expression of the α_4-chain of the LPAM integrin and upregulated expression of the α_{IEL}-chain of the epithelial-specific integrin. Furthermore, intraepithelial DP T cells suppressed expression of the costimulator molecules CD2 and CD28. These data indicate that competent, peripheral T cells from the gut lamina propria adopt a strikingly different phenotype when they enter the microenvironment of the intestinal epithelial layer.

J1-137 INTERFERON-GAMMA INDUCED IMMUNE MARKERS ON INTESTINAL EPITHELIAL CELLS AND THEIR MODULATION BY LIPOPOLYSACCHARIDE. Eduardo J. Schiffrin, Rosanna Di Guglielmo, Sylviane Cochet, Patrick Serrant, Lorna Henderson and Anne Donnet-Hughes. Department of Immunology, Nestec Ltd., Nestlé Research Centre, Vers-chez-les-Blanc, P.O. Box 44, CH-1000 Lausanne 26 (Switzerland).

Intestinal epithelial cells are now recognised as important immunocompetent cells which can present antigen in an MHC-Class II dependent fashion. Examination of their immune reactivity upon interaction with bacterial or cellular products would be useful for the development of oral vaccines, the induction of oral tolerance and/or the control of inflammatory intestinal conditions. For this reason, we examined the effect of IFN-γ in the presence or absence (<50pg/ml) of LPS on the intestinal epithelial cell (IEC) line, HT-29. Production of IL-8 and expression of MHC-related molecules were studied using the PCR amplification of reverse-transcribed RNA and/or flow cytometry. IFN-γ induced expression of the message for IL-8 but this induction was reduced in the presence of LPS. HT-29 cells did not constitutively express message for HLA-DRβ or invariant chain but expression of both was induced in the presence of IFN-γ. Similar results were obtained for the expression of HLA-DR protein on the cell surface. However, LPS inhibited the induction of HLA-DR at the molecular level. Moderate levels of HLA-ABC on HT-29, were maximal in the presence of IFN-γ, were unchanged in the presence of LPS. Taken together, our results confirm that IEC contribute to the immunological integrity of the gut and that this contribution is modulated by factors of both bacterial and cellular origin.

J1-138 MURINE M290 EXPRESSION MODULATED BY MAST CELL DEGRANULATION Tracey J. Smith and John H. Weis, Division of Cell Biology and Immunology, Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84132

Intraepithelial T lymphocytes (IEL) possess a transforming growth factor-β (TGF-β) inducible integrin complex which consists of the β_7 integrin chain in association with a novel α chain, murine α_{M290}. The potential of mast cells to express the same integrin complex was demonstrated using antibodies directed against the murine α_{M290} chain. IL-3 dependent murine bone marrow derived mast cells demonstrated positive staining only after induction with TGF-β. Transcription of the gene encoding the α_{M290} integrin subunit by bone marrow derived mast cells was also found to be induced *de novo* by treatment of cells with TGF-β. IgE-mediated crosslinking of the high affinity IgE receptor of mast cells, FcεR1, also induced expression of the α_{M290} gene by activated mast cells. Furthermore, exposure of murine TK-1 cells to supernatants recovered from such activated mast cells induces the expression of the α_{M290} gene by the target T cells. This indicates that at least one mast cell mediator can act in a paracrine manner to induce expression of this integrin complex by other cells.

J1-139 *Abstract Withdrawn*

J1-140 REPOPULATION OF SCID MICE WITH MUCOSAL LYMPHOCYTES, Beate C. Sydora, Victoria N. O. Camerini, and Mitchell Kronenberg, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024.

In an effort to study the development and homing of the intestinal lymphocyte population, we have investigated the ability of gastrointestinal mucosal lymphocytes to repopulate severe combined immuno-deficient SCID mice. Intraperitoneal injection of peripheral lymphocytes (splenocytes, lymph node lymphocytes, Peyer's patch lymphocytes) and thymocytes into SCID mice leads to reconstitution of most lymphoid organs including the intestinal mucosae. Repopulation with gastrointestinal mucosal lymphocytes (small and large intestinal intraepithelial lymphocytes and small and large intestinal lamina propria lymphocytes) however, primarily reconstituted the intestinal mucosae. Even though there are phenotypic differences between lymphocytes of the small and large intestine, there were no regional barrier in repopulation, i.e. lymphocytes from the large intestine can repopulate the small intestine and vice versa. Furthermore lymphocytes of the lamina propria repopulated the lamina propria as well as the intestinal epithelium. Kinetic studies revealed that repopulation of the large intestine begins at about two weeks while the small intestine begins at four to five weeks. In addition, repopulation of the lamina propria usually proceeds that of the intestinal epithelium. All heterologous subpopulations of mucosal lymphocytes can be recovered from reconstituted SCID intestine, however the percentage of the different subpopulations varied between individual animals. Repopulation is likely oligoclonal as the usage of certain Vβ genes dominates in individual animals. Lymphocytes recovered from the intestinal tissue of SCID mice that have been repopulated with nonmucosal lymphocytes acquire a phenotype that is specific for mucosal lymphocytes, for example loss of the lymph node homing receptor L-selectin and expression of the mucosal lymphocyte specific integrin $\alpha_{IEL}\beta_7$. Moreover, reconstitution with sorted populations of intestinal lymphocytes as well as studies employing mucosal lymphocytes isolated from *nu/nu* mice suggests that repopulation is due to homing and expansion of mature CD3 positive lymphocytes, as opposed to an immature CD3 negative precursor.

J1-141 RESISTANCE OF INTESTINAL INTRAEPITHELIAL LYMPHOCYTES TO APOPTOSIS INDUCED BY IMMUNOSUPPRESSIVE AGENTS, Nancy Van Houten and Gordana Gasic. Departments of Internal Medicine and Pediatrics, University of Texas Medical Branch, Galveston, TX 77555-0366.

The immune system associated with the gastrointestinal tract is distinct from the systemic immune system, and consists of diverse sequestered compartments. Intestinal intraepithelial lymphocytes (IEL) are incompletely characterized with regard to their functional responses to immunosuppressive agents. Both phenotypic diversity as well as microenvironmental differences define a uniqueness of these populations. Many phenotypic characteristics of IEL are similar to immature lymphocytes in the thymus, in contrast to peripheral T lymphocytes from the spleen, lymph nodes (LN) or blood. Clearly the signalling mechanisms of IEL differ from those of peripheral T cells, yet the underlying mechanisms have not been defined. Data is presented to show that IEL do not respond to the same immunosuppressive signals as their peripheral counterparts. When mice were treated with 4 mg hydrocortisone acetate for 12, 24, or 48 hr prior to harvest of thymus, LN, or IEL, minimal deletion of $CD4^+CD8^+$ IEL was observed. Other signalling molecules involved in induction of differentiation and death of IEL were examined. Although IEL have many characteristics of activated lymphocytes, $CD4^+$ IEL fail to express the CD40 ligand , and also do not express Fas, the mouse homologue of APO-1. Together these data demonstrate that activation and signalling mechanisms of IEL differ from peripheral T lymphocytes as do their responses to immunosuppressive agents.

J1-142 CLONING AND GENOME STRUCTURE OF THE MURINE POLYMERIC IMMUNOGLOBULIN GENE,

Martin Ph. Verbeet[1], Ellen Vollebregt[1], Shirley van Amersfoorth[1], Charlotte Kaetzel[2], Sang He Lee[1], Herman A. de Boer[1], 1) Medical Biotechnology Dept., Leiden Institute of Chemistry, Leiden University, P.O. Box 9502- 2300 RA, Leiden, The Netherlands; 2) Dept. of Pathology, University of Kentucky, U.S.A.

The polymeric Ig receptor [pIgR], which binds to polymeric IgA and IgM and mediates their transepithelial transport into the mucosal and glandular secretions, plays a keyrole in the protection of mucosal surfaces against environmental pathogens. The receptor is also involved in the transfer of passive immunity from the mother to the offspring via milk. To study the regulation of pIgR expression in the mammary gland of the mouse, we isolated the complete murine gene. We screened a genomic library of mouse strain #129. Using the murine pIgR cDNA as a probe, we obtained three clones containing the whole gene on overlapping fragments. Upon resolving its genomic structure, we found the 11 exons (I through XI) of the gene spanning 24 kb. Exons II to XI contain the complete coding region. They range in size from 59 bp (exon X) to 797 bp (exon XI containing the polyadenylation site). All intron/exon bounderies meet the consensus sequence motifs for splicing signals. The putative transcription start of the gene lies 200 bp upstream of the exon I splice site. In addition, 3 kb of the promoter region and 2 kb of 3' flanking sequences were obtained. Comparison of the murine gene organisation with that of the human pIgR gene [Kracji et al., Eur. J. Immunol. 22 (1992) 2309] shows a similar intron/exon structure with the exception of a 7 kb long intron II in the murine gene corresponding to a much smaller (0.8 kb) intron in the human gene.

J1-143 **DISEASE EVOLUTION IN SPONDYL-ARTHROPATHY PATIENTS IN RELATION TO THEIR GUT HISTOLOGY ON ILEOCOLONOSCOPY.**

Eric M. Veys, Dirk Elewaut, Filip De Keyser, Herman Mielants, Claude Cuvelier, Martine De Vos, Stefaan Goemaere. Dept. of Rheumatology, Ghent University Hospital, Belgium.

The gut plays an important role in the pathogenesis of the disorders fitting into the concept of spondylarthropathies (SPA). Previously, we demonstrated gut inflammation in 60% of the patients with different forms of SPA. One-hundredtwenty-three patients with SPA who underwent an ileocolonoscopy were reviewed after 2 to 9 years (mean 5 years and 9 months). Patients with psoriatic arthritis and patients with inflammatory bowel disease (IBD) were excluded. They were all subjected to a clinical, radiological and laboratory examination. Forty-nine of them underwent a second ileocolonoscopy at the follow-up examination. For 94 other patients the evolution was obtained through a telephone interview. At the time of the first ileocolonoscopy 53 patients (43%) had ankylosing spondylitis (AS), the remaining 70 patients presented another form of SPA. Inflammatory gut lesions were found in 67% of the patients.

At review 53 patients (43%) no longer complained of axial or peripheral inflammation. Eighty-five (47%) were diagnosed as having active AS, 9 had active undifferentiated SPA and 3 patients had developed rheumatoid arthritis. In 8 patients (6.5%) clinical and histological evidence of IBD was obtained; all of them had AS. Three other patients who participated in the telephone interview mentioned a diagnosis of IBD. In all, 11 of 217 patients with SPA had developed ideopathic IBD; all of them had presented inflammatory gut lesions at the first ileocolonoscopy (2 acute and 9 chronic inflammatory gut lesions). Of the 49 patients who underwent a second ileocolonoscopy, 17 patients did not complain of inflammatory symptoms; all of these had a normal gut histology at the second examination.

Gut inflammation in SPA is related to the inflammatory locomotor symptoms and can in some cases progress to IBD.

J1-144 NITRIC OXIDE SYNTHASE IN THE DEVELOPING RAT INTESTINE, G.T.M. Wagenaar, M.W.M. Markman, P.A.J. de Boer, A.F.M. Moorman and W.H. Lamers, Dept. Anatomy & Embryology, Univ. Amsterdam, The Netherlands.
When studying the expression pattern of ornithine cycle enzymes in the rodent intestine, we observed that all enzymes *except* arginase, the last enzyme of the cycle, are expressed at elevated levels up to weaning. After weaning, enzymes converting citrulline to arginine disappear, suggesting a switch from arginine to citrulline production in the gut. We speculated that the infantile intestine synthesizes arginine in stead of citrulline (as in the adult) to produce substrate for nitric oxide synthase (NOS). Using monoclonal antibodies, we found that the neonatal intestine hardly expresses NOS, but that high levels subsequently accumulate in the enterocytes of the ileum, with a peak in the 2nd postnatal week. NOS expression colocalizes with the "supranuclear vacuole", a giant lysosome and a landmark organelle of the suckling ileocyte. NOS was further found in the neurons of the myenteric plexus. No immunoreactivity was seen in the developing Peyer's patches. Striking functional features of the suckling intestine are the immaturity of the mucosal immune system (MIS) and the ability to absorb luminal contents via pinocytosis. Since the contents of pinocytotic vesicles are delivered to the supranuclear vesicle, the presence of NOS in this temporary organelle fits perfectly with the idea that a high cellular NO production serves to defend this cell against intracellular pathogens. The development of a "physiological inflammation" in the gut at weaning suggests that the transition from protection by an innate passive immunity that is based on NO synthesis in the cells lining the gut, towards one that is based on acquired immunity, is not seamless. The temporal coordination between both processes is therefore critical. A tight temporal coupling between the decline in the intestinal NO synthesis and the activation of the MIS may be accomplished if the NO produced by the suckling ileum also suppresses immune system activation prior to weaning. Such an active immune suppression in the suckling period may well be essential for the development of oral tolerance to dietary substances.

J1-145 ANALYSIS OF COSTIMULATION-REQUIREMENTS FOR IG ISOTYPE- AND TERMINAL DIFFERENTIATION OF PEYER'S PATCH B LYMPHOCYTES, [1,2]Yoshio Wakatsuki, [1]Yoichi Matsunaga, [1]Takashi Usui, [1]Toru Kita and [2]Warren Strober, [1]Faculty of Medicine, Kyoto University, Japan and [2]Mucosal Immunity Section, NIAID, NIH, Bethesda, MD
For B cells in the Peyer's patches to go through cell-activation, proliferation, differentiation and to evade cell-death, stimulations through antigen receptors and costimulation-receptors in appropriate combinations and sequences are presumed to be essential, which still remains to be proved yet. For instance, molecules like CD40, B7-1, B7-2 and their counter receptors are playing central roles for B cells to class-switch, to differentiate to memory cells and antibody-production competent cells, and to activate both cognate and non-cognate T cells. In these context, to study these costimulation-requirements, we have created cell lines expressing CD40 ligand, B7-1 and B7-2, together with Fc gamma receptor respectively, which allowed us to costimulate B and T cells using anti-antigen receptor antibodies in coculture of these recombinant cells and lymphocytes. Anti-IgD stimulation of Pp B cell in the absence of costimulation lead to cell death, whereas it showed weak mitogenicity to spleen B cells. Stimulation of Pp B cells by anti-IgD, CD40 ligand and IL-4/-5 increased more mIgM positive cells than mIgA positive cells, whereas stimulation by CD40 ligand, IL-2 and TGF-ß induced 3-5 % of mIgA positive cells in the resting spleen B cell culture. Moreover, we have compared the effect on B cells for emergence of PNA^{hi} cells by stimulation of B cells with Anti-IgD + CD40 ligand + cytokines and by indirect stimulation of B cells with prestimulated T cells with anti-CD3 and B7-1. The indirect stimulation induced relatively more PNA^{hi} cells than the direct stimulation. More details will be discussed in the meeting.

