DENDRITIC CELLS: ANTIGEN PRESENTING CELLS OF T AND B LYMPHOCYTES

Organizers: Jacques Banchereau and Ralph Steinman

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Dendritic Cells: Antigen Presenting Cells of T and B Lymphocytes

Origin, Maturation, Migration of Dendritic Cell in Vit

C1-001 IMMUNOBIOLOGY OF MOUSE DENDRITIC CELL CLONES M.B. Lutz, F. Granucci, GP. Girolomoni*, C.U. Aßmann, M. Rescigno, C. Winzler, and P. Ricciardi-Castagnoli, *University of Modena, Department of Dermatology, Via del Pozzo 71, Modena, Italy, CNR Center of Cytopharmacology, Via Vanvitelli 32, University of Milano, Milano, Italy.

Similarly to other leukocytes, dendritic cell precursors acquire full accessory functions apparently only after passage through several differentiation stages. The signals and the genetic programs that drive these maturation steps are still under investigation and are a matter of debate. The molecular approach in these studies is often limited by the difficulties in generating dendritic cell-restricted reagents. Our contribution to the study of the differentiation pathway of dendritic cells was the generation of mouse dendritic cell clones using the retroviral vector MIBΨ2-N11, which carries an env^{AKR}-myc^{MH2} fusion gene (1), previously shown to immortalize phagocytic cells. Several dendritic cell clones were obtained from both lymphoid (spleen) and nonlymphoid tissues (skin). These clones exhibit an immature phenotype. They were characterized by low, but constitutive MHC class II expression and high levels of costimulatory and adhesion molecules such as B7.1, B7.2, HSA, CD11a, CD11b, CD11c, CD49d, and ICAM-1; they also show ER-MP12, Sca-1 and Sca-2 expression, further indicating their precursor-stage of differentiation. Functionally, the clones stimulated a primary allogeneic MLR and, in contrast, to macrophage clones, also a syngeneic MLR. Presentation of nominal antigens in vitro was weak, but could be augmented by pretreatment with IFN-γ. Some other dendritic cell clones required activation with GM-CSF alone or GM-CSF and Il-4 in order to efficiently present antigen in vitro; interestingly, these latter cytokines enhanced antigen uptake and induced a two-fold increase in cell size. When tested for cytokine gene expression, several exogenous signals were shown to activate the dendritic cell clones. GM-CSF triggered the production of TGF-β1 and TNFα. In contrast, induction of IL-12 was observed after viral infections of the clones, but was not promoted by GM-CSF or LPS. However, in vivo the cell clones had a behaviour similar to mature dendritic cells in terms of migration and T cell priming. These results indicate that, using immortalized dendritic cell progenitors, the relevant signals that lead to dendritic cell maturation and the molecular events occuring in this process could be more easily investigated.

(1) P. Paglia, G. Girolomoni, F. Robbiati, F. Granucci and P. Ricciardi-Castagnoli. Immortalized dendritic cell line fully competent in antigen presentation initiates primary T cell responses in vivo. J. Exp. Med. 178:1893-1901, 1993.

C1-002 PRODUCTION AND PROPERTIES OF LARGE NUMBERS OF DENDRITIC CELLS FROM HUMAN BLOOD, Gerold Schuler, Daniela Brang, Paul Cameron, and Nikolaus Romani, Department of Dermatology, University of Innsbruck, A-6020 Innsbruck, Austria

The first detailed descriptions of the formation of dendritic cells (DCs) from hematopietic progenitors were obtained using mouse blood and marrow. Under the aegis of GM-CSF distinctive, proliferating aggregates evolved which gave rise to typical nonproliferating DC cell progeny. We have found that similarly DCs can be grown from human blood without any need to purify rare CD34+ cells (J.Exp.Med. 180: 83-93, 1994). It is sufficient to add GM-CSF (to promote DC development) and IL-4 (to inhibit macrophage colony formation) to lymphocyte-depleted peripheral blood mononuclear cells (PBMC). This induces the formation of proliferating DC aggregates from loosely adherent precorsors that include CD34+/CD14- as well as CD34-/CD14low cells. From these aggregates many nonproliferating typical DC progeny are released within 1 - 2 weeks (about 5 million per 40ml of blood). In addition in such cultures the firmly adherent, CD14++ nonproliferative monocytes become nonadherent, develop many processes, and come to resemble DCs in their phenotype as well (about 3 million per 40ml of blood). Despite their similarity to mature DCs these monocyte-derived accessory cells were, however, far less potent stimulators of resting T cells on a per cell basis upon direct comparison to mature DCs. The DCs that can be grown from *proliferating* precursors are not only potent stimulators of resting T cells but can also produce substantial amounts of bioactive IL-12. These DCs appear, therefore, promising to stimulate T cell-mediated resistance against certain infections and tumors.

C1-003 HUMAN DENDRITIC CELL HEMATOPOIESIS: LINEAGE FIDELITY AND FUNCTIONAL MATURATION FROM $CD34^+$ BONE MARROW PROGENITORS, Paul Szabolcs[1,2], David Avigan[1,3], Malcolm A.S. Moore[4,5], and James W. Young[1,3], [1]Laboratory of Cellular Physiology and Immunology, The Rockefeller University; and [2]Dept. of Pediatrics, [3]Dept. of Medicine, [4]James Ewing Laboratory of Developmental Hematopoiesis, [5]Cell Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center; New York, NY 10021.

$CD34^+$ bone marrow cells, cultured in c-*kit*-ligand, GM-CSF, and TNF*α*, generate large numbers of phenotypically mature and functionally potent dendritic cells among the myeloid progeny. After 12-14d primary culture in these cytokines, approximately half of the resultant cells remain $HLA\text{-}DR^+$, but they are either $CD14^-$ or $CD14^+$. Despite shared expression of costimulatory ligands among the $HLA\text{-}DR^+$ progeny, only the $CD14^-$ $HLA\text{-}DR^+$ cells are immunostimulatory for resting, unprimed allogeneic T cells. The lineage fidelity of these bone marrow-derived dendritic cells has been further explored in semi-solid colony assays, in which we have identified a committed progenitor for dendritic cells and monocytes/macrophages which we have termed the CFU-DC/mono. c-*kit*-ligand, in conjunction with GM-CSF and TNF*α*, supports two and three logs greater expansion of CFU-DC/mono after two and three weeks' culture respectively, compared to the CFU-DC/mono expansion supported by GM-CSF and TNF*α* alone. c-*kit*-ligand, however, has little direct effect on the differentiation of dendritic cells from committed progenitors. To ascertain whether dendritic cells and monocytes share additional precursors or develop independently from the CFU-DC/mono, class II MHC positive, CD34-depleted intermediates have been sorted from bulk suspension cultures of $CD34^+$ bone marrow cells after 6-7d culture in c-*kit*-ligand, GM-CSF, and TNF*α*. These have been further separated according to their expression of CD14 or lack thereof. In contrast to the more mature progeny, among which there is a clear segregation of phenotype and immunostimulatory activity, both $CD14^-$ and $CD14^+$, $HLA\text{-}DR^+$ intermediates express some dendritic cell properties. More importantly, however, these $CD14^+$ $HLA\text{-}DR^+$ intermediates are bipotential and can mature into either dendritic cells in the presence of GM-CSF and TNF*α*, or monocytes under the aegis of M-CSF. We are investigating whether parallel maturational changes in antigen processing and costimulatory ligand expression by dendritic cells occur, and whether additional glycoprotein cytokines may influence these later stages of differentiation. The answers to these important unknowns should prove useful in the application of dendritic cells to the manipulation of T cell-mediated immune responses.

Dendritic Cells: Antigen Presenting Cells of T and B Lymphocytes

Origin, Maturation, Migration of Dendritic Cells in Vivo

C1-004 LYMPH-BORNE DENDRITIC CELLS AND ANTIGEN PRESENTATION IN VIVO. G. Gordon MacPherson, LiMing Liu and Natasha Kushnir, Sir William Dunn School of Pathology, Oxford University, England.

Dendritic cells (DC) acquire antigen in peripheral tissues, transport it to lymph nodes and present MHC-associated peptides to recirculating T cells in the paracortex. In mesenteric-lymphadenectomized rats, DC which would have been retained in the nodes enter thoracic duct lymph and can be purified by simple density gradient centrifugation. KLH or ovalbumin introduced into the intestine by injection or gavage is acquired by DC in the intestinal wall within a few hours, and these DC migrate into lymph from which they can be purified. Such DC do not carry detectable native antigen but can stimulate sensitized T cells "in vitro" and prime naive CD4+ T cells "in vivo" in a MHC class II-restricted manner, showing direct presentation [1,2]. Lymph DC are mature in their ability to stimulate a strong MLR but can still process exogenous antigens and, in contrast to murine Langerhans cells (LC), this ability is not lost after 72h in culture. Also in contrast to murine LC, DC freshly extracted from the small intestine by enzymatic digestion can stimulate a moderate MLR but as with LC, overnight culture markedly increases their potency.

The regulation of DC release from the intestine in steady state conditions is not understood but DC release is stimulated by 2-5-fold within 6-12h of giving IV LPS and this effect is blocked by an anti-TNF-α antibody. DC released by LPS do not differ significantly from those released in steady state conditions.

Lymph DC can interact with small, resting, recirculating B lymphocytes to form short-lived clusters "in vitro". This interaction is energy- and cytoskeletal-dependent, is stimulated by PMA and inhibited by PKC inhibitors, suggesting the involvement of integrins, but the available anti-integrin antibodies do not block cluster formation. The ability of DC to cluster B cells is down-regulated after overnight culture. Spleen and lymph node B cells bind DC more actively than lymph or blood B cells, suggesting that adhesion molecule expression is activated or up-regulated after B cells cross endothelium. DC/B cell clusters can also be seen in fresh lymph. B cell activation in primary responses occurs in T cell areas and we suggest that this B cell-DC interaction may allow time for B cells to process antigen they encounter so that they can present it to T cells that have already been activated by the same DC.

1. Liu L.M. & MacPherson G.G. (1991) Lymph-borne (veiled) dendritic cells can acquire and present intestinally administered antigens. *Immunology*, **73,** 281.
2. Liu L.M. & MacPherson G.G. (1993) Antigen acquisition by dendritic cells: intestinal dendritic cells acquire antigen administered orally and can prime naive T cells in vivo. *J Exp Med*, **177,** 1299.

C1-005 *Abstract Withdrawn*

C1-006 THYMIC DENDRITIC CELLS: DEVELOPMENTAL AND FUNCTIONAL RELATIONSHIPS TO T CELLS, Ken Shortman, Li Wu, Carlos Ardavin, Gabriele Süss, Ken Winkel, Dolores Saunders and David Vremec. The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia.

The earliest T-precursor population isolated from the adult mouse has the potential to form both T-lineage and B-lineage cells but not erythroid or myeloid cells. This precursor population also forms dendritic cells (DC) on intrathymic transfer, suggesting that thymic dendritic cells are formed within the thymus itself, and may be related to the lymphoid lineage. When an irradiated thymus is reconstituted with the thymic precursor, each cohort of developing T cells is accompanied by a parallel cohort of DC, with a ratio of T-lineage cells to DC of around 1000:1, the normal ratio within the thymus. Both the T-lineage cells and the DC have a short intrathymic lifespan. This may be a mechanism of ensuring that negative selection is restricted to self-antigens presented by short-lived, endogenously generated DC.

Thymic DC also express certain lymphoid markers (e.g. CD8α and BP-1), further evidence of a lymphoid relationship. A subset of DC in peripheral lymphoid organs shares some of these markers with thymic DC (in particular CD8α). When the thymic DC precursor is used to reconstitute splenic DC by intravenous transfer, the progeny DC retain some (e.g. CD8α) but not all (e.g. BP-1) of the characteristic thymic DC markers. When splenic DC are separated into $CD8\alpha^+$ (thymic-like) and $CD8\alpha^-$ subpopulations, the $CD8^-$ DC are more effective stimulators of $CD4^+$ allogeneic T cells, $CD8^+$ DC apparently causing a degree of cell death. The results suggest that there are separate sublineages of DC, differing both in origin and function.

Dendritic Cells: Antigen Presenting Cells of T and B Lymphocytes

Antigen Processing and Presentation

C1-007 FORMATION AND FUNCTION OF PEPTIDE: MHC MOLECULE LIGANDS FOR THE TCR, Ronald N. Germain[1], Flora Castellino[1], Paola Romagnoli[1], Joaquín Madrenas[1], Ronald Wange[2], Noah Isakov[2], and Lawrence E. Samelson[2]
[1]Lymphocyte Biology Section, Laboratory of Immunology, NIAID, and [2]Cell Biology and Metabolism Branch, NICHD, NIH, Bethesda, MD 20892

Major histocompatibility complex (MHC) class I and class II molecules are peptide binding and transport proteins that provide an extracellular representation of intracellular antigen content for recognition by the clonotypic receptors of T lymphocytes. The two classes of MHC proteins have evolved distinct biochemical and cell biological strategies for peptide acquisition, providing a cell with the means to capture peptides derived from proteins in the cytoplasm [class I] or in intracellular membrane-bound vesicles [class II]. For both classes of MHC molecule, peptide binding affects the stability of subunit association, a property that helps regulate the intracellular trafficking and lifespan of the proteins. Conserved binding pockets for the free N- and C-termini of peptides 8-10 residues in length helps class I discriminate between peptides imported from the cytosol, and free loops and strands of intact self-proteins present in the ER. For class II, which can associate with such intact proteins, binding site interaction with a peptide-length internal segment of invariant chain [the CLIP segment] helps fulfill the occupancy and folding requirements for ER to Golgi transport while preventing occupancy with a diverse array of self-proteins. Invariant chain also contains targeting signals that guide class II to the endocytic pathway, which the complexes enter via early endosomes. These complexes transit to more distal endocytic organelles during which time invariant chain is proteolytically degraded and removed from class II, and fragments of denatured proteins are captured by the class II binding site. The peptide:MHC class II complexes then move to the cell surface for recognition by CD4+ T cells. This recognition event induces oligomerization among the TCR, the peptide:MHC class II complex, and the CD4 coreceptor, which in turn generates intracellular signals leading to T cell activation. Alteration in the structure of the peptide:MHC ligand from that normally capable of activating a T cell can generate partial agonists or antagonists. Recent data suggest that such variant ligands transduce a qualitatively distinct set of biochemical signals compared to full agonists; the dissection of these changes is providing new insight into the relationship between the molecular organization of the antigen-engaged TCR complex and the early biochemical events necessary for eliciting T cell effector functions.

Accessory Molecules

C1-008 A COMPARISON OF B CELL AND DENDRITIC CELL ANTIGEN PRESENTATION TO NAIVE T CELLS, Delanie J. Cassell and Ronald H. Schwartz, Laboratory of Cellular and Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda.

Activated B cells can clearly stimulate T cell clones and memory T cells. In contrast, the question of whether these cells can stimulate naive T cells remains controversial. We have examined purified $CD44^{lo}$, $V\alpha11^{+}$, $CD4^{+}$ T cells from a cytochrome *c*-specific T cell receptor transgenic mouse for their ability to secrete IL-2 *in vitro* when presented with antigen by either interdigitating dendritic cells or activated B cells. These T cells require both costimulation and T cell receptor occupancy to make IL-2. Both activated B cells and dendritic cells could stimulate IL-2 production; however, a quantitative analysis of the response by single T cells revealed a 4-fold lower production when poly I-poly C-stimulated B cell blasts were used compared to activated splenic dendritic cells. This relative deficiency was shown to result mainly from suboptimal costimulation, although T cell receptor occupancy was also slightly deficient. Unactivated B cells did not stimulate at all, because of profound deficiencies in both costimulation and antigen presentation. These results will be discussed as to their relevance to the controversy surrounding B cell antigen presentation to naive T cells *in vivo*.

Tolerance and Autoimmunity

C1-009 ROLE OF CELLS IN DENDRITIC CELLS IN DIRECTING T HELPER CELL RESPONSES, Anne O'Garra, DNAX Research Institute, Palo Alto.

Cytokine production by subsets of $CD4^{+}$ T cells regulates the effector response to invading organisms. The selective induction of $CD4^{+}$ T helper cells with these distinct cytokine profiles may be dictated by factors that include: the nature and dose of antigen, the APC, or cytokines made early in the response by the APC/accessory cell or the T cell itself. We have recently shown that dendritic cells (in contrast to B cells and macrophages) induce strong antigen specific proliferation of $CD4^{+}$ T cells from mice expressing an OVA-specific transgenic TCR. Addition of Listeria-activated macrophages producing significant levels of IL-12, or IL-12 itself, was required for development of a Th1 phenotype producing high levels of IFNg, and addition of exogenous IL-4 was required for development of Th2 cells producing high levels of IL-4 upon restimulation. Dendritic cells induced IL-12-dependent Th1 development occurred only upon removal of endogenous IL-4.

We now show that appropriately activated B cells can induce significant antigen-specific proliferation of naive $CD4^{+}$ T cells, comparable to that induced by dendritic cells. We further show that regardless of the APC, the dose of antigen is critical in determining whether naive $CD4^{+}$ T cells develop into a Th1 or Th2 phenotype.

C1-010 HUMAN PERIPHERAL BLOOD DENDRITIC CELL SUBSETS: ISOLATION AND CHARACTERIZATION OF PRECURSOR AND MATURE ANTIGEN-PRESENTING CELLS, Ranjeny Thomas[1*†] and Peter E. Lipsky[2*], [*]The Harold C. Simmons Arthritis Research Center and The Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, TX 75235; and [†]Department of Medicine, University of Queensland, Princess Alexandra Hospital, Brisbane, Australia.

Dendritic cells (DC) are the major APC capable of stimulating resting T cells in human peripheral blood. Recent evidence suggested that various subsets of DC and monocytes might circulate in human PB, but their exact phenotype and function had not been delineated. We have previously characterized a population of human PB DC precursors that express the myeloid marker CD33, but not the monocyte marker CD14. To identify and characterize further functional myeloid APC subsets, triple color FACS analysis and sorting was used. A $CD33^{dim}CD14^{dim}CD16^{+}$ monocyte subset, with similar APC function but less efficient accessory function than $CD14^{bright}$ monocytes, was isolated. In addition to the $CD33^{+}CD14^{dim}CD16^{-}$ DC precursors, a smaller population (0.1 to 0.2% of PBMC) of $CD33^{bright}CD14^{dim}CD16^{-}$ cells with potent APC function was identified. This DC population expressed greater amounts of MHC class II, adhesion, and accessory molecules, and demonstrated a great costimulatory capacity when freshly isolated than $CD33^{dim}CD14^{dim}$ DC precursors, and therefore had the characteristics of mature, possibly tissue-derived DC. When freshly isolated, however, these DC did not express B7, and up-regulation of accessory function occurred after in vitro differentiation. These data demonstrate multiple circulating myeloid accessory and APC subsets in human peripheral blood. Phenotypic and functional differences suggest that they are at different stages of differentiation, and have specialized roles in Ag presentation in vivo. Furthermore, full functional DC differentiation, associated with B7 expression and the capacity to activate T cells maximally, is likely to occur only in specific physiologic circumstances.

Dendritic Cells as Adjuvants

C1-011 DENDRITIC CELLS STIMULATE STRONG PROLIFERATIVE AND CYTOLYTIC RESPONSES FROM HUMAN CD8+ T CELLS. Nina Bhardwaj, Long Kim Bui, Mary Feldman, Marie Larsson, Armin Bender. The Rockefeller University, 1230 York Avenue, New York, N.Y. 10021.

Antigen specific CD8+ cytolytic T lymphocytes [CTLs] are thought to be important mediators of resistance in a number of infectious diseases and malignancies. Vaccines that preferentially induce the formation of these effector cells could be highly efficacious in the establishment of effective host immunity. We have developed approaches to generate strong CD8+ CTL responses from fresh human T cells, using dendritic cells as antigen presenting cells. Using influenza A virus as a model agent, we find only a few dendritic cells [APC:T cell ratio of 1:100-200] induce specific CTL responses from most donors in 7 days of culture, while monocytes are weak or inactive. Whereas large numbers of dendritic cells [>90%] and monocytes [>70%] are infected with influenza, the former serve as effective APCs for CTL induction while the latter act as targets for the CTLs that are induced. The strong CD8+ response to influenza virus-infected dendritic cells is accompanied by extensive proliferation of the CD8+ T cells, but the response can develop in the apparent absence of CD4+ helpers or exogenous lymphokines. CD4+ influenza virus-specific CTLs can also be induced by dendritic cells, but cultures must initially be depleted of CD8+ cells. Influenza A virus can be attenuated by heat treatment [56°, 30 min] or by exposure to UV light, which reduces infectivity by greater than 10^4 fold, as assessed by standard plaque forming assays. Surprisingly, these attenuated forms also generate virus specific CD8+ CTL responses, when presented on dendritic cells. The CTL responses that are generated are as potent as those elicited by live virus. Cytofluorography and immunohistochemistry has verified that the attenuated viruses are deficient in synthesizing new viral protein synthesis. Fewer than 2% of dendritic cells infected with nonreplicating virus demonstrate the synthesis of NS1 [which is only synthesized by replicating virus] or the highly conserved protein NP [nucleoprotein]. Thus attenuated forms of influenza virus can be immunogenic as long as the virus is delivered to dendritic cells. These findings suggest that such forms of virus may be potentially useful as vaccines where CTL responses are the desired features. Furthermore, since influenza A virus can be engineered to express foreign CTL epitopes, it may be possible to pulse dendritic cells with such vectors to generate human CD8+ CTLs to other target antigens.

C1-012 REGULATION OF ANTIGEN-PRESENTING-CELL FUNCTION IN VIVO, T. De Smedt[1], G. De Becker[1], E. Muraille[1], M. Van Mechelen[1], L. Lespagnard[1], T. Sornasse[1], V. Flamand[1], F. Tielemans[1], K. Thielemans[2], E. Heinen[3], J. Urbain[1], O. Leo[1] and M. Moser[1], [1]Laboratoire de Physiologie Animale, Université Libre de Bruxelles, Rhode-Saint-Genèse, [2]Laboratorium of Hematologie-Immunologie, Vrije Universiteit Brussel, Brussels, [3]Institut d'Histologie humaine, Université de Liège, Liège, Belgium.

The specificity of an immune response is determined by the peptide/MHC complex that occupies the T cell receptor. Besides this antigenic signal (signal 1), the antigen-presenting-cells (APC) may provide a second signal, the costimulatory signal, which seems to determine the amplitude and the orientation of the response. The population of APC is heterogeneous: B cells, macrophages and dendritic cells (DC) provide signals 1 and 2 that differ quantitatively and qualitatively, and may therefore induce distinct immune responses *in vivo*. We and others have previously shown that it was possible to prime mice using elements of the immune system. In particular, a single injection of syngeneic DC, which have been pulsed *in vitro* with an antigen, induces a strong antibody response in mice, upon challenge with a low dose of soluble antigen. The humoral response is quantitatively and isotypically similar to the response induced classically, i.e., with complete Freund's adjuvant. Similarly, the injection of DC pulsed with the immunoglobulin expressed by BCL1 lymphoma induces an antiidiotypic humoral response, and leads to tumor resistance *in vivo*. We compared the adjuvant capacity of DC, B cells and macrophages in the same experimental system. The results show that antigen-pulsed DC induce the synthesis of IgG1 and IgG2a antibodies, whereas peritoneal macrophages favor the production of specific IgG1 and IgE antibodies. By contrast, B cells appear to induce a state of hyporesponsiveness in the injected mice. The distinct isotypic profile induced by DC and macrophages suggests that the nature of the APC may determine the Th1/Th2 balance *in vivo*, a hypothesis that is currently being tested by directly measuring the lymphokines produced by T cells.

The potent adjuvant capacity of DC correlates with a specialization of function over time, and therefore we compared the expression of B7 molecules on fresh versus cultured DC. The costimulatory signal is strongly upregulated during culture, a step that is independent of T cell activation or external stimuli, and correlates with an increase in their capacity to sensitize naive T cells. Of note, the addition of IL-10 or dexamethasone during overnight culture seems to prevent full maturation of DC and modify their immunostimulatory capacity *in vivo*.

We further tested whether the APC function could be modulated by pathogens *in vivo*. The injection of bacterial lipopolysaccharide, an activating agent for B cells and macrophages, results in transient immunodeficiency and represents a model of septic shock. We found that spleen cells isolated from animals previously injected with LPS display a defective capacity to sensitize naive T cells *in vitro*, a defect that correlates with a loss of splenic DC.

Taken together, these observations suggest that the antigen presenting function of DC can be modulated *in vitro* and *in vivo*, and could be a target of immune regulation.

C1-013 GENES ENCODING HUMAN TUMOR ANTIGENS RECOGNIZED BY CYTOLYTIC T LYMPHOCYTES. Pierre van der Bruggen, Benoit Van den Eynde, Pierre Coulie, Vincent Brichard, Aline Van Pel, Etienne De Plaen, F. Brasseur, and Thierry Boon. Ludwig Institute for Cancer Research, Brussels Branch, 74 avenue Hippocrate - UCL 7459, B-1200 Brussels, Belgium.

Transplantation experiments have demonstrated that most mouse tumors express antigens that can constitute targets for rejection responses mediated by syngeneic T lymphocytes. For human tumors, autologous cultures mixing tumor cells and blood lymphocytes have produced cytolytic T cell (CTL) clones that recognize autologous tumor cells. We used gene transfection approaches in order to identify the target antigens borne by these tumor cells.

Human melanoma MZ2-MEL presents several antigens. The gene coding for one of those, MZ2-E, has been isolated (1). This gene, named MAGE-1, belongs to a family of at least 12 closely related genes, located on chromosome X (2). These genes are not expressed in normal tissues except for testis. Gene MAGE-1 is expressed in 40 % of melanoma tumors, 20% of breast tumors and 30% of non small cell bronchial tumors. Antigen MZ2-E consists of a nonapeptide encoded by MAGE-1 and presented by HLA-A1 (3). MAGE-1 also codes for a peptide presented by HLA-Cw*1601 (4). Gene MAGE-3 codes for antigens recognized by CTL on HLA-A1 and on HLA-A2 (5, 6). Gene MAGE-3 is expressed in 73% of metastatic melanoma . Two additional genes that code for tumor antigens and are expressed only in tumors and in testis have been isolated. These genes are unrelated to each other and to the MAGE family.