J1-146 **Induction of the Proinflammatory Cytokine IL-6 by Human Intestinal Epithelial Cells After *Salmonella typhi* Invasion. Debra L. Weinstein, Barbara L. O'Neill, and Eleanor S. Metcalf. Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.**
Typhoid fever is a communicable disease which occurs as a consequence of ingestion of *S. typhi*-contaminated food or water. In the host, penetration of the epithelial barrier of the intestinal mucosa is an essential, initial step in the pathogenesis of this organism. Understanding the mechanisms of this interaction between host and pathogen is the main goal of our studies. In the present investigation, the role of the intestinal epithelial cell in the induction of proinflammatory cytokines after adherence/invasion by *S. typhi* is analyzed. Previous studies from this laboratory have demonstrated the capacity of *S. typhi* to adhere to and invade the human intestinal epithelial cell line, Intestine 407 (Int407), *in vitro*. In this study, Int407 cells were cultured in the presence or absence of *S. typhi* or other enteric bacteria, and standard invasion assays were performed. The supernatants from these cultures were collected, centrifuged or filtered, and analyzed for the presence of IL-6 by ELISA or the B9 cell proliferation bioassay. In addition, the total RNA from the infected epithelial cells was extracted and quantitated for specific IL-6 mRNA by RT-PCR. The results show that: 1) *S. typhi* strains ISP 1820, Ty-2, and Quaile stimulate the secretion of IL-6 by Int407 cells, 2) bacterial entry is required for induction and secretion of IL-6 by Int407 cells, 3) *S. typhi* vaccine strains (double *aro* mutants) stimulate the secretion of biologically active IL-6 by Int407 cells, and 4) under culture conditions in which all strains of *S. typhi* stimulate the secretion of IL-6, the secretion of biologically active IL-6 by *S. typhimurium*, enteroinvasive *E. coli*, *S. flexneri*, and *Y. enterocolitica* is significantly less. Taken together, these results suggest a role for epithelial cell-derived IL-6 in the pathogenesis of *S. typhi*.

J1-147 ORAL ADMINISTRATION OF MURAMYL DIPEPTIDE (MDP) MODULATES IgA SYNTHESIS, CELL PROLIFERATION AND CYTOKINE mRNA ACCUMULATION IN MICE, Melita Zunic, Franz Kricek, Peter Dukor, Dorian Bevec and George M. Bahr, Sandoz Forschungsinstitut, A-1235 Vienna, Austria.
Muramyl peptides are synthetic immunostimulants endowed with the ability to potentiate non specific resistance to infections following perenteral administration into a host. We have studied the effect of oral treatment of mice with MDP on the lymphoproliferative responses, immunoglobulin secretion and cytokine induction in gut associated lymphoid tissues (GALT). Cells derived from Peyer's patches (PP) of mice orally primed with MDP, were found to have enhanced proliferative responses to different mitogens and to secrete significantly higher IgG, IgM and IgA antibodies as compared to cells from unprimed mice. These effects were not observed with cells derived from mesentheric lymph nodes (MLN) or spleens of MDP-primed mice. On the other hand, oral administration of MDP resulted within 12-24 hrs in the up-regulation of interleukin-6 (IL-6) mRNA accumulation and a concomittent down-regulation of IL-4 mRNA accumulation in both MLN and spleens of treated mice. These findings reveal an important immunomodulatory activity of orally administered MDP on the GALT and point to a potential role of this immunostimulant in the enhancement of mucosal immunity.

J1-148 **INTERLEUKIN 1 IS RELEASED BY MURINE MACROPHAGES DURING APOPTOSIS INDUCED BY *Shigella flexneri*.** Arturo Zychlinsky*, Catherine Fitting+, Jean-Marc Cavaillon+ and Philippe J. Sansonetti, Unité de Pathogénie Microbienne Moléculaire, INSERM U389 and +Unité d'Immuno-Allergologie. Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France. The Skirball Institute and Department of Microbiology, New York University Medical School, 540 First Avenue, New York, NY 10016.

Peritoneal macrophages undergoing apoptosis induced by *Shigella flexneri* infection release the inflammatory cytokine Interleukin 1 (IL-1), but not IL-6 or Tumor Necrosis Factor α (TNFα). Wild type shigella causes a very fast and significant release of IL-1 from pre-stimulated peritoneal macrophages, before the cell's integrity is compromised. Both IL-1α and IL-1β are released, IL-1β in its mature processed form. IL-1 is released from pre-synthesized cytoplasmic pools. These results demonstrate that bacteria-induced apoptosis of macrophages may play an active role *in vivo* by releasing IL-1 which in turn mediates an early inflammatory response in epithelial tissues.

Immune Responses to Mucosal Pathogens and Novel Mucosal Vaccines

J1-200 **ORAL IMMUNIZATION WITH RECOMBINANT NORWALK VIRUS PARTICLES,** J.M. Ball, D. Graham, A. Opekum, M. Hardy, C. Barone, M.E. Conner, and M.K. Estes, Baylor College of Medicine, Houston, TX 77030

Norwalk virus (NV) is a calicivirus which causes acute non-bacterial epidemic gastroenteritis in all age groups. The NV capsid is composed of 180 copies of a single 58 kd protein which spontaneously form virus-like particles (VLP) when expressed in the baculovirus system. These recombinant NV (rNV) particles have been utilized as an oral immunogen in mice and volunteers to test their potential as an oral vaccine. Mice were orally inoculated 4 times with rNV particles in concentrations ranging from 5 to 500 ug in the absence of adjuvant or from 5 to 200 ug with 10 ug of cholera toxin (CT). A serologic immune response was obtained with all concentrations of VLPs in the presence or absence of CT. In the absence of CT, 100% serological reactivity was observed with doses of 200 ug and above, and 8 of 11 mice demonstrated an immune response with only 5 ug of rNV particles. In the presence of CT, 100% serological reactivity was induced with a lower dose (50 ug) of VLPs. We are currently examining fecal IgA antibody responses. In a phase I human trial, volunteers with pre-existing NV-specific antibodies were given 2 oral doses of 100 ug of rNV at a 3 week interval in the absence of CT. We observed a significant rise in titer with 3 of the 5 volunteers following the first dose of rNV VLPs; there were no additional rise in titers following the second dose. No adverse reactions were observed or reported by the volunteers from the vaccine. These studies show rNV particles are an excellent model to test the oral delivery of mucosal immunogens.

J1-201 **INTRANASAL VACCINATION WITH SUBUNIT INFLUENZA VACCINE COMBINED WITH THE ADJUVANTS LTK63 OR MF59,** G.L. Barchfeld[1], A.L. Hessler[1], D.A. Higgins[1], D. Cataldo[1], P. Traquina[1], M. Pizza[2], M.T. De Magistris[2], G. Douce[3], G. Dougan[3], G. Van Nest[1], [1]Chiron Corporation, Emeryville, CA, [2]Istituto Ricerche Immunobiologiche Siena, Siena, Italy, [3]Imperial College of Science, Technology and Medicine, London, UK

Existing influenza vaccines are administered intramuscularly without an adjuvant. Protection is correlated with a threshold serum HAI titer, but populations at high risk for influenza have a poor antibody response. Influenza begins as an upper respiratory infection, with increased incidences of deep lung involvement in high risk patients. A more effective influenza vaccine should provide both mucosal immunity to aid resistance to initial upper respiratory infections and systemic immunity to protect against possible deep respiratory disease. We are screening candidate adjuvanted influenza vaccines in the mouse intranasal model for their ability to elicit both mucosal and systemic immune responses. Balb/c mice were immunized two times four weeks apart by intranasal instillation of subunit A/Taiwan alone or in combination with a non toxic mutant of heat labile enterotoxin, LTK63, or an oil-in-water emulsion adjuvant, MF59. Two weeks after the second immunization, serum anti-HA Ig and nasal wash anti-HA IgA had been elicited by vaccines with either adjuvant but were very low for HA alone. We found a threshold HA dose for serum Ig and nasal wash IgA between 0.1 and 1.0 ug. An optimum LTK63 dose for anti-HA IgA was 1 ug, with no maximum serum Ig titer observed with up to 50 ug of LTK63. An optimum MF59 dose was 10 ul. Control intramuscular experiments with HA/MF59 generated greater than ten fold higher serum anti-HA Ig, but no nasal wash anti-HA IgA. The immune responses to intranasal vaccination of both previously infected and intramuscularly immunized HA seropositive mice will be compared. We will also test intranasal vaccination in HA seropositive baboons.

J1-202 EXPRESSION OF *B. PERTUSSIS* ANTIGENS IN THE *S. TYPHI* LIVE ORAL VACCINE STRAIN CVD908. Eileen M. Barry[1], Oscar Gomez-Duarte[1], Stephen Chatfield[2], Rino Rappuoli[3], James E. Galen[1], and Myron M. Levine[1]. Center for Vaccine Development, University of Maryland at Baltimore, Baltimore, Md 21201[1], Medeva Vaccine Research Imperial College of Science, Technology and Medicine, London, SW7 2AZ, UK[2] and Biocine-Sclavo, 53100 Siena, Italy[3].

Ideally, protection against *Bordetella pertussis* infection should involve a systemic as well as mucosal immune response within the respiratory tract. The concept of the common mucosal immune system allows that elicitation of such a response could be stimulated by the *Salmonella typhi* vaccine strain CVD908, which has been developed to allow delivery of heterologous antigens to the immune system via oral inoculation. CVD908 may be exploited to serve as a carrier of several foreign antigens simultaneously. In order to test the ability of CVD908 to stimulate a protective mucosal response at a site distant to that of delivery, a series of constructs of *Bordetella* antigens have been engineered in this strain. An antigen that can elicit neutralizing antibodies against pertussis toxin (the virulence factor responsible for most systemic effects caused by *B. pertussis*) is considered essential for any new pertussis vaccine. Stable expression of the enzymatically active S1 subunit of pertussis toxin was achieved by engineering transcriptional fusions with tetanus toxin Fragment C, driven either by the constitutive *lac* promoter or the anaerobically-induced *nirB* promoter. In addition vectors were developed to allow antigen fusion to either the amino terminus or carboxy terminus of Fragment C. The S1 subunit and truncated peptides of S1 were fused to both ends of Fragment C. Expression of the hybrid S1-Fragment C or Fragment C-S1 proteins was directed by the anaerobically-induced *nirB* promoter in CVD908. Fusion proteins were recognized on Western blots by both antibody to Fragment C as well as pertussis toxin neutralizing antibody indicating that a functional conformation was maintained. CVD908 derivatives harboring the tetanus and pertussis antigen constructs are being evaluated in the mouse model to compare their capacities to stimulate serum and mucosal immune responses.

J1-203 ***LACTOBACILLUS* AS VECTORS WITH INTRINSIC ADJUVANTICITY FOR SAFE LIVE MUCOSAL VACCINES**

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Single dose live oral vaccines are most urgently needed to meet the prerequisites as phrased in the "Children's Vaccine Initiative". The use of a GRAS (safe) micro-organism as *Lactobacillus* as oral vaccine carrier is of advantage in that these bacteriae have probiotic or health beneficial properties and are normal commensals in most mammals. In mice, using model antigens like haptens (TNP), chicken gamma-globulin and synthetic peptides we have investigated the properties of various *Lactobacillus* strains as a carrier for antigens and as an adjuvant in classical parenteral immunization and after oral administration. It was shown that some *Lactobacillus*-strains do express adjuvant properties to a level as observed with water-in-oil adjuvants whereas other strains do not provide immuno-enhancing effects. Haptenated *Lactobacillus* provide a carrier function in serum antibody as well as DTH responses. Experiments with synthetic peptides, containing at least one B-cell and one T-cell epitope, free or coupled to the surface of the bacteriae indicate that surface expression best supports immune response induction (antibodies, DTH). New expression vectors for *Lactobacillus* with surface anchored viral- and bacterial peptide-antigens were developed for application in vaccine studies. For anchoring the proteinase of *L.casei* was used whereas β-glucuronidase was used as a reporter molecule. Allexpression cassette vectors have a rho-independent-like terminator sequence. With the constitutive l-*ldh*-promoter successful expression of epitopes of the haemagglutinin of influenza was obtained. Alternatively vectors were prepared with the α-amylase promoter from *L.amylovorus* to obtain regulatable expression of epitopes which dependent on the design of the vectors may be surface anchored or excreted.

J1-204 MACAQUE RECTAL MUCOSA: A MODEL SYSTEM FOR HIV RECTAL TRANSMISSION, Cécile Butor, Anne Couëdel-Courteille, Jean-Gérard Guillet et Alain Venet, INSERM U152, ICGM, 22 rue Méchain, 75014 Paris, France

The most common route of transmission of HIV is the mucosal route. There are theoretically four ways for a lymphotropic virus to cross the epithelial barrier: lesion in the epithelium, infection of the epithelial cells, transcytosis of virions in epithelial cells and uptake of virions by cells of the mucosal immune surveillance (M cells or Langerhans cells). Each route of entry will lead initially to the infection of a different subset of immune cells, implying a different mechanism for viral dissemination, a different host immune response and a different strategy for protection.

We are currently establishing the SIV-macaque system as an animal model for the study of HIV transmission by the mucosal route, and of the local immune response to infection. We chose to study rectal transmission because cell biology and immunology have been more extensively studied in the gut than in other epithelia.

We show that the overall morphology of the macaque rectal mucosa is very similar to that of humans. Differenciation markers follow the same pattern as in the human system. The carcino-embryonic antigen, for example, is apical in cells of the villi, and the epithelial cells express a basolateral marker. Dipeptidyl-peptidase IV (CD26) is not detectable by immunofluorescence in rectocytes. Galactocerebroside, whose expression correlates with infectability of human colon carcinoma cells by HIV, is abundant in macaque rectocytes, including the apical domain. We found few intraepithelial lymphocytes, but numerous lamina propria lymphocytes. Up to 80% of IEL and 55% of LPL expressed the $\alpha_E\beta_7$ integrin (detected with the HML-1 antibody). Functional activity of both populations was similar to their human counterparts.

These observations are very similar to published data on the human rectal mucosa. We believe therefore that the macaque rectum-SIV system is a good model for HIV rectal transmission.

J1-205 SALIVARY, GUT, VAGINAL & NASAL ANTI-BODY RESPONSES AFTER ORAL IMMUNISATION WITH BIODEGRADABLE MICROPARTICLES. S.J.Challacombe, D.Rahman & D.T.O'Hagan*. Department of Oral Medicine & Pathology, UMDS, Guys Hospital, London and *United Biomedical Inc. Hauppauge, NY 11788

Previous studies have shown that oral immunisation with polylactide co-glycolide microparticles containing antigen can efficiently induce salivary and systemic antibody responses. The aims of this study were to determine whether oral immunisation with biospheres might lead to a common mucosal response including vaginal secretions. Female Balb/c mice were immunised orally with biospheres containing ovalbumin at 0 and 4 weeks. Antibody responses were assayed by ELISA in saliva, gut washings, vaginal washings and serum, and antibody producing cells were assayed by ELISPOT in salivary glands and the nasal associated lymphoid tissue (NALT).

After primary immunisation with biospheres, IgA antibodies were detected in saliva and in vaginal and gut washings which were significantly greater than those detected with soluble antigen alone ($p<0.05$). Secondary immunisation greatly enhanced antibody titres in all fluids examined. Oral immunisation with biospheres also induced antibody forming cells in salivary glands and NALT particularly after the secondary immunisation. Immunisation with soluble antigen resulted in detectable antibodies in saliva and gut washings but these were significantly less than with biospheres. There was no direct correlation between individual secretory and serum antibody titres. The results suggest that oral immunisation with antigen in biospheres induce common mucosal immune responses and should prove useful in immunisation against infections affecting mucosal surfaces.

J1-206 **Polymeric Ig Receptor gene expression in the mammary gland during pregnancy and lactation and hormonal regulation**

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The polymeric immunoglobulin receptor (poly Ig-R) mediates transcytosis of IgA and IgM antibodies produced by local plasma cells across epithelial cells of mucosal and glandular tissues. Gene expression of the poly-Ig R was analyzed in rabbit mammary gland during pregnancy and lactation. The poly Ig-R was expressed as early as day 8 (G8) of gestation and mRNA level remained low until about G18. From G21, the mRNA levels increased and reached steady state levels 5-fold higher at day 15 of lactation (L15) when compared to basal levels at G8. The hormonal regulation of poly-Ig receptor gene expression was assessed in mammary organ cultures. Poly-Ig R mRNA levels in mammary explants cultured for 24 or 48 h in presence of increasing concentrations of ovine prolactin (oPRL) was dose-dependent with a 2.5 fold increase with 50 ng ml^{-1} oPRL, and a maximun 4 fold increase at 1 $\mu g\ ml^{-1}$. Oestradiol (100 pg ml^{-1}) or progesterone (1 $\mu g\ ml^{-1}$) did not further stimulate poly-Ig R expression. In contrast, their combination resulted in a significant 30-50% decrease of poly-Ig-R mRNA levels, which was similar to that generated by the addition of 1 $\mu g\ ml^{-1}$ of cortisol to medium containing oestradiol or progesterone. The results suggest that until mid-pregnancy, polyIg-R expression is inhibited in the mammary gland by elevated progesterone-oestradiol concentrations and that the subsequent increase is due to the concomitant decrease of the two circulating steroids and the increase of serum prolactin levels.

J1-207 IMMUNOMODULATION OF GASTROINTESTINAL BARRIER FUNCTIONS BY LACTOBACILLUS REUTERI. Walter J. Dobrogosz, Holly J. Dunham, Carl Williams, Frank W. Edens, Ivan A. Casas, Departments.of Microbiology and Poultry Science, NC State University, Raleigh, NC 27695.

Lactobacillus reuteri is the major Lactobacillus component of the 'normal microbiota' which colonizes in the proximal gastrointestinal (GI) tract of animals and humans. We have obtained evidence that L. reuteri effects the host animal's mucosal immune response. Under natural conditions this species is transferred from mother to offspring at birth - a process precluded in modern poultry brooding. Because young chicks and poults are hatched and brooded in isolation from their mothers and other adults, their GI tract is poorly colonized with L. reuteri. Using these animals as a model system, we have shown that peroral inoculation with viable pure cultures of host-specific strains of L. reuteri can significantly moderate the severe mortality and morbidity effects resulting from Salmonella and enteropathogenic Escherichia coli infections. In similar experiments using rats, prophylactic colonization of the GI tract by L. reuteri has been shown to reduce the incidence of experimental bacteremia and to protect colonic tissue from acetic acid-induced colitis. We have obtained evidence indicating that L. reuteri confers these benefits by potentiating the host's immune response. For example, in comparison to untreated controls, L. reuteri-treated chicks at 3 days of age exhibit significantly longer villi and deeper crypts, specifically in the ileal region of the gut, a response reported to be associated with enhanced T cell function. When challenged with Salmonella, the L. reuteri-treated animals also exhibit increased production of serum anti-Salmonella IgM antibodies. Also, GI tissue sections obtained from 5 day old control and L. reuteri-treated chicks have been immunoperoxidase stained using chick monoclonal antibodies to CD4+ and CD8+ lymphocyte subsets. These data showed that the treated animals have significantly more CD4+ T cells present in the lamina propria of the ileum; whereas, the CD8+ T cell population was unaffected by the treatment. Thus, the treatment increased the ileal CD4+ / CD8+ T cell ratio from approximately 2 to 3.5. This effect is deemed an important clue concerning L. reuteri's mode of action in protecting the host animals from microbial stressors.

J1-208 IMMUNOGENICITY OF BACTERIAL RIBOSOMES USED AS ORAL VACCINES. Gilbert C Faure, Carole Zanin, Philippe Perrin, Bernard Gobert, Anne M Perruchet, Marie C Béné. CDPF Toulouse - Immunologie & ORL, Faculté de Médecine & CHU de Nancy, 54500, Vandoeuvre les Nancy, France

The immunogenic properties of bacterial ribosomes were initially demonstrated for mycobacteria. In Europe, a widely prescribed immunostimulant, D53, composed of ribosomal fractions from *Klebsiella pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae* and *Streptococcus pyogenes* has been used for over 10 years. Clinical studies have demonstrated the efficacy of D53 in reducing the number of infectious episodes of the upper respiratory tract, suggesting that it could act as an oral vaccine. Immunohistological studies later demonstrated a significant increase in specific-immunoglobulin producing plasma-cells on tonsil sections from treated children. We further investigated the immunogenic properties of this compound by comparing the reactivity of B-cells towards either whole extracts of the relevant bacteria or purified ribosomal fractions, and by performing immunochemical studies of the ribosomal proteins. Specific B-cells were enumerated in parallel using the ELISAspot method in the peripheral blood and tonsils of treated and untreated children and in an animal model involving three days treatment in sheep. Similar numbers of spots were obtained towards both types of antigenic preparations. The data obtained in untreated children or animals demonstrate that activated B-cells triggered spontaneously by wild strains of these common bacteria can indifferently recognize whole bacterial antigens or ribosomes. Data in treated children or animals, where increased numbers of both types of cells were evidenced, suggest that oral administration of the ribosomal preparation can trigger the proliferation of B-cells recognizing whole bacteria antigens. Comparative SDS-PAGE analysis of whole bacteria extracts and ribosomal proteins showed the presence of several bands of similar molecular weight. The presence of antigenic proteins was further explored by preparative isoelectric focusing (IEF) of the ribosomal proteins, which were found to have mostly low pI values, a feature previously reported for antigenic peptides from mycobacteria. IEF fractions were finally tested in immunochemistry, and two of the 20 fractions collected, with respective pI of 2.42 and 7.33 were found to react strongly with specific antibodies in dot-blot. These data support the efficacy of bacterial ribosomes to trigger protective mucosal immune reponses after oral administration.

J1-209 HOST DEFENSE MECHANISMS IMPORTANT FOR PROTECTION AGAINST VAGINAL CANDIDIASIS. Fidel, P.L., M.E. Lynch, and J.D. Sobel, Division of Infectious Diseases, Wayne State University School of Medicine, Detroit, MI 48201.

Mucosal candidiasis is a significant problem in immunocompromised hosts with reduced cell-mediated immunity (CMI). Recurrent vulvovaginal candidiasis (RVVC) is similarly postulated to result from reduced or deficient CMI. However, recent evidence suggests that systemic CMI in women with RVVC is normal. We have used a murine model of vaginal candidiasis to study the host defense mechanisms at the vaginal mucosa. Results indicate that a localized vaginal Candida infection stimulates Candida-specific systemic Th1-type CMI that is indistinguishable from that induced by systemic immunization with Candida antigen. However, preinduced Candida-specific Th1-type systemic CMI did not protect mice against vaginal candidiasis. In contrast, mice given a primary vaginal infection that was allowed to spontaneously resolve, had enhanced DTH reactivity and were partially protected from a second vaginal infection. Suppression of Candida-specific systemic CMI by tolerization with Candida antigen, or depletion of peripheral CD4 and CD8 T cells by neutralizing antibodies, abrogated DTH reactivity but did not affect vaginal Candida burden during the primary infection or protection against a secondary infection. Taken together, our results suggest that local (compartmentalized), rather than systemic CMI represents a protective host defense mechanism at the vaginal mucosa. In support of this, flow cytometric analyses show that the vaginal mucosa of mice contains $CD3^+$ T cells (alpha/beta and gamma/delta) in percentages distinct from those in the periphery.

J1-210 USE OF RECOMBINANT COMMENSAL BACTERIA EXPRESSING FOREIGN SURFACE PROTEINS AS LIVE VACCINE VECTORS FOR THE INDUCTION OF A MUCOSAL AND SYSTEMIC IMMUNE RESPONSE

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Our approach uses gram-positive commensal bacteria as live vaccine vectors to deliver antigens to mucosal niches invaded by pathogens. As a model, we developed a system allowing the stable expression of heterologous antigens on the surface of *Streptococcus gordonii,* a commensal organism of the human oral cavity. To prove that a wide array of antigens may be delivered in this way, a *S. gordonii* strain was engineered to chromosomally express an allergen (Ag5.2) of the white-faced hornet venom as a fusion with M6 protein of *S.pyogenes*. The fusion protein consisted of the total 204 amino acids of Ag5.2 inserted between the N-terminal and anchor region of the M6 protein, after deleting a 179 amino acid segment of the surface exposed region of the M6 molecule. The immunogenicity of the M6-Ag5.2 fusion protein expressed on the surface of the recombinant strain was assessed in mice inoculated orally and intranasally with a single dose of recombinant bacteria (about 10^9 CFU). The recombinant strain efficiently colonized the oropharyngeal mucosa of the mice for 10-12 weeks. Ag5.2-specific IgA levels in saliva, lung and intestinal fluids were determined with relation to the total IgA. Significantly elevated titers of Ag5.2-specific IgA was detected in saliva when compared with control mice colonized with the wild type *S. gordonii*. Comparable increases of Ag5.2-specific IgA was also detected in lung lavage with levels three to four times that of controls. Lower levels of Ag5.2-specific IgA were found in intestinal lavages demonstrating that the IgA response was limited primarily to the colonized mucosa. A serum IgG response was also induced after oral colonization which peaked between 4 and 6 weeks with titers comparable to intradermal immunization using the same antigen in adjuvant. Animals inoculated with killed recombinant bacteria showed no immune response in the mucosal compartments tested. Our results indicate that non-pathogenic gram-positive commensal bacteria may be used as a safe and effective method of inducing both a mucosal and systemic immune response to a variety of proteins and peptides.

J1-211 EXPRESSION OF A STABLE TETANUS TOXIN FRAGMENT C-DIPHTHERIA TOXIN PROTEIN FUSION IN THE ATTENUATED *Salmonella typhi* CVD 908 VACCINE STRAIN.

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The current parenteral diphtheria-tetanus-pertussis (DTP) vaccine requires 3 doses to confer protective immunity, and suffers from reactogenicity of the inactivated whole cell pertussis component of the vaccine. These disadvantages can be overcome through expression of relevant protective DTP antigens in the well-tolerated and highly immunogenic single-dose *Salmonella typhi* CVD 908 vaccine strain. Here we demonstrate that the protective yet non-toxic carboxy-terminal fragment of tetanus toxin (Fragment C) is efficiently expressed from plasmids within CVD 908. However, expression from plasmids of the carboxy-terminal receptor-binding domain of diphtheria toxin (DT) was not detected using Western immunoblot analysis, although Northern blot analysis demonstrated synthesis of the mRNA encoding this domain. The binding domain of DT was therefore genetically fused through a Gly-Pro-Gly-Pro hinge region to the carboxy-terminus of Fragment C, creating a 611 amino acid fusion protein with a predicted molecular mass of 67 kDa. Expression of this plasmid-encoded fusion protein within CVD 908 was readily detected by Western immunoblot analysis using monoclonal antibodies specific to both Fragment C and the DT receptor-binding domain. This Fragment C-DT fusion is now being mobilized into the chromosome of CVD 908, and studies are underway to evaluate the immunogenicity and efficacy of this fusion protein using the BALB/c mouse model.