We have also identified two additional genes that code for differentiation antigens recognized by CTL on most melanomas of HLA-A2 patients. The first gene codes for tyrosinase, the enzyme that synthesizes DOPA in the melanin pathway (7). This gene is expressed in most melanoma and, among normal tissues, only in melanocytes. The second gene, named Melan-A, is unrelated to presently known sequences (8). Its expression is also restricted to most melanoma and melanocytes.

The identification of human tumor antigens shared by a significant proportion of human tumors opens new possibilities for systematic approaches of cancer immunotherapy. To establish whether immune responses will be generated, it will be necessary to compare CTL frequencies before and after immunization.

1. P. van der Bruggen, et al., *Science* **254**, 1643-1647 (1991).
2. E. De Plaen, et al., *Immunogenetics*, In press. (1994).
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Follicular Dendritic Cells

C1-014 CELLULAR INTERACTIONS LEADING TO GERMINAL CENTER RESPONSES, Marie H. Kosco-Vilbois[1/3], Michael Wiles[1], Doris Scheidegger[1], Georges Köhler[2], and Manfred Kopf[1/2], [1]Basel Institute for Immunology, Grenzacherstr. 487, CH-4005 Basel, Switzerland, [2]The Max-Planck Institute for Immunobiology, Stuebeweg 51, D-7800, Freiburg, Germany and [3]Glaxo Institute for Molecular Biology, 14, chemin des Aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland.

Follicular dendritic cells (FDC) are located in lymphoid compartments that undergo dynamic changes in response to an antigenic challenge. These sites, called germinal centers (GC), appear transiently and are essential for generating complete antibody responses. The generation of mouse models where specific proteins are either over-produced as soluble chimeric molecules or rendered inactive by gene targeting has facilitated inquiries into the molecules regulating GC responses. Since IL-6 is known to influence growth and differentiation of B cells towards antibody production, we investigated its role using IL-6 deficient mice. While these mice appear to develop normally, when challenged with a T-cell dependent antigen (i.e. dinitro-phenylated ovalbumin), they produced significantly lower IgG titers as compared to non-deficient littermate controls. Interestingly, while IgG_{2a}, IgG_{2b} and IgG_3 remained low or absent even after a secondary immunization, with time IgG_1 titers began to approach that found in normal controls. Immunohistochemical evaluation of GC size in situ revealed that similar to controls, small foci of peanut agglutinin (PNA) binding B cells formed in association with the FDC network. However, with time post antigenic challenge, the ability to form the characteristic light and dark zone was impaired. Using PNA binding as a means to delineate the borders of a GC, morphometric analysis quantitated this size dissimilarity. Non-deficient mice produced GC whose size reached 3.0 mm^2 while the maximum measured in IL-6 deficient mice was no larger than 1.0 mm^2. In an attempt to dissect further what was happening in vivo, single cell suspensions containing the GC population were prepared from lymph nodes seven days post a primary antigenic challenge. The GC population, displaced into a culture system, revealed that the cytokine pattern produced by the T cells from the IL-6 deficient mice was skewed towards a higher production of interferon-γ and a lower amount of IL-5. When IL-6 was added to the cultures, the impaired levels of the various IgG isotypes could be rescued to within the amounts produced by the cells from the non-deficient mice. Furthermore, the cultures derived from the deficient mice incorporated more ^{3}H-thymidine in the presence of exogenous IL-6. These data demonstrate that IL-6 is essential for producing the profile of immunoglobulin isotypes seen during an antibody response. The defect occurring in the IL-6 deficient mice appears to be at least partly due to an inability to form the microenvironments in vivo where B cells receive signals to expand and differentiate. The ability to augment the amount of an isotype produced and the level of proliferation achieved by adding IL-6 to the in vitro GC population reinforces the proposal that the cells within the GC are being directly affected in this deficient model. Additional information as to the source and role of IL-6 in GC will be discussed.

C1-015 ICCOSOMES AND FOLLICULAR DENDRITIC CELLS: ANTIBODY FORMATION AND B CELL PROLIFERATION, John G. Tew[1], Dahui Qin[1], Shirley T. Helm[1], Chin-Lo Hahn[1], Jiuhua Wu[1], Ying Xiong[1], Andras K. Szakal[2] and Gregory F. Burton[1], [1]Departments of Microbiology and Immunology and [2]Anatomy, Division of Immunobiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

Follicular dendritic cells (FDC) trap Ag in the form of immune-complexes and are capable of presenting this Ag to specific B cells. The Ag in the immune-complexes may be presented to B cells in the form of immune-complex coated bodies (iccosomes) which are liposome like structures produced by the FDC. B cells find iccosomes palatable and after endocytosis the B cells process the iccosomal Ag and can present it to specific T cells. These events occur in germinal centers where B cells proliferate, memory B cells are produced and antibody forming cells are generated which home to the bone marrow where they terminally differentiate. We believe that FDC are the critical accessory cell in the germinal center and hypothesize that they provide not only specific Ag (the primary B cell signal) but also additional primary and co-stimulatory signal(s) which enhance the ability of Ag to stimulate B cells. To begin testing this hypothesis, B cells were activated by polyclonal B cell activators [sIg dependent (anti-μ-dex) or independent (LPS)] and the effects of adding FDC or iccosomes were determined. The B cell responses measured were: antibody production, B cell proliferation and B cell chemotaxis. FDC or iccosomes had the following accessory activities: (A) FDC markedly increased mitogen induced B cell proliferation. (B) FDC increased mitogen induced IgG production. (C) FDC stimulate B cell chemotaxis. In contrast to the augmentation of proliferation and antibody production, the FDC's ability to stimulate chemotaxis was a primary signal and did not require a signal from mitogen or antigen. (D) FDC promoted long term B cell survival and function in culture. In the presence of FDC B cells could continue to produce antibody in culture for at least a month. In the absence of FDC, B cells rapidly died in culture. (E) In contrast to FDC, iccosomes did not promote B cell proliferation. However, iccosomes were potent stimulators of specific IgG production when loaded with appropriate Ag. In further contrast to the intact FDC, iccosomes could not sustain B cell cultures and maintaining Ab production for weeks to months in culture. The molecular mechanisms responsible for these FDC accessory activities are not yet clear but the ability of the FDC to augment B cell proliferation and maintain viability in culture for weeks appears to relate, in part, to an ability to protect B cell from oxidative stress which leads to apoptosis. In short, these data indicate that FDC provide not only specific Ag but also other signals which contribute to make the microenvironment in the germinal center favorable for B cell survival, proliferation and maturation. Supported by NIH Grant # AI 17142.

Dendritic Cells in AIDS

C1-016 VIRAL AND CELLULAR DETERMINANTS OF INFECTION AND TRANSMISSION OF HIV-1 BY LYMPHOID DENDRITIC CELLS. Paul Cameron, Alison Coughlan, Jeanette Reece, *Frank Sotzik, Suzanne Crowe and *Ken Shortman. AIDS Pathogenesis Research Unit , Macfarlane Burnet Centre for Medical Research, Fairfield, Victoria, Australia 3078, *Walter and Eliza Hall Institute, Parkeville, Victoria, Australia

Two views of the frequency and functional consequences of HIV-1 infection of dendritic cells are current. The earlier view holds that blood DCs are frequently infected *in vivo* and readily infected *in vitro*. Infection and dysfunction or transmission from infected DCs are thus responsible for abnormalities of APC function and defects in immune responses to recall and neoantigens. The alternative view that the frequency of infection *in vivo*, and *in vitro* is much lower than in T cells is based on studies that have used blood DCs purified by cell sorting after culture. Such cells express low levels of CD4 and are infected with macrophage tropic or T cell line tropic isolates at a low frequency. We have shown that such cells although infrequently infected by PCR are able to carry virus and can infect resting T cells that cluster with the DC during a cognate interaction. Transmission of T-cell-line-tropic isolates by $CD4^-$ DCs , but not the MLR stimulatory capacity, decreases rapidly with time and there is low level of transmission if T cells are added to virus pulsed DCs after 24 hours of culture in conditioned medium.

In order to determine the effect of high levels of CD4 expression on the interaction of virus with DCs we have isolated DCs from thymus and tonsil. These cells constitutively express CD4. Cultured tonsil DCs are functionally similar to the cultured blood DCs but express high levels of CD4 and are $CD11c^-$. Fresh thymic DCs express high levels of CD4, MHC Class I and Class II, and CD11c. Both tonsil and thymic DCs are infected with macrophage tropic isolates of HIV-1 at a frequency similar to cultured macrophages. The DCs, like macrophages, are infected with T cell line tropic isolates of HIV-1 at low frequency. Productive infection of DCs by macrophage-tropic isolates occurs. In transfer experiments the $CD4^+$ DCs were able to transfer both macrophage-tropic and T-cell-line-tropic isolates but transfer after 24 hours was proportionally greater with macrophage tropic isolates. By immunofluorescence the DCs and T cells in clusters expressed p24 after pulsing with the macrophage tropic virus but only the T cells after the DCs have been pulsed with T cell tropic virus.

Selective infection of $CD4^+$ DCs by macrophage-tropic virus may be responsible for preferential transmission of macrophage tropic virus to T cells during the early stages of infection and could account for the predominance of such strains in seroconverters. In established infection the ability of DCs to cluster and activate T cells and provide a favourable microenvironment for T cell-T cell transmission is likely of much greater pathogenic significance than direct DC infection. This virus carriage by $CD4^+$ DCs may allow transmission to responding T cells that is independent of viral tropism.

C1-017 CUTANEOUS DENDRITIC CELL-T CELL CONJUGATES AS A SITE FOR ACTIVE INFECTION WITH HIV-1, Melissa Pope[1], Nancy Gallo[2], Lloyd Hoffman[3], Stuart Gezelter[1], and Ralph M. Steinman[1]. [1]Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021, [2]New York Firefighters Skin Bank, New York Hospital, New York, NY 10021, [3]Division of Plastic Surgery, Cornell University Medical College, New York, NY 10021

Both dendritic cells and memory T cells [$CD4^+$ and $CD8^+$] migrate from organ cultures of split thickness skin that has been removed from donors undergoing reductive plastic surgery[1]. The skin cell emigrants consist of free dendritic cells, free T cells, and dendritic cell-T cell conjugates. The three cell subsets can be separated by cell sorting; conjugates continue to form when sorted dendritic cells and T cells are recultured together. The skin cell emigrants are permissive to infection with several HIV isolates[2]. Multinucleated syncytia are the major site of virus production. Infection with $HIV\text{-}1_{IIIB}$ is dependent on the presence of both dendritic cells and memory T cells, which fuse together to form the syncytia.

In addition to furthering our infection studies using the primary patient-derived isolates, we have investigated cadaver skin as a source of dendritic cells. Comparable numbers of dendritic cells and memory T cells [$CD4^+$ and $CD8^+$] migrate from cadaver skin relative to fresh skin. Some of the dendritic cells and T cells also form stable conjugates, and the three cell subsets are able to separated by cell sorting. The dendritic cells and T cells are functionally competent and susceptible to infection with HIV-1. Cadaver skin taken within 24 hours of death has provided access to a much greater number of cells, assisting in our studies of the infectibility of cutaneous dendritic cells with patient-derived isolates.

Infection of skin cell emigrants with either syncytia-inducing or non-syncytia-inducing HIV isolates requires both dendritic cells and $CD4^+$ T cells together. AZT blocks infection with all HIV isolates tested. Cutaneous dendritic cells and memory T cells alone show little if any sensitivity to infection with HIV-1, but together they create a microenvironment that is permissive to productive and cytopathic infection. This cellular milieu may play an important part in the transmission and production of virus in vivo.

1. Pope, M., Betjes, M.G.H., Hirmand, H., Hoffman, L. & Steinman, R.M. *J. Invest. Dermatol.* **In Press**, (1994).
2. Pope, M., Betjes, M.G.H., Romani, N., Hirmand, H., Cameron, P.U., Hoffman, L., Gezelter, S., Schuler, G. & Steinman, R.M. *Cell* **78**, 389-398 (1994).

C1-018 MEMBERS OF THE DENDRITIC CELL SYSTEM IN HIV INFECTION, Paul Racz[1], Irma Gigli[1/2], Andrea von Stemm[1], Julia Ramsauer[1], Jörn Schmitz[1], Klara Tenner-Racz[1/3], [1]Bernhard-Nocht-Institut for Tropical Medicine, Hamburg, [2]University California, San Diego, [3]AK St. Georg, Hamburg, Germany.

Organized lymphoid tissues, especially the B dependent zones are important reservoirs for HIV. The virus persists in the germinal centers (GC) for years, even during the stages of clinical latency. In vitro studies have shown that although HIV are found mainly in CD4 T cells, members of the dendritic cells family may also support virus replication. Three distinct dendritic cell types are of interest: follicular dendritic cells (FDC), interdigitating cells (IDC) and Langerhans cells (LC) of the squamous epithelium. Analysis of HIV gene expression in these immunologically important cells may be of releveance in the pathogenesis of the disease in vivo. Electron microscopic, immunohistochemical as well as nucleic acid hybridization studies have shown that FDC capture and retain HIV. In acute HIV infection the virus can be detected associated with FDC. During the course of the disease intrafollicular accumulation occurs in those areas in which FDC coexpresses the CD23 antigen. As noted with CD4 T cells, constant finding in HIV infection is the progressive loss of FDC. The mechanism leading to FDC death may be the cytopathic effect of HIV. However, virus budding from FDC membranes is only rarely seen. In situ hybridization for HIV RNA and DNA by nested PCR, and electron microscopy on isolated FDC show that FDC are not major targets for the virus in lymph nodes with follicular hyperplasia. It is likely that several factors may contribute to the destruction of FDCs. In lymph nodes with follicular hyperplasia or mixed follicular pattern neither IDC expressing HIV RNA nor preferential localization of productively infected cells were noted around IDC. LC in the skin and squamous epithelium overlying lymphoid tissues seems to have distinct behaviour. No HIV RNA were detected in LC of 52 skin biopsies, and rarely p24 positivity was found in these cells. In contrast, squamous ephitelium lining the wall of lymphoepithelial cysts of the parotid gland showed some LC expressing viral RNA. These findings suggest that LC in the skin are not a significant reservoir for HIV, but these cells in an infected microenvironment may be targets for HIV.

C1-019 **ROLE OF DENDRITIC CELLS IN THE IMMUNOPATHOGENESIS OF HIV DISEASE.** Drew Weissman[1], Jean Ananworanich[1], Tobias D. Barker[1], James A. Daucher[1], Yuexia Li[1], Jan M. Orenstein[2], Anthony S. Fauci[1]. [1]Laboratory of Immunoregulation, NIAID, NIH, Bethesda, MD 20892, [2]George Washington University Medical Center, Washington, DC

Dendritic cells are the primary antigen presenting cells involved in initiating new T cell immune responses. They are also the first immune competent cells to arrive at sites of inflammation in mucous membranes. Their role in the initiation and propagation of HIV disease is currently under intense investigation. We have isolated 2 populations of dendritic cells from peripheral blood. One population, which has been described previously, are precursor cells that most likely have left the bone marrow and are traveling to become tissue dendritic/Langerhans' cells. When allowed to mature in culture these precursors express the DC marker, CD83. A second population of mature DC are found bound to T cells after ficoll-hypaque density gradient centrifugation. This second population of DC, when detached from the T cells and purified, express CD83, CD86 and high levels of CD80. They can efficiently bind HIV to their surface and when cocultured with unstimulated, autologous CD4 positive T cells can lead to high levels of viral replication. When conjugates of mature DC and T cells were isolated and very small amounts of HIV (1 $TCID_{50}$) were added, an efficient infection resulted in the absence of exogenous mitogens or cytokines. Thus, one population of DC in peripheral blood can efficiently bind HIV to their surface and infect unstimulated CD4 positive T cells. This same population is found as a conjugate with T cells that are highly susceptible to infection with HIV. These observations suggest a role for DC in the initiation of HIV infection where, after entry through mucous membranes, HIV may bind to DC and either travel directly to lymph nodes, or the DC may bind to T cells and lead to a local productive infection prior to disseminating to lymphoid organs.

Dendritic Cells and Allergy

C1-020 LANGERHANS CELLS AND THE EPIDERMAL CYTOKINE MILIEU IN CONTACT SENSITIVITY, Alexander H. Enk, Clinical Res. Unit, Dept. Dermatol., Univ. of Mainz, Germany.

Contact sensitivity (CS) has served as a model for T cell-mediated primary immune responses for some time. Langerhans cells (LC) as the representatives of the dendritic cell lineage in the epidermis are known to be of critical importance for the induction of contact sensitization, in that they are leaving the epidermis following contact with allergen to sensitize T cells in the regional lymph node. As epidermal cells (EC) are also a rich source of various cytokines, our laboratory has been interested in studying the effects of cytokines on LC-immune functions during the induction phase of contact sensitivity for several years. In this regard, especially two cytokines, IL-1β and IL-10, were of interest to us. IL-1β is the first cytokine whose mRNA signal is enhanced within 15min after allergen is applied to the epidermis. The fact that the IL-1β signal is derived from LC lead us to investigate whether we could causally link epicutaneous sensitization to IL-1β production by LC. Indeed we demonstrated that IL-1β injection into skin mimicked the epidermal cytokine pattern of epicutaneously applied allergen, that is enhancement of signals for TNFα, IL-1α, IL-10, MIP-2, IP-10, I-Aα, and IL-1β itself. Additionally as shown by staining for MHC class II on epidermal sheets and by FACS, injection of IL-1β (but not TNFα, or IL-1α) induced an upregulation of MHC class II molecules, a change in LC morphology and a rarefication of LC similar to that induced by epicutaneously applied allergen. Functionally LC derived from IL-1β-injected skin were also more potent accessory cells than cells derived from control skin. The essential function of IL-1β was furthermore emphasized by the fact that injection of anti-IL-1β mAb prior to hapten application prevented sensitization in mice.
On the other end of the spectrum KC-derived IL-10 induced late in the induction phase of CS inhibited LC-APC function for Th1 cell clones by inhibiting the expression of a costimulatory molecule on freshly prepared LC. LC accessory functions for Th2 cell clones remained unaffected by IL-10. Thus LC were converted from potent inducers of T cell responses to tolerizing APC as could be demonstrated in classical anergy experiments using the AE7 Th1 cell clone. This tolerizing function was also demonstrated in vivo when IL-10 was injected locally prior to allergen application and hapten-specific tolerance ensued. This anergizing effect could also be observed in the regional lymph nodes of IL-10-treated animals in hapten-specific lymph node proliferation assays. As a possible mechanism, suppression of the epidermal cytokine pattern was observed following intradermal IL-10-injection in allergen-treated mice. This lack of stimulation by proinflammatory cytokines might prevent the maturation of LC into potent immunostimulatory APC.
In aggregate the data demonstrate that the local cytokine milieu and its homeostasis is critical for the function of LC governing the development of either a potent immune response or the induction of allergen-specific tolerance.

C1-021 THE ROLE OF DENDRITIC CELLS IN REGULATION OF IMMUNOINFLAMMATORY RESPONSES IN THE LUNG AND AIRWAYS, Patrick G. Holt, Delia J. Nelson, Andrew S. McWilliam, Jenny A. Thomas, Louise J. Hamilton, and Amanda M. Marsh, Division of Cell Biology, Institute for Child Health Research, Perth, Australia.

Epithelial surfaces in the upper and lower respiratory tract are under continuous exposure to a wide range of both pathogenic and intrinsically inert environmental antigens, necessitating an effective local "surveillance" system. Under normal steady-state conditions, in most species which have been tested in detail, the only resident cell population which expresses efficient antigen presenting cell (APC) activity, displays the characteristic hallmarks of Dendritic Cells (DC), notably dendriform morphology, and high level surface expression of class II MHC together with lack of expression of markers characteristic of mature tissue macrophages. These cells are especially prevalent at sites of frequent contact with inhaled particulates e.g. the base of the nasal turbinates, and the epithelium of the large conducting airways, and taper off in density towards the periphery of the lung, reflecting the normal *in vivo* gradient of airway mucosal stimulation from the natural environment. Recent studies have highlighted the sensitivity of the respiratory tract DC network to both acute and chronic inflammatory stimuli, and in particular demonstrate its capacity for rapid expansion during the earliest phase of the acute inflammatory response to inhaled bacteria or LPS, in which transient increases of up to 300% in airway intraepithelial DC density occur over a time frame comparable to that of the neutrophil response. Inhalation of non-microbial antigens (such as OVA) by pre-immunised animals, also elicits rapid DC recruitment into the airway wall. Studies on respiratory tract DC population dynamics employing a radiation chimera model indicate that >85% of the airway population are normally renewed every 48 hrs, from bone-marrow derived precursors; in contrast, the DC population in the peripheral lung are significantly more long-lived, and exhibit a steady-state half-life of in the order of 4-5 days, whilst those in the epidermis are >15 days. Exposure of normal animals to anti-inflammatory steroids by inhalation, reduces airway DC density by up to 40% within 48 hrs, while reductions of up to 80% can be achieved with high dose systemic dexamethasone. The exquisite sensitivity of these cells to steroids in the steady-state appears to be due to the capacity of these drugs to block the recruitment of incoming blood-borne precursors that are required constantly to replenish the resident population, which normally is being depleted continually by the migration of mature resident DC to regional lymph nodes. This sensitivity is most obvious during inflammation, where the acute DC response in the airways can be inhibited completely by inhaled topical steroids. Ongoing studies in our lab are focussing on the heterogeneity of airway intraepithelial DC populations, particularly at the level of MHC gene expression. In particular, we have defined a major subset present in normal rat airway epithelium, which expresses high level of class I MHC but is class II MHC "low" or negative. This subset is particularly prominent in large airway epithelial tissues during early infancy, a period during which the immature immune system is first exposed to airborne non-pathogenic protein antigens from the natural environment, which can cause T-cell mediated allergic disease. The accumulating epidemiological evidence argues strongly that patterns of T-cell "memory" generation against these airborne antigens are set during this early phase of life, which hints at a potentially important determinant role for these APC in the process; accordingly functional characterisation of these neonatal airway DC populations represents an important focus for ongoing research in our lab.

Origin, Maturation, Migration of Dendritic Cells in Vitro and in Vivo;

C1-100 CSF-1 SECRETED BY FIBROBLASTS PROMOTES THE GROWTH OF DENDRITIC CELL LINES (XS SERIES) DERIVED FROM NEWBORN MOUSE EPIDERMIS, Paul R. Bergstresser, Dale Edelbaum, Toshiyuki Kitajima, Shan Xu, Kiyoshi Ariizumi, Akira Takashima, Dept. of Dermatology, U.T. Southwestern Medical Center, Dallas, TX 75235.

As members of the dendritic cell (DC) family, epidermal Langerhans cells (LC) possess a potent capacity to present antigens to T cells, thereby playing a central role in the induction of T cell-mediated immunity in skin. Although current concepts of the skin immune system have addressed the relevance of cytokines produced in the epidermis on LC maturation and on leukocyte traffic and inflammation in the dermis, little attention has been paid to mechanisms by which dermal cells might provide regulatory influence in the epidermis. Recently, we established from newborn BALB/c mouse epidermis long-term cell lines (XS series) that resemble resident epidermal LC by their surface phenotype, antigen-presenting profile, and cytokine mRNA profile. Importantly, XS cell growth was promoted maximally by a factor(s) secreted by NS lines, which are stromal cell lines also established from newborn mouse skin. Herein we report that: a) by phenotype and function, NS lines resemble dermal fibroblasts, b) NS lines and dermal fibroblasts both express constitutively mRNA for colony-stimulating factor (CSF)-1, c) XS lines and epidermal LC both express mRNA for the CSF-1 receptor (R), d) other epidermal cells and cell lines, including keratinocytes, dendritic epidermal T cells, express mRNA for neither CSF-1 nor CSF-1R, e) recombinant CSF-1 mimics NS culture supernatants in its ability to promote XS cell growth, and f) NS supernatant-dependent XS cell growth is blocked completely by anti-CSF-1R antibodies. We conclude that CSF-1 produced by fibroblasts is a relevant growth-promoting factor for the XS lines of DC. This study gives rise to the testable hypothesis that the survival and growth of resident LC (and their precursors) is sustained by CSF-1 produced by closely neighboring dermal fibroblasts.

C1-101 EXTRACELLULAR MATRIX AND THE MATURATION OF HUMAN PERIPHERAL BLOOD DENDRITIC CELLS
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The differentiation of peripheral blood dendritic cell precursors into tissue dendritic cells (DC), and the maturation of these into potent antigen presenting cells must occur in the interstitial tissues. The protein and carbohydrate structures of the extracellular matrix (ECM) are likely to play a role in maintaining and regulating these processes. At the cell surface, the β_1-integrins bind the protein component ECM and can transduce activation and growth signals to the intracellular compartment. Changes in the levels of expression or activation states of these molecules may not only affect adhesive and motile cellular behaviour, but also confer altered differentiation and activation signalling. Antigen presentation occurring outside of the lymphoid ECM environment, as in the rheumatoid synovium, may as a result, be different from that in a conventional lymphoid setting.

We have examined the expression of β_1-integrins on putative dendritic cells derived from human peripheral blood. PBMC adherent to plastic after 2 hours incubation were cultured in the presence of human recombinant GM-CSF and IL-4 for 7 days. Loosely adherent clusters were seen to develop after 3-4 days. Both the clustered cells and single cells showed dendritic processes and veils and were similar in size to monocytes. These cells were further analysed using indirect immunofluorescence and flow cytometry. CD14 expression was low or negative, and expression of HLA-DR, DQ and B7 were increased compared to cells not exposed to cytokines (on which CD14 expression was high). Cytokine treated cells expressed increased levels of CD29 (the β1 subunit) which was complexed predominantly with CD49d and CD49e (α-4 and α-5 subunits respectively). Where adherence and culture was performed on plates coated with human fibronectin, collagen I or collagen II, an increased proportion of tightly adherent cells were seen but dendritic morphology was not affected in the remaining non-adherent population. Tightly adherent cells in such cultures express high levels of CD14. These changes in behaviour in vitro may be a reflection of altered DC immunophenotype and functional antigen presenting capacity in different tissue environments.

C1-102 FUNCTIONAL ACTIVATION OF CORNEAL LANGERHANS CELLS *IN VITRO* AND *IN VIVO*, William E. Bowers*†, V. Al Pakalnis†* and Aniruddha Choudhury*, Departments of Microbiology & Immunology* and Ophthalmology†, University of South Carolina School of Medicine, Columbia, SC 29208

Dendritic cells (DC) found in the periphery of the cornea have been known to be important regulators of immune responses in the anterior segment of the eye. In this study, DC have been immunomagnetically isolated from rat corneal epithelium and assayed for their ability to stimulate lymphocytes. DC enriched immunomagnetically from rat corneal epithelium comprised about 1.1 ± 0.6% (n=14) of the total corneal cells and were able to stimulate allogeneic or periodate-treated lymphocytes, and present myelin basic protein and ovalbumin to antigen-primed T cells. Other cells had negligible lymphostimulatory activity. The functional activities of corneal DC did not increase significantly after culture for 1 or 3 days. Lymphostimulatory activity was also examined after trauma to the cornea, which causes DC to migrate into the central cornea. Confocal microscopy of corneal epithelium immunofluorescently stained with a panel of anti-DC mAb confirmed that traumatized eyes contained DC in the central cornea, whereas DC in the normal contralateral eye were located only in the peripheral corneal epithelium. DC isolated immunomagnetically from traumatized corneas had significantly greater activity in stimulating allogeneic or periodate-treated lymphocytes than did those from normal cornea. DC from the periphery and central cornea of traumatized eyes showed the same increased activity. In an attempt to delineate the factors that influence DC activity, immunomagnetically purified cells were cultured with a variety of cytokines or metabolic inhibitors for 72 hours. IL-1β and GM-CSF increased DC accessory activity by 3- and 13-fold respectively. These functional increases were inhibited with cycloheximide. These findings suggest that cytokines such as IL-1β and GM-CSF may be involved in the functional activation of DC that occurs when DC migrate to the central cornea after trauma.

C1-103 PHENOTYPIC CHARACTERISATION AND FUNCTIONAL ACTIVITY OF RAT DENDRITIC CELLS. Melissa Chen-Woan, Conor P. Delaney, Veronique Fournier, Yoshitaka Wakizaka, Ricardo Moliterno, Noriko Murase, John J. Fung, Thomas E. Starzl, Anthony J. Demetris. Pittsburgh Transplantation Institute, University of Pittsburgh, Pittsburgh, PA 15213.

In a recent report we demonstrated that large numbers of dendritic cells (DC) can be propagated from rat bone marrow (BM) by supplementing cultures with murine rGM-CSF, and further enriched by depleting non-DC on serum coated plates. The current study examines the phenotypic expression and Ag presenting ability of these BM-derived DC.

Expression of phenotypic markers closely correlated with time spent in culture. MHC class II and co-stimulatory markers gradually increased with DC maturity, whereas the specific marker OX-62 only stained relatively immature DC. By d8, mature DC were strongly positive for OX-6 (MHC class II), OX-7 (thy 1) and the co-stimulatory molecules B7.1, B7.2 and CTLA4-Ig. Enriched d8 DC were found to have 2-300 times the stimulatory ability of fresh splenocytes in mixed lymphocyte reactions (MLR). Blocking studies of enriched d8 DC showed that allostimulatory ability in MLR could be inhibited by CTLA4-Ig (68% inhibition at 2.5μg/ml and 58% at 1.25 μg/ml) or OX-6 (89% inhibition at 2.5μg/ml and 72% at 1.25μg/ml). Furthermore, a combination of CTLA4-Ig and OX-6 at 1.25 μg/ml almost completely blocked (>96%) allostimulatory ability. Presentation of soluble ovalbumin and HSP-70 to syngeneic T cells by enriched d8 DC was 100 times greater than that of fresh splenocytes. However, no difference was found between d4 and d8 enriched-DC at presenting soluble heat-shock protein (HSP) 70 to a HSP-specific cell line. In contrast, d4 DC were less effective than d8 cells at stimulating allogeneic T cell proliferation in MLR. In conclusion, mature rat BM-derived DC, which are potent stimulators of naive lymphocytes in MLR, strongly express the costimulatory molecules found on their human and murine counterparts and these molecules are essential for their potent stimulatory ability in vitro. These studies provide a basis for further studying the role of DC in well defined rat models of transplantation tolerance and autoimmune diseases.

C1-104 PHENOTYPIC AND FUNCTIONAL PROPERTIES OF RAT SKIN LANGERHANS CELLS DIFFERENTIATING *IN VITRO*, Aniruddha Choudhury and William E. Bowers, Department of Microbiology & Immunology, University of South Carolina School of Medicine, Columbia, SC 29208

Langerhans cells (LC) are known to be the principal accessory cell within mammalian skin. Confocal microscopy of rat skin stained with a panel of five mAb that detect rat lymphoid dendritic cells (A3C, B6G, C11B, F280, and G489) indicated that LC *in situ* expressed class II MHC (G489), but not the other markers. LC immunomagnetically isolated with G489 from fresh preparations of skin cells did not stimulate periodate-treated and allogeneic T cells, but did show some ability to process and present myelin basic protein and ovalbumin, as well as their peptides, to antigen-primed T cells. After 24 hours of culture LC still showed no activity with periodate-treated or allogeneic T cells, but robust activities were measured after 72 hours. The ability to process and present antigen unexpectedly continued to increase in culture up to 72 hours, at which time LC showed strong staining with A3C, B6G, C11B and F280. The cytokines GM-CSF and IL-1β increased the functional activity of LC in culture, while cycloheximide inhibited the functional differentiation of LC and blocked the expression of DC-specific markers. These findings indicate that rat LC differentiate functionally in culture to a more competent accessory cell and acquire some phenotypic characteristics of lymphoid dendritic cells.

C1-105 **STIMULATORY CAPACITIES OF HUMAN BLOOD-DERIVED DENDRITIC CELLS AND THEIR PHENOTYPICAL ALTERATIONS IN THE PRESENCE OF CONTACT SENSITIZERS,** Joachim Degwert, Friedhelm Steckel, and Udo Hoppe, Paul Gerson Unna Skin Research Center, Beiersdorf AG, Unnastrasse 48, D-20245 Hamburg, Germany

Dendritic cells (DSs) are highly specialized antigen presenting cells (APCs) initiating primary T-lymphocyte associated immune responses. DCs are located in many non-lymphoid tissues and a specialized form of DCs - the Langerhans cell (LC) - is found in the skin. The functionality of LCs as APCs is crucial for the induction of an allergic contact dermatitis. For long time LC research has been hampered by the limiting numbers of functional active LCs which could be isolated from human skin. The addition of GM-CSF and IL-4 to the non-adherent fraction of mononuclear cells generated a large amount of $CD1a^+$ $HLA\text{-}DR^+$ DCs. These in vitro generated DCs exhibited the morphology, phenotype and T-lymphocyte stimulating capacity of the human DC/LC system. Beside analyzing their antigen presenting capacity to stimulate allogeneic T-lymphocytes, we also tested their stimulatory capacity towards autologous T-lymphocytes in the presence of various stimulants (mitogens Con A or PHA; superantigen staphyloccocal aureus enterotoxin B (SEB)). Furthermore, we had tested phenotypical alterations of our in vitro generated DCs under the influence of subtoxic concentrations of different chemicals and contact sensitizers. In vitro stimulation with the contact sensitizers urushiol, primin, C10- and C11-primin analogues, alantolactone, isoalantolactone and $NiSO_4$ resulted in a decrease of HLA-DR expression on the surface of these cells under certain conditions. Incubation with irritants like SDS and benzalkoniumchloride induced an increase or no change of HLA-DR surface expression. With regard to the adhesion molecule ICAM-1 there was no clear difference between irritants and allergens. ICAM-1 expression was always slightly increased or not changed under our conditions.

C1-106 UPTAKE OF DNP-ALBUMINE BY PRECURSOR DENDRITIC CELLS CORRELATES WITH CYTOPLASMATIC MHC CLASS II LABELING
A.J. Engering, I.L. Schadee-Eestermans, D.M. Broekhuis-Fluitsma and E.C.M. Hoefsmit, Department of Cell Biology, Faculty of Medicine, Vrije Universteit, Amsterdam, The Netherlands.

One of the functions of antigen presenting cells (APC) is the uptake of exogenous antigen, the degradation into peptides and the loading of these peptides on MHC class II molecules. The MHC class II-peptide complexes are transported to the plasmamembrane and are able to activate antigen specific T cells.

The dendritic cell (DC) family is known as the main APC. Recently, a method was described by O'Doherty et al. to isolate precursor DC from blood. These cells mature during 40 to 48 hrs of culture in monocyte conditioned medium, showing a dendritic morphology and high capacity in MLR.

Our aim was to establish the difference between these phenotypes concerning antigen uptake and presentation. Using the soluble antigen DNP-albumine, we established that precursor DC were capable to take up antigen. This uptake correlates with the intracellular labelling of MHC class II in immunoEM. Mature DC show abundant MHC class II on the plasmamembrane. We will present results concerning the ability of mature DC to present DNP-albumine to autologous T cells.

C1-107 INDUCTION OF DENDRITIC CELL MIGRATION BY SYNOVIAL FLUID AND TRANSFORMING GROWTH FACTOR β, Michael P. Everson and William J. Koopman, Dept. of Med., Univ. of Ala. at B'ham and VAMC, Birmingham, AL 35294.

Recent data suggest that dendritic cells (DC) are found in increased numbers in synovial effusions of patients with rheumatoid arthritis (RA). The increased density of this extremely potent stimulatory cell may play a role in the chronicity of RA disease. Since increased levels of transforming growth beta (TGF-β) are found in RA synovial fluid, we asked whether this or other small molecules could cause migration of DC from one compartment to another. To elucidate the mechanism(s) underlying the movement of DC into the synovial compartment, we devised a novel method to determine DC migration using a sterile, Transwell system fitted with a membrane (5 μ pores) through which DC could pass. Mouse (C3H/HeJ) spleen cells enriched for DC (60-70% purity) were applied to the upper chamber of a Transwell and the soluble factors were placed in the lower chamber. Following varying time intervals, DC were enumerated using an inverted microscope equipped with a camera. The Transwell system was validated using human peripheral blood leukocytes and 10^{-7}M FMLP as a positive control; all the cells passed into the lower chamber in 2 hours. We then tested rhIL-8 (3.5 ng/mL, a dose known to be chemoattractive for T lymphocytes and neutrophils), recombinant human TGF-β (1 ng/ml), and synovial fluid (1:10 dilution) for induction of DC migration. In media control wells, ≈36% of the 46,500 cells placed in the upper chamber were found in the lower chamber after 3.5 hours. Relative to this control value, the results for the soluble factors (reported as % of control with control value arbitrarily set at 100%) were: IL-8, 100%; TGF-β, 131%, and synovial fluid, 175%. In a confirmatory experiment using neutralizing monoclonal anti-TGF-β antibodies, the results were: synovial fluid, 146%, TGF-β, 155%, and TGF-β plus 100 μg/mL anti-TGF-β, 73%. Further experiments are planned to ascertain chemokinetic versus chemotactic movement in these cultures.

C1-108 DENDRITIC CELL LINES FROM HUMAN BONE MARROW AND CORD BLOOD DERIVED CD34$^+$ CELLS. T. Venkat Gopal and Abha A. Saini, Clonexpress, Inc., 504 E. Diamond Ave, Suite G, Gaithersburg, MD 20877.

Dendritic cells are strong antigen presenting cells, and play an important role in several immune response reactions. Recently it has been shown that dendritic cells also act as a reservoir for human immunodeficiency virus (HIV). Unlike CD4 positive T cells, HIV infected dendritic cells do not show any cytopathic effect and remain viable without loss of cell number while maintaining a high level of HIV replication. This may play an important role in transmitting HIV to T cells during routine antigen presentation in lymphoid tissues.

Methods have been established to isolate a fairly pure population of dendritic cells from human peripheral blood, and also to produce dendritic cells from human cord blood derived CD34$^+$ cells. Since the dendritic cells isolated by these methods have a limited life span, we resorted to the development of immortalized human dendritic cell lines to understand the role of dendritic cells in HIV pathogenesis. Human bone marrow mononuclear cells and human cord blood derived CD34$^+$ cells were transfected with different combination of oncogenes, and selected in growth medium that supports the development of dendritic cells. Growing populations of cells displaying dendritic cell specific markers, but lacking T,B, and monocyte markers, were obtained with both transfections. These cell lines express high level of HLA-DP, DQ, and DR antigens, and possess characteristic veils that are rapidly changing. Further studies on functional properties, such as antigen presentation function and secretion of various cytokines, of these cells lines will also be presented.

C1-109 **USE OF CD1a$^+$ DENDRITIC CELLS AND KERATINOCYTES TO CHARACTERIZE CELLULAR REACTIONS INVOLVED IN THE ALLERGIC CONTACT DERMATITIS,** Udo Hoppe, Joachim Degwert, and Friedhelm Steckel, Paul Gerson Unna Skin Research Center, Beiersdorf AG, Unnastr. 48, D - 20245 Hamburg, Germany

Topical application of consumer products (e.g. cosmetics) to the skin of sensitive individuals can result in a contact dermatitis. There are two forms of contact dermatitis: the irritant and the allergic type (DTH IV). At present neither form can be clearly differentiated by clinical and histological parameters. The critical step during the induction phase (sensitizing step) is the presentation of the allergen to the T-lymphocytes by the antigen-presenting cells (APCs). This activates the T-lymphocytes and they generate an immunological memory. During the antigen presentation step the allergen is located on the cell surface structures (HLA molecules) of the APCs. In the past, Langerhans cells, the predominant APCs of the human skin, could only be isolated from skin biopsies in extremely low numbers using difficult technical procedures. We are now able to generate functional CD1a$^+$ HLA-DR$^+$ Langerhans cells like cells from the peripheral blood of healthy human volunteers with the aid of the cytokines GM-CSF and TNF-α or IL-4. These CD1a$^+$ HLA-DR$^+$ cells are potent APCs as documented in different systems and they can also induce significant in-vitro primary sensitization reactions. This result could be clearly documented using the contact allergens TNP and urushiol, making it possible to evaluate an in-vitro system that simulates the induction phase of ACD. Using of the polymerase chain reaction (PCR), a technique that analyzes differential gene activity, it was also possible to discriminate in-vitro irritants from contact-allergens. When the contact allergen urushiol was incubated with CD1a$^+$ HLA-DR$^+$ APCs for 30 minutes, IL-1β expression could be determined. The irritant SDS did not have this potential.Not only the classical APCs of the skin - the Langerhans cells - but also the keratinocytes are able to express the molecules necessary for antigen presentation (HLA-DR) and cellular communication (ICAM-1). We are able to show that keratinocytes can function as APCs by presenting the superantigen staphylococcal enterotoxin B (SEB) to autologous T-lymphocytes, resulting in T-lymphocyte proliferation. This result suggests that KCs can function as APCs during the elicitation phase of an ACD. Furthermore, all of the contact allergens analyzed have the potential to induce or enhance the expression of ICAM-1 molecules on the cell surface of KCs, making them able to interact directly with T-lymphocytes.

In summary, we had established a set of cellular systems that could serve as the basis for an in vitro pre-screening system for the evaluation of allergic and irritant potentials of raw materials and cosmetic consumer products.

C1-110 GENERATION OF DENDRITIC CELLS FROM MOUSE SPLEEN CELL CULTURES IN RESPONSE TO GM-CSF: IMMUNOPHENOTYPIC AND FUNCTIONAL ANALYSES, M Hsieh, L Lu, TB Oriss, PA Morel, TE Starzl, AS Rao, AW Thomson, Pittsburgh Transplantation Institute and Departments of Surgery and Medicine, University of Pittsburgh, Pittsburgh, PA 15213-2582

Within a few days of liquid culture in GM-CSF, B10 BR (H-2^K I-E$^+$) mouse splenocytes formed loosely-adherent myeloid cell clusters. Mononuclear progeny released from these clusters at and beyond 4 days exhibited distinct dendritic morphology and expressed leukocyte common antigen (CD45), CD11b, heat stable antigen, Pgp-1 (CD44) and intercellular adhesion molecule-1 (ICAM-1; CD54). The intensity of expression of the DC-restricted markers NLDC 145 and 33D1, the macrophage marker F4/80, and FcγRII (CDw32) was low to moderate, whereas the cells were negative for CD3, CD45RA and NK1.1. High and moderate levels respectively, of cell surface staining for MHC class II (I-E^k) and B7 antigens were associated with potent stimulation of unprimed, allogeneic T cells (B10; H-2^b I-E$^-$). DC propagated similarly from DBA/2 mouse spleen were strong antigen-presenting cells (APC) for MHC-restricted, syngeneic Th2 cell clones specifically responsive to sperm whale myoglobin. Footpad or intravenous injection of GM-CSF-stimulated B10.BR spleen-derived DC into B10 (H-2^b I-E$^-$) recipients resulted in homing of the allogeneic cells to T cell dependent areas of lymph nodes and spleen, where they strongly expressed donor MHC class II antigen 1-2 days later. This indicates that cells can be propagated from fresh splenocyte suspensions that exhibit distinctive features of DC, namely morphology, motility, cell surface phenotype, potent allogeneic and syngeneic APC function and in vivo homing ability. This propagation of DC from progenitors present in lymphoid tissue provides an alternative and convenient source of high numbers of otherwise difficult to isolate but important APC.

C1-111 A PHASE I TRIAL OF TUMOR NECROSIS FACTOR (TNF) AND GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) TO ENHANCE DENDRITIC CELL MATURATION, John E. Janik, Langdon L. Miller, William Kopp, Barry Gause, Brendan Curti, Dan L. Longo, Clinical Research Branch, Biological Response Modifiers Program, National Cancer Institute, Frederick, MD 21701

The combination of TNF and GM-CSF stimulates the maturation of dendritic cells from primitive hematopoietic progenitors in vitro. We are conducting a Phase I trial of TNF and GM-CSF to determine whether this combination increases dendritic cell numbers after in vivo administration. Patients receive TNF by continuous intravenous infusion for seven days and GM-CSF by subcutaneous injection twice daily for seven days; after seven days rest, patients are retreated. The dose of TNF is escalated in cohorts of three patients (25, 50, 100, 150 and 200 mcg/m^2/d), with the same GM-CSF dose (125 mcg/m^2 every twelve hours). Four patients have been treated, three at 25 mcg/m^2/d and one at 50 mcg/m^2/d of TNF. The endpoints of the trial are the toxicity of the combination and to determine whether the number of dendritic cells or dendritic cell precursors are increased in the blood or skin. No dose limiting toxicity was observed at the first dose level of TNF, 25 mcg/m^2/d or in the one patient who has completed therapy at the second dose level. All patients had a significant drop in platelet counts but no significant bleeding complications occurred and no platelet transfusions were needed. Microscopic hematuria has been seen in two of the four patients treated. One patient treated at the first dose level had a significant increase in S100 positive cells in the skin after treatment, consistent with an increase in dendritic cells. No tumor responses have been observed; the patient who had an increase in dendritic cells had a drop in the serum carcinoembryonic antigen level during therapy but the level returned to baseline one month after therapy.

C1-112 STEROIDS AND UV-B ARE POTENT INDUCERS OF PROGRAMMED CELL DEATH IN IMMATURE AND MATURE DENDRITIC CELLS. Eckhart Kaempgen, Ralf Gold*, Andreas Eggert, Petra Keikavoussi, Oliver Grauer, Klaus Toyka* and Eva-B Broecker. Departments of Dermatology and Neurology*, Univ. of Wuerzburg, 97080 Wuerzburg, Germany.

We have recently provided first evidence that lymphoid dendritic cells (DC) and tissue DC like epidermal Langerhans cells (LC) may undergo apoptosis, unless active suppression of the death program is signaled by cytokines such as GM-CSF or TNFα. We now report on potent inducers of apoptotic cell death in DC populations. Quantification of apoptotic cells was performed by intracellular in situ nick translation (ISNT) labeling of DNA fragments and FACS analysis. When highly purified murine epidermal LC or DC (obtained from spleen or generated from bone marrow cultures) were incubated with dexamethasone [10^{-7}M] rapid onset of apoptotic cell death occurred after 6h culture and affected about 90% of the cells at 12h. Comparable results were obtained when DC/LC were irradiated with UV-B exceeding 0,01 J/cm^2, whereas treatment with pure UV-A up to 20 J/cm^2 did not enhance the percentage of apoptotic DC/LC over basal levels (~20% after 12h). Interestingly, UV-induced apoptosis was significantly delayed by preincubation of the cells with retinoic acid [10^{-7}M]. To evaluate in-vivo responses of epidermal LC, ears from Balb/c mice were treated with 0.1% dexa or 0,1 J/cm^2 UV-B. When ears were taken off 12h later, intraepidermally located apoptotic LC could be demonstrated by double staining with ISNT and anti-class II mab. 12h later apoptotic class II positive cells appeared in the dermis and in addition pronounced apoptosis of basal keratinocytes was seen.

Our in vitro and in vivo studies provide evidence that steroids and UV-B are potent inducers of apoptotic cell death in DC populations. These data strongly favour real LC depletion following UV-B / steroid treatment of the skin over solely loss of surface markers and thus may settle this longstanding debate.

C1-113 ULTRASTRUCTURE OF FRESHLY ISOLATED AND CULTURED HUMAN BLOOD DENDRITIC CELLS

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In this investigation the ultrastructure of freshly isolated human dendritic cells were compared with those after culture. The freshly isolated cells were obtained from a human buffy coat using a modified isolation procedure of O'Doherty et al. (see abstract Richters, this symposium). They were relatively small, more or less rounded, by which the nucleo/cytoplasmic ratio was about 1 : 1-1.2. The cytoplasm contained scattered mitochondria and (poly)ribosomes. Some vesicles were present in a perinuclear position. Most of the cells showed 5-7 strands of rough endoplasmic reticulum (rer) following the contours of the cell membrane.

After a culture period of 40 hr in monocyte conditioned medium the cells were enlarged, by which the cell membrane was always irregular. Some of them possessed moderate, small surface folds, others possessed many blunt pseudopodia of varying length. The nucleo/cytoplasmic ratio varied from 1:2 till 1:3. Frequently the indented (euchromatic) nuleus had an eccentric position. The cells contained bundless of microfilaments near the nucleus and relatively small mitochondria. The number of rer was decreased but still present. Sometimes a cytocentre was seen surrounded by large numbers of small surfaced vesicles with electron lucent contents. Only a few cells showed small (phago) lysosomes in a perinuclear position. However distinct signs of phagocytic activity was never observed. Birbeck granules, the characteristic cell organelle of the epidermal Langerhans cells, were absent.

C1-114 FRESHLY ISOLATED AND CULTURED BLOOD DENDRITIC CELLS DISPLAY AN IMMATURE, RESPECTIVELY MATURE PHENOTYPE. Monique J. Kleijmeer*, Hans W. Nijman#, Viola M.J. Oorschot*, Miriam A. Ossevoort#, W. Martin Kast#, Cornelis J.M. Melief#, Hans J. Geuze*. * Department of Cell Biology, School of Medicine, Utrecht University, The Netherlands. # Immunoheamatology and Blood Bank, Academical Hospital Leiden, The Netherlands.

Dendritic cells (DC) are thought to exist in two stages of maturation: as immature cells in non-lymphoid tissues and as mature cells in the T cell areas of lymphoid organs. To investigate whether we could obtain immature and mature DC from blood, from which DC are distributed to both lymphoid and non-lymphoid tissues, we isolated fresh (fDC) and cultured DC (cDC) from human peripheral blood. Long cytoplasmic processes and a high MHC expression were characteristic for cDC, which suggests that they have a mature phenotype. fDC showed only small processes and a low MHC expression, but their endocytic capacity was higher than that of cDC. In addition, we found that fDC were superior in processing and presenting the soluble 65kD protein of M. Leprea, which all together suggests that fDC resemble immature DC. However, cDC were still capable of processing this antigen and induced a higher T cell response than PBL. To further delineate the differences between fDC and cDC, we compared both the MHC-II and invariant chain distribution in fDC and cDC from blood and resident Langerhans cells. Both blood DC and LC display MHC-II enriched compartments (MIIC), with similar lysosomal characteristics as MIIC previously described in B cells and macrophages. LC showed a high expression of MHC-II and invariant chain in the endoplasmic reticulum, whereas both in fDC and cDC from blood this expression was lower. Apparently, fDC from blood do not display a phenotype that is completely similar to the one of resident LC.

C1-115 ULTRASTRUCTURAL AND IMMUNOCYTO-CHEMICAL CHARACTERIZATION OF DISTINCT TYPES OF DENDRITIC CELLS EXPANDED FROM CD34$^+$ HEMATOPOIETIC PROGENITOR CELLS, Gabriele Köhler and Hans E. Schaefer, Department of Pathology, University of Freiburg, 79104 Freiburg, Germany.