J1-212 EXPRESSION OF FOREIGN PROTEINS BY INFLUENZA VIRUS VECTORS,

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The development of a ribonucleoprotein transfection method allows us to genetically manipulate influenza virus, a segmented negative strand RNA virus. Using this technique we have been able to generate influenza virus vectors expressing foreign polypeptides. We have rescued into infectious virus synthetic viral RNAs which encode both an essential viral protein as well as a foreign protein. The expression of the foreign polypeptide has been achieved in two different ways. The first approach is based on the expression of a polyprotein which self-cleaves into two different proteins. Self-cleavage is accomplished by the 2A protease motif of foot-and-mouth disease virus. This strategy has been used to construct an influenza virus which expresses a polyprotein that is cleaved into the viral neuraminidase and a reporter protein (CAT). The second approach involves the use of a bicistronic gene containing an internal ribosome entry site (IRES). The first cistron is translated by the usual cap-dependent mechanism, and the second cistron is translated via internal initiation. Influenza viruses have been generated which contain bicistronic genes for the expression of foreign polypeptides derived from the gp41 of HIV-1. The ability to express foreign proteins from influenza virus recombinants may allow the use of this virus as an efficient vector for vaccination against different diseases.

J1-213 DIFFERENCES IN IMMUNE RESPONSES OF ORALLY VACCINATED MICE WITH FIVE BCG STRAINS.

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The induction of mucosal immune response is important for protection against diseases which are clearly related to the mucosal system such as tuberculosis, AIDS or salmonellosis.In previous studies we have found increased immune responses in guinea pigs vaccinated via respiratory route with Pasteur BCG vaccine strain and with a Pasteur recombinant *Lac Z* BCG orally administered.

In this study , five BCG strains (Pasteur, Glaxo, Russian, Japanese and Prague), orally administered were investigated for their capacity to translocate the gut mucosa, disseminate in target organs and induce cellular and humoral immune responses.

Results demonstrate, the BCG orally administered is largely eliminated through faeces but it disseminates in Payer's Patches and target organs.

We found significant differences among the five BCG vaccine strains in their capacity to induce T-cell proliferation, DTH and protection against a late mycobacterial challenge. We observed also differences in the local and systemic production of IgG and particularly IgA antibodies.

In conclusion after oral administration, the BCG disseminate in target organs and induces strong cellular and humoral immune responses which vary among the BCG strains. Thus, the Pasteur, Russian and Glaxo prouve similar immunogenicity but significantly higher than that induced by Japanese and Prague vaccine strains.

J1-214 **PROTEIN COCHLEATES: UNIQUE SUBUNIT VACCINES FOR MUCOSAL AND PARENTERAL IMMUNIZATION** Susan Gould Fogerite, Masoumeh Kheiri, Yvette Edghill-Smith, Zheng Wang, Eleonora Feketeova and Raphael J. Mannino, Dept. of Laboratory Medicine and Pathology, UMD, New Jersey Medical School, Newark, NJ 07103-2714

Protein cochleates are a new type of highly effective subunit vaccine. Protein cochleates are stable protein-phospholipid-calcium precipitates which are structurally distinct from liposomes. They have a unique structure consisting of a large, continuous, solid, lipid bilayer sheet rolled up in a spiral, with no internal aqueous space. Cochleates can be stored in calcium-containing buffer, or lyophilized to a powder, stored at room temperature, and reconstituted with liquid prior to administration. Cochleates are capable of multivalent presentation of several protein and peptide antigens from several pathogens.

Oral administration, (simply by drinking), leads to strong, long lasting circulating and mucosal antibody responses, and long term immunological memory. Cell mediated immune responses, including proliferative and cytolytic activities, are also generated. Protection from replication of virus in the trachea and lungs following intranasal challenge with live influenza virus has been demonstrated.

Protein cochleates are also highly effective immunogens when administered intramuscularly. IM immunization stimulates strong circulating and mucosal antibody, proliferative, and cytolytic responses to viral glycoproteins, including recombinant gp160 from HIV 1. In addition, peptides are also strongly immunogenic when formulated as protein cochleates and given perorally or intramuscularly.

We hypothesize that protein cochleates slowly unroll producing proteoliposomes which may be a major structure interacting with cells of the immune system. Long term persistence of immune responses (in many cases increasing over several months) indicates a high probability of slow release characteristics for protein cochleate formulations. This would be consistent with their solid multilayered structure.

J1-215 **PRODUCTION OF AN ORALLY IMMUNOGENIC BACTERIAL PROTEIN IN TRANSGENIC PLANTS** Tariq A. Haq*, Hugh S. Mason*, Charles J. Arntzen*, and John Clements†. *Plant Biotechnology Program, Institute of Biosciences and Technology, Texas A&M University, 2121 West Holcombe Blvd., Houston, TX 77030-3303. †Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA.

We have produced genetically transformed plants that express the binding subunit of the heat-labile enterotoxin of *Escherichia coli* (LT-B). Data will be presented to show that LT-B expressed in plants retains its antigenic epitopes and ganglioside binding ability. Protein extracts from transgenic plants expressing the protein were tested for immunogenicity in mice. When fed to mice, they evoked both systemic as well as mucosal immune responses. *In vitro* cell protection assays demonstrate that the antibodies of serum and gut extractions from immunized mice are effective in neutralization of the enterotoxin. This verifies our concept that transgenic plants expressing candidate oral vaccine antigens can be utilized to provide a very inexpensive manufacturing and delivery system for vaccines that is especially appropriate for developing countries.

J1-216 **MUCOSAL VACCINATION USING A NOVEL MICROPARTICLE DELIVERY SYSTEM,** Philippa L. Heritage, Jianxiong Jiang, Lesley M. Loomes, Brian J. Underdown and Mark R. McDermott, Molecular Virology and Immunology Programme, Department of Pathology, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5

Oral vaccination can be accomplished using replicating organisms, certain enterocyte receptor binding immunogens, e.g. cholera toxin or its B subunit, or large and often repeated doses of nonviable or soluble antigens. Although recent reports demonstrate that oral administration of poly(lactide-coglycolide) (PLG) microparticles (MP) can enhance the immunogenicity of soluble antigens, likely by facilitating uptake and controlled release of antigen in the gut-associated lymphoid tissue, this delivery system may not be suitable to all antigens. We have developed an MP fabrication technique in which soluble antigen was entrapped in starch MP (mean dia. = 5μ). MP could be covalently grafted with a 3-(triethoxysilyl)propyl-terminated polydimethylsiloxane (TS-PDMS), a novel biocompatible silicone polymer. The fabrication process did not appreciably alter the immunogenicity of several entrapped antigens. When given intraperitoneally, both grafted and ungrafted MP dramatically enhanced serum IgG responses. When delivered orally, both TS-PDMS grafted and ungrafted MP elicited antigen-specific IgA responses in gut secretions, in contrast to orally-administered soluble antigen. However, TS-PDMS grafted MP given orally stimulated stronger serum IgG responses, compared to ungrafted MP and soluble antigen. These results indicate that TS-PDMS grafted MP have considerable potential as a mucosal and systemic vaccine delivery vehicle. Supported by Connaught Laboratores Limited, Medical Research Council of Canada and Ontario Ministry of Education and Training.

J1-217 **NOVEL MUCOSAL IMMUNOGEN GENETICALLY CONSTRUCTED FROM A PROTEIN ANTIGEN AND CHOLERA TOXIN A2 AND B SEGMENTS,** Susan K. Hollingshead, George Hajishengallis, Toshihiko Koga, Pamela White, Hong-Yin Wu, Suzanne M. Michalek and Michael W. Russell, Department of Microbiology, University of Alabama at Birmingham, AL 35294, and Department of Preventive Dentistry, Kyushu University Dental School, Fukuoka, Japan.

Potent mucosal immunogens that, upon intragastric (i.g.) or intranasal (i.n.) administration, induce IgA antibodies in various secretions as well as circulating IgG and IgA antibodies, can be constructed by chemically conjugating protein antigens to the nontoxic B subunit of cholera toxin (CT). Construction of antigen-CTB conjugates by direct gene fusion readily disrupts the G_{M1}-ganglioside-binding properties of CTB, which are deemed important for mucosal immunogenicity. To develop a general approach that avoids this problem, we fused PCR-amplified DNA encoding the 42-kDa saliva-binding region of the *Streptococcus mutans* fibrillar protein adhesin (AgI/II) to the PCR-amplified A2-B region of the *ctx* gene, and ligated these into a vector constructed from pET20b(+), which was verified by restriction and sequence analysis. The chimeric protein expressed in *E. coli* BL21(DE3) contained the saliva-binding region of AgI/II fused to CTA2 and assembled with CTB pentamers, as demonstrated by SDS-PAGE, western blotting with antibodies to AgI/II and CTB, and G_{M1}-ELISA. I.g. or i.n. immunization of mice with this chimeric protein induced IgA antibodies in saliva and other secretions, and circulating IgG and IgA antibodies to AgI/II, similar to those found after immunization with chemical conjugates which gave protection against oral colonization with *S. mutans*. Secretory IgA antibodies to AgI/II have also been found effective in inhibiting the adherence of *S. mutans* to in vitro models of the saliva-coated tooth surface. Similar chimeric protein-CTA2/B constructs have been made from two other bacterial antigens and one viral antigen, and are being evaluated for immunogenicity and protective potential.

Supported by US-PHS grant DE06746 and an American Heart Association grant-in-aid to SKH.

J1-218 **ORAL IMMUNIZATION OF MICE WITH A LIVE ATTENUATED *SALMONELLA* VECTOR EXPRESSING RECOMBINANT GP120 OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 ON THE SURFACE OF THE VECTOR, INDUCES MUCOSAL AND SYSTEMIC IMMUNITY AGAINST GP120.** **David M. Hone[1], Timothy R. Fouts[1,2], Shaoguang Wu[1], David W. Pascual[3], John Van Cott[3], Jerry McGhee[3], and George K. Lewis[2].** [1]Center for Vaccine Development, [2]Department of Microbiology and Immunology, University of Maryland, Baltimore, MD 21201 and [3]Mucosal Immunization Research Group, University of Alabama.

One approach toward stimulating HIV-specific mucosal and systemic immunity is the use of live oral *Salmonella* vectors. Using recombinant DNA techniques we constructed an expression cassette which comprises sequences encoding recombinant gp120 (rgp120). When mice were immunized orally with a *Salmonella aro* mutant carrying a single copy of this expression cassette integrated into the chromosome of the vector, no detectable humoral or cellular immune responses were observed. In contrast, mice immunized orally with a *Salmonella aro* mutant carrying multiple copies of the rgp120-expression cassette developed measurable gp120-specific IFN-γ secretion and T cell proliferative responses in the spleen. Recently, we constructed an expression cassette that expresses a chimeric outer membrane protein A, OmpA, fused to sequences of gp120 necessary for CD4 binding. This fusion protein, called OmpA::tgp120, forms an ~65 kD protein that is located in the outer membrane of the vector. In a pilot experiment, mice immunized orally with a *Salmonella aro* mutant carrying a single copy plasmid expressing OmpA::tgp120, developed gp120-specific IgA-producing B cells in the lamina propria, mesenteric lymph nodes and spleen. These result provide encouraging support that *Salmonella* vectors will provide a safe and inexpensive means for delivery of HIV antigens to, and the elicitation of HIV-specific humoral and cell-mediated immunity in, the mucosal and systemic compartments.

J1-219 ALCOHOL CONSUMPTION AFFECTS RESISTANCE TO INTESTINAL PATHOGENS AND IS ASSOCIATED WITH CELL DEPLETION IN THE GALT, Thomas R. Jerrells, Don Sibley, and John Fuseler, Department of Cellular Biology and Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130

Consumption of large amounts of ethanol (ETOH) is associated with an increased incidence of infectious diseases in human beings, including tuberculosis, opportunistic pathogens, and gastrointestinal pathogens. Results of studies have shown that ETOH is associated with depletion of lymphocytes from primary and secondary lymphoid organs and decreased lymphocyte function. The purpose of the present study was to define the effects of ETOH on host defense mechanisms operating in the gastrointestinal tract. Male C57Bl/6 mice were either fed a liquid diet containing 7% (V/V) ETOH or pair-fed an isocaloric liquid diet containing dextrin maltose. After 1, 3, 5, and 7 days of feeding, the number of lymphocytes in mesenteric lymph nodes (MLN) and Peyer's patches (PP) was determined. The proportion of T and B cells and T-cell subpopulations was determined by using flow cytometric techniques. Animals were challenged orally with *Salmonella typhimurium* to assess the ability of ETOH-fed mice to resist a bacterial challenge. ETOH feeding resulted in depletion of lymphocytes from MLN and PP. B cells were the predominant cell lost from MLN and PP; however, the B-cell numbers were recovering at 7 days of feeding. T-cell loss was greatest at this time. Histologic evidence of apoptosis was correlated with B-cell loss. ETOH feeding resulted in a significant increase in translocation of *Salmonella* from the intestinal tract and high numbers of bacteria in the blood, liver, and spleen compared with the control animals. The results of these studies support the suggestion that ETOH affects the ability to resist pathogenic bacteria, and this may be due to cell loss from the MLN and PP. Supported by NIAAA grants AA 09803 and AA 07731.