Purified CD34$^+$ hematopoietic progenitor cells from cancer patients were expanded with various hematopoietic growth factors *in vitro*. These cells were analyzed by immunocytochemistry [avidin-biotin-complex (ABC) method] and electron microscopy at various time points of *in vitro* expansion. Under culture conditions including granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4), dendritic cells (DC) were generated that contained a peculiar type of granules, with a lamellar substructure, composed of parallel stacked or circular components or trilamellar membranes. Such DC were obtained by two to three weeks of *in vitro* culture, while DC examined later in the course of these cultures appeared as interdigitating reticulum cells lacking these granules. Similarly, these lamellar structures were absent in macrophage-like cells, expanded from the same purified CD34$^+$ cells under different culture conditions *in vitro*. Birbeck-like granules were occasionally detected in the DC, mostly composed of short tubular remnants. Immunocytochemistry revealed that the dendritic cells expressed more CD1a, S100-protein and LAC-antigen, but less MAC387, lysozyme and α1-chymotrypsin than macrophage-like cells. In summary, the lamellar granules appear characteristic for the DC expanded in our *ex vivo* cultures. They might be associated with the presence of Birbeck-like granules and/or related to the powerful antigen presenting abilities of these DC.

C1-116 DENDRITIC CELLS OF SKIN AND ORAL MUCOSA. MIGRATORY CAPACITIES AND THEIR ROLE IN ORAL TOLERANCE

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The oral mucosa is an important site to induce immunological tolerance to protein antigens. Because of the importance of tolerance induction as a possible way to modulate allergic reactivity we wished to study the mechanisms involved in efficient tolerance induction via the oral mucosa. Dendritic langerhans' cells in both skin and oral epithelium are the first cells to encounter antigen. By applying fluoresceinated hapten on skin or oral mucosa of the mouse we could demonstrate similar migratory activities of the Langerhans from both sites to the draining lymph nodes. To see if any functional differences between the Langerhans cells deriving from the two different sites would exist we studied the antigen presentation capacity of the two cell types. However, no differences in antigen presenting capacity were seen after stimulation of the dendritic cells via skin or oral mucosa. Transfer in vivo of dendritic cells from either site did not result in tolerance induction but instead both cell types were able to induce delayed type hypersensitivity reactions. Analyzing the cytokines produced in the draining lymph node a skewing towards Th2 was seen after oral antigen application. The results indicate that dendritic cells do not directly influence the direction of the generated immune responses, but that local factors in the microenvironment of the draining lymph node are of crucial importance for the induction of oral tolerance.

C1-117 THE CONDITIONAL V-relER ONCOGENE TRANSFORMS A PROGENITOR OF DENDRITIC CELLS

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We describe a powerful in vitro system for studying dendritic cell differentiation in culture. A conditional v-rel estrogen receptor fusion protein, v-relER, is used which causes hormone-dependent v-rel-specific transformation of chicken bone marrow cells. Such transformed cells exhibit B-lymphoid determinants in line with previous studies on v-rel.

However, following inactivation of v-relER activity by administration of an estrogen antagonist, cells differentiate into fully competent antigen-presenting dendritic cells. These cells exhibit an elongated, bipolar morphology, form lamellipodia or veils, and are highly motile in culture. In addition, they have low to moderate phagocytic activity, express high levels of MHC class II and stimulate T cell proliferation in a primary mixed lymphocyte reaction. Yet, under different culture conditions, v-relER cells also differentiate into cells resembling polymorphonuclear neutrophils.

Our studies therefore suggest that the conditional v-relER transforms a common progenitor of dendritic cells, neutrophils and possibly also B cells, and provide new insights into the disease caused by v-rel virus.

C1-118 FRESH TONSIL DENDRITIC CELLS REQUIRE EXTRINSIC SOLUBLE FACTORS FOR T CELL STIMULATION AND SURVIVAL.

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Human blood dendritic cells (DCs) include two functionally and phenotypically distinct populations. Dendritic cells in secondary lymphoid organs are considered to represent the final stage in the life cycle of bone marrow-derived DCs and may be similar to the CD11c+ population in blood. Tonsil DCs have been reported to be more mature and express higher levels of costimulatory molecules following culture than the DCs isolated directly from blood. In order to determine the functional properties of lymphoid DCs isolated without culture, we examined the morphology, phenotype and functional properties of freshly isolated tonsil DCs and the maturational changes that occur during their subsequent culture.

Fresh tonsil DCs did not express the costimulatory molecule CD80 (B7-1) and were 1-2 log less efficient than cultured tonsil DCs in stimulating allogeneic CD4+ T cells in a MLR. Neither fresh nor cultured DCs expressed surface CD11c. Expression of HLA class-I and class-II and CD80 was increased after overnight culture. The viability of fresh sorted DCs rapidly decreased when cultured in non-conditioned medium. Death was by apoptosis and was reduced by the addition of conditioned medium. GMCSF-receptor was found on fresh DCs but not on cultured DCs. When added to allogeneic T cells in a MLR the viability of fresh tonsil DCs was reduced compared to cultured tonsil DCs.

These data indicate that the reduced stimulatory capacity of tonsil DCs purified without culture may be attributed to both lower expression of MHC and costimulatory molecules such as CD80 and to increased death by apoptosis. Maturation of tonsil DCs is accompanied by reduced expression of the GMCSF receptor and reduced dependence on soluble factors derived from other accessory cells.

C1-119 PHENOTYPICAL CHANGES IN DENDRITIC CELLS DURING FUNCTIONAL MATURATION

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It has been shown by O'Doherty et al. that dendritic cells freshly isolated from human blood undergo functional maturation during a 36-40h culture period. We used a slightly modified isolation procedure; depletion of T cells was done with SRBC, depletion of monocytes, NK cells and B cells by panning with monoclonal antibodies against CD11b, CD21, CD3 and CD16. After the last purification step, FACS sorting for HLA-DR positive and CD5, CD19, CD14 negative cells, a 95% pure population of cells remains. These proposed precursor dendritic cells showed no or low capacity in the allogeneic MLR. After 40h culture in monocyte conditioned medium, the cells were capable to induce a high response in the MLR.

We studied the phenotype of these cells before and after culture using immuno-cytochemistry. Before culture, cells were relatively small. After culture, cells were enlarged and showed cell processes. Using the monoclonal EBM 11 on cytospins, we detected that the cells before culture had labelled the peri-nuclear area. After culture, the labelling intensity was lower but the staining was also as a spot near the nucleus. Acid phosphatase reactivity was observed in only 10 % of the cells before culture but after culture in 40 % of the cells. The reaction product was always present in a spot near the nucleus.

To determine whether these immature cells might be precursor Langerhans cells we tested the cells before and after culture for CD1a, -b, and -c expression. We could not detect any CD1 positivity, not even after co-culture with human keratinocytes.

C1-120 IN VITRO DIFFERENTIATION OF DENDRITIC CELLS FROM CD34+ PROGENITOR CELLS. M. Rosenzwajg, B. Canque, M. Yagello, and J.C. Gluckman. CNRS URA 1463, Hôpital Pitié-Salpêtrière, 75651 Paris Cedex 13, France.

Dendritic cells (DC) are the most potent antigen-presenting cells, especially as regards priming of naive T lymphocytes. However, the DC lineage is still incompletely characterized in humans. We examined the in vitro differentiation of DC from cord blood purified CD34+ cells cultured with GM-CSF and TNF-α. Two populations appeared after 5 days, 1/3 of cells being $CD13^{lo}$ and 2/3 being $CD13^{hi}$; most of the latter cells were $CD38^{lo}$HLA-DR+ CD4+, 30% also were CD14+ and 10% CD1a+. Differentiation of these cells was examined by day-5 sorting of $CD13^{hi}CD1a^{-}$ vs $CD13^{lo}$ cells and further culture: $CD13^{hi}$ cells — CD1a+ or CD1a− — were then produced from $CD13^{lo}$ cells, while CD1a+ cells emerged from $CD13^{hi}$ cells. By day 12, 30% of the cells were large and granular, with high forward/90° scatters. These cells were $CD13^{hi}$, either CD1a+ (30-50%) or CD1a−, and all of them expressed equally HLA-DR, CD4, CD14 as well as CD80, CD40 and adhesion molécules CD18, CD29, CD54 and CD58. Despite different CD1a expression, all these cells displayed characteristic DC morphology. Although CD14+, they were nonspecific esterase− nonphagocytic cells in contrast to mono/macrophages. As assessed after day-12 sorting, whether $CD1a^{+}$ or $CD1a^{-}$, these cells stimulated allogeneic lymphocytes to the same extent, and much stronger than their macrophage counterparts cultured under the same conditions. At day 20, the % of CD1a+ cells decreased, though most still had intracellular CD1a, while that of CD14+ cells with or without surface CD1a increased. Changing culture conditions by adding SCF to TNF-α and GM-CSF considerably augmented cell yields without modifying the phenotypic evolution. These data point to an in vitro DC differentiation pathway: (i) increased CD13, DR, CD4 expression; (ii) acquisition of CD1a and/or CD14 as well as of CD80 and CD40; (iii) at a later stage, decreased surface CD1a and increased CD14 expression, which is at variance with published data. One may consider that DC progenitors differentiate in vitro first into cells that present membrane CD1a, like Langherans cells or lymph node DC, and then to cells close to $CD1a^{-}$ peripheral blood DC.

C1-121 DENDRITIC CELL (DC) HEMATOPOIESIS REQUIRES IL-6
F Santiago-Schwarz, J Tucci, LE Thorpey and SE Carsons. Winthrop-Univ Hosp, Mineola, NY and SUNY, Stony Brook, NY.

DC hematopoiesis from normal CD34+ progenitors is facilitated in vitro by exogenous TNF, GM-CSF and SCF (stem cell factor). Recently, we showed that development of mature DCs from myelodendritic leukemic blasts is further dependent on the exogenous addition of IL-6. We hypothesized that during DC development from normal CD34+ cells, there is endogenous IL-6 production by the myeloid progenitors as they differentiate into DCs and that this process is crucial for DC development. Here we (1) employ an ELISA to quantitate endogenous IL-6 under conditions supporting monocyte (mono)-DC hematopoiesis versus those favoring mono development and (2) study the neutralizing effects of αIL-6 antibodies (ab) during distinct phases of mono-DC growth. IL-6 was detected in cultures favoring mono development (GM-CSF) by day 4 with the levels of IL-6 remaining constant (~100 pg/ml) throughout an 18 day period. In comparison, in cultures promoting DC development, the pattern of IL-6 production was linked with the presence of DCs. IL-6 levels increased until ~day 11 and declined by day 18, when typical DC features were no longer present. During peak DC development (day 11) IL-6 levels in GM-CSF+TNF+SCF cultures were at least doubled, compared to GM-CSF cultures (83 vs 213 pg/ml, $P<.02$). In long term DC cultures repeatedly supplemented with GM-CSF+TNF+SCF, levels were even higher on day 11 (671 pg/ml, $P<.05$). High levels of IL-6 in these cultures were maintained beyond day 18. Polyclonal αIL-6 ab profoundly inhibited DC growth. When added to GM-CSF+TNF+SCF cultures at the onset, αIL-6 decreased peak proliferation by ~58% ($P<0.02$), as measured by thymidine uptake. Decreases in cell content were also noted, and microscopic analysis revealed that few, if any, DC progeny developed. Proliferation was also decreased when αIL-6 was added 5 days after cultures were initiated with GM-CSF+TNF+SCF (by ~50%, $P=.05$). In this instance, the total number of mature progeny was reduced, but there was no apparent selective inhibition of mono-DC progeny. These studies establish the importance of IL-6 as a secondary cytokine during distinct phases of DC development and provide insight into another important control point in DC hematopoiesis.

C1-122 MODULATION OF LANGERHANS CELL E-CADHERIN EXPRESSION DURING THE INITIATION PHASE OF CONTACT SENSITIVITY REACTIONS, Kathryn Schwarzenberger and Mark C. Udey, Dermatology Branch, NCI, NIH, Bethesda, MD 20892

E-cadherin (E-cad) mediates adhesion of murine epidermal Langerhans cells (LC) to keratinocytes (KC) in vitro, and presumably is also important for LC-KC adhesion in vivo. To determine if levels of E-cad expressed by LC are modulated during antigen-induced activation of LC prior to emigration from epidermis, we applied the contact allergen 2,4,6-trinitrochlorobenzene [TNCB, 1% in acetone:olive oil (4:1)] to the ears of BALB/c mice and prepared single cell suspensions of epidermal cells using 0.5% trypsin in 1 mM calcium-containing medium (to protect E-cad from degradation) at various intervals. Cells were subsequently assayed for simultaneous expression of class II MHC (I-A/E) antigens and E-cad by two color flow cytometry using directly-labeled anti-I-A/E (M5/114) and anti-E-cad (DECMA-1) mAb. As expected, TNCB induced increased expression of I-A/E antigens by a subpopulation (~25%) of epidermal LC 12-48 hrs after application of the contact allergen. Approximately 24 hrs after TNCB treatment, E-cad levels were reproducibly (15/15 experiments) and selectively decreased on a subpopulation of activated LC [41.9 ± 9.8% (mean ± S.D.) of LC that expressed increased levels of I-A/E antigens]. LC exhibiting decreased E-cad expressed levels that were ~6-fold lower than those expressed by vehicle control and untreated LC. These levels were similar to those present on LC that had been maintained in culture for 72 hrs (which do not adhere avidly to KC) and to those present on the surfaces of LC that emigrate from skin explants. Identical results were obtained with BALB/c nude (nu/nu) mice suggesting that modulation of I-A/E antigens and E-cad by contact allergens is T cell-independent. Similar, but less dramatic, alterations were also observed with the contact sensitizer 2,4-dinitrofluorobenzene (DNFB, 0.5% in acetone:olive oil), but they did not occur with the tolerogenic congener 2,4-dinitrothiocyanobenzene (DNTB). Changes in LC E-cad expression also were not evident 24 hrs after treatment of murine skin with irritants such as sodium lauryl sulfate (10% in DMF), and benzalkonium chloride (10% in water). We propose that modulation of LC E-cad expression preceeds emigration of antigen-activated LC from epidermis and speculate that this is an essential event in the initiation of primary immune responses to epicutaneously applied antigens.

C1-123 MICROENVIRONMENTAL FACTORS DICTATE FUNCTIONAL PROPERTIES OF ANTIGEN-PRESENTING DENDRITIC CELLS, J. Wayne Streilein and Shigeki Okamoto, Schepens Eye Research Institute, Harvard Medical School, Boston, MA

Dendritic cells have been found to be functionally plastic, able to display disparate antigen processing and presenting capabilities, as well as co-stimulatory properties. As a consequence, functionally diverse naive, primed and memory T and B lymphocytes can be served. The plasticity displayed by dendritic cells is dictated, at least in part, by factors in their microenvironment. In the eye, indigenous dendritic cells endocytose exogenous antigens, process and present them as immunogenic peptides on surface MHC molecules (chiefly class I), and migrate to the spleen where they activate CD8+ effector and regulatory T cells. The induced systemic immune response, that is selectively deficient in delayed hypersensitivity, has been termed Anterior Chamber Associated Immune Deviation (ACAID). ACAID is believed to play a major role in providing the eye with a form of immune protection that avoids immunogenic inflammation, and therefore preserves vision. Dendritic cells within the eye are bathed in fluids rich in factors with potent immunomodulatory properties, especially transforming growth factor-beta (TGF-β). It has recently been shown that when non-ocular dendritic cells are pulsed with exogenous antigen in an in vitro"intraocular" microenvironment (containing TGF-β), the cells acquire functional properties similar to those displayed by eye-derived antigen presenting cells. This system has enabled us to determine whether pro-inflammatory cytokines (gamma-interferon - γ-IFN, IL-1β) which interfere with ACAID induction in vivo can alter TGF-β-dependent changes among dendritic cells. Cells that were first treated with γ-IFN or IL-1β and then pulsed with OVA in the presence of TGF- β were unable to evoke down-regulatory T cells. These data indicate that the eye regulates the type of immunity expressed locally by creating a novel microenvironment containing immunomodulatory factors. These factors mandate the unique functional properties of intraocular dendritic antigen presenting cells. We suggest that regional immunity in various organs and tissues is governed by locally-produced cytokines and growth factors that modify indigenous dendritic, antigen presenting cells.

C1-124 DENDRITIC CELLS OR MONOCYTES MATURE FROM AN INTERMEDIATE CLASS II MHC POSITIVE CELL THAT COEXPRESSES HIGH LEVELS OF CD14, P. Szabolcs, D. Avigan, M.A. Rabin, K. Shido, M.A.S. Moore, J.W. Young, The Rockefeller University and Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Because dendritic cells [DCs] share a committed progenitor with monocytes, the CFU-DC/mono, we evaluated intermediates in their expansion and maturation from $CD34^+$ bone marrow precursors. Under the influence of c-*kit*-ligand [KL], GM-CSF [GM], and TNFα in serum-replete suspension cultures, ~ 10-fold expansion was achieved over 6-7d. The majority of cells were class II MHC positive and could be segregated into two distinct populations based on CD14 expression. After panning depletion of $CD34^+$ precursors that persisted in culture after 6-7d in KL, GM, and TNFα, the class II MHC positive cells were sorted into $CD14^+$ $HLA\text{-}DR^+$ and $CD14^-$ $HLA\text{-}DR^+$ intermediates. The majority of both sorted $HLA\text{-}DR^+$ subpopulations were NSE-negative and exhibited the typical veiled morphology of DCs, irrespective of CD14 expression. Both subpopulations could also stimulate resting, unprimed allogeneic T cells in MLRs. To evaluate the developmental potential of the $CD14^-$ vs $CD14^+$, class II MHC-positive sorted intermediates, the cells were recultured for an additional week in cytokines that promoted mixed myeloid, dendritic, or monocytic maturation, *i.e.*, GM, GM+TNFα, or M-CSF, respectively. A striking and unexpected finding was that the $CD14^+$ $HLA\text{-}DR^+$ intermediates acquired much more potent allostimulatory activity and lost expression of CD14 under the influence of GM+TNFα, whereas they retained CD14 positivity and lost allostimulatory potency when recultured in the presence of M-CSF. These cells exhibited limited expansion, only doubling in yield under the combined influence of cytokines. We conclude that an immature DC/mono intermediate exists that has acquired some mature DC features yet also expresses CD14. These cells have the potential to mature into either $CD14^+$ $HLA\text{-}DR^+$ monocytes or potent $CD14^-$ $HLA\text{-}DR^+$ DCs depending on specific cytokine support.

C1-125 GROWTH OF DONOR-DERIVED DENDRITIC CELLS FROM THE BONE MARROW OF MURINE LIVER ALLOGRAFT RECIPIENTS IN RESPONSE TO GRANULOCYTE/MACROPHAGE COLONY-STIMULATING FACTOR Angus W. Thomson, Lina Lu, William A. Rudert, Shiguang Qian, Delbert McCaslin, Fumin Fu, Abdul S. Rao, Massimo Trucco, John J. Fung, and Thomas E. Starzl. Pittsburgh Transplantation Institute and Departments of Surgery and Pediatrics, University of Pittsburgh, Pittsburgh, PA 15213

It has been postulated that the inherent tolerogenicity of the liver may be a consequence of the migration and perpetuation within host lymphoid tissues of potentially tolerogenic donor-derived ("chimeric") leukocytes, in particular the precursors of chimeric dendritic cells (DC). In this study, we have used granulocyte/macrophage colony-stimulating factor (GM-CSF) to induce the propagation of progenitors that give rise to DC ($CD45^+$, $CD11c^+$, $33D1^+$, NLDC 145^+, MHC class II^+, $B7\text{-}1^+$) in liquid cultures of murine bone marrow cells. Using immunocytochemical and molecular methods, we show that in addition to cells expressing female host (C3H) phenotype ($H\text{-}2K^{k+}$; $I\text{-}E^+$; Y chromosome$^-$), a minor population of male donor (B10)-derived cells ($H\text{-}2K^{b+}$; $I\text{-}A^+$; Y chromosome$^+$) can also be grown in 10d DC cultures from the bone marrow of liver allograft recipients 14 d post transplant. Evidence was also obtained for the growth of donor-derived cells from the spleen but not the thymus. In contrast, donor cells could not be propagated from the bone marrow or other lymphoid tissues of unmodified C3H mice rejecting B10 cardiac allografts. These novel findings provide a mechanistic basis for the establishment and, more importantly, for the perpetuation of cell chimerism following organ transplantation. They are also congruent with the possibility that bidirectional leukocyte migration and donor cell chimerism (highlighted herein regarding DC lineage) play important roles in organ allograft acceptance and acquired transplantation tolerance.

C1-126 PULMONARY DENDRITIC CELL IMMUNOSTIMULATORY ACTIVITY IS REGULATED BY POLAR SECRETION OF ALVEOLAR EPITHELIAL CELL-DERIVED GM-CSF. Galen B. Toews, Paul J. Christensen, Lori R. Armstrong, John J. Fak, Gwo-Hsiao Chen, Rod A. McDonald, Robert Paine III, Division of Pulmonary Medicine and Critical Care Medicine, University of Michigan, Ann Arbor, MI 48109-0360

The presentation and recognition of foreign antigen is the critical initial event in the development of local immunity. In the lung, antigen presenting cell activity is largely attributable to pulmonary dendritic cells (DC) that are located in the pulmonary interstitium beneath the alveolar epithelium. *In vitro*, DC antigen presenting cell activity can be modulated by cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF). Because of the close anatomic relationship between pulmonary DC and alveolar epithelial cells, we formed the hypothesis that the immunostimulatory capacity of pulmonary DC is controlled by alveolar epithelial cell-derived GM-CSF. To test this hypothesis, rat type II cells were cultured as confluent monolayers on porous Transwell filters. Conditioned media (CM) was collected from the apical and basolateral compartments and tested for effects on the capacity of highly purified rat pulmonary DC to stimulate T-lymphocyte proliferation in an allogeneic mixed leukocyte response (MLR). Both apical and basolateral CM enhanced a DC-driven MLR; however, the basolateral CM was significantly more potent in this regard (apical CM 190% of control, basolateral CM 275% control). Proliferation also was enhanced when pulmonary DC were transiently preincubated with epithelial cell CM prior to the addition of allogeneic T-lymphocytes, confirming that the observed effect was on *DC* activity. Polyclonal antibodies raised against recombinant murine GM-CSF partially abrogated (70% reduction) the effect of basolateral-CM on DC stimulatory activity. To confirm the presence of GM-CSF mRNA in alveolar epithelial cell cultures, RT-PCR was performed on rat epithelial cell RNA. GM-CSF mRNA was found in alveolar epithelial cells cultured on both tissue cultured plastic and on Transwell filters. We conclude that alveolar epithelial cells in primary culture produce GM-CSF, which is capable of enhancing pulmonary dendritic cell function capacity. Furthermore, alveolar epithelial cell GM-CSF is secreted in a polar fashion towards the basolateral cell surface. Thus, the regulated production of GM-CSF by alveolar epithelial cells plays an important role in the development and compartmentalization of local pulmonary immune responses.

C1-127 DENDRITIC CELLS DERIVED FROM CORD BLOOD AND FETAL LIVER CD34+ CELLS: EFFECT OF GROWTH FACTORS ON ANTIGEN EXPRESSION, M. Kim Warren, Wendy L. Rose and Norma L. Graber, Otsuka America Pharmaceutical, Inc., Rockville, MD 20850

Dendritic cells (DC) are known to develop from CD34+ hematopoietic progenitor cells in culture with the cytokines GM-CSF and TNF. In this study, CD34 cells from cord blood and fetal liver were cultured in flasks or 96-well plates for 10 days in multiple combinations of growth factors to evaluate DC growth. Expression of a panel of antigens was measured by flow cytometry and a novel 96-well-based ELISA method. Flow cytometry of CD34 cells cultured with GM-CSF+TNF for 10 days revealed that 15% of the cells were CD1a+, a marker found on DC and T cells. However, there are no T-lineage cells in these cultures (CD3-). CD1a+ cells increased to 28% with the addition of IL-4 to the CD34 cell cultures. CD11c+ cells, primarily monocytes and DC, remained constant at 88%, but the CD11c+CD14- non-monocyte subset increased from 53% to 80% with the addition of IL-4. CD34 cells were also grown in FBS, human plasma or in serum-deprived medium, and antigen expression was measured by ELISA on cells fixed after 10 days in culture. High levels of expression of CD1a were measured from cells grown in FBS or plasma with GM-CSF+TNF. The addition of c-kit ligand (SCF) greatly increased expression of CD1a in FBS or plasma-containing cultures, and was required for expression of this antigen in serum-deprived cultures. The addition of IL-4 increased CD1a expression, while inhibiting expression of the monocyte marker CD14, in agreement with flow cytometry. Expression of markers common to both DC and monocytes, such as HLA-DR, HLA-DQ and CD11c, remained high with the addition of IL-4. IL-3, in combination with TNF±IL-4, was nearly as effective as GM-CSF in inducing DC antigens.

C1-128 DNA-MEDIATED IMMUNIZATION TO THE HEPATITIS B SURFACE ANTIGEN: POTENTIAL INVOLVEMENT OF INTERSTITIAL DENDRITIC CELLS. Robert G. WHALEN[1], Marie-Louise MICHEL[2], Maryline MANCINI[2], Martin SCHLEEF[1], Simon C. WATKINS[3], Reinhold SCHIRMBECK[4], Jörg REIMANN[4] and Heather L. DAVIS[5]. [1]Dept. Molecular Biology and [2]INSERM U163, Pasteur Institute, 75724 Paris, FRANCE, [3]Dept. Cell Biology, Univ. of Pittsburgh, PA, [4]Inst. of Immunology, Univ. of Ulm, FRG, and [5]Loeb Medical Research Inst., Ottawa Civic Hospital, CANADA.