J1-220 **MUCOSAL IMMUNOGENICITY AND EFFICACY OF PROTEOSOMES AND PA ADJUVANTS FOR HIV, INFLUENZA, SHIGELLA & STAPH. ENTEROTOXIN B (SEB) VACCINES.** **G. Lowell[1], R. Kaminski,[1,2] C. Colleton,[1] N. Orr,[3,4] C. Mallett,[1] R. Levi,[3] E. Aboud-Pirak,[3] J. Estep,[5] L. Pitt,[5] L. Loomis,[1] K. Kersey,[1] T. Vancott[1], W. Baker[1], D. Frost,[1] R. Hunt,[5] J. Hatch,[1] S. Dean,[1] S. Amselem,[2] G. Smith,[6] D. Cohen,[4] R. Arnon,[3] R. Redfield,[1] D. Birx,[1] T. Hale,[1] and W. Baze.[1]** **[1]Walter Reed Army Institute of Research, Washington, DC; [2]Pharmos, Inc., NY; [3]Weizmann Institute of Science, Rehovot, Israel; [4]Israel Defense Forces Medical Corps, Israel; [5]US Army Medical Research Institute for Infectious Diseases, Fort Detrick, Fredrick, MD; [6]MicroGeneSys Inc., Meriden, CT.**

We have used meningococcal outer membrane protein proteosomes as a mucosal vaccine delivery system and Pharmos adjuvants (PA) (either alone or with proteosomes) to enhance mucosal and systemic immunity to microbial proteins, peptides or LPS. With these systems, nasal immunogenicity of HIV gp160 for IgG and IgA in sera and in lung, intestinal and vaginal fluids was enhanced. Nasal or oral proteosome-*Shigella* LPS vaccines induced lung and intestinal IgA and IgG in rodents and protected against lethal pneumonia and keratoconjunctivitis. Nasal proteosome vaccines with influenza B and T peptides protected against respiratory challenge. Formulating SEB toxoid with proteosomes and/or PA enhanced mucosal immunogenicity and synergistically protected against lethal respiratory or i.m. SEB challenge in a murine model. Furthermore, i.m. and intratracheal immunization of monkeys with proteosome-toxoid vaccines induced serum IgG, bronchial lavage IgA and 100% protection against aerosol challenge with lethal doses of SEB. These data show that the proteosomes and PA enhance mucosal immunogenicity of many acellular antigens and enhance protection against mucosal challenge with infectious diseases or toxins in both rodents and non-human primates and hence are prime vaccine candidates for human use.

J1-221 WORM INDUCED SUPPRESSION OF IMMUNITY TO INTRACELLULAR AND EXTRACELLULAR COLONIC BACTERIA IN SWINE, L. S. Mansfield[1], D. E. Hill[2], and J. F. Urban[2], [1] College of Veterinary Medicine, Michigan State Univ., East Lansing, MI 48824, [2]Parasite Immunobiology Lab, LPSI, ARS, USDA, Beltsville, MD 20705

Mucohemorrhagic enteropathies of the intestinal tract of swine have complex and interrelated etiologies of largely unknown pathogenesis. Pigs inoculated with <2,500 Trichuris suis (whipworm) eggs exhibited diarrhea, poor weight gain, intestinal adenomatosis associated with intracellular Ileal symbiont intracellularis bacteria, enlarged colonic lymphoglandular complexes (LGCs) containing numerous extracellular bacteria, lymphocytes, eosinophils, macrophages and neutrophils. At the site of worm attachment in the proximal colon there was mucosal edema, inflammatory cell infiltrates, and bacterial accumulation. Other pigs inoculated with T. suis and treated with antibiotics gained weight normally and had pathologic lesions localized to the site of worm attachment, and histologically normal LGCs with no resident bacteria. The association between bacteria, lymphocytes and macrophages in the LGCs of pigs infected with T. suis suggest an antigen processing role for these structures in the colon. In addition, the complex pathogenesis of necrotic proliferative colitis in pigs can be linked to worm induced suppression of mucosal immunity to resident bacteria.

J1-222 EVALUATION OF HIV-1-SPECIFIC CYTOTOXIC T LYMPHOCYTE (CTL) RESPONSES IN SEMEN FROM HIV-INFECTED MEN. M. Juliana McElrath, Yuxiang Hu, and Luwy Musey. Department of Medicine, University of Washington School of Medicine, Seattle, WA 98195.

Transmission of HIV infection occurs predominantly through mucosal routes. CD8+ MHC class I-restricted CTL are likely to play a critical role in HIV mucosal immunity by controlling cell-associated infection. The goal of this study was to identify the T cell responses in mucosal compartments that may contribute to immune surveillance.

We examined HIV-specific CTL responses in semen at 1-3 time points in 15 HIV-infected men, and as controls, in 2 HIV-uninfected men. The seminal cell pellet was layered over Histopaque, centrifuged and washed. The resulting mononuclear cells were plated 10 cells per well or in limiting dilution concentrations in 96-well plates and amplified with either autologous macrophages expressing HIV gene products or anti-CD3 mAb with rIL-2. Expanded wells were tested for HIV-1-specific CTL responses using autologous EBV-transformed B cell lines (LCL) infected with a panel of recombinant vaccinia viruses expressing HIV Env, Gag, or Pol.

On average, we recovered 100,000 mononuclear cells using this isolation method. No HIV-specific lytic activity by seminal T cells from the HIV-uninfected control donors was demonstrated. In contrast, we detected HIV-specific lytic activity in seminal T cells in nearly 50% of HIV-infected men. Specific lysis in a split well analysis ranged from 10-28%. The predominant gene product recognized in these studies was Gag, and the effector response was mediated by CD8+ T cells. We are currently comparing precursor frequencies of CTL from semen to those in peripheral blood and determining the presence and quantity of HIV from the seminal plasma.

Our findings indicate that HIV-specific CTL are present in semen of infected men. We speculate that these effector cells may have a direct impact on the ability of men to transmit HIV by sexual intercourse.

J1-223 DEVELOPMENT OF LACTIC ACID BACTERIA AS LIVE VECTORS FOR ORAL OR LOCAL VACCINES, Annick Mercenier, Philippe Slos, Philippe Dutot, Patricia Kleinpeter, Jacqueline Reymund, Patricia Paris-Coussinier and Marie-Paule Kieny, Department of Bacteriology, Transgène S.A., Strasbourg, F67082

Dietary lactic acid bacteria offer a number of advantages as potential live vaccine vehicles: they are GRAS organisms, widely used in the preparation of food and feed products; their health beneficial role has been documented; some strains, especially of the genus *Lactobacillus*, are normal commensals of the gastro-intestinal and urogenital tracts; they can be administerd by oral or local routes. These properties altogether render lactic acid bacteria attractive as potential live vectors for the delivery of antigens to mucosal surfaces such as the gut, the vagina and the oro-nasal cavity. We thus initiated a research program which is aimed at the construction and immunological evaluation of recombinant lactic acid bacterial strains expressing model antigens in different cellular locations (intracellular, secreted or cell-surface exposed). So far, immunizations by different routes (intragastric, oro-nasal, vaginal) have been conducted in mice, mainly with a *Lactobacillus casei* strain, LbTGS1.4, isolated from the vagina of mice. This strain was shown to be able to persist in both the intestine and the vagina of inbred mice. Vaginal colonization was observed only after careful optimization of a prior oestrogen treatment. A series of expression/secretion/anchoring vectors were constructed for this bacterial host. Model antigens included the B subunit of cholera toxin, the C subunit of tetanus toxin and HIV epitopes linked to carrier proteins. The expression of most heterologous proteins in the host strain LbTGS1.4 was detectable only by Western blotting. This has hampered the induction of clear local immune responses so far. Present work includes improvement of the production level of foreign antigens in lactobacilli as well as the use of a more genetically amenable lactic acid bacteria, *Lactococcus lactis*.

J1-224 ORAL VACCINATION OF RHESUS MACAQUES WITH RECOMBINANT *SALMONELLA TYPHIMURIUM* EXPRESSING SIV P27 ANTIGEN. K.K. Meyer, P. Hinds, P. Valentine*, F. Heffron*, M. So* and C.D. Pauza. Dept. of Pathology and Laboratory Medicine and Wisconsin Regional Priamte Center, University of Wisconsin, Madison, WI., *Dept. of Microbiology and Immunology, Oregon Health Sciences University, Portland, OR.

Attenuated enteric bacteria expressing viral antigens are attractive candidates for live, oral vaccines. *Salmonella* species have the capacity to cross the mucosal epithelium and are favored vaccine vectors because of the many well characterized attenuated mutants and their persistent infection of macrophages. aroA-attenuated *Salmonella typhimurium* strains were engineered to express the SIV p27 gag antigen from plasmid, or chromosomally-integrated prokaryotic expression constructs. Rhesus monkeys vaccinated with live, attenuated *Salmonella* expressing the plasmid p27 expression construct did not develop serum antibodies to SIV. Analysis of the bacteria in the stool indicated that >99% of *Salmonella* isolates lost the recombinant plasmid within 24 hours after oral administration. These recombinant *Salmonella* are not useful for eliciting immunity to SIV antigens because of rapid plasmid loss. p27 expression constructs, regulated by the inducible groEL promoter were integrated into the bacterial chromosome at the aro A locus to overcome the problem of plasmid loss. Chromosomally integrated expression constructs were stable following oral inoculation of macaques.

Balb/c mice were immunized with a single dose ($1\text{-}5x10^9$) of the attenuated, recombinant *Salmonella* expressing p27 from a chromosomally integrated locus (PV4570). Sera tested at 10 days after oral administration, were negative for both IgG and IgA antibodies to the SIV p27 antigen. Day 10 gut wash samples, however, were positive for IgA p27-specific antibodies. Cellular immune responses to the recombinant viral protein were detected at 42 days following oral administration in the form of MHC-restricted, p27-specific cytotoxic T-lymphocytes.

Rhesus macaques were inoculated by gastric intubation with PV4570. Doses of up to 10^{10} *Salmonella* were tolerated. Coproculture studies showed that two of four animals had a high degree of bacterial shedding. Serum IgG antibodies to SIV p27 were detected in these two animals by western blot assays. Analysis of mucosal IgA responses and CTL activity in this cohort of animals is continuing. Our data show initial success in efforts to utilize recombinant enteric bacteria as a means to elicit immunity to virus proteins.

J1-225 B7 INTEGRIN UP-REGULATION ON MESENTERIC T LYMPHOCYTES INDUCED BY LW50020, Michael L. Misfeldt, Department of Molecular Microbiology and Immunology, University of Missouri, School of Medicine, Columbia, MO 65212

The mucosal immune system represents an important host defense mechanism which can interfere with microbial invasion. Induction of mucosal immune responses, therefore, can be effective in preventing infection at mucosal surfaces. In this study, we have examined the induction of an immune response after oral administration with a particulate antigen preparation, LW50020. LW50020, a bacterial immunomodulator, represents a preparation which contains lysates of seven different bacteria. The experiments performed in this study were focused on determining if LW50020 could stimulate mucosa-associated lymphoid tissue. Mice were orally administered the lysate preparation and mesenteric lymph nodes were removed after completion of the immunization scheme. Mesenteric lymphocytes were prepared from the mesenteric lymph nodes and evaluated for their responsiveness to LW50020 stimulation *in vitro*. Results from these experiments indicate that oral immunization with LW50020 results in the stimulation of mesenteric lymphocytes. In addition, we observed that mesenteric T lymphocytes were stimulated by LW50020 and the optimal lymphoproliferative response was observed at 5 days post stimulation. Furthermore, we have observed that *in vitro* stimulation with LW50020 induced the expression of the B7 integrin molecule on mesenteric T lymphocytes isolated from mice fed LW50020. Current experiments are exploring the induction of specific cytokine production by LW50020 as it relates to the up-regulation of the integrin subunit B7 on mesenteric T lymphocytes.

J1-226 INDUCTION OF MUCOSAL AND HUMORAL ANTI-HIV RESPONSES WITH HIV *ENV*-DNA, William M. Mitchell and Trent Rosenbloom, Department of Pathology, Vanderbilt University, Nashville, TN 37232

Balb-c mice were exposed to a replicative-permissive HIV *env*-DNA by an intranasal/pulmonary aerosol or intramuscular route. Experimental animals were further segregated by the number of exposures at three-week intervals and whether the DNA was complexed to dioctadecylamidoglyclspermine (DOGS) (5:1::DOGS:DNA). Naked DNA (100 µg) yielded HIV-specific serum IgG responses in 2 of 5 animals with a single IM exposure and 3 of 5 animals with 2 or 3 IM exposures. Naked DNA (10 µg) produced HIV-specific responses in 40% of the animals with one, two, or three IM exposures. Naked DNA (1 µg) produced no HIV-specific immune responses with IM exposure. DNA (10 µg or 1 µg) complexed with DOGS produced HIV-specific immune responses in 80% of IM-treated animals after one, two, or three exposures. Aerosol exposure to 10 µg DNA complexed with DOGS produced systemic HIV-specific immune responses in 20% of mice with one exposure and 40% of mice exposed two or three times. Aerosols with 1 µg DNA complex produced HIV-specific IgG responses in 20% of mice after one or two exposures and 80% of mice following the third exposure. Western blots revealed gp160 reactivity for serum IgG, IgA, and IgM. Frozen sections of lung demonstrated HIV-specific IgA responses in bronchioles of aerosol-exposed animals with humoral responses. Complexes of DOGS and replicative-permissive DNA decrease the amount of DNA required for a gene-mediated immune response and are capable of inducing specific mucosal responses with aerosol administration.

J1-227 USE OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* AS A VACCINE VECTOR, Robert J. Moore, Mary Tachedjian, Jolanta Krywult, Linda J. Rothel, and Adrian L.M. Hodgson, CSIRO Division of Animal Health, Private Bag No. 1, Parkville 3052, Victoria, Australia.

Corynebacterium pseudotuberculosis is the Gram-positive bacterium that causes caseous lymphadenitis (CLA) in sheep and goats. We have previously shown that inactivation of the chromosomal phospholipase D (PLD) gene substantially reduces the virulence of the organism in sheep. The rationally attenuated PLD-negative derivative (Toxminus) retained the capacity to stimulate strong humoral and cellular responses and therefore had potential for use as a live vaccine vector. In order to investigate the potential of *C. pseudotuberculosis* as a live vaccine vector, for veterinary use, we have developed the basic tools for strain manipulation and foreign antigen expression. Vaccine antigen genes from a number of pathogenic bacteria and parasites of economic importance have been cloned and expressed in the Toxminus strain. A number of these constructs have been used in sheep vaccination trials and have been shown to stimulate immune responses specific for the foreign proteins. In some cases immune responses to the foreign antigen were not seen. To further improve foreign gene expression a range of promoters have been investigated, some of which have the potential for a level of *in vivo* control. Plasmid based expression cassettes can be somewhat unstable *in vivo* and so we have improved the stability of foreign gene expression by site-specific integration of vaccine antigen genes into the chromosome. The development of these tools and methods and the results of vaccination trials have shown that *C. pseudotuberculosis* has good potential as a live vaccine vector. Future work will be aimed at further enhancing foreign gene expression and enhancing appropriate immune responses.

J1-228 **CHOLERA TOXIN B STIMULATES NEUTRALIZING ANTIBODIES AFTER INTRANASAL CO-IMMUNIZATION WITH MEASLES VIRUS**

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The oropharyngeal cavity represents the main portal of entrance for the MV and mucosal tissues are important sites of MV-related pathology. However, after intranasal immunization the MV induces only a weak antibody response. CTB is a potent mucosal adjuvant when combined with protein antigens, much less is known about its effect when combined with whole virus. In the presence of CTB higher titers of HI antibodies developed after fewer boosts than after immunizing with MV alone. Consistent titers of neutralising antibodies were found only after co-immunization with CTB. These were directed against the native MV hemagglutinin protein and were mainly of the IgG_1 isotype. The cytokine pattern of the T cell response suggested that these were induced by Th_1 cells in an IL-4 independent manner. CTB was also required to induce elevated levels of local IgA in the nasal wash. In the lung wash and the saliva no difference was observed whether the antigen preparation contained CTB or not. The low titers of IgA in comparison to IgG are compatible with transsudation of antibodies from plasma into the alveoli and the salivary glands while the nasal wash antibodies were of local origin. In the gut washes no MV-specific IgG or IgA activity was detected after nasal immunization. No consistent MV-specific reactivity was detected after intragastric immunization. The analysis of the T cell response suggested that regional differences exist whether animals were immunized with or without CTB. This model is suitable for studying the T cell response to the MV in the presence of passively acquired antibodies.

J1-229 RECOMBINANT CHOLERA TOXIN B SUBUNIT IS NOT AN ADJUVANT FOR ORAL IMMUNIZATION AGAINST HELICOBACTER FELIS IN MICE . John Nedrud, Thomas Blanchard, Steven Czinn and Nils Lycke, Institue of Pathology and Department of Pediatrics, Case Western Reserve University, Clevleand, Ohio 44106 and Department of Medical Microbiology and Immunology, University of Göteborg, 41346 Göteborg, Sweden.

Cholera toxin (CT) is one of the most effective mucosal adjuvants yet discovered but because of it's capacity to cause severe diarrhea, its usefulness as an adjuvant for humans is questionable. In order to overcome the toxicity associated with CT some groups have suggested using the non-toxic cholera toxin B subunit (CTB) as an adjuvant. The CTB used by these groups which has been purified from holotoxin is invariably contaminated with small amounts of holotoxin and it is not clear whether the adjuvant activity is dependent on the contaminating holotoxin or is an intrinsic property of CTB. To address this issue we compared recombinant CTB (rCTB, a gift from Dr. Jan Holmgren), purified commercial CTB (cCTB) and holotoxin as adjuvants to enhance antibody responses and generate protection from infection after oral immunization with Helicobacter felis sonicate. The results, summarized below, indicate that while both holotoxin and cCTB were effective adjuvants, rCTB was not.

Anti-H. felis antibody titers

adjuvant	serum IgG	gut IgA	protection
rCTB	676	< 2	0%
cCTB	1978	170	45%
CT	4240	70	57%
naive	<256	< 2	0%

Methods: C57BL/6 mice were orally immunized 4 times with 2 mg of H. felis sonicate plus either rCTB, cCTB, or CT and were then challenged with live H. felis.

Conclusion: It appears that at least in rodents, a small amount of pharmacologic activity associated with CT A subunit is necessary for adjuvant activity.

J1-230 DEVELOPMENT, SAFETY AND IMMUNOGENICITY OF A *ΔaroA,ΔvirG* SHIGELLA VACCINE STRAIN, Fernando R. Noriega, Karen Kotloff, Jin Yuan Wang, Myron M. Levine, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD 21201.
Our strategy was to engineer a *Shigella* oral vaccine prototype capable of invading intestinal epithelial cells but incapable of extensive intracellular replication or of extension to adjacent cells. In frame deletion mutations were introduced in chromosomal *aroA* (aromatic biosynthesis pathway) and invasion plasmid *virG* (intracellular and cell to cell spread) of wild type *S. flexneri* 2a. Strain *ΔaroA,ΔvirG* CVD 1203 was highly attenuated in the Sereny test. In a blind, placebo-controlled experiment in guinea pigs, two spaced 10^9 cfu orogastric doses of CVD 1203 administered two weeks apart stimulated SIgA antibodies to *S. flexneri* 2a LPS and conferred 80% protection against conjunctival sac challenge with virulent *S. flexneri* 2a. In a double-blind, randomized study, 32 adult volunteers received 1.5×10^9 cfu, 1.5×10^8 cfu, or 1.2×10^6 cfu of CVD 1203. This vaccine elicited short-lived, self-limited adverse reactions (fever, diarrhea, or blood in stools) in 72%, 18%, and 0%; an IgA antibody-secreting cell (ASC) response to LPS in 100%, 91% and 60% and to invasiveness plasmid antigens (IPA) in 91%, 64% and 40% of the volunteers that received 10^9 cfu, 10^8 cfu, and 10^6 cfu of CVD 1203 respectively.

J1-231 **ENHANCEMENT OF ROTAVIRUS-SPECIFIC HUMORAL IMMUNE RESPONSE IN GALT BY MICROENCAPSULATION.** **Paul A. Offit, Charlotte A. Moser, Christian A. Khoury, Kurt A. Brown, Joon E. Kim, and Tully J. Speaker. The Children's Hospital of Philadelphia, The University of Pennsylvania School of Medicine, and Temple University School of Pharmacy, Philadelphia, PA 19104.** Rotavirus is the leading cause of acute gastroenteritis in infants and young children both in developed and developing countries. The staggering impact of rotavirus infections has generated interest in disease prevention by immunization. However, development of a consistently effective vaccine has been elusive. Formulation of an adjuvant strategy which enhances the immunogenicity of orally-administered rotaviruses may help in the development of a successful vaccine.

We determined the capacity of microencapsulation to enhance the humoral immune response to rotavirus in GALT of mice. Using a system of microencapsulation based on the ionic linkage of aqueous anionic polymers and aqueous amines we found that microcapsules 1) captured infectious rotavirus, 2) penetrated into and persisted in GALT after oral inoculation, 3) delivered rotavirus antigen to GALT at levels greater than those detected after oral inoculation with free virus, and 4) enhanced both the frequencies of virus-specific IgA-secreting cells in the lamina propria by ELISPOT assay, the quantities of virus-specific IgA produced in GALT by intestinal fragment culture, and the quantities of virus-specific IgA detected at the intestinal mucosal surface. In addition, an enhanced virus-specific immune response was associated with enhanced production of presumably polyclonal, non-rotavirus-specific antibodies in GALT and at the intestinal mucosal surface.

J1-232 STRUCTURAL REQUIREMENTS OF CANDIDATE SYNTHETIC VACCINES FOR THE INDUCTION OF MEASLES SPECIFIC CTL RESPONSES AFTER INTRANASAL ADMINISTRATION. Partidos C.D, Vohra P, Steward M.W. Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K.

The induction of CTL responses are of great importance for the clearance of measles infection. Although several studies have suggested the requirement of help for the in vivo induction of CTL responses, others have provided evidence for their induction independent of help. We investigated the structural requirements for the induction of CTL responses in vivo after intranasal vaccination with an immunodominant CTL epitope from the nucleoprotein of measles virus. Using a previously identified Th epitope from the fusion protein, chimeric peptides were constructed by co-linear synthesis. A 9mer CTL epitope was synthesized at the amino or carboxyl-terminus of one or two copies of the Th epitope. After intranasal vaccination with CTB as an adjuvant, spleen cells from CBA immunized mice exhibited CD8 CTL and CD4 Th activity. In contrast, weak CTL responses were observed after intranasal vaccination with the CTL epitope alone or a mixture of the CTL epitope with the Th epitope. The orientation of epitopes in chimeric peptides did not affect their immunogenicity. However, the presence of two copies of the Th epitope resulted in enhanced CTL response when compared with one copy of the Th epitope. These results suggest that for effective priming of measles specific CTL responses via the upper respiratory tract, linkage of the Th and CTL epitopes is required. Also they highlight the importance of the amount of help provided for successfully raising CTL responses in vivo.

J1-233 γδ T CELL SPECIFICITY IN INFLUENZA INFECTED MICE. S. Ponniah, M. Eichelberger, and P. Doherty, St. Jude Children's Research Hospital, Memphis, Tennessee. A well defined role for γδ T cells in immunity remains to be clearly established. A major piece of information missing in the puzzle of γδ T cell function is a lack of well defined antigen specificity for these cells. In our studies we attempt to answer this question by investigating the specificity of γδ T cell hybridomas that were generated from mice infected with influenza virus. Significant numbers of CD4-8- γδ T cells accumulate in the lungs of conventional C57BL/6 mice infected with an H3N2 influenza A virus, X-31. mRNA for Hsp60, a common antigen able to stimulate all murine Vγ1 T cells, is increased in macrophages from this inflammatory site. Thus it has previously been postulated that Hsp60 is a candidate antigen for γδ T cells at this site. Cells from the mediastinal lymph nodes of mice homozygous for a disruption of the TCR β gene (TCR β(-/-)) or the TCR α gene (TCRα(-/-)) were infected with X-31 and used to generate hybridomas which were then tested for their ability to specifically secrete IL-2 when cultured with influenza-infected cells. In this way a panel of hybridomas was obtained whose responses were independent of MHC-restriction and cross-reactive for both influenza A and B virus strains. While influenza A strains A/PR/8/34(H1N1) and X-31(H3N2) as well as an influenza B strain B/HK/8/73(HG) all induced lymphokine production, no such response was observed when the hybridomas were cultured with cells infected with vaccinia virus or Sendai virus. Expression of Hsp60 was observed to be increased in cells infected with influenza A or B viruses, but not with Sendai or vaccinia viruses indicating that this may be the virus-induced antigen which interacts with the TCR. In preliminary studies, hybridomas that respond to virus-infected cells appear to also be able to respond to a peptide from Hsp60 even in the absence of presenting cells. While our observation may help to explain the cross-reactive responses to different strains of influenza viruses, it also raises the possibility that γδ T cells responding to over-expressed endogenous Hsp60 may indeed play some role in viral pneumonia .

J1-234 CHARACTERISATION OF MONONUCLEAR CELLS IN SEMEN FROM HIV+ MEN: VIABILITY, FUNCTION AND EVIDENCE FOR MUCOSAL DERIVATION. Alison J Quayle, Kenneth H Mayer* and Deborah J Anderson. Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Womens Hospital, Harvard Medical School, Boston, MA 02115 and *Fenway Community Health Center, Boston, MA 002115.

Semen is presumed to be the primary vehicle for transmission of HIV-1. Virus has been isolated from both seminal plasma and from the leucocytes present in ejaculate, and elevated numbers of leukocytes have been reported in HIV+ men. The aim of this study was to investigate whether these cells are viable, capable of immune function, and whether their phenotype gives any indication to their origin. Viable mononuclear cells were isolated from all six semen samples obtained from HIV+ individuals so far examined. Macrophages were the most common cell [mean965/ejaculate], followed by CD8+ [mean 698] and CD4+ [mean 385] cells. A high proportion of the lymphocytes expressed the $\alpha^E\beta7$ integrin, which is a molecule almost exclusively found on mucosal T cells, thus indicating their mucosal origin. Successful cloning of the T cells from these samples indicated their functional capacity. Both CD4+ and CD8+ T cell clones were obtained, but the latter were in the majority. All CD8+ and some CD4+ clones stably expressed the $\alpha^E\beta7$ integrin, and TIA-1 which is a cytolytic granule-associated protein. T lymphocytes and macrophages from reproductive tract mucosa are hypothesised to play an important role in transmission of virus, and in host defence; further studies in this area may contribute to our understanding of these processes.

J1-235 NOVEL VACCINE STRATEGIES FOR MUCOSAL IMMUNITY USING RECOMBINANT FOWLPOX VIRUSES EXPRESSING CYTOKINE GENES

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We have studied avipox viruses encoding cytokine genes, both alone and in combination with naked DNA plasmids, as potential vaccine strategies. As these viruses abortively infect mammalian cells, yet still effectively present foreign genes to the immune system, they offer a safer but effective alternative to other live vectors. We have examined the effect of co-expressing the cytokines interleukin-6 (IL-6) or interferon-γ (IFNγ) on immune responses to fowlpox virus (FPV) expressing influenza hemagglutinin (HA) as a vaccine antigen. IL-6 augmented antibody responses while IFNγ inhibited these responses without affecting the generation of cell-mediated immunity. Mucosal IgA and IgG responses were also signicantly enhanced by the expression of IL-6. IFNγ on the other hand suppressed the mucosal antibody response without affecting the mucosal cytotoxic T cell response induced.

Direct injection of DNA plasmids encoding foreign genes as a means of vaccination is a subject of great current interest. We have combined this approach with fowlpox vectors, both expressing common HA antigen, to examine the effects of priming with the former intramuscularly and boosting with recombinant FPV. With this combination, both systemic and mucosal antibody responses, respectively, were greatly elevated after boosting at the relevant site. Levels of systemic antibody achieved using this strategy approximated those in convalescent serum. In summary, FPV vectors encoding cytokines represent a safe and effective vaccine strategy which may be used to selectively manipulate the immune response. Greatly enhanced immune responses can be generated when this approach is used in combination with naked DNA immunisation. These findings have implications for the development of improved vaccination strategies.