Intramuscular DNA-mediated immunization has been shown to be an effective means of inducing both humoral and cytotoxic immune responses in various animal models, in spite of the very small amounts of protein (nanograms) generated. The foreign protein encoded by plasmid DNA appears abruptly in muscle fibers of a healthy animal and in an environment in which a strong inflammatory response has not been induced. This unusual mode of antigen delivery may therefore allow processing of the antigen by interstitial dendritic cells present in an antigen acquisition state. To characterize the environment in which the immune reaction is initiated and to investigate the strength of the humoral and cytotoxic responses, we have used DNA expression vectors encoding the hepatitis B surface antigen (HBsAg). Immunohistochemistry on sections of C57BL/6 mouse muscle tissue shows that muscle fibers accumulate the HBsAg and after 5 days a mild cellular infiltrate is present. In BALB/c mice, introduction of HBsAg expression vectors gives rise to high levels of CTLs. Spleen cells of immunized mice are capable of 80% specific lysis at effector:target ratios as low as 2.5:1 after specific restimulation with antigen-presenting cells. After non-specific stimulation, it was also possible to obtain nearly 40% specific lysis at effector:target ratios of 200:1. We also analyzed HBsAg antibody production in haplotype-restricted poor responsiveness to HBsAg. Congenic mice of the haplotypes H-2^b (B10 strain), H-2^s (B10.S) and H-2^b (B10.M) were injected intramuscularly with plasmid DNA vectors expressing HBsAg or intraperitoneally with one of the proteins itself. B10.S and B10.M mice both produced good levels of HBsAg antibodies after a single injection of DNA but responded poorly to protein unless a second protein injection was performed. The strong humoral and cytotoxic responses obtained after DNA-based immunization suggest that interstitial dendritic cells are involved in mediating the high efficiency of antigen presentation at nanogram levels.

C1-129 REGULATION OF LANGERHANS CELL FUNCTION VIA BLOOD BORNE FACTOR(S), Yong Xie, J. Wayne Streilein, Schepens Eye Research Institute, Harvard Medical School, Boston, MA 02114

Langerhans cells (LC) are functionally labile epidermal dendritic cells. When freshly obtained, LC constitutively express class II MHC molecules, CD1a (humans) and receptors for Fc of IgG and for C3b. Fresh LC (fLC) readily activate allogeneic T cells, but not autologous T cells; even when pulsed with antigen, they fail to activate naive, antigen-specific T cells. When fLC are cultured for 2-3 days in the presence of GM-CSF, LC swiftly up-regulate expression of class I and II MHC molecules, express de novo the co-stimulatory molecules B7 and ICAM-1, and acquire the novel functional property of activating autologous naive T cells, in addition to displaying enhanced ability to activate allogeneic T cells. It is believed that GM-CSF is the driving force behind the conversion of fresh to cultured LC, yet recent studies have documented that in vivo administration of GM-CSF failed to induce LC to undergo functional transformation in situ. Moreover, despite a high level of GM-CSF in the circulation, fLC from mice bearing GM-CSF-producing tumors fail to activate syngeneic naive T cells. These observations suggest that a factor that antagonizes the effect of GM-CSF may be present in vivo. To test this possibility, we have examined the functional properties of LC prepared from mouse skin that had been explanted in vitro for three days. We found that the functional and phenotypic features of these cells closely resembled those of LC cultured in single cell suspension: strong expression of B7-1, B7-2, enhanced display of class II MHC molecules, capacity to activate naive T cells. However, when cultured in the presence of 10% mouse serum, LC failed to acquire full T cell activating properties; and surface expression of co-stimulatory molecules was low. If mouse serum was only added during the last 24 hours of culture, the LC displayed full functional transformation. Human, rabbit and bovine serum showed no inhibitory effect on LC functional transformation. Addition of exogenous GM-CSF to cultures containing mouse serum failed to reverse the inhibitory effect on LC functional transformation. We conclude that mouse serum contains a species-specific soluble factor that antagonizes the effects of GM-CSF, thereby inhibiting epidermal LC from automatically undergoing functional transformation in vivo.

C1-130 **EVALUATION OF DENDRITIC CELLS IN ALLOGENEIC MARROW GRAFTS.** Zhou Ye, Syedur Khandkar, Carlos Lee, William Bowers & Adrian Gee, Department of Microbiology & Immunology, University of South Carolina & Division of Transplantation Medicine, Richland Memorial Hospital, Columbia, SC 29203.

Allogeneic bone marrow (BM) transplants are the treatment of choice for patients with certain refractory cancers. BMT is limited by failure to engraft & graft-versus-host disease, which are attributed to recipient & donor T cells respectively. Dendritic cells (DC) possess potent antigen-presenting activity & their role in graft/host interactions is unknown. We developed methods for culture, characterization & quantitation of DC in allogeneic BM harvests. $CD34^+$ cells were enriched using the MiniMACS immunomagnetic system & set up in liquid culture (RPMI + 10% fetal bovine serum + TNFα/SCF/GM-CSF) & in colony-forming (CFU) assays ± cytokines. Cells in liquid culture were fed every 4 days & enumerated (Table 1) & phenotyped at day 8 (Table 2).

Table 1(n=4)

Day	Total Cell # (±SE)	%DC
0	5.0x10e5±0.0	0.0±0
4	1.5x10e6±0.24	4.4±0.9
8	4.5x10e6±2.1	6.8±3.1
12	7.7x10e6+3.4	6.0+3.8

Table 2 (n=4)

% (+SE)	Day 0	Day 8
CD34	88.4±3.8	1.9±0.7
CD1a	1.3±0.8	9.7±1.4
CD14	1.9±1.1	31.6±6.8
CD4	2.3±0.1	68.3±10.2
HLA-DR	66.4±24.2	53.3±9.8
CD11a	48.3±2.7	81.9±6.7
CD54	2.1+0.2	61.6+10.0

Addition of cytokines to CFU assays decreased growth of CFU-GM from 38.9±14.7 / 500 $CD34^+$ cells plated to 22.1±7.7; whereas CFU-DC increased from 7.6±3.3 to 14.6±9.2. DC colonies were plucked & stained positively by immunocytochemistry for CD1a, HLA-DR & CD4 but were $CD14^{dim\ or\ -ve}$. Ex vivo depletion of BM T cells for partially-matched BMT using anti-T cell receptor monoclonal antibody & complement had no effect on the proportion of CFU-DC. Two patients who failed to engraft stably received the highest numbers of CFU-DC. These assays are being used to define the role of DC in graft host interactions in BMT.

C1-131 **STUDY OF CD4+ AND CD8+ CELLS SUBSETS IN PATIENTS WITH FIBROADENOMATOSIS BEFORE AND IN DYNAMICS OF TRADITIONAL, HOMEOPATHIC AND BIORESONANCE THERAPY,** **Penezina O.P.[1], Goroshnicova T.V.[1], Nosa P.P.2, G. Lednyczky[3] and Fomovskaya G.N.[1]. 1- Molecular Immunology Dept., Institute of Biochemistry, Kiev, Ukraine; 2 - Kavetzky Institute of Oncology, Kiev, Ukraine; 3 - Applied Logic Laboratory, Budapest, Hungary.**

Investigation of T-cell subsets (CD4+ and CD8+ cells) and their rate is the traditional method of immunological status characterization used in clinic. Wide statistic matherial about those parameters in healthy donors also is published. It is obvious that the status of immune system and probability of cancer development have to correlate. Especially important to know that correlation on the stage of precancer diseases. Knowleges about that changes in immune responce may also help to understand the molecular mechanisms of cancerogeneses.

Fibroadenomatosis is one of the most widespread deseases both in Ukraine and abroad considered as a precancer state. Investigation of immunologycal status of that patients before, during and after treatment is very important for diagnostic and prognostic clinical purposes. We investigated T-cell subsets and their rate in patients before treatment and in dynamics of traditional (with remedies), homeopathic and bioresonance ("Bicom")-treatmant. The studies were carried out by means of flow cytometry. Peripheral blood of 18 donors and 21 patients (both women) were analysed. Analysis of healthy donors mainly were in the same range as in published data. Analysis of patients with fibroadenomatosis has shown that the rate of T-helper and T-supressor lymphocytes was abnormal in 10 cases (48%), level of T-helpers - in 10 cases (48%), level of T-supressors - in 12 cases (57%). These results shows significant disbalance of immune system in studied patients. It was shown that in dynamics of treatment numbers of T-helper and T-suppressor lymphocytes in many cases significantly changed. Analysis of that changes will help to show advantages of different types of treatment.

C1-132 EXPRESSION AND FUNCTION OF B7.1 (CD80) AND B7.2 (CD86) ON HUMAN PERIPHERAL BLOOD DENDRITIC CELLS. JW Dekker, AD McLellan, GC Starling, LA Williams, BD Hock, DNJ Hart. Haematology/Immunology Research Laboratory, Christchurch Hospital, Christchurch, New Zealand

The interaction of the CD28 molecule on T lymphocytes with its ligands on antigen presenting cells (APC) initiates a critical costimulatory pathway in the induction of T lymphocyte proliferation and cytokine secretion. Potential CD28/CTLA-4 ligands on dendritic cells (DC) are particularly relevant and we therefore examined the expression and function of the CTLA-4 Ig ligands B7.1 (CD80) and B7.2 (CD86) on human blood DC. Resting DC populations lacked cell membrane CD80 and CD86 and detectable CTLA-4 Ig binding, although CD86 but not CD80 transcripts were detected by RT-PCR. In contrast, conventionally prepared DC, isolated by gradient separation and FACS sorting after a period of in vitro culture, expressed both CD80 and CD86 mRNA and had high surface CD86 but limited CD80 expression. Likewise short term in vitro culture of fresh DC induced both CD80 and CD86 coincidental with upregulation of the DC-associated early activation antigens CD83 and CMRF-44. Analysis of the kinetics of CD28/CTLA-4 ligand induction showed that CD86 surface expression was detectable with 8h of culture, whereas CD80 antigen was first detected after DC were cultured for 24h. RT-PCR analysis confirmed that CD86 mRNA expression precedes that of CD80 in the course of MLR. The functional importance of CD86 upregulation in DC - T lymphocyte interactions was suggested by the ability of CTLA-4Ig, but not by the mAb BB-1, to block an allogeneic mixed lymphocyte reaction (MLR) mediated by DC populations, initially negative for these ligands. These results demonstrate that CD28/CTLA-4 ligand expression must be upregulated on human DC and that CD86 is the earliest and perhaps functionally the predominant CD28/CTLA-4 ligand on DC.

C1-133 GENERATION OF NEW MONOCLONAL ANTIBODIES REACTING WITH HUMAN BLOOD DENDRITIC CELLS. Hart DNJ, Hock BD, McLellan A, Boyce A, Haematology/Immunology Research Laboratory, Christchurch Hospital, Christchurch, New Zealand

No dendritic cell (DC) specific surface marker has been identified in man to date and this makes studies on the putative DC haemopoietic lineage and the isolation of DC for functional studies difficult. After investigating several different methods for generating mouse mAb reactive with human DC, our laboratory has now established a method which has generated new mAbs recognising apparently novel antigens on human DC. The first mAb produced by this method, CMRF-44, recognises an early activation expressed by human DC. The antigen is not expressed on blood DC preparations obtained by negative immunoselection from SRBC rosette negative peripheral blood mononuclear cells but is expressed strongly on blood DC purified by conventional techniques, involving separation of low density cells over a Nycodenz gradient, after a period of in vitro tissue culture, as well as by tonsil DC. The activation of DC preparations obtained by negative immunoselection generates a subpopulation of CMRF-44 positive cells, which overlaps with the CTLA-4 ligand positive population. Double labelling with biotinylated CMRF-44 can be used to follow DC activation whilst monitoring the expression of other antigens. A further fusion has generated additional mAbs, which react with standard human DC preparations. The application of new mAbs to DC may provide further delineation of DC subsets and insight into the events of DC differentiation and activation.

Antigen Processing and Presentation; Accessory Molecules

C1-200 FAILED ANTIGEN PRESENTATION AFTER UVB RADIATION MAY RESULT FROM MODIFICATIONS OF LANGERHANS CELL CYTOSKELETON, Stefano Bacci, J. Wayne Streilein, Schepens Eye Research Institute, Harvard Medical School, Boston, MA 02114

Cutaneous antigen presenting cells, especially Langerhans cells (LC), play a central role in induction of contact hypersensitivity (CH). Acute, low dose ultraviolet B radiation (UVB) impairs CH induction in genetically defined strains of mice, and genetic evidence suggests that susceptibility to UVB is mediated by polymorphism at the TNF α locus. Intracutaneous injections of TNFα and cis-urocanic acid similarly impair CH induction, and both UVB and cis-UCA effects are abolished by anti-TNF α antibodies. These findings imply that TNFα is the principal mediator of the deleterious effects of UVB on CH induction. UVB radiation, injections of TNFα, cis-UCA, and epicutaneous hapten (dinitrofluorobenzene-DNFB) alter the morphology of epidermal LC, suggesting that these agents may impair CH induction by altering the LC's cytoskeleton. To address this issue, epidermal sheets were obtained from mice after cutaneous treatment with UVB, TNFα, cis-UCA or DNFB at optimal sensitizing (10 μg/ml) and toxic (189 μg/ml) doses. The sheets were double labeled with anti-IA and anti-vimentin antibodies for immunohistochemical analysis. Except for 10 μg/ml DNFB, LC from skin treated with the other agents displayed reduced Ia expression, partial loss of dendrites, and deficient expression of vimentin. By contrast, LC from skin receiving an optimal sensitizing dose of DNFB retained their dendritic shape and intracellular vimentin expression. These findings indicate that UVB, TNFα, cis-UCA and toxic doses of hapten induce among LC a co-ordinated loss of class II MHC expression from the plasma membrane, and rearrangement of cytoskeletal intermediate filaments. We suspect that the latter effect explains the shape changes displayed by these cells (loss of dendrites), and may either impair the ability of LC to process and present haptens in association with MHC molecules, or render them unable to migrate to draining lymph nodes where the critical first encounter with hapten-specific T cells occurs during CH induction.

C1-201 USE OF DENDRITIC CELLS TO AUGMENT THE IMMUNE RESPONSE TO TUMOR-ASSOCIATED MUCIN IN TWO ANIMAL MODELS. Simon M. Barratt-Boyes, Robert A. Henderson and Olivera J. Finn, Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Mucin is a cell surface glycoprotein encoded by a gene Muc-1, and is associated with certain adenocarcinomas in humans. Humoral and cellular immune responses to mucin have been described, however, as expressed on tumors mucin is poorly immunogenic. In an effort to enhance the immunogenicity of mucin,we are using dendritic cells as immunogens in two animal models. We have transduced a previously published immortalized murine dendritic cell line, D2SC/1, with a retroviral vector containing Muc-1. Transduced cells express high levels of mucin and have been sorted to purity. Studies in mice are being performed to assess the immunogenicity of these cells and to compare them to tumors expressing Muc-1. We are applying similar techniques in chimpanzees for eventual use in preclinical studies. Little is known about chimpanzee dendritic cells and we are using various protocols to cultivate dendritic cells in vitro. Addition of recombinant human GM-CSF and IL-4 to freshly isolated chimpanzee peripheral blood mononuclear cells for 6-9 days induces proliferation of cells with typical dendritic morphology. Phenotype and function of these cells are being evaluated to confirm their lineage as dendritic cells. Prior to immunization, chimpanzee dendritic cells will be transduced with the Muc-1 containing retroviral vector.

C1-202 ATTENUATED INFLUENZA A VIRUS WHEN PULSED ON DENDRITIC CELLS ELICITS STRONG CYTOLYTIC RESPONSES FROM HUMAN CD8+ T CELLS. Armin Bender, Long Kim Bui, Mary A.V. Feldman and Nina Bhardwaj, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, NY, NY 10021

CD8+ cytolytic T cells (CTLs) are considered to be important mediators of resistance to several infectious diseases, e.g. influenza, CMV and malaria. We have recently developed an in vitro system to generate strong influenza virus-specific CD8+ CTL responses from fresh human T cells, provided peripheral blood derived dendritic cells are used as the antigen presenting cells (N. Bhardwaj et al. 1994, J. Clin. Invest. 94, 797-807). We now demonstrate that heat-inactivated (56°C, 30 min) or UV-treated virus can also be used to stimulate CTL responses with similar potency to live influenza virus. Several approaches were taken to characterize these forms of attenuated viruses. While the binding ability of physically intact virions was not altered in standard hemagglutination assays, infectivity was markedly diminished (more than 10^4 fold) as determined in plaque forming assays using the MDCK-II cell line. This loss of infectivity was confirmed in the more sensitive egg infectious dose test (EID_{50}) by injecting serially diluted virus into embryonated hen eggs. The reduced infectivity of attenuated virus was not due to abolishment of viral fusogenic capacity since these virus preparations could still lyse chicken erythrocytes at low pH equally well as live virus. Studies with monocytes, which are permissive for influenza virus, indicate similar binding and uptake kinetics for both the live and attenuated virus forms. Attenuated viruses demonstrated little new protein synthesis in infected cells. Cell surface hemagglutinin could be detected on the majority of cells (monocytes, dendritic cells, MDCK-II cells) pulsed with live virus, but only on a few cells treated with attenuated virus using cytofluorography. Furthermore, in immunohistochemical studies with virus-susceptible dendritic cells only few cells were detected with intracellular staining for viral nucleoprotein. Therefore attenuated, virtually non-replicating influenza virus maybe usefull as a vaccine tool when presented in concert with human dendritic cells to elicit CD8+ CTL responses.

C1-203 THE RELATIVE ROLE OF B7-1 AND B7-2 IN STIMULATION OF NAIVE AND MEMORY CD4 T LYMPHOCYTES BY VARIOUS CELLS PRESENTING DIFFERENT ANTIGENS, Delanie J. Cassell and James P. Allison, Cancer Research Laboratory, University of California, Berkeley, Berkeley, CA 94720

Freshly explanted, naive CD4 T cells, like T cell clones, require costimulation in addition to TCR occupancy in order to produce IL-2. Ligation of CD28 receptors on TCR-triggered T cells by either B7-1 or B7-2 transfected cells can provide the necessary costimulatory signals. The relative contributions of these two molecules to the costimulatory function of normal antigen-presenting cells (APC) are not completely understood. Different APC vary in their ability to stimulate IL-2 production by naive CD4 T cells. Peptide-pulsed, activated B cells induce IL-2 production by naive CD4 T cells, but are inferior to peptide-presenting dendritic cells in the amount of lymphokine they elicit per T cell. The relative deficiency of activated B cells is largely due to their inability to provide optimal costimulation (Cassell and Schwartz, 1994). B7-2 is the major costimulatory ligand on activated B cells. By contrast, B7-2 specific mAb fail to block stimulation of naive CD4 T cells by dendritic cells (Cassell and Schwartz, unpublished). This finding raises the possibility that optimal activation of unprimed CD4 T cells may rely on different costimulatory ligands depending on the type of APC with which the T cell interacts and further suggests that B7-2 does not play a critical role in the costimulatory function of dendritic cells for primary IL-2 responses.

It has been reported that the activation requirements of naive and memory cells differ. It is not yet known, however, whether the two T cell subsets differ in their costimulatory requirements in particular. Finally, the relative roles of each costimulatory ligand in responses to different types of TCR stimuli such as superantigens and peptide/MHC complexes has yet to be fully determined. Using highly purified APC and T cell subsets in single cell and limit dilution cultures together with reagents specific for the various costimulatory molecules, we are currently examining these issues. Results of experiments designed to evaluate the relative role of B7-1 and B7-2 will be presented.

C1-204 **A DEFECTIVE MATURATION AND FUNCTION OF DENDRITIC CELLS IN TYPE 1 DIABETICS**

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Type 1 diabetics is an autoimmune disease in which β cells are destroyed by autoreactive T cells and macrophages. The disease is based on a imbalance in immunoregulation leading to an array of humoral and cellular autoimmune responses to islet cell antigens. There are numerous indications that dendritic cells and macrophages play an important role in the initiation of the islet cell directed autoimmune response.

In type 1 diabetic patients (n = 6) we found:

1. a lowered maturation of dendritic cells from their precursors in the blood, and
2. a lowered function of these blood-derived dendritic cell populations, viz. a lowered capability of the cells to act as stimulator cells in autologous and allogeneic T cell stimulation and a lower capability to form cellular clusters with other lymphoid cells.

These defects were independent from the duration of the disease. Similar and innate abnormalities in the function of APC have been demonstrated in the animal models of type 1 diabetes (NOD mouse, BB rat).

Since DCs are of crucial importance in the triggering of naive T cells, and since optimal APC function is earlier required for immunization, we hypothesize that the found suboptimal maturation and function of dendritic cells form the basis for a disturbed regulation of T cell activation in which induction of tolerance is affected more severely than induction of immunization.

C1-205 USING HUMAN DENDRITIC CELLS TO AUGMENT THE IMMUNE RESPONSE TO TUMOR-ASSOCIATED MUCIN

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Except for melanoma and some other tumors, T lymphocyte mediated immune responses to human tumors has been very difficult to demonstrate. Despite these difficulties, our laboratory has shown that epithelial cell mucin encoded by the MUC-1 gene and expressed by adenocarcinomas of the pancreas and breast can be recognized directly by human cytotoxic T cells in an MHC-unrestricted fashion. Despite the presence of these T cells, the tumors are not eliminated and the patients eventually die. We propose that if the mucin molecule were to be transferred from the tumor to a more potent antigen presenting cell (APC), such as a dendritic cell, then it may be possible to greatly enhance the immune response to the mucin molecule.

We have been successful in generating human dendritic cells from $CD34^+$ hematopoietic stem cells. Purified $CD34^+$ cells were isolated from umbilical cord blood, bone marrow, or G-CSF mobilized peripheral blood and cultured with varying combinations of the cytokines GM-CSF, TNF-α, and stem cell factor. By day 12 of culture, approximately 50 to 70% of the non-adherent cells in these cultures expressed CD1a. These CD1a positive cells also expressed high levels of class II MHC molecules, adhesion molecules, and the T cell co-stimulatory molecule B7.1. In addition, these cells stimulated allogeneic $CD4^+$ T cells to a much greater extent than autologous macrophages.

In order to transfer the mucin molecule to the dendritic cells described above, the MUC-1 gene was cloned into the retroviral vector MFG. MFG-MUC1 transduced 3T3 cells expressed mucin at high levels as judged by FACS analysis. In addition, the mucin protein appears to be full length as judged by Western blot analysis. This vector will be used to transduce human CD34-derived dendritic cells and the ability of these APC's to induce mucin-specific T cell responses will be evaluated. Thus, these transduced dendritic cells may be a novel and powerful immunotherapeutic tool for the elimination of mucin-expressing adenocarcinomas.

C1-206 REANALYSING THE TISSUE DISTRIBUTION OF THE 205 Kd PROTEIN THAT IS ABUNDANT ON DENDRITIC CELLS AND IDENTIFIED WITH THE NLDC-145 MONOCLONAL ANTIBODY.

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Prior work found that the NLDC-145 monoclonal reacted primarily with dendritic cells and thymic cortical epithelium. We have found that the size of the corresponding molecule is 205 kD and have raised rabbit polyclonal antibodies to the immunoselected "DC-205" protein. Using polyclonal and monoclonal antibodies, and flow cytometry, we find that B cells from several organs [but not pre B cells] express DC-205. The levels do not increase on B cells stimulated with a panel of activators. Trace but clear staining is observed on T cells, granulocytes, and some elicited macrophages [thioglycollate]. By immunoblotting, the levels of DC-205 protein in dendritic cells are >10-30 times more than in B cells. The same antibodies were applied to sections of most organs. We found clear cut staining of B cells in follicles, brain capillaries, and both airway and intestinal epithelium. We conclude that the DC-205 protein is more broadly expressed than previously was apparent. Leukocytes other than dendritic cells, and nonleukocytes other than thymic epithelium, express DC-205.

* Contributed equally to the work

C1-207 A NOVEL METHOD FOR ISOLATING EUKARYOTIC cDNA CLONES ENCODING SECRETED PROTEINS,

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We have developed a comparatively simple technique for isolating cDNA clones that encode secreted proteins. The method has been tested by isolating clones from a cDNA library derived from activated human peripheral blood mononuclear cells. Approximately half the clones isolated encode secreted proteins. Examples of clones that have been isolated are a T cell receptor α chain, a IgM antibody chain, the MET protooncogene, IL-8, and IL-10. The method can be applied to any source of eukaryotic mRNA.

C1-208 MOLECULAR CHARACTERIZATION OF A 205 KD PROTEIN THAT IS ABUNDANT ON DENDRITIC CELLS AND IDENTIFIED WITH THE NLDC-145 MONOCLONAL ANTIBODY. Wanping Jiang, *William J. Swiggard, Asra Mirza, Michael Peng, Ralph M. Steinman, and Michel C. Nussenzweig. Laboratories of Cellular Physiology and Immunology, and Molecular Immunology, The Rockefeller University, New York, NY 10021.

Over the decade since its initial isolation by Kraal, the monoclonal antibody NLDC-145 has been utilized as a histochemical and flow cytometric marker for mouse dendritic cells [DCs] in several lymphoid organs and skin. We have set out to clone the molecule bound by NLDC-145. To begin, we purified the protein from mouse thymus, at a scale that permits direct biochemical study. The molecule proves to be an integral membrane glycoprotein with a mildly alkaline pI of 7.5 and an electrophoretic molecular weight of 205 kD, not 145 kD as originally reported. About 7 kD of the mass is contributed by covalently-bound carbohydrates. The aminoterminus is not blocked, and the sequence of its first 25 amino acids is not significantly homologous to any known protein. A polyclonal anti-peptide antibody blotted to a 205 kD species in crude thymus and dendritic cell extracts. Two dozen additional peptides have been obtained and sequenced. We are using this information to clone the corresponding cDNA.

* Contributed equally to this work.

C1-209 DENDRITIC CELL INVOLVEMENT IN PATHOGENESIS OF DIABETES IN A T CELL RECEPTOR TRANSGENIC MOUSE. Pieter J.M. Leenen[1], Jonathan D. Katz[2], Jane S.A. Voerman[1], Diane Mathis[2], Hemmo A. Drexhage[1]. Dept. Immunology[1], Erasmus University, Rotterdam, The Netherlands, and IGBMC[2], BP163, Illkirich, C.U. de Strasbourg, France.

NOD mice spontaneously develop a disease similar to type 1 diabetes in humans. In addition, a transgenic mouse strain, expressing rearranged T cell receptor (TCR) α and β genes from the BDC2.5 diabetogenic T cell clone and crossed onto NOD background, has been shown to develop extensive insulitis, followed by diabetes (Katz et al., 1993, Cell 74:1089).

To investigate the involvement of dendritic cells (DC) and macrophages in the inflammatory process, we have carried out a detailed immunohistochemical analysis of the islet infiltrates in both TCR transgenic NOD mice and regular NOD mice. In regular NOD mice, DC and macrophages (MHC class II$^+$, ER-MP23$^+$, and MOMA-1$^+$) were the first inflammatory cells appearing at the circumference of the islets at 4-7 weeks of age. These cells clearly preceded the later influx of T cells. At later stages (7-10 wks.), macrophages (BM8$^+$, F4/80$^+$, Mac-1$^+$) infiltrated into the islets, particularly in mice prone for diabetes (females and castrated males). True insulitis, characterized by an infiltrate of lymphocytes, DC, and macrophages, occurred predominantly after 15 weeks, and in this process pancreatic β cells were destroyed.

In the TCR transgenic NOD mice, we found a fast developing inflammatory process starting already at 3 weeks of age. Initially, islets were either heavily infiltrated, or still unaffected. This infiltrate consisted primarily of CD4$^+$ T cells, joined by B cells in a later stage. DC (MHC class II$^+$, CD11c$^+$, NLDC-145$^+$) were found in all compartments of the inflammatory infiltrate, but especially at the interface of intact β cells and the T lymphocytic infiltrate. In contrast to the regular NOD insulitis, BM8$^+$ macrophages were virtually absent. The DC-T-B cell peri-insulitis protruded into the islets, and from week 20 almost no insulin-producing β cells could be detected.

In conclusion, the patterns of infiltration found in the regular NOD mouse and in the NOD mouse, expressing a transgenic T cell receptor from a diabetogenic T cell clone, are clearly distinct. In both models of type I diabetes, however, DC play a likely role in the pathogenesis of the disease, given their appearance in the earliest phases of inflammatory infiltration.

C1-210 IMMUNOLOGICALLY IMMATURE DENDRITIC CELLS PROPAGATED IN VITRO INDUCE ALLOANTIGEN-SPECIFIC ANERGY AND PROLONG CARDIAC ALLOGRAFT SURVIVAL

Lina Lu, Delbert McCaslin, Shiguang Qian, Fumin Fu, Thomas E. Starzl, John J. Fung, and Angus W. Thomson, Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15213-2582

Recently, we reported (*J Exp Med* 179, 1823, 1994) that liver DC progenitors home to T-dependent areas of the lymphoid tissue of non-immunosuppressed allogeneic recipients. In these sites, they express donor MHC class II but persist for months, recapitulating the fate of donor-derived DC following orthotopic liver transplantation. The functional significance of these "chimeric" DC has yet to be established, but we have speculated that the migration of such immunologically immature DC from liver grafts may explain their inherent tolerogenicity. Functional maturation of DC is now thought to reflect the upregulation of cell surface MHC class II and other T cell costimulatory molecules, especially the CD28 ligands B7-1 (CD 80) and B7-2 (CD86). We have propagated GM-CSF stimulated DC from B10 mouse (H-2^b; I-A$^+$) bone marrow in liquid culture. DC (NLDC 145$^+$, 33D1$^+$, N418$^+$) harvested from 10-day cultures were CD45$^+$, heat stable antigen$^+$ (HSA$^+$), CD45$^+$, CD44$^+$, MHC class II$^+$, B7-1$^+$ but B7-2$^-$. These cells induced alloantigen-specific hyporesponsiveness (anergy) of allogeneic T cells (H-2^k; I-E$^+$) in MLR in contrast to B7-1$^+$, B7-2$^+$ spleen-derived DC propagated similarly in vitro, which were potent allostimulators. When injected into C3H (H-2^k) mice 7 days before transplant, the bone-marrow derived immature DC (2 x 10^6 i.v.) prolonged B10 (H-2^b) cardiac allograft mean time survival from 9.5 to 22.0 days. These findings provide further evidence of the importance of B7-2 as an accessory molecule for T cell stimulation and suggest that immature (B7-2$^-$) DC may induce alloantigen-specific unresponsiveness in vitro and in vivo.

C1-211 *IN VITRO* ACTIVATION OF MURINE Vβ5$^+$ CELLS BY SYNGENEIC SPLENIC DENDRITIC CELLS, Vivian L. MacKay and Pamela J. Fink, Department of Immunology, University of Washington, Seattle, WA 98195

C57BL/6 mice (I-E$^-$, H-2^b) exhibit an age-dependent deletion of peripheral CD4$^+$ Vβ5$^+$ T cells that is not dependent on Mtv-6 or Mtv-9; such deletion occurs both in mice that are transgenic for a rearranged Vβ5$^+$ TCR gene and in nontransgenic B6 mice. Peripheral CD8$^+$ Vβ5$^+$ T cells are also deleted with age and the CD8$^+$ population includes a distinct CD8^{low} Vβ5^{low} fraction that appears to have been activated as an early event in the deletion pathway. In order to test several hypotheses for the mechanism(s) of deletion of peripheral CD4$^+$ and CD8$^+$ Vβ5$^+$ T cells, we undertook to develop an *in vitro* assay that would detect the generation of CD8^{low} cells, the activation of T cells, and the deletion of CD4$^+$ and CD8$^+$ Vβ5$^+$ T cells.

Splenic dendritic cells enriched by density fractionation on BSA columns and overnight plastic adherence/detachment specifically activate and induce the proliferation of syngeneic Vβ5$^+$ T cells when co-cultured at dendritic cell:responder ratios of $\geq$1:100. This assay can be used to test such parameters as the age-dependence of dendritic cell-mediated stimulation, the response of separated CD4$^+$ and CD8$^+$ Vβ5$^+$ T cells, the correlation with deletion of CD4$^+$ Vβ5$^+$ cells, and the generation of CD8^{low} Vβ5^{low} cells.

C1-212 FcεRI MEDIATES IgE-DEPENDENT ALLERGEN PRESENTATION, Dieter Maurer, Christof Ebner, Bärbel Reininger, Edda Fiebiger, Dietrich Kraft, Jean-Pierre Kinet and Georg Stingl, DIAID and Gen. and Exp. Pathol., Univ. of Vienna Med. Sch., Vienna, Austria, Mol. All. and Immunol. Sect., NIAID, NIH, Rockville, MD

The discovery that professional antigen presenting cells (APCs) of atopic individuals express the high affinity IgE receptor (FcεRI) raised the possibility that the functional importance of FcεRI in atopy may extend beyond its role in immediate-type allergic reactions. Using hapten (4-hydroxy-3-nitrophenacetyl, NP)-specific, monomeric IgE (cIgE) and NP-conjugated recombinant birch (*rBet v* I) and grass pollen (r*Phl p* II) allergens, we observed that cIgE bound to monocytes of atopics allows efficient allergen targeting to these cells. FcεRI was found to be the pivotal receptor mediating this event since allergen/IgE-binding to monocytes was abolished by a mAb to FcεRIα, but neither by an anti-CD23 (FcεRII) mAb nor by lactose-elution of the IgE-binding protein εBP. Even more importantly, cIgE-dependent targeting of r*Bet v* I-NP or r*Phl p* II-NP to monocytes from atopics resulted in a 100-1000-fold amplification of allergen presentation to autologous *Bet v* I- or *Phl p* II-specific T cell clones (TCCs, n=15). The addition of an anti-FcεRIα-chain mAb, but not of an anti-FcεRII mAb, reduced this cIgE-enhanced TCC response to levels seen in the absence of cIgE. These results demonstrate that FcεRI, but not FcεRII, is critically involved in IgE-dependent allergen uptake, processing, and presentation by peripheral blood APCs from atopics. Taken together, these findings demonstrate a yet unknown function of FcεRI and provide evidence that the presence of this receptor on professional APCs critically lowers atopic individuals' threshold to mount allergen-specific T cell responses capable of perpetuating or, even, eliciting allergic tissue reactions.

C1-213 BONE MARROW-DERIVED DENDRITIC CELLS SERVE AS POTENT ADJUVANTS FOR PEPTIDE-BASED ANTITUMOR VACCINES, Jose I. Mayordomo, Walter J. Storkus, W.Martin Kast*, Tatiana Zorina, Albert B. DeLeo, Michael T. Lotze. Depts of Surgery, Pathology and Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA 15261,and * Dept of Immunohematology and Bloodbank, University of Leiden, The Netherlands.

We have addressed the ability of bone marrow-derived dendritic cells (DC) to present E7(49-57) peptide, a known H-2D^b-binding viral tumor CTL-recognized epitope derived from human papilloma virus-16. DC were grown from C57BL/6 mice by culturing lymphocyte-immunodepleted bone marrow in a combination of GM-CSF plus either TNF-alpha or IL-4. We evaluated the ability of synthetic E7(49-57) peptide emulsified in adjuvants or presented on the surface of DC, unfractionated splenocytes or RMA-S TAP-deficient cells to elicit a protective antitumor response in C57BL/6 mice. Mice were immunized twice, 14 days apart,with 100 μg E7(49-57) emulsified in incomplete Freund's adjuvant (IFA) or ethiodol injected s.c. or 10 μg of peptide pulsed *ex vivo* onto DC (i.v.) or RMA-S (s.c. or i.p.) .They were challenged with 2.5x10^6 C3 cells (B6 mouse embryonic cells transformed with E6 and E7) s.c. 14 days after the second immunization.

Progressive tumors grew in all control mice and in those receiving adjuvant only. Tumors regressed over the next 2-5 weeks in all animals immunized with peptide plus ethiodol or pulsed onto DC (GM-CSF+ IL-4) or RMA-S and 40-60% of those receiving peptide plus IFA, DC(GM-CSF+TNF) or splenocytes. DC generated with GM-CSF+IL-4 outperformed those generated with GM-CSF+TNF, although tumors in most animals receiving either vaccine eventually regressed. These preliminary results support the use of peptide-pulsed DC as a cancer vaccine.

C1-214 NEONATAL TOLERANCE IS A NUMBERS GAME, John Paul Ridge, Ephraim Fuchs, and Polly Matzinger, Labratory of Cellular and Molecular Immunology, National Insitutes of Health, Bethesda, Md 20892

For many years it has been thought that neonatal T cells are in a special state which makes them easily tolerizable, however we find that they can actually be immunized if an appropriate APC is used. In this study, we injected either male spleen cells or purified male dendritic cells into neonatal female mice. We showed that spleen cells induced tolerance whereas dendritic cells immunized. We conclude that neonatal T cells are not in an inherently tolerizable state but, because they are virgin cells and their numbers are small, they are easily turned off by encounter with non professional Antigen Presenting Cells(APC) present in spleen cell populations.

C1-215 REGULATION OF IMMUNOSTIMULATORY FUNCTION AND B7 MOLECULE EXPRESSION ON MURINE DC. Shannon C. Ritchie, Thomas C. Pearson, David A. Gerber, Rose Hendrix, Diane Z. Alexander, Peter S. Linsley*, Christian P. Larsen. Department of Surgery, Emory University School of Medicine, Atlanta, GA 30322; and *Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.

Cytokines regulate phenotypic and functional properties of DC. In vivo, DC may encounter cytokines produced by injured nonlymphoid cells or by T cells (e.g., GMCSF, IFNγ, IL2, TNFα). We have recently reported that GMCSF upregulates expression of B7-1, B7-2 and immunostimulatory capacity of DC. In contrast, IFNγ upregulates expression of B7-2 without upregulation of B7-1 or enhancement of immunostimulatory capacity.

Further studies of the stimulatory properties of DC cultured in GMCSF, IFNγ or a combination of both cytokines have been undertaken using CD4$^+$ and CD8$^+$ T cell subsets in allogeneic MLR. GMCSF enhances the capacity of DC to stimulate each subset, whereas IFNγ fails to augment stimulatory activity and blocks the ability of GMCSF to enhance DC stimulatory ability. Flow cytometric analysis demonstrated that IFNγ prevented GMCSF-induced B7-1 expression on splenic DC and LC. Studies are underway to define the kinetics of GMCSF- and IFNγ-induced B7-1 and B7-2 in epidermal cell suspensions and skin grafts.

Cytokine-cultured DC were compared as stimulators in MLR and blocked with various anti-B7 reagents. CTLA4 Ig, anti B7-1 and anti B7-2 each inhibited T cell proliferation in a dose dependent manner. This was seen even using IFNγ-cultured DC as stimulators suggesting that B7-1 is induced and utilized though it is not expressed at the outset of the assay. Further, the combination of anti B7-1 and anti B7-2 resulted in inhibition of T cell proliferation equal to that seen with CTLA4 Ig, indicating that B7-1 and B7-2 comprise the principle CTLA4 ligands utilized in the MLR.

These results suggest that optimal activation of unprimed T cells requires both B7-1 and B7-2. A detailed analysis of the expression and function of the B7 molecules should assist in the development of rational strategies to block this critical pathway in efforts to alter T cell responses in vivo.

C1-216 CONDITIONAL ABLATION OF DENDRITIC CELLS IN TRANSGENIC AND CHIMERIC MICE. B. Salomon,[1] C. Pioche,[1] P. Lores,[2] J. Jami,[2] P. Racz,[3] and D. Klatzmann[1]
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Dendritic cells (DC) knockout in mice should be a powerful mean to appreciate the role of these cells in physiological or pathological situations. In order to generate such an animal model, we used a stategy based on the DC-specific expression of a suicide gene in transgenic mice. We used the herpes simplex virus type 1-thymidine kinase (HSV1-TK) which allows conditional ablation of dividing HSV1-TK expressing cells by converting the non toxic ganciclovir (GCV) into a toxic metabolite. DC expression of HSV1-TK in transgenic mice was attempted with the HIV-LTR promoter which had been previously shown to preferentially direct the expression of a CAT transgene in Langerhans cells (J. Leonard et al., 1989, AIDS Res. Hum. Retovir. 5:421). We generated LTR-TK transgenic mice expressing the HSV1-TK gene under the control of the HIV-LTR promoter. We showed a low but preferential expression of the transgene in DC, leading to DC depletion in spleen and thymus following GCV administration (B. Salomon et al., 1994, J. Immunol. 152:537). This depletion was often associated with a thymic atrophy and a wasting syndrome. To rule out the possibility that a transgene expression leakiness in non hematopoietic cells could be responsible for some of these pathological findings, we generated chimeric mice: normal irradiated mice were engrafted with transgenic bone marrow cells. The GCV treatment resulted in a splenic DC depletion comparable to the depletion observed in treated transgenic mice. However, thymic atrophy, and wasting syndrome were not observed in treated chimeric mice. We are currently using these two animal models to study the role of DC in allograft rejection, thymocytes selection, and immune responses.

C1-217 DENDRITIC CELLS WITHIN THE IRIS OF THE RAT: POTENTIALLY POTENT APCs WITHIN AN 'IMMUNE-PRIVILEGED SITE', Raymond J. Steptoe[1,2], Patrick G. Holt[1] and Paul G. McMenamin[2]. [1]Department of Cell Biology, Institute for Child Health Research, Subiaco, W.A. 6008, [2]Department of Anatomy and Human Biology, The University of Western Australia, Nedlands, W.A. 6009, Australia

Previous studies from this group have demonstrated the presence, in mice, rats and humans, of distinct and separate networks of Ia+ dendritic cells and resident tissue macrophages co-existing within the iris, ciliary body and aqueous outflow pathways, tissues bordering the anterior chamber of the eye, an 'immune-privileged site'. Recent studies have found that during post-natal development, Ia+ DC within the iris increase in number and in the intensity of surface Ia expression. X-irradiation and bone marrow reconstitution studies indicate that iris DC are bone marrow derived and demonstrate a $T_{1/2}$ in the order of 4-5 days, compared with 12-14 days for LC in the same animals. In contrast, resident tissue macrophages within the iris exhibited a $T_{1/2}$ of approximately 10-12 days. For functional analysis, Ia+ DC were isolated from iris by positive selection using immunomagnetic bead sorting. Freshly isolated DC were moderate stimulators when assessed in allogeneic MLR cultures. However, following isolation and 48h culture with GM-CSF iris DC expressed elevated levels of Ia and were potent stimulators in allogeneic MLR cultures, comparable with GM-CSF cultured LC isolated from the same animals. Demonstration of the rapid turnover of iris DC and the potent GM-CSF inducible APC activity suggest a key role for these cells in immune surveillance within the anterior chamber of the eye.

These findings have important implications for the understanding of immune-surveillance and immune regulation in the anterior chamber of the eye.

C1-218 ENHANCEMENT OF MLR STIMULATION BY HUMAN DENDRITIC CELLS WITH MAB TO CD40 AND CD40 LIGAND. Sun-sang J. Sung and Shu Man Fu, Department of Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

CD40 is expressed on dendritic cells and has been shown to be important for the maintenance of dendritic cell proliferation and viability. In this report, the effects of anti-CD40 mAb 626 and the soluble CD40 ligand on the dendritic cell morphology and T cell stimulatory capacities were examined. Dendritic cells were cultured from peripheral blood adherent cells in the presence of GM-CSF and IL-4. After five days, the majority of the cells appear to be dendritic cells by morphological and phenotypic criteria. These cells potently stimulated allogenic MLR (alloMLR). They were more than an order of magnitude more potent than isolated monocytes or B cell blasts in alloMLR. Anti-CD40 mAb 626 caused dendritic cells to adhere and acquire a spindly morphology within 24 hours when 10μg/ml mAb was added to dendritic cell cultures at day 5. In the presence of 626, dendritic cell stimulation of alloMLR increased two- to three-fold, and its stimulation of syngeneic MLR increased by two-fold over control. Soluble CD40L also caused a two-fold increase in T cell proliferation in alloMLR. The alloMLR was inhibited by anti-B7-1 and anti-B7-2 mAb, and the two anti-B7 mAb showed additive inhibitory effects. Little inhibition was seen with the anti-CD45 mAb A1. It is of interest to note that the mAb A1 is a potent inhibitor of alloMLR when the stimulator cells were freshly isolated non-T mononuclear cells. These results indicate that CD40 plays an important role in antigen presentation by dendritic cells.

C1-219 DENDRITIC-CELL TRANSFORMATION AND EXPRESSION OF OX-6 BY A PHOSGENE-SENSITIVE PROGENITOR GROWN FROM RAT TRACHEAL EXPLANTS, Robert J. Werrlein, Janna S. Madren-Whalley and Stephen D. Kirby, Biochemical Pharmacology Branch, U.S.A. Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425

Pulmonary edema and immunosuppression of the lung are generally regarded as the primary causes of debilitation and death following phosgene inhalation. Even doses of $\leq$ 240 ppm•min have been shown to decrease host resistance and enhance susceptibility to pulmonary infection and dysfunction. Using laser cytometry, FITC-phalloidin and immunofluorescent techniques, we have discovered that F-actin is a sensitive and early target of phosgene; and that dendritic-cell progenitors, isolated and grown from rat tracheal explants, are exquisitely sensitve to the gas. Sublethal doses of 54 and 348 ppm•min, delivered to progenitor cells during a 20 min period, produced immediate, post-exposure, F-actin decreases of 8 % ($p < .05$) and 50 % ($p < .01$) respectively. Phosgene produced corresponding changes in the F-actin organization of progenitor cells and potentiated their transformation to a dendritic-cell phenotype. Time-lapse photographs show that spontaneous transformations were fast acting (10-30 min) and completely reversible. Transformed dendritic cells expressed OX-6, a class II major histocompatibility antigen, and were, therefore, capable of serving as antigen presenting cells. The OX-6 epitope was not expressed by progenitors. Results indicate that phosgene's effects on F-actin can alter the expression of antigenic surface markers and may alter specific cell involvement in immune response and post-exposure response to drug treatments.

C1-220 T CELL ACTIVATION BY SPOROZOITE VS CSP PROTEIN SPECIFIC PEPTIDES. Katherine L. White and Urszula Krzych. Department of Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20307 and The Catholic University of America, Washington, D.C. 20026

Immunization with radiation attenuated *Plasmodium berghei* induces protection against the sporozoite (SPZ) stage parasite. The mechanism of protection is not fully understood but it is known that both CD4 and CD8 T cells are required. These T cells are directed against the circumsporozoite protein (CSP), the major surface protein covering the SPZ. Our laboratory has been working to understand protection by studying T cell activation by the CSP. Lymphocytes primed with CSP in the context of the intact SPZ are unusual in that: 1. they are presented by B cells and not macrophages; 2. they do not require processing; 3. bind outside the MHC groove; 4. do not recognize CSP peptides; 5. require MHC but are loosely restricted; 6. can be presented by both IA and IE. For technical reasons it is not at this time possible to obtain lines or clones of SPZ-specific T cells. Alternatively, priming with CSP peptides and recall with the priming peptide has revealed several peptides that have conserved epitopes throughout several murine strains. One of these, peptide #6 a.a.51-70, is of particular interest because specific T cell reactivities mimic those of the SPZ-specific T cells. Currently we are characterizing these cells and analyzing T cell activation by CSP in the context of the whole SPZ as well as CSP peptide #6.

C1-221 CD24 EXPRESSION ON ANTIGEN PRESENTING CELLS. GJ Clark, LA Williams, AB McLellan, BD Hock, AJ Boyce, DNJ Hart. Haematology/Immunology Research Laboratory, Christchurch Hospital, Christchurch, New Zealand

The human CD24 antigen is a GPI-anchored glycoprotein consisting of 30-35 aa core protein, which is extensively glycosylated resulting in a 35-45 kD molecule. The mouse homologue of this molecule, heat stable antigen (HSA), is expressed on spleen dendritic cells (DC) and Langerhans cells (LC). Functional studies suggest that HSA may have co-stimulator activity and a synergistic effect with CD80 (B7) in primary activation of mouse T lymphocytes has been proposed. In this study we investigated the expression and function of the CD24 antigen on human cell lines and normal leucocyte populations including DC. The binding of a panel of CD24 mAb to human cell lines identified two groups of antibodies. The IgG mAb ALB-9 and SWA-11, reacting with the core protein labelled the myeloid K562, HEL and U937 cell lines as well as the B lymphoid lines Nalm 6 and Nalm 16, all of which contained CD24 mRNA estimated by Northern blot and RT-PCR. The same mAb did not bind bind to EBV lymphoblastoid cell lines. In contrast the IgM mAb VIB-E3, BA-1, HB-8, HB-9 reacted strongly with three EBV lymphoblastoid cell lines which were shown to lack CD24 mRNA by RT-PCR. These latter antibodies also showed a broader specificity on normal haemopoietic cells binding, CD24 mRNA negative T lymphocytes, in addition to B lymphocytes and granulocytes. The CD24 mAb binding to T lymphocytes and EBV lines was shown to relate to a neuraminidase and pronase sensitive epitope apparently distinct from the CD24 epitope on K562. The expression of these CD24 epitopes on human DC varied with individual DC preparations and time in culture. The human CD24 cDNA has been isolated and transient and stable transfectants generated to investigate CD24 antigen co-stimulatory potential.

Tolerance and Autoimmunity; Dendritic Cells as Adjuvants

C1-300 ANTIGEN-PRESENTING ACTIVITY OF DENDRITIC CELLS IN HEPATITIS B VIRUS TRANSGENIC MICE, S.M.F.Akbar, K. Kurose, T. Masumoto, K.Inaba,* M.Onji,Third Department of Internal Medicine,Ehime University School of Medicine,Ehime, Japan.*Department of Zoology,Kyoto University,Kyoto,Japan.

We have reported the impaired immune response in hepatitis B virus(HBV) transgenic mice to keyhole limepet hemocyanin (KLH) and it is attributable to a defect of antigen-presenting dendritic cells, probably due to the substantial decrease of MHC class II antigen expression(Akbar et al. Immunology 1993;78:468).Since other investigators have reported that immunological unresponsiveness to HBV-related antigens in HBV-carriers is primarily due to the tolerance of T cells, we immunized transgenic mice with graded doses of 2 ip injections of hepatitis B surface antigen(HBsAg) in CFA and found that these mice could not produce specific antibody upon challange with antigen in vivo and in vitro. However, when a group of mice, having 2^8titre of HBsAg by Reverse Passive hemoagglutination Method were immunized with 10ug of HB vaccine,ip for 6 months,60% of them reduced the HBsAg titre in sera to 2^2(responder),no change was seen 40% of the immunized mice(non responder), and in control mice during the experimental period.The dendritic cells from the responder showed significantly higher Con-A dependent T cell proliferation than the same from the non responder and the control mice. On the other hand,T/B cells from HBsAg-primed transgenic mice produced anti-HBs when cultured with dendritic cells from the normal mice, but not with the same from the transgenic mice.Treatment of transgenic mice-derived dendritic cells resulted in increased MHC class II expression and these cells were found to be competent to induce specific antibody production to KLH-or HBsAg-primed T/B cells. These results indicate that dendritic cells play an important role in the immune response of mice infected with virus, and suggest the possible use of dendritic cells in immunotherapy.

C1-301 ANALYSIS OF THE MECHANISMS OF CTLA4-IG PLUS BONE MARROW INDUCED TRANSPLANTATION TOLERANCE. Diane Z. Alexander, Thomas C. Pearson, Shannon C. Ritchie, Rose Hendrix, David A. Gerber, Peter S. Linsley†, Christian P. Larsen. Emory University School of Medicine, Department of Surgery, Atlanta, GA 30322 and †Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.

CTLA4-Ig, a fusion protein which blocks the B7/CD28/CTLA4 costimulatory pathways, has been shown to inhibit allograft rejection. We have shown that CTLA4-Ig combined with donor bone marrow transfusion can induce donor specific transplantation tolerance in the adult murine cardiac allograft model. The purpose of the current study was to investigate the mechanisms of tolerance induction in this model.

C3H/He recipients of BALB/c vascularized cardiac allografts were treated intravenously with 2 x 10^7 donor bone marrow cells and human CTLA4-Ig (200 μg) at the time of transplant and then with intraperitoneal CTLA4-Ig (200 μg) on post-transplant days 2, 4 and 6. These tolerant recipients were compared to control recipients which were treated with 4 doses of CTLA4-Ig alone or no treatment.

Analysis of tolerant recipients at 30 days after transplantation, showed no donor specific peripheral T cell hyporesponsiveness in the MLR. RT-PCR data demonstrate similar expression of IL-2 and γIFN message in the allograft of tolerant and control animals at days 1, 3, 5, 8, 12 and 15 post transplant. However, IL-4 expression is markedly decreased in the tolerant recipients. Finally, using RT-PCR we have found expression of donor specific MHC class II message in the skin of tolerant recipients 30 days following transplantation.

These results demonstrate that multiple mechanisms may be important for the induction and maintenance of tolerance in the CTLA4-Ig plus bone marrow murine cardiac transplant model.

C1-302 CHARACTERIZATION AND GENE MODIFICATION OF DENDRITIC CELLS TO BE USED FOR ANTIGEN PRESENTATION, Susan Alters, Jose Gadea, Elisa Brunette, Lydia Kilinski, Mohan Philip, and Ramila Philip, Applied Immune Sciences, Santa Clara, CA 95054

Dendritic cells are potent accessory cells which have been shown to present antigen to $CD4^+$ and $CD8^+$ T cells. We are interested in using dendritic cells, either transfected with the gene for a defined tumor associated antigen, or pulsed with a tumor antigen specific peptide, as antigen presenting cells to generate tumor antigen specific CTLs from peripheral blood or TIL of cancer patients. Dendritic cells have been isolated from peripheral blood by culturing precursors with GMCSF and IL4 for 5-7 days. The isolated dendritic cells express high levels of HLA class I, DR, CD1a, CD11c, CD33, CD40, CD54, CD58 and moderate levels of CD4, CD45R0, and CD80 (B7). As expected they do not express CD3, CD8, CD14, CD15, CD16 or CD19. Functionally, the dendritic cells give a potent MLR response; the number of cells required to give 50% response is <1000 and a response is seen with as few as 100 cells. Furthermore, dendritic cells are approximately 100 fold more effective at stimulating a response to a soluble antigen, tetanus toxoid, when compared with PBMC.

Having shown that functionally active dendritic cells can be isolated, we have recently begun to optimize conditions for transgene expression in dendritic cells using plasmid DNA constructs containing the inverted terminal repeats of adeno-associated virus (AAV) complexed to cationic liposomes (Avectin). We have achieved 10-30% efficiency of transgene expression using reporter genes such as chloramphenicol acetyl transferase (CAT) and nerve growth factor receptor (NGFR), as well as therapeutic genes such as IL-2. We are currently investigating the antigen presentation capability of dendritic cells expressing mutated K-ras and p53 for CTL generation in vitro.

C1-303 GENERATION OF IMMUNOSTIMULATORY DENDRITIC CELLS FROM HUMAN CD34+ HEMATOPOIETIC PROGENITOR CELLS OF THE BONE MARROW AND PERIPHERAL BLOOD. Helga Bernhard*, Mary L. Disis*, Eric S. Huseby*, Susan Hand*, Julie R. Gralow*, Grant Risdon**, Shelly Heimfeld**, and Martin A. Cheever*; Department of Medicine*, Division of Oncology, University of Washington, Seattle, WA 98195; CellPro Inc**, Bothell, WA 98021, USA.

The use of dendritic cells (DC) has been proposed to generate therapeutic T cell responses to tumor antigens in cancer patients. One limitation is that the number of DC in peripheral blood is exceedingly low. Dendritic cells originate from CD34+ hematopoietic progenitor cells (HPC) which are present in the bone marrow and in small numbers in peripheral blood. CD34+ HPC can be mobilized into the peripheral blood by *in vivo* administration of G-CSF. The aim of the current study was to determine whether substantial numbers of functional dendritic cells could be elicited and grown *in vitro* from CD34+ HPC derived from bone marrow or G-CSF mobilized peripheral blood. Culture of CD34+ HPC with GM-CSF and TNF-alpha yielded a heterogeneous cell population containing cells with typical dendritic morphology. Phenotypic studies demonstrated a loss of the CD34 molecule over one week and an increase in cells expressing surface markers associated with dendritic cells, CD1a, CD80, CD4, CD14, HLA-DR, and CD64. Function was validated in experiments showing that cultured cells could stimulate proliferation of allogeneic CD4+ and CD8+ T lymphocytes. Antigen presenting capacity was further confirmed in experiments showing that cultured cells could stimulate specific responses to exogenous protein and peptide, but responses were weak. Others have shown that the ability of DC to present antigen may diminish over time in culture. Current experiments are directed towards defining culture conditions which can expand DC which retain the ability to process and present exogenous antigen. The derivation and expansion of DC from cultured bone marrow or G-CSF mobilized CD34+ HPC may provide adequate numbers for testing of DC in clinical studies, such as vaccine and T cell therapy.

C1-304 **PRESENTATION WITHOUT PROCESSING OF ENDOGENOUS PRECURSORS BY MHC CLASS I**

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The antigen presentation pathway yields peptide/MHC class I complexes on the antigen presenting cell (APC) surface. Expression of the peptide/MHC complex on APC surface is preceded by several steps that include the generation of peptide fragments in the cytoplasm and assembly of peptide/MHC complexes in the ER. Here, we addressed the question of whether there is an obligatory relationship between peptide cleavage and the generation of peptide/MHC complexes by analyzing the processing of ovalbumin (aa257-264, SL8) or influenza nucleoprotein (aa366-374, AM9) analogs. We examined the generation of naturally processed peptides using precursors that did, or did not, contain residues flanking the MHC-binding optimal peptides. By characterizing the peptides generated from these precursors by T-cell stimulation assays and by HPLC analysis, we established that intracellular assembly of peptide/MHC complexes and their expression on the cell surface can occur without removal of any residue from the precursor polypeptide. In addition, the presentation of endogenously synthesized perfect *fit* peptides demonstrates that the cleavage of precursor polypeptides is an independent step in this pathway.

This research was supported by grants to NS from the National Institutes of Health (AI-26604), and the Tobacco Related Disease Research Grant. DB and PS were supported by the pre-doctoral fellowships from the Howard Hughes Medical Institute and UC System-wide Biotechnology Research and Education Program Training Grant, respectively.

C1-305 DENDRITIC CELL TRANSFERS PROTECT NON-OBESE DIABETIC (NOD) MICE FROM DIABETES; DEPENDENCE ON ISLET ANTIGEN EXPOSURE AND MODIFICATION OF CYTOKINE PROFILES OF ISLET INFILTRATING CELLS. Michael Clare-Salzler, P. Robinson, J. Cornelius, J. Anderson, A. Peck, Department of Pathology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL 32610-0275

We reported that a single footpad injection of dendritic cells (DCs) isolated from the draining lymph node of the pancreas (PanLN) of NOD mice with islet inflammation protects recipient mice from diabetes, promotes the generation of regulatory cells, and reduces the degree of insulitis whereas the transfer of splenic or axillary (ALN) DCs provides no protection (J.Clin.Invest;90:741). We postulated that DC exposure to, and presentation of islet antigens may be critical in the transfer paradigm. To evaluate this possibility we pulsed ALN DCs with a isolated islet antigen preparations (800ug/ml), or with purified or recombinant antigens(insulin, glutamate decarboxylase (GAD_{65}), and heat shock protein (HSP_{65}), (500ug/ml) for 2 hours. DCs were washed and injected into the footpad of 4 wk old NOD female mice. Other NOD recipients also received PanLN DCs and unpulsed ALN DCs. The diabetes incidence at one year of age was; un-pulsed ALN DCs 13/15 (86%), GAD pulsed 13/15 (86%), HSP pulsed 13/15 (86%), insulin pulsed 14/15 (93%). In contrast there was a marked reduction in diabetes incidence in mice receiving ALN DCs pulsed with islet antigen 2/10, and in the PanLN DC group (0/4). We also examined the cytokine mRNA profile of islet infiltrating cells from mice receiving PanLN DC transfers and unmanipulated NOD mice at 16 wks of age by RT-PCR. In comparison to un-manipulated mice, mice receiving PanLN DCs demonstrated a loss of mRNA for IFN-g, TNF-a, and a reduction in IL-1, IL-4 and IL-7. IL-2 and IL-6 mRNA was uniquely expressed in mice receiving PanLN DC transfers. These results demonstrate that, 1) exposure to islets antigens is critical for DC generated regulation of autoimmunity in the NOD mouse, and that 2) prevention of autoimmune disease is associated with a reduction in islet inflammation and modification of the character or activation state of the infiltrating cells.

C1-306 **INTRACELLULAR TRANSPORT AND ANTIGEN PRESENTATION FUNCTION OF MURINE MHC CLASS II MOLECULES WITH OR WITHOUT INVARIANT CHAINS.**
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The Ii p31 and p41 chains associated with newly synthesized class II molecules, direct αβIi complexes to endosomal compartments where they are degraded. We have found that Ii p31 and Ii p41 transfection into L cells influences drastically the conformation of murine IAk class II molecules, but marginally their intracellular distribution in multivesicular body containing vesicles, the structure of which is closely related to the MHCII compartment identified in B cells. Ii chains are required there, to induce a compact conformation of class II molecules, suggesting a chaperone function for the association of peptides with the αβ dimers. We have analyzed in the same cells, the contribution of various endogenous antigens to the class II presentation pathway. A *bona fide* class II restricted endogenous presentation pathway exists, allowing proteins translocated within the lumen of the ER in general, to be presented by class II molecules. The expression of the Ii chains favors this endogenous presentation pathway even for antigens retained in the ER.

The cytoplasmic domain of Ii contains specific motives involved in the targeting of class II molecules. Having dissected the C terminal amino acid sequences of α and β IAk chains, deletion of α or β C terminal sequences induces a partial retention of class II molecules in the ER, but also trigger for some constructs a massive surface expression and a loss of endosomal resident αβ dimers visualized with mAb in L cells. Using a synchronized wave of intracellular transport, class II molecules are targeted from the Golgi complex to Golgi-derived endosomal vesicles. Class II molecules lacking their C terminal domains are constitutively exported to the cell surface as class I molecules, suggesting that C terminal domains of class II molecules may also control their intracellular trafficking.

Humbert, M.et al., (1993) **Eur. J. Immunol.**, 23, 3158-3166.
Humbert, M.et al., (1993) **Eur. J. Immunol.**, 23, 3167-3172.
Wade W. et al., (1993) **Immunol. Today**, 14, 539-546.

C1-307 **MODULATION OF IMMUNE RESPONSES IN VIVO BY POLYCLONAL ACTIVATORS.**
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Optimal T cell activation requires at least two signals delivered by the antigen presenting cell (APC). The first signal, antigen specific, results in the interaction of the TcR molecules with the MHC/peptide complex on the APC. The second signal, also called costimulatory signal, is delivered by the interaction of costimulatory molecules (mainly the B7 molecules) on the APC with counter receptors on T cell. Lipopolysaccharides (LPS) have been shown to be potent inducers of the B7 family costimulatory molecules on macrophages and B cells in vitro. We have studied the effect of a single injection of LPS on the capacity of APC to induce a T cell response. Surprisingly, a pretreatment with LPS results in a decrease in the T cell response induced in vivo by the bacterial superantigen SEB. This study shows that this phenomenon is due to an APC-deficit and that the T cell function seems to be unchanged. Indeed, APCs from LPS injected animals show a decrease in the ability to activate T cell by alloantigens or superantigens in vitro. This correlates with a decrease in these animals in the number of dendritic cells, the most potent APC able to induce a T cell activation. This immunomodulatory phenomenon is also observed after a single injection of a T cell activator (SEB or anti-CD3 mAbs), suggesting that a negative feedback mechanism targeted on APC could occur after an overstimulation of the immune system.

C1-308 DENDRITIC CELLS ISOLATED FROM THE MEDIASTINAL LYMPH NODES OF INFLUENZA-INFECTED MICE ARE ABLE TO STIMULATE ANTIGEN-SPECIFIC T CELLS.
Maryna Eichelberger and AnnMarie Hamilton-Easton, Department of Immunology, St Jude Children's Research Hospital, Memphis, TN 38105.

To understand the requirements necessary to initiate a protective immune response to viral infections, it is important to identify the principal antigen presenting cell (APC). As with other mucosal antigens, it is believed that priming of naive T cells occurs in the draining lymph nodes. Kinetic experiments demonstrated the presence of influenza virus in the mediastinal lymph node (MLN) soon after C57BL/6 mice were infected intra-nasally with X-31. Virus-specific cytotoxic T cells were demonstrated at this site by day 3 post-infection. This precedes the response at the inflammatory site, suggesting that this is indeed the site of initiation. When professional APC populations were isolated from the MLN, virus was only present in the dendritic cell population, whereas virus was associated with macrophage, dendritic cell, and B cell populations in the lung. The dendritic cell population from the MLN was able to stimulate class I-restricted, influenza virus-specific T cell hybridomas, suggesting that there is sufficient antigen present in the correct form to generate a response. The activation of naive $CD8^+$ T cells is currently under investigation.

C1-309 REGULATION OF THE PERIPHERAL IMMUNE RESPONSE BY ANTIGEN PRESENTING CELLS
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The ability of peptidic antigen to induce a variety of functionally distinct peripheral immune responses, depending on the dose and route of administration, suggests that factors other than TCR binding to peptide-MHC complexes are crucial in determining the outcome of an encounter with antigen in the periphery. We are using T and B cell receptor transgenic models in which the frequency of lymphocytes responding to cognate antigen is artificially high to examine at the single cell level the effect of APC subpopulations on phenotypic and functional aspects of the primary immune response *in vivo*. Our data suggest that, for T cells, immunogenic encounters with antigen are characterised by a peak of production of IL2, IL3, IL4 and IFNγ which precedes the peak in T cell numbers by 3-4 days. The Th1/Th2 balance, as measured by the ratio of IFNγ to IL4, is a complex function. Once the threshold needed to stimulate a measureable response is reached, higher doses of antigen bias the response in favour of IL4 production whilst depressing IL2 and IFNγ production, consistent with a dominant role for antigen-dependent, primary T cell-derived IL4 in controlling Th1/Th2 ratios by positive feedback. We are determining whether APC type or activation state is crucial in regulating Th1/Th2 immune responses via initial rates of IL4 production and, in particular, which APC's are required for the stimulation of such a Th2 response *in vivo*. To compare the role of dendritic cells and B cells, we have set up a model of B cell antigen presentation using a hybrid antigen (hen egg lysozyme (HEL) linked to cytochrome C) to allow specific presentation by anti-HEL immunoglobulin transgenic B cells to anti-cytochrome TCR transgenic T cells. We are also examining the effect of specific antibody binding to Fc receptors on dendritic cells and B cells on T cell activation and priming in this model.

C1-310 ROLE OF B70/B7-2/CD86 IN CD4+ T CELL IMMUNE RESPONSES INDUCED BY DENDRITIC CELLS,
Francesco F. Fagnoni, Masaru Takamizawa, Wayne R. Godfrey, Alberto Rivas, Miyuki Azuma, Ko Okumura and Edgar G. Engleman, Department of Pathology, Stanford University School of Medicine, Stanford, California 94305
Dendritic cells (DC) are potent APC. However, the molecular basis underlying this activity remains incompletely understood. To address this question we generated murine monoclonal antibodies against human peripheral blood derived DC. One such antibody, designated IT209, stained differentiated DC, but failed to stain freshly isolated PBMC. The antigen recognized by IT209 was identified as B70 (human B7-2, also recently identified as CD86). Using this mAb we performed functional in vitro studies on the role of B70 in CD4+ T cell activation by DC. IT209 partly inhibited the proliferative response of CD4+ T cells to allogeneic DC and to recall antigens, such as tetanus toxoid and purified protein derivative of tuberculin, presented by autologous DC. The mAb had a more potent inhibitory effect on the primary response of CD4+ T cells to autologous DC pulsed with HIV gp160 or keyhole limpet hemocyanin. Adherent monocytes, despite acquired expression of B70, failed to induce T cell responses to these antigens. IT209-mediated inhibition of CD4+ T cell responses was equivalent to that produced by an anti-CD28 mAb, whereas an anti-CD80 mAb was only marginally inhibitory and did not augment the effect of IT209. These findings indicate that B70 antigen plays an important role in DC-dependent CD4+ T cell activation, especially in the induction of primary CD4+ T cell responses to soluble antigens. However, since activated monocytes, despite their expression of B70, failed to prime naive T cells, our results indicate that additional molecules contribute to the functions of DC in CD4+ T cell activation.

C1-311 *EX VIVO* GENERATION OF FUNCTIONALLY ACTIVE ANTIGEN PRESENTING CELLS FROM PERIPHERAL BLOOD $CD34^+$ HEMATOPOIETIC PROGENITOR CELLS IN CANCER PATIENTS,
Paul Fisch, Gabriele Köhler, Birgit Herbst, Annette Garbe, Wolfram Brugger, Roland Mertelsmann, Lothar Kanz, Departments of Hematology/Oncology and Pathology, University of Freiburg Medical Center, 79106 Freiburg, Germany.
Peripheral blood $CD34^+$ progenitor cells (PBPC) mediate hematopoietic reconstitution in cancer patients after autologous transplantation. *Ex vivo* expansion of these $CD34^+$ PBPC with hematopoietic growth factors produced highly potent antigen presenting cells (APC) for soluble protein antigens that require antigen processing. We cultured $CD34^+$ cells *ex vivo* in medium containing either (1) SCF, IL-1ß, IL-3, IL-6, and EPO, (2) SCF, granulocyte-macrophage CSF (GM-CSF) and tumor necrosis factor-α (TNF-α), as well as (3) SCF, GM-CSF, TNF-α, and IL-4. Under the culture conditions (1) and (2), the main population of expanded APC resembled macrophages ($CD14^+$, $HLA\text{-}DR^+$) while under the culture conditions (3) the predominant APC were dendritic cells (DC) ($CD14^-$, $HLA\text{-}DR^+$). The cells generated *ex vivo* from all types of cultures were powerful APC of tetanus-toxoid (TT) and *Mycobacterium tuberculosis* (PPD) antigens, with both antigens requiring processing and presentation. The antigen presenting capacity of these cells was maintained for at least 38 days of culture. Our data demonstrate that DC can be expanded from peripheral blood $CD34^+$ cells of cancer patients and suggest that such cells might be able to present tumor antigens to the immune system. Thus, these cells could be useful for vaccination against various malignancies. The immunogenicity and therapeutic potential of the PBPC-derived DC might be further enhanced upon transfer of various cytokine genes into autologous DC.

C1-312 **BINDING OF A NATURALLY PROCESSED HETERODIMERIC DISULFIDE-LINKED INSULIN PEPTIDE TO MHC CLASS II: IMPLICATIONS FOR T CELL TOLERANCE**
Frédérique Forquet, Mirko Hadzija, John W. Semple, Edwin Speck and Terry L. Delovitch. Banting and Best Department of Medical Research and Department of Immunology, University of Toronto, Toronto, Ontario M5G 1L6 Canada.
We have shown that disulfide-linked insulin peptides are immunogenic *in vitro* for MHC class II restricted T cells. To determine whether such peptide binds to MHC class II *in vivo*, biosynthetically labeled recombinant human insulin (rHI) was injected into BALB/c mice and naturally processed radiolabeled HI peptides bound to MHC class II ($I\text{-}A^d$) on different thymic APCs were characterized. The major $I\text{-}A^d$-bound HI peptides isolated from thymic epithelial cells (TNC) are the A6-A11/B7-B19 (A6) and A19-A21/B14-B21 (A19) heterodimeric disulfide-linked peptides, respectively. Neither of these nor other rHI peptides could be eluted from $I\text{-}A^d$ molecules on dendritic cells (DC). While both TNC and DC present rHI to $HI/I\text{-}A^d$-specific T cells, these APCs do not present the reduced or non-reduced forms of the synthetic A6 and A19 HI peptides. The inability of these peptides to be presented is not due to their failure to bind to $I\text{-}A^d$, since they compete for the presentation of rHI by TNC and DC as well as by fixed B cells. Thus, a naturally processed heterodimeric disulfide-linked peptide can bind to an MHC class II molecule *in vivo*.

The inability of the A6 peptide to be presented may be due either to its lack of or weak immunogenicity. Although it consists of an immunodominant A-chain loop containing T cell epitope, it lacks some residues flanking the A-chain loop that are required for its immunogenicity. This peptide differs from its self mouse insulin homolog only at residue B9, and this may result in either the absence of A6-specific T cells in the periphery or the presence of such T cells at a very low frequency. T cell unresponsiveness to HI peptides A6 and A19 peptides may also arise from the striking structural similarity between the B-chain peptide components, B7-B19 and B14-B21, of these HI peptides and those HI B-chain peptides, B8-B18 and B10-B22 able to suppress the incidence of type I diabetes in nonobese diabetic mice after oral administration of HI (Weiner, H (1993) *Autoimmunity* **15**, S6-7.). Thus, processed insulin B-chain linear peptides or heterodimeric disulfide-linked peptides that contain these linear B-chain peptides may elicit the T cell unresponsiveness to HI observed here and the induction of oral tolerance to pork insulin and suppression of type I diabetes.

Our observations illustrate the importance of examining the T cell immunogenicity of MHC-bound peptides physiologically processed *in vivo*, since only a fraction of these peptides may be immunogenic and involved in positive T cell selection. They may also explain why a non-immunogenic peptide(s) of an antigen is bound to and co-transported with MHC class II molecules to the surface of a given APC in the thymus and/or periphery, i.e. to induce tolerance. Finally, they may identify naturally processed peptides of a given autoantigen (e.g. HI) that could prove efficacious in the prevention of an autoimmune disease.
(Supported by MRC of Canada MT-5729 and JDFI).

C1-313 DENDRITIC CELLS CAN NOT PRESENT TYPE II COLLAGEN TO T-CELL HYBRIDOMAS,
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Dendritic cells are potent antigenpresenting cells and are regarded as crucial for the priming of an immune response. We have studied the capacity of dendritic cells for presentation of type II collagen (CII)- a protein which can induce autoimmune arthritis (type II collagen induced arthritis). CII immunization of $H\text{-}2^q$ mice leads to a proliferative response of CII-reactive T-cells and subsequently development of arthritis. We have analyzed the capacity of freshly isolated Langerhans cells (LC) compared to spleen cells (SC) and peritoneal macrophages (PEC), to stimulate CII-specific T cell hybridomas. We have previously determined the immunodominant peptide in the CII molecule (1) and showed that this can be recognized in two forms; with or without naturally O-linked disaccharides (2). Surprisingly, we found that LCs are unable to present CII. Neither of the two distinct T-cell determinants on CII could be presented unless the antigen was added as a synthetic peptide. Both of the T-cell determinants could be presented by PEC and SC. In contrast, the Langerhans cells could present other protein antigens, i.e. myelin basic protein, ovalbumin and pepsin to antigen-specific T hybridomas. So far we have found no way to stimulate the Langerhans cell to present CII by using cytokines (GM-CSF, gIFN, IL-4, IL-5, IL-10) or by supplying CII in vivo.
These data suggest that other APCs than dendritic cells are initiating the immune response to CII.
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C1-314 ANTIGEN-PULSED DENDRITIC CELLS IN THE TREATMENT OF PATIENTS WITH B-CELL LYMPHOMA

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B cell lymphomas display unique immunogloulin receptors on their cell surface, and the idiotypic determinants (Id) of these proteins can be effectively targeted by the immune system. In prior animal studies and in human clinical trials, we have demonstrated that vaccination with Id determinants can induce both humoral and cellular immunity which can confer both protection against tumor challenge and can cause regression of pre-existing disease. We describe here the initial results using antigen-pulsed dendritic cells as a vaccine in humans. Human dendritic cells can be isolated from blood, and these cells retain their ability to process and present antigens. In a pilot study, we have infused three patients (pts) with follicular low grade lymphoma with autologous dendritic cells that were pulsed in vitro with idiotype-specific tumor protein or a control protein, keyhole limpet hemocyanin (KLH). All pts had previously been treated with chemotherapy but still had measurable disease. Peripheral blood leukocytes were harvested by leukapheresis. Autologous DC were purified by differential gradient centrifugation and negative selection culture techniques. During the purification, half of the DC were pulsed separately with either Id protein or KLH. The purified antigen pulsed cells were then reinfused intravenously as a vaccine. Two weeks later, the pts received a boost of soluble Id protein and, in a separate site, KLH subcutaneously. Two pts have completed the 3 planned cycles of DC vaccine and one pt is undergoing therapy. All 3 pts have developed strong peripheral blood mononuclear cell (PBMC) proliferative and antibody responses to the KLH protein. The last 2 pts developed intense cellular proliferative responses immediately after the first reinfusion of DC and before the soluble boost. So far, pts who completed all treatments (2 pts) have developed PBMC proliferative responses against the tumor Id proteins. No significant side effects were associated with DC vaccine therapy. Our first pt, who had measurable disease, subsequently underwent tumor regression after completing vaccine treatments and is in complete remission approximately one year later. These results demonstrate that autologous antigen pulsed DC are an effective method of stimulating cellular and humoral immune responses, and in one case, a significant clinical response has been induced.

C1-315 HUMAN EPIDERMAL LANGERHANS CELLS *IN SITU* CONTAIN A REGULAR MHC-CLASS II COMPARTMENT AND FORM A CLASS II POSITIVE RETICULO-EPITHELIAL NETWORK FOR EFFICIENT ANTIGEN CAPTURE AND PRESENTATION.

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MHC class II molecules expressed on the cell surface of antigen presenting cells play a crucial role in the presentation of foreign peptide antigens to CD4 positive T cells. In the skin, epidermal Langerhans cells (LC) exert this specialized function and they are known to be very potent initiators of specific immune responses. Exogenous antigens need to be internalized and degraded to immunogenic peptide fragments that can then bind to class II in an intracellular compartment that has recently been identified. Using a sensitive immunogold assay coupled to electron microscopic analysis we performed double-labelling experiments with mAbs to class II and to LAMP-1, CD63 and α-glucosidase, specific markers for organelles of the endosomal/lysosomal system. Our results show expression of class II molecules on intracellular, electron dense vesicular structures, and co-localization of class II molecules and the markers for late endosomes and early lysosomes in human LC *in situ*. Expression of these markers was never found on Birbeck granules, a LC specific organelle. This suggest the presence of an MHC class II compartment in LC that is similar to that found in other antigen presenting cells and that the Birbeck granules are not involved in antigen processing or presentation. In the epidermis LC expressed class II molecules on the plasmamembrane of the dendrites, resulting in a class II positive reticulo-epithelial network, but not on the surface of the cell body. In contrast, isolated LC as well as activated LC *in situ* displayed an even and strong expression of class II molecules on their entire cell surface. These results indicate that the isolation procedure and/or activation results in redistribution and upregulation of class II molecules on the cell surface and they are in contrast with the current concept that LC constitutively express high amount of class II on their entire cell surface. These results however, are consistent with the view that exogenous peptide antigens bind to newly synthesized class II molecules in an endosomal/lysosomal compartment and are subsequently expressed on the cell surface.

C1-316 NAIVE VERSUS PREACTIVATED T CELLS EXHIBIT DIFFERENT SUSCEPTIBILITY TO IL-1ß OR IL-6 DURING ACTIVATION INDUCED BY PARAFORMALDEHYDE-TREATED LANGERHANS CELLS,

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Activated Langerhans cells (LC) represent a paradigm for dendritic cells (DC), the principal antigen presenting cells for the initiation of primary immune responses. We have recently shown that LC produce substantial amounts of IL-1ß and IL-6 upon activation in vitro and that paraformaldehyde (PFA)-treated LC can induce strong proliferation in allogeneic T cells in the presence but not in the absence of these cytokines.

To further analyze the role of IL-1ß, or IL-6 in T cell activation, PFA-treated LC were cocultured with allogeneic $CD4^+$ or $CD8^+$ T cells, either depleted of CD44 expressing (memory) cells or not. When cocultures with CD44 non-depleted $CD4^+$ or $CD8^+$ T cells were supplemented with rIL-1ß (250 pg/ml) and/or rIL-6 (50 U/ml), we found that either cytokine costimulated vigorous proliferation in the responders and that both cytokines together were active in an additive fashion. In contrast, IL-6 but not IL-1ß was able to costimulate proliferation of naive ($CD44^-$) $CD4^+$ and $CD8^+$ T cells. Again, the response was further enhanced when both cytokines were present in the cultures. In the absence of stimulatory cells, neither T cell subset was able to respond by proliferation to the cytokines administered. Our data strongly suggest that IL-6 but not IL-1ß may serve as an important soluble costimulatory molecule during activation of naive ($CD44^-$) T cells by LC/DC, and that IL-1ß augments proliferation of preactivated responder T cells.

C1-317 COMPARATIVE ANALYSIS OF TRANSPORT ASSOCIATED PROTEIN ALLELES IN GORILLA: A PHYLOGENETIC APPROACH.

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Transport Associated Proteins (TAPs), which are necessary for the cell surface display of class I molecules, are encoded within the Major Histocompatibility Complex by two genes, TAP1 and TAP2. They form a heterodimer which is involved in the transport of intracellular peptide antigens into the lumen of the endoplasmic reticulum for subsequent presentation by class I molecules. Allelic variants of TAP genes impart specificity to the process of peptide acquisition by class I.

In our laboratory highly divergent TAPs have been identified in a gorilla cell line which also expresses a highly divergent class I molecule. This suggests the use of the gorilla as a model system in which to understand how allelic variants of TAPs affect peptide selectivity. RNA prepared from EBV-transformed gorilla B-cell lines was used to synthesize first strand cDNA and TAP coding sequences were amplified by PCR using primers based on human TAP sequences. The resulting PCR products were subcloned into appropriate vectors and sequenced.

In this work, TAP1 sequences from gorilla cell lines are presented and the divergence between gorilla TAP1 and TAP1 from other species is examined. Polymorphisms between gorilla and human TAP1 sequences are compared for possible effects on protein function. Experiments are also being designed to transfect full-length TAP cDNAs into TAP1/2 negative cell lines to test the ability of divergent gorilla TAP alleles to supply peptides to normal class I molecules. This will test the hypothesis of coevolution and coordinate function of TAP and class I genes.

C1-318 **MHC CLASS I^+/II^- DENDRITIC CELLS SENSITIZE FOR TRANSPLANTATION IMMUNITY,** P. Lenz , A. Elbe, G. Stingl and P.R. Bergstresser*, DIAID, Dept. of Dermatology, VIRCC, University of Vienna, Austria, *Dept. of Dermatology, UT Southwestern Medical Center, Dallas, TX.

MHC molecules expressed by dendritic cells (DC) play a critical role during allostimulation, although the relative contributions of Class I and Class II determinants have not been fully discerned. Recently a unique DC cell line (80/1), derived from C3H ($H\text{-}2^k$) fetal skin, was established in our laboratory, enabling us to study the role of MHC Class I molecules. 80/1 DC express Class I determinants but lack constitutive or cytokine inducible Class II expression, and they are potent stimulators of highly purified allogeneic, naive $CD8^+$ T cells in vitro. 80/1 DC were employed in an allogeneic skin transplantation system to immunize BALB/c ($H\text{-}2^d$) recipients. 20 days later, skin from C3H donors was grafted and inspected daily until rejected.

Conventional skin grafts were rejected with a mean survival time (MST) of 10.1 days (first set), while second grafts had accelerated (second set) rejections (MST 7.3 d; $p<0.001$). Mice that had received 10^4 80/1 DC subcutaneously (s.c.) rejected C3H skin grafts in a second set fashion (MST 7.7 d; $p<0.001$), indicating that 80/1 DC are immunogenic. This effect was shown to be specific, because 80/1 cells failed to affect rejection of third party grafts from C57BL/6 ($H\text{-}2^b$) mice. As few as 10^2 80/1 DC injected s.c. were effective (MST 8.6 d; $p<0.05$), indicating their high in vivo potency. This conclusion was supported further by the observation that BALB/c mice immunized with 10^4 3 day cultured Langerhans cells, which represent the most effective cutaneous stimulators of T cell responses, rejected C3H skin grafts with a similar second set tempo (MST 7.8 d; $p<0.001$). In contrast, non-APC, e.g., the fibroblast cell line L929 (C3H origin), failed to accelerate skin graft rejection when administered in the same numbers (10^4) s.c. (MST 9.3 d; $p=0.3$), indicating that 80/1 DC are professional APC cells.

We conclude that MHC Class I^+/II^- 80/1 DC induce transplantation immunity, and that in vivo, DC-driven MHC Class I-restricted T cell activation can occur in the absence of Class II expression.

C1-319 GM-CSF OVERRIDES THE IMMUNOSUPPRESSIVE FUNCTION OF CORTICOSTEROIDS ON RAT PULMONARY DENDRITIC CELLS, Tao-Keang Lim, Gwo-Hsiao Chen, Rod McDonald, Galen B. Toews, Division of Pulmonary and Critical Care Medicine, University of Michigan, Ann Arbor, MI 48109-0360

Pulmonary dendritic cells (DC) are potent antigen presenting cells. Pure populations of DC can be isolated from the lung following collagen digestion, Percoll gradient centrifugation, removal of phagocytic cells and fluorescence activated sorting for cells which exhibit high levels of class II MHC. Type II airway epithelial cells (AEC) and exogenous granulocyte-macrophage colony stimulating factor (rmGM-CSF) enhance while alveolar macrophages (AMø) suppress the immunostimulatory capacity of the pulmonary DC demonstrating that the function of DC may be regulated by neighboring cells and locally produced cytokines. Corticosteroids inhibit T cell proliferation and macrophage activation and are widely used immunosuppressive agents in pharmacotherapy. In this study, we examined the effect of dexamethasone (Dex) on the immunostimulatory activity of pulmonary DC. Rat lung DC were isolated and their immunostimulatory activity in the allogenic mixed lymphocyte reaction measured after incubation for 1 or 20 hours in either culture media alone or media plus various concentrations of Dex. Dex (10^{-6}M) pretreatment for 20h, but not 1h, resulted in 50-75% reduction in the stimulatory activity of DC [37,657 (control) vs 9,567 ± (Dex) cpm H^3-thymidine incorporation in culture containing 500 DC as stimulating cells]. This was associated with reduced intensity of surface MHC II staining. The inhibitory activity of Dex on DC was abrogated by 4ng/ml of GM-CSF [38,938 cpm]. Dex pretreatment of AEC and AMø did not modify their respective stimulatory and inhibitory effects on DC. Corticosteroids reduce the expression of class II MHC molecules and antigen presenting capacity of pulmonary DC in a dose- and time-dependent manner. However, GM-CSF overrides the immunosuppressive activity of corticosteroids on pulmonary DC. The immunosuppressive activity of corticosteroids on DC is likely modified by the local cytokine milieu.

C1-320 ANTIGEN PRESENTATION MEDIATED BY RECYCLING OF SURFACE HLA-DR MOLECULES, Eric O. Long, Oddmund Bakke*, and Valérie Pinet, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, NIH, Rockville, MD 20852, and *Department of Biology, University of Oslo, Oslo, Norway.

Class II molecules of the major histocompatibility complex associate with peptides derived from antigens that are processed in endocytic compartments. Antigen presentation to class II-restricted T cells generally requires newly synthesized class II molecules1, associated invariant chain, and HLA-DM. Exceptions to these rules have been reported but without description of an underlying mechanism. Here we show that presentation of an immunodominant epitope in the hemagglutinin protein of influenza virus correlates strictly with recycling of surface HLA-DR molecules. Truncation of either one of the α or β cytoplasmic tails virtually eliminated internalization of HLA-DR molecules and presentation of hemagglutinin from inactive virus particles. In contrast, the invariant chain-dependent presentation of matrix antigen from the same virus particles was unaffected by these truncations. Thus, HLA-DR cytoplasmic tails are not required for the conventional presentation pathway but jointly contribute a signal for an alternative pathway involving internalization of HLA-DR molecules.

C1-321 T-CELL AND B-CELL RESPONSES IN RESISTANT AND SUSCEPTIBLE CATTLE DURING PRIMARY INFECTION WITH TRYPANOSOMA CONGOLENSE, Vittoria Lutje and Katharine A. Taylor, International Laboratory for Research on Animal Diseases, Nairobi, Kenya

Bovine trypanosomiasis, caused by tsetse fly-transmitted Trypanosoma congolense, T.brucei and T.vivax, is endemic in sub-Saharan Africa and is characterized by anemia, weight loss and progressive cachexia. Certain cattle breeds are able to control parasite growth and anemia by mechanisms which are not yet well characterized and which might involve a superior immune response. The main features of the immune response elicited during infection are antibodies to the trypanosome variable surface coat (VSG) and to invariant antigens, and a general suppression of T-cell responses. IgM responses are similar in resistant and susceptible cattle, whereas more antigen-specific IgG1 are detected in resistant animals; this might be caused by an impairment of T-cell help. We analyzed T-cell and B-cell responses during primary infection with T.congolense in an attempt to elucidate differences in immune functions. Proliferative responses, IL-2 and gamma-IFN production were measured in lymph node cells draining the site of infection in a group of six susceptible Boran cattle and six resistant N'Damas. Antigen- and isotype-specific antibody responses were measured in spleen cells by an ELISPOT assay. Surface phenotype of LN cells was analyzed by cytofluorimetry. Proliferation to trypanosome antigens was detected in LN cells of cattle from both breeds; responses were higher 11 days post-infection and greatly reduced by 35 days p.i. This was paralleled by a reduction in responses to Con A. B cells from LN had surface activation markers such as IL-2 receptor and transferrin receptor, and antibody responses to VSG were detected in spleen cells, confirming a differential isotypic response between breeds. We don't know yet which subsets of T-cells are proliferating and how this short-lived response in the lymph node affects B-cell responses. However, we did not detect major quantitative differences in T-cell responses between the two cattle breeds.

C1-322 PRIMARY IMMUNE RESPONSE INDUCTION BY DENDRITIC CELLS

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Recently, we and others have identified antigens recognized by anti-melanoma cytotoxic T cells (CTL). These tumor-associated antigens provide potential targets for immunotherapy against melanoma. A key question is how immune responses against these antigens can be induced most efficiently. The potent antigen presentation capacity of dendritic cells renders them suitable for exploitation in the induction of these responses. We investigated the capacity of dendritic cells obtained from healthy donors to induce CTL responses against peptides derived from the melanocyte differentiation antigens gp100, tyrosinase or melan-A/Mart-I. For these purposes dendritic cells were generated by culturing highly enriched monocytes, obtained by centrifugal elutriation, in the presence of GM-CSF and IL-4 as described by Romani *et al.* (1994). When pulsed with peptides derived from the aforementioned antigens, these cells were able to induce a primary CTL response *in vitro*. Furthermore these CTL were capable of lysing melanoma cells, indicating that they recognized peptides derived from the melanoma differentiation antigens. These data demonstrate that CTL reactive with melanocyte differentiation antigens can be induced in healthy donors using dendritic cells.

While the powerful antigen presentation capacity of dendritic cells has been well documented, the mechanisms by which this is achieved have yet to be elucidated. We have employed the technique of differential display PCR in an attempt to identify novel proteins which are specific to dendritic cells and thus may account for the functional properties of these cells. This method allows for the selection of mRNAs which are differentially expressed in different cell types. Dendritic cell RNA, from at least three healthy donors, was compared to RNA from a panel of monocyte, B cell and T cell lines. Using multiple primer sets, numerous PCR products have been identified which appear to be specific to dendritic cells. Characterization of these cDNAs is ongoing and may provide insights into the role of dendritic cells in the induction of primary immune responses.

Romani, N. *et al.* (1994) *J. Exp. Med.* **180**:83.

C1-323 INDUCTION OF ANTIGEN SPECIFIC CD4+ T LYMPHOCYTES BY SENSITIZATION OF NAIVE PRECURSORS WITH ANTIGEN PRESENTED BY DENDRITIC CELLS, Anita Mehta-Damani, Sergiusz Markowicz and Edgar G. Engleman, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

The conditions required for sensitizing naive T cells to nominal antigens are poorly understood. In this report we describe an in vitro system for generating antigen specific CD4+ T cells from previously unprimed individuals. Freshly isolated CD4+ T cells were cultured with keyhole limpet hemocyanin (KLH), sperm whale myoglobin (SWM), or HIV gp160, antigens to which most persons have not been sensitized, in the presence of either dendritic cells (DC) or monocytes (Mϕ). In short-term (< 8 days) cultures, CD4+ T cells or their CD4+,CD45RA (naive) subpopulation mounted significant proliferative responses to KLH, SWM, and HIV gp160 but only if the antigens were presented by DC. In contrast, CD4+,CD45RO (memory) T cells responded poorly to these antigens, although they responded vigorously to tetanus toxoid, a recall antigen, presented by either DC or Mϕ. KLH and SWM specific CD4+ T cell lines were established from the in vitro sensitized starting population following repeated stimulation with antigen and Mϕ in medium supplemented with IL-2 and IL-4. Despite the continued presence of these cytokines during T cell expansion, the expanded lines retained their ability to respond to the priming antigen in the absence of exogenous cytokines. When the CD45RA and CD45RO subpopulations were sensitized and expanded separately, the CD45RA cells alone gave rise to antigen specific T cell lines, while the CD45RO cells proliferated nonspecifically. These results demonstrate that human naive CD4+ T cells can be sensitized, in vitro, to nominal antigens presented by DC and that the sensitized cells can be expanded into long-term lines that retain their antigen specificity.

C1-324 HALF-LIFE OF ANTIGEN/MHC COMPLEXES ON ANTIGEN PRESENTING CELLS (APC) IS REGULATED BY THE MICROENVIRONMENT *IN SITU*, Klaus-Peter Mueller and B. Kyewski, German Cancer Research Center, Tumor Immunology Program, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

We recently described that the half-life *in vivo* of antigen/MHC-class-II complexes on subsets of APCs [e.g. dendritic cells, B-cells, epithelial cells] differs between organs. No such differences however, could be observed when the half-lives were analyzed *in vitro*. Furthermore, pulsing APCs with peptide or intact protein yielded similar half-life values indicating that this parameter is independent of antigen processing (Eur. J. of Immunol., 1993, *23*: 3203). Extension of these studies show that the absolute values of the half-life differ according to the particular antigen/MHC complex studied, but the relative differences among distinct APC subsets remain the same. These results indicate that the half-life *in situ* is tightly regulated by the intact organ-specific microenvironment. We will report on parameters which influence the antigen/MHC half-life in the microenvironment *in situ*.

C1-325 *Abstract Withdrawn*

C1-326 DENDRITIC CELLS (DC) LOADED WITH SOLUBLE β-GALACTOSIDASE (β-GAL) PRIME CTL AGAINST β-GAL TRANSDUCED TUMOR CELLS, Paola Paglia, Monica Rodolfo, Giorgio Parmiani and Mario P. Colombo,Istituto Nazionale Tumori, Milano, Italy.

The priming of an immune response against an MHC-I restricted antigen expressed in nonhemopoietic cells involves the transfer of that antigen to a host bone-marrow-derived APC before its presentation to $CD8^+$ T lymphocytes. DC, as most efficient bone-marrow derived APC, are strong candidate even for tumor associated antigen presentation. The aim of our study was to induce tumor resistance following vaccination with DC which have processed a soluble antigen in vitro. Lacking well defined mouse tumor associated antigens, we took the advantage of β−gal transduced colon carcinoma C26 cells (C26-bag) as tumor model . For in vivo priming a murine DC line either β-gal transduced or in vitro loaded with soluble protein was used. Experiments were run in parallel by using fresh DC obtained in vitro from bone-marrow precursors. A β-gal antigen specific activation of $CD8^+$ T cells, measured as IFN-γ production and CTL induction, as well as a $CD4^+$ activation, observed as antigen specific proliferation, occurs in vitro following priming with either DC lines or fresh bone marrow derived DC loadedwith soluble β-gal. CTL which recognize $\beta\text{-gal}^+$ targets in MHC-I context, develop in MLTC of splenic lymphocytes from mice immunized with β-gal transduced DC cell line or fresh DC pulsed in vitro with the soluble protein. Partial in vivo protection (35%) against C26-bag challenge was obtained following vaccination with fresh DC loaded in vitro with the soluble protein. These results confirm that MHC-I restricted presentation of exogenous antigen could be mediated by DC and that these cells may play an essential role in the immunosurveillance of tumors.

C1-327 CTLA4-Ig + BONE MARROW INDUCES TRANSPLANTATION TOLERANCE IN THE MURINE MODEL. Thomas C. Pearson, Diane Z. Alexander, Rose Hendrix, Shannon C. Ritchie, Peter S. Linsley†, Christian P. Larsen. Emory University School of Medicine, Department of Surgery, Atlanta, GA 30322 and †Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA.

Allograft rejection is dependent on T cell activation, which requires both the engagement of the T cell receptor by antigen in the context of the MHC molecule and costimulatory signals delivered by cell surface molecules such as the B7/CD28/CTLA4 pathway. CTLA4-Ig is a fusion protein which blocks this pathway. We and others have shown that perioperative CTLA4-Ig treatment can prolong allograft survival and in some models produce transplantation tolerance. However, as a mono therapy, it is not universally successful. The current study was designed to test a protocol using CTLA4-Ig combined with donor bone marrow (BM) in the murine cardiac allograft model.

C3H/He recipients of BALB/c vascularized heterotopic cardiac allografts were treated intravenously with 2×10^7 donor bone marrow cells and 200 μg CTLA4-Ig at the time of transplant and then with three intraperitoneal doses of CTLA4-Ig (200 μg) given every other day. Our results demonstrate a quantitative and qualitative improvement in cardiac allograft survival and function using the combination therapy compared with CTLA4-Ig alone. All recipients (n=11) treated with BM + CTLA4-Ig had indefinite allograft survival (>100 days) as compared to the median survival time in untreated controls (14.5 ± 5.3 days, n=6), recipients treated with CTLA4-Ig alone (48.5 ± 10.5 days, n=10) or BM alone (11 ± 2 days, n=3). To test for transplantation tolerance recipients treated with CTLA4-Ig and BM were grafted simultaneously with donor (BALB/c) and third party (C57) skin 50 days following cardiac transplant without further treatment. While the third party grafts were rejected promptly, the donor specific skin grafts went on to indefinite survival without evidence of rejection.

These results demonstrate a significant improvement in cardiac allograft survival in the murine model when bone marrow transplant is combined with CTLA4-Ig treatment and raise the question of a functional role for stable hematopoietic chimerism in the establishment of CTLA4-Ig induced transplantation tolerance.

C1-328 **MONITORING ANTIGEN PROCESSING AND PRESENTATION IN MURINE DENDRITIC CELLS USING ANTIBODIES SPECIFIC FOR A T CELL DETERMINANT.**

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Short term culture of murine bone marrow in the presence of GM-CSF generates a mixture of adherent cells, including those with dendritic morphology, expressing class II MHC molecules. The intracellular location of class II MHC molecules in these cells is also heterogenous. Thus, while some cells have predominantly cell surface expression, others show pronounced accumulation in large cytoplasmic vesicles. It seems most likely that these different cell populations represent stages of differentiating dendritic cells.

A series of antibodies have been generated to IE^k associated with a peptide representing residues 95-103 of moth cytochrome c. Several of these monoclonals are highly specific for this MHC/peptide combination as shown by FACS analysis of peptide pulsed cells, ELISA on soluble IE^k bound to different peptides, or by immuno-precipitation. In addition, immuno-fluorescence studies show that these antibodies specifically stain IE^k expressing cells only after addition of antigen.

We are using these antibodies to determine the kinetics and intra-cellular location of IE^k/MCC complex formation in bone marrow derived dendritic cells following administration of peptide or intact antigen. In particular, we are investigating whether the observed class II MHC positive vesicles also act as a peptide loading sub-compartment.

C1-329 DENDRITIC CELLS USE TWO DIFFERENT PHAGOCYTIC MECHANISMS: POSSIBLE CONSEQUENCES FOR ANTIGEN PROCESSING

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The role of dendritic cells (DC) in the phagocytosis of microbes is a matter of debate. In this study we investigated the phagocytic capacity of human blood-derived DC and the murine spleen-derived DC line D2SC/1 by electron microscopy. The DC were incubated with the spirochete *Borrelia burgdorferi*, a microbe which is particularly difficult to internalize. It was found that only the minority of the DC engulfed the spirochetes, but these active cells readily started within minutes. Like other phagocytes, the DC used two different uptake mechanisms simultaneously, conventional and coiling phagocytosis. Conventional phagocytosis resulted in the membrane-bound intralysosomal degradation of the microbes, whereas coiling phagocytosis transported the engulfed spirochetes into the cytosol. Here, the microbes disintegrated without obvious participation of lysosomal vesicles. It is concluded that the use of these two different uptake mechanisms in parallel may lead to a dual processing of microbial antigens: the endosome-derived antigens will associate with MHC class II molecules and the cytosolic antigens will load MHC class I molecules, respectively. In summary, DC turned out to be effective phagocytes even with a microbe which is difficult to internalize. However, the reason for the dual antigen presentation to both T helper cells as well as cytotoxic T cells is not understood, since this will lead to the subsequent lysis of the antigen-presenting cell.

C1-330 INVARIANT CHAIN KNOCKOUT MICE A TOOL TO STUDY AUTOIMMUNE DISEASES

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Invariant Chain (Ii) is a glycoprotein that shortly after its synthesis, in the Endoplasmic Reticulum (ER), combine with MHC class II heterodimers. The resulting complexes exit the ER, travel through the Golgi apparatus and end up in the endosomal compartment where Ii is then proteolytically cleaved. Several functions have been proposed for the Ii: function in assembling or folding class II heterodimers, a role in segregating antigen presentation by MHC class I and class II molecules by blocking peptide binding to the latter the ER, and various roles in the transport of class II dimmers. To study the physiological role of Ii we had generated a mouse lacking this gene using homologous recombination in embryonic stem cells (ES Cells).

Recently, deficient mice for the Ii have been generated. Although, all the functions that have been proposed for the Ii were not yet studied, it appears that cells from mutant animals show aberrant transport of MHC class II molecules, and these do not have the typical compact formation, indicative of tight peptide binding. This finding might suggest that in the absence of Ii, MHC class II molecules may bind and present more often self peptides leading consequently to more susceptibility in developing autoimmune diseases. In this respect, we are using our deficient mice for the Ii to test this hypothesis. Our results will be discussed at the meeting.

C1-331 HLA-DM ACCUMULATES IN THE MULTILAMINAR MHC CLASS II COMPARTMENT,

Frances Sanderson, Monique J. Kleijmeer, Claire Thomas, Hans J. Geuze and John Trowsdale, Human Immunogenetics Laboratory, Imperial Cancer Research Fund, London WC2A 3PX, UK and Department of Cell Biology, School of Medicine, Utrecht University, The Netherlands.

HLA-DMA and *-DMB* encode an unconventional class II molecule that is required for the appropriate binding of peptide to classical HLA class II products. Sequence analysis suggests that *DM* originated at around the same time as class I and class II sequences split from one another. Computer modelling in collaboration with Paul Travers and Chris Thorpe based on the co-ordinates of DR1 crystals, indicates that the protein structure may be similar. Here we demonstrate that DMA and DMB associate as a heterodimer, which like its class II counterpart is stable in SDS. Unlike the classical class II molecules DM is not found in significant