J1-236 ASPECTS OF IMMUNOPATHOGENESIS OF HEMORRHAGIC ENTERITIS VIRUS INFECTION IN TURKEYS, Silke Rautenschlein and Ulrich Neumann, Clinic for Poultry, School of Veterinary Medicine, 30559 Hannover, Germany

Infection with Hemorrhagic Enteritis Virus of turkeys (HEV), an adenovirus, results in immunosuppression, usually accompanied by hemorrhagic enteritis (HE). Pathogenesis factors leading to HE, however, are not fully elucidated yet. On the other hand, the anatomical dichotomy of the adaptive avian immune system represented by the two anatomical separate primary lymphoid organs, the thymus and the bursa of Fabricius, offers the chance to study the effect of HEV on cell-mediated immune functions and humoral immune reactions, respectively. These particularities may favour comparative studies of adenovirus induced immunopathogenesis of an enteric disease. Previous findings suggest that target cells for HEV infection belong to the mononuclear phagocytic system as well as to B- and T-lymphocyte populations of the spleen and the gut. We were able to show immunohistochemical changes in cell surface determinants of spleen B-lymphocytes and ellipsoid-associated non-lymphoid cells following HEV-infection. Further immunohistochemical findings on the distribution and expression of macrophage and lymphocyte surface determinants employing monoclonal antibodies in HEV-infected and uninfected turkeys may now provide further insights into the role of intestine associated immune cells involved in HEV pathogenesis.

J1-237 INTRANASAL ADMINISTRATION OF FILAMENTOUS HEMAGGLUTININ INCORPORATED IN BIODEGRADABLE MICROPARTICLES PROTECTS MICE AGAINST BORDETELLA PERTUSSIS INFECTION, Keith Redhead[1], Edward S. Cahill[2], Derek T. O'Hagan[2], Lisbeth Illum[2], Amanda Barnard[1] and Kingston H.G. Mills[3], [1] Divisions of Immunology and Bacteriology, NIBSC, Potters Bar, EN6 3QG, UK; [2] Dept. of Pharmaceutical Sciences, Nottingham University, NG7 2RD, UK; [3] Biology Department, St Patrick's College, Maynooth, Ireland.

It has been shown that mice can be protected against *Bordetella pertussis* respiratory infection by i.p. or i.n. immunization with solutions of the adhesin filamentous haemagglutinin (FHA). We have now demonstrated that similar protection can be elicited when FHA is incorporated in poly (lactide-co-glycolide) biodegradable microparticles prior to i.n. administration. In this form the antigen was found to be stable and immunogenic. This method of antigen delivery resulted in good systemic IgG responses to FHA and high levels of anti-FHA IgA and IgG antibodies in the lungs, which were shown to inhibit the binding of *B.pertussis* to tissue-culture cells. Antigen-specific *in vitro* spleen cell proliferation and IL-2 secretion, indicative of a Th1-type T-cell response, were also detected. Six months later, the immunized mice still had significant antibody and CMI responses to FHA. The i.n. immunised mice displayed the presence of FHA-specific antibody secreting cells in their lungs and were significantly more resistant to *B.pertussis* respiratory challenge than naive controls.

J1-238 ***STAPHYLOCOCCUS CARNOSUS* WITH SURFACE-DISPLAYED RECOMBINANT IMMUNOGENS, A NOVEL LIVE VECTOR FOR SUBUNIT VACCINE DELIVERY** Stefan Ståhl[1], Patrik Samuelson[1], Marianne Hansson[1], Thien N. Nguyen[2], Christine Andréoni[2], Diana Haddad[3], Alain Robert[2], Hans Binz[2] and Mathias Uhlén[1]. Department of Biochemistry and Biotechnology, Royal Institute of Technology, S-100 44 Stockholm, Sweden[1]. Centre d'Immunologie et de Biotechnologie Pierre Fabre, F-74 164 Saint-Julien en Genevois, France[2], Department of Immunology, Stockholm University, S-106 91 Stockholm, Sweden[3].

Immunization experiments using whole cells displaying heterologous proteins on the surface have been shown to elicit antibodies reactive with the expressed antigenic determinants and the corresponding native antigen. Efficient surface exposure of the heterologous peptides has been demonstrated for Gram-negative bacteria, such as *E. coli* and *Salmonella*. The surface display has been shown to be important to obtain antibody responses in this way. Recently, Gram-positive bacteria have started being investigated for surface-display of heterologous immunogens for the purpose of developing live bacterial vaccine vehicles. Cell-surface display systems for non-pathogenic streptococci and staphylococci have been used for surface expression of different antigenic determinants and upon immunization, both local and systemic antibody responses to the hybrid receptors have been induced.

Here, we describe a novel system for surface display of heterologous proteins on *Staphylococcus carnosus*, a gram-positive bacteria widely used in food industry for the ripening process of dry sausages and as starter culture for the fermentation of meat and fish products, and thus considered safe for oral use in humans. Antigenic determinants of different origin, bacterial (streptococci), viral (respiratory syncytial virus) and protozoan (from a *Plasmodium falciparum* malaria antigen), have been expressed on the bacterial cell surface, as determined by immunofluorescence, immunogold and FACS analyses. The antibody responses, including isotype studies and the effects of booster immunizations, after delivery of live recombinant *S. carnosus* to mice, using different routes of immunization, is described.

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J1-239 CYTOTOXIC T LYMPHOCYTES FOLLOWING ORAL IMMUNIZATION WITH ATTENUATED VACCINE STRAINS OF SALMONELLA TYPHI IN HUMANS, Marcelo B. Sztein, Michael K. Tanner, Yuri Polotsky, Jan M. Orenstein and Myron M. Levine, Departments of Pediatrics and Medicine, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland 21201

Although the presence of cytotoxic T lymphocytes (CTL) able to lyse *S. typhi* -infected cells could potentially be of paramount importance in protection against *S. typhi* infection, this phenomenon has not previously been reported. We investigated the presence of CTL activity against *S. typhi*-infected cells in volunteers immunized with attenuated vaccine strains of Salmonella typhi. Target cells were prepared by incubating *S. typhi* with autologous Epstein-Barr virus (EBV)-transformed lymphoblastoid cells. *S. typhi* was found to invade EBV-transformed cells, remaining inside the vacuolar compartment. These infected cells expressed *S. typhi* antigens on the cell membrane, as determined by flow cytometry. Oral immunization with *S. typhi* strain CVD 908 elicited the appearance in circulation of CTL effectors able to kill *S. typhi*-infected autologous EBV-transformed cells. CTL activity was only observed after a 6-8 days in vitro expansion in the presence of *S. typhi*-infected autologous EBV-transformed cells. The use of heat-phenol-killed whole-cell *S. typhi* particles, gentamicin-killed *S. typhi* particles or *S. typhi* flagella during expansion did not result in measurable CTL activity. Maximum CTL activity was observed 29 days after immunization. The CTL effector cell population appears to express the $CD8^+$ $CD11a^+$ phenotype. The observation that oral immunization of volunteers with attenuated *S. typhi* elicits CTL responses raises the possibility that CTL activity might play an important role in protection during typhoid fever.

J1-240 ORAL DELIVERY OF AN ANTI-IDIOTYPIC ANTIBODY TO CHLAMYDIAL EXOGLYCOLIPID IN MICROSPHERES PROTECTS AGAINST OCULAR CHLAMYDIAL INFECTION IN MICE. Judith A. Whittum-Hudson, W. Mark Saltzman, Robert A. Prendergast, and A. Bruce MacDonald. Depts. of Ophthalmology and Chemical Engineering, Johns Hopkins University, Baltimore, MD 21205 and Dept. of Microbiology, Univ. Massachusetts, Amherst, MA 01003

Chlamydia trachomatis infections of mucosal tissues pose enormous public health problems. Ocular chlamydial infections represent the leading cause of infectious blindness and affect over 8 million individuals worldwide. Our laboratories have extended studies on the immunopathogenesis of ocular chlamydial infections to investigations of potential anti-chlamydial vaccine candidates. We have identified a monoclonal anti-idiotypic antibody (Ab_2) raised against the mAb_1 to chlamydial exoglycolipid (GLXA). This novel vaccine candidate induces both an anti-anti-idiotypic antibody response and protects BALB/c mice against ocular infection by a human biovar of *C. trachomatis* in a dose-dependent fashion. After immunizations, mice were tested for Ab_3 serum responses and then topically challenged in the conjunctivae with 5000 IFU of infectious *C. trachomatis* (C serovar; TW-3). Sixty-90% of eyes were protected from infection by immunization with 50-100 μg of soluble Ab_2 in Maalox delivered subcutaneously. In addition, Ab_2 incorporated into poly(lactic acid) microspheres delivered subcutaneously or orally (2-6 μg/dose) resulted in reduced infectious yields and more rapid clearing of infection compared to controls receiving normal IgG1. Protective immunity persisted in Ab_2 recipients; at rechallenge, only some eyes became infected, and those that did, became culture-negative faster than rechallenged controls.Experiments are in progress to further characterize the immunologic basis of the protection induced by the anti-idiotypic antibody vaccine candidate. This is the first time that significant protection from microbiologic disease has been induced by any anti-chlamydial vaccine candidate and, to our knowledge, the first time an anti-idiotypic antibody has been protective against disease caused by a replicating infectious organism.

J1-241 COLD-ADAPTED, TEMPERATURE SENSITIVE AND WILD-TYPE RESPIRATORY SYNCYTIAL VIRUSES PRIME COMPARABLE LOCAL IMMUNE RESPONSE, Laura J. York, Giuseppe Palladino, David Giorgio, Sharon Adams and Eric M. Mishkin, Viral Vaccine Research, Lederle-Praxis Biologicals, Pearl River, NY 10965

A high incidence of vaccine-enhanced disease has been associated with RSV infection following immunization with a formalin-inactivated, alum-precipitated respiratory syncytial virus (RSV) vaccine preparation. This raised concerns regarding the pattern of immune responses induced by the priming antigen. Because enhanced illness has not been associated with clinical trials with live virus preparations, cold adaptation has used to isolate attenuated strains of RSV as potential live virus vaccine candidates. BALB/C mice were inoculated intranasally with parental and mutant viruses representing both subgroups of RSV to compare their ability to elicit appropriate local immune responses. While the mutant viruses could not be isolated from the murine lungs 5 days post inoculation, these attenuated viruses still elicited comparable levels of pulmonary T cells exhibiting virus-specific cytolyic activity. Cytokine secretion patterns of the regional lymph node cells indicate that priming with either wildtype or attenuated viruses elicited a dominant virus-specific Th1 response. B ELISPOT methods used to investigate induction of RSV fusion protein-specific, IgA secreting B cells indicated that the reduced growth of the attenuated viruses resulted in a lower frequency of these specific B cells resident in the lungs. While the magnitude of some immune parameters elicited by the attenuated vaccine candidates may be reduced, the spectrum of the mucosal immune response remains unaltered and is equivalent in protecting against virus growth in the lungs of challenged mice. Data support the use of these vaccine candidates in clinical trials.

Late Abstracts

FALL-39, A PUTATIVE NOVEL HUMAN PEPTIDE ANTIBIOTIC, CYSTEINE-FREE AND EXPRESSED IN BONE MARROW AND TESTIS. Birgitta Agerberth[1,4], Hans Gunne[1], Jakob Odeberg[2], Per Kogner[3], Hans G Boman[1] and Gudmundur H Gudmundsson[1]. 1)Department of Microbiology, Stockholm University, S-10691 Stockholm, Sweden 2)Department of Biochemistry, The Royal Institute of Technology, S-10044 Stockholm, Sweden 3)Departments of Pediatrics and Clinical Chemistry, Karolinska Institute, S-17177 Stockholm, Sweden 4)Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-17177 Stockholm, Sweden

A clone from a human bone marrow cDNA library codes for a putative novel human peptide antibiotic, designated FALL-39 after the first four residues. FALL-39 is a 39 residue peptide lacking cysteine and tryptophan. All human peptide antibiotics previously isolated (or predicted) belong to the defensin family with three disulfide bridges. The clone for the prepro-FALL-39 encodes a cathelin-like precursor protein with 170 amino acid residues. We have postulated a dibasic processing site for the mature FALL-39 and chemically synthesized the putative peptide. In the presence of the basal Medium E, synthetic FALL-39 was highly active against *Escherichia coli* and *Bacillus megaterium*. Residues 13-34 in FALL-39 can be predicted to form a perfect amphiphatic helix and CD spectra showed that medium E induced 30% helix formation in FALL-39. Northern blot analyses showed that the gene for FALL-39 is expressed mainly in human bone marrow and testis.

LOCAL AND SYSTEMIC IMMUNE RESPONSE IN YOUNG RABBITS INTRAGASTRICALLY IMMUNIZED WITH AN *Aeromonas hydrophila* EXTRACELLULAR PROTEIN WITH AFFINITY FOR MUCOSAL ANTIGENS, Ascencio F., Sierra A., Ruiz-Bustos E., Romero M.J., and Greene Y., Department of Marine Pathology, Centre for Biological Research, La Paz, B.C.S., 23000, México.

We recently isolated and purified a cell-associated and extracellular protein from the fish and human pathogen *A. hydrophila* that binds mucosal antigens (MABP) such as mucin, lactoferrin and immunoglobulins. Since putative adhesins play a significant role in the tissue adhesion and colonization processes, it may be advantege for a further vaccine design to evaluate whether, and to what extent, this extracellular protein can elicits an immune response in secretions and tissues of the mucosal organs, bilis, and the blood, as measured by levels of antibodies and antibody spot-forming cells (SFC) of different isotypes after immunization by intragastric route with *A. hydrophila* MABP.

White New Zealand rabbits were given MABP, covalently coupled to the B subunit of cholera toxin (CTB) by intragastric route; Cholera toxin (CT) was administrated as an adjuvant with the MABP-CTB conjugates. Two additional immunizations were given at about 2-week intervals. One week after each immunization, individual serum, bilis, and intestinal mucus samples were collected and assayed immediately for antibody activity to MABP and CT by ELISA. Part of the immunized rabbits were sacrificed after each immunization and samples of blood, small intestine and spleens were collected to isolate and assay for specific and non-specific antibody SFC by ELISPOT. The results indicate that intragastric administration of micrograms amounts of MABP-CTB, elicits a significant mucosal as well as extramucosal immunologic antibody response in rabbits. However, levels of antibody titters to CT were significantly increased in mucosal secretions.

CONSTITUENTLY EXPRESSED HEAT SHOCK PROTEINS (Hsps) AND INTESTINAL INTRAEPITHELIAL LYMPHOCYTES (iIEL) TcRαβ, TcRγδ IN MURINE INTESTINAL IMMUNOGLOBULIN (Ig) PRODUCTION, Clara G.H. Bell, UIC, Dept. Microbiology/Immunology, College of Medicine, Chicago, IL 60612

IgA, the major Ig of intestinal secretion, is sought to play a defense role at mucosal surfaces, the mucosal immune system being one of the first line of defense against bacterial and viral infection. Whether IEL TcRαβ/TcRγδ T-cells -that are likewise anatomically positioned to be the first line of defense against enteric pathogens- regulate this function and handle the myriad antigens (Ags) interfacing with the gut environment is an open question, because, while, historically, the T-cells present in secondary lymphoid organs and blood and involved in protection are characteristically TcRαβ T, even the IEL TcRαβ T have a unique character. In previous studies of the mouse gut immune system, I delineated B-cells specific for IgA to be principally localized in the Peyer's patches (PP) -which comprise the thymic-dependent characteristic TcRαβ T-cell component, functioning as T help for these B-cells. I dissected the gut lamina propria (LP) preponderance of cytoplasmic (c)IgA-bearing-cells (~90%) and showed by combined autoradiography/immunofluorescence studies these to principally consist of nondividing IgA plasma cells that were only partially amenable to further mitogen/Ag activation, and, presumably, represented end differentiation programs. Herein, I focus on the gut T-cell compartment and present a dissection of the T relative to the B, to ascertain the correlation between the cellular lymphocyte (LC) population composition of the gut and the immune activity. I show that purified IEL stripped with dithiothreitol of epithelium, and with the PP removed (in order not to contaminate the gut LC), and dissociated with EDTA in Ca^{2+} & Mg^{2+} free HBSS, comprise a heterogenous $CD3^+$ population distinguished by the TcR αβ or γδ expression and by cluster differentiation markers CD4, CD8. A cytofluorometric dissection for cell-surface phenotypes depicted: (i) $CD4^-CD8^+$ phenotypes (~80% of the T IEL), the majority (58-69%) of which exhibit the unique $CD8\alpha\alpha$(Lyt-2)$^+\beta$(Lyt-3)$^-$ homodimeric form of CD8 associated with either the thymus independent TcRαβ (42-50% of the CD3) or TcRγδ (50-58% of the CD3), **that mature without a thymus**, as ascertained by dissections of nude mice. (ii) $CD8\alpha\beta$(Lyt-2^+Lyt-3^+) heterodimeric form of CD8 TcRαβ IEL, that essentially are the PP emigrant component of TcRαβ of thymic origin, as are the $CD4^+CD8\alpha^+\beta^+$ (~19-29% of the IEL TcRαβ) and the $CD4^+CD8^-$ (~5-11% of the IEL TcRαβ), that **are the thymus dependent (TD) component and that resemble the PP T-cells**. Few IEL $CD3^+$TcRγδ$^+$ were of the $CD4^-CD8^-$ phenotype. And, by also being mostly Thy-1^-, these differed from lymph node (LN) and spleen (SpL) $CD3^+$TcRγδ$^+$ -that were essentially of the $CD4^-CD8^-$ phenotype and exhibited the cell-surface phenotype typical of naive, mature, Thy-1^+CD5(Lyt-$1)^{+dull}B220^-$(some were Pgp-1^+ and exhibited the marker of memory T-cells). Functionally, many IEL $CD3^+$TcRγδ$^+$ T-cells reacted with bacterial Hsp65 family proteins that bear similarity to the murine endogenously synthesized Hsp60 family, that may be presented in association with MHC-like molecules. Many, even when freshly isolated as $CD3^+CD4^-CD8^-$TcRγδ$^+$, predominantly Thy-1^-CD5(Lyt-$1)^{+dull}B220^-$, could kill Fc receptor positive targets in presence of anti-TcRγδ mAb. These and the $CD3^+CD4^-CD8^+CD5$(Lyt-$1)^{+dull}$ could be activated in vitro with anti-CD3 (145-2C11) mAb coated degalan beads to $CD8\alpha\alpha$(Lyt-2)$^+\beta$(Lyt-3)$^-$ expression, to $B220^{+dull}$ expression, and to proliferative responsiveness to Hsp65 family, and to activation-induced cytolytic activity. A dissection of the gut lamina propria (LP) depicted Ig^+ and Thy-1 (40.6%) cells: surface (s)IgM (45% of the B-cells); sIgM,sIgD (43%), sIgA (~7%); sIgMcIgM (81%); sIgAcIgA (~30%). The B-cells residing in the peritoneal (coelomic) cavity (PerC), were principally of the B1-lineage ($CD5^{+dull}B220^{dull}IgM^{hi}$ [43%]) Some B1 as also the conventional B2-cells were $CD5^-B220^{hi}IgM^{med-hi}$ (26%), while the T-cells (18.9%) were $CD5^{+hi}$. PerC lavage-collected cells transferred i.v. to total-body-irradiated mice generated high frequency of Ig-polyreactive secreting precursors. The characteristic pattern of this reactivity and the parallel increase in frequency of the IEL Hsp65/Hsp70 family TcRγδ reactivity and increase in the constituent overexpression of the endogenous Hsp70 family, leads to the speculation that a selection occurs on account of the reactivity with the highly conserved Hsp60/70 family. However, whether Hsp expression is causal in promoting TcRγδ or whether TcRγδ is an independent consequence of the Hsp elevated expression remains to be defined.

RETROVIRAL VECTOR MEDIATED INDUCTION OF HIV AND SIV SPECIFIC CTL IN A MURINE MODEL

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The cytotoxic T lymphocyte (CTL) response is critical for preventing the establishment and mediating recovery from viral infections. Using a mouse model, we have demonstrated that antigen specific, MHC Class I restricted CD8+ CTL are elicited after injection of syngeneic cells expressing HIV-1 or SIV envelope genes. We have also demonstrated that direct administration of non-replicating retroviral vectors encoding HIV-1 env can elicit potent CTL responses in mice, baboons and macaques. The CTL responses generated using this novel gene transfer approach are cross-reactive between divergent HIV-1 strains. Mice have been immunized via various routes and we are currently examining the mechanism of induction of systemic CTL responses. Because of the importance of mucosal immunity in HIV infection, we have initiated experiments to immunize animals via mucosal routes and analyze the resulting immune responses.

THE EFFECT OF NEONATAL APPENDECTOMY ON MUCOSAL IMMUNITY IN RABBITS, Joe F. Dasso and Mark D. Howell*, Department of Biology, University of Northern Colorado, Greeley, CO 80631, *Department of Microbiology, Colorado State University, Fort Collins, CO 80523
Like the Peyer's patches, the rabbit appendix is an induction site for B cell activation and dissemination in gut associated lymphoid tissues. In particular, it is an enriched source of IgA B cell precursors. Previous studies with rabbits have shown neonatal appendectomy impairs peripheral antibody responses to various antigens but have not tested its effect on mucosal responses. We are testing the effect of neonatal appendectomy on secretory Ig production in the gut in response to mucosal immunization with ovalbumin (OVA). OVA is known to promote a strong secretory IgA response. Litter mates of 18 newborn rabbits were randomly divided into appendectomy and sham-surgery control groups. Surgery was performed within one week of birth. After six weeks both groups will receive an intraperitoneal injection of OVA emulsified in Freund's complete adjuvant. Two weeks later they will receive an intraduodenal injection of OVA in saline. At about nine weeks, animals will be sacrificed and the small intestine rinsed to collect secretory Ig. An enzyme-linked immunosorbent assay will be performed to compare anti-OVA antibody levels in the intestinal secretions of the two groups. Preliminary results from a pilot study involving 3 appendectomized and two sham-surgery control animals suggest neonatal appendectomy may reduce secretory IgA response to OVA. ELISA results showed the intestinal fluids of the appendectomized animals had half the anti-OVA antibody titer of the controls. Thus we hypothesize the high population of IgA B cell precursors observed in the appendix are a major source of IgA plasma cells within the lamina propria of the gut.

A PORCINE cDNA CLONE CORRESPONDING TO NK-LYSIN, AN EFFECTOR PEPTIDE OF T AND NK CELLS AND ITS IL-2 DEPENDANT INDUCTION. Hans Gunne[1], Birgitta Agerberth[1,2], Berit Olsson[3], Åke Dagerlind[4], Hans Wigzell[3], Hans G Boman[1], and Gudmundur H Gudmundsson[1] 1)Department of Microbiology, Stockholm University, S-106 91, Stockholm, Sweden 2)Departments of Medical Biochemistry and Biophysics, 3)Microbiology and Tumour Biology, and 4)Neuroscience, Karolinska Institutet, S-171 77, Stockholm, Sweden
NK-lysin, a 78-residue antimicrobial peptide, was isolated from pig small intestine (Andersson et al., 1994). Standard methods identified the peptide as basic, with six half-cystine residues in three intrachain disulphide bonds. The sequence showed 33 % identity with a part of a putative gene product (NKG5) from activated T and NK cells. NK-lysin showed good antibacterial activity against *Escherichia coli* and *Bacillus megaterium* as well as a marked lytic activity against two NK sensitive tumour cell lines. We now report the sequence of a cDNA clone corresponding to NK-lysin and we have analyzed the cell and tissue specific expression and the induction of the gene. A lymphocyte fraction enriched in T and NK cells, stimulated by human interleukin-2 (IL-2), showed a 30 fold increase in the NKL transcript. NK-lysin specific mRNA is also detectable in spleen, bone marrow and colon. Immunostaining showed NKL to be present in different types of lymphocytes. Our results strongly suggest that NK-lysin is involved in the inducible cytotoxicity of T and NK cells.

INFLUENCE OF HUMAN EPITHELIAL CELL LINES ON B CELLS DIFFERENTIATION, Zina Moldoveanu[1], Itaru Moro[2], and Jiri Mestecky[1], [1]University of Alabama at Birmingham, Birmingham, AL 35294 U.S.A., and [2]Nihon University, School of Dentistry, Tokyo, Japan.
Due to the difficulties in obtaining and maintaining human intestinal epithelia in culture, cell lines derived mainly from colon carcinoma are used as models for studies involving various physiological and immunological aspects of the intestine. The proximity of epithelial and lymphoid cells in the mucosal tissues prompts the question of a functional interrelation between these cell types. We have therefore investigated the *in vitro* secreted products of several human epithelial cell lines and their influence on the immunoglobulin (Ig) synthesis by lymphocytes. When PCR technique was employed, human colonic carcinoma lines HT-29, CaCo2, T84, LIM 1863, and SW-480 differed in their ability to secrete cytokines. IL-6 production differ also with the cell line and time in culture, as determnined by bioassay. Mononuclear cells isolated from human peripheral blood or tonsils were cultured with supernatants derived from epithelial cell cultures, or in some cases with the corresponding amounts of recombinant human IL-6, in the presence or absence of PWM. The Ig production was evaluated by immunofluorescence, ELISA and ELISPOT assay. Our results indicate that secreted products of epithelial cells induce the differentiation of B cells into Ig-producig cells. The Ig isotype distribution differed according to the source; B lymphocytes from some donors differentiated into cells secreting either all major isotypes, or preferentially IgA. These results suggest that the epithelial cell should be considered as a sources of soluble factors that promote differentiation of lymphocytes in the mucosal microenvironment. Supported by USPHSE grant DK 28537.

ONTOGENY OF A HUMORAL AND CELLULAR MUCOSAL IMMUNE RESPONSE TO A COMMENSAL GRAM NEGATIVE BACTERIA, Khushroo E. Shroff, Keith Meslin, Fan Lee, Sangeeta Bhargava Periwal, John J. Cebra. Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018.
We have employed a germ free mouse model to study the development and persistence of a humoral and cellular mucosal immune response to Morganella morganii, over a period of 316 days. The bacteria succesfully translocate to the mesentric lymph node and spleen early after the monoassociation. They also cause the hypertrophy of the Peyer's patches and germinal center reaction (GCR) which peaks 14 days after infection. The GC reaction begins to wane thereafter and the number translocating bacteria begin to drop simultaneously. A clonal B cell microculture technique was done to determine the frequency of specific IgA plasmablasts and IgA memory cells. We suggest that a successful secretory IgA response can attenuate chronic stimulation of germinal center reactions even though, the bacteria persists in the gut.

Differential Binding Properties of Gal/GalNAc Specific Lectins Available For Characterization of Glycoreceptors

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The binding properties of Gal and/or GalNAc specific lectins, studied by quantitative precipitation and precipitin-inhibition, competitive-binding,and hemagglutinin inhibition assays, were grouped as follows: Galβ1→3GalNAc specific (*Arachis hypogaea, Bauhinia pupurea alba, Maclura pomifera, Sophora japonica, Artocarpus integrifolia* and *Artocarpin* lectins and ricin; Galβ1→4GlcNAc specific (*Ricinus communis, Datura stramonium* (thom apple), *Erythrina cristagalil* and *Geodia cydonium* lectins); GalNAcα1→3GalNAc (Forssman specific), *Dolichos biflorus, Helix pomatia, Amphicarpoea bracteata,* and *Wistaria floribunda;* GalNAcα1→3Gal (Human blood group A) specific lectins, *Griffonia simplicifolia* A_4. *Phaseolus lunatus* agglutinin (lima bean), *Glycine max* agglutinin (soybean), *Vicia villosa* (a mixture of two isolectins, B_4 and A_4) and *Wistaria floribunda,* and GalNAcα1-ser or thr of protein core (*Vicia villosa* B_4, and *Maclura pomifera*). Many of them demonstrate dual specificities such as *Maclura pomifera* which is specific for Galβ1→3GalNAc and GalNAcα1→linked residues and *Bauhinia purpurea* alba for both Galβ1→3GalNAc and *N*-acetyllactosamine. Grouping of lectins will aid the selection of lectins for carbohydrate residues studied as well as for the interpretation of the distribution and the properties of carbohydrate chain residues on the cell surface.

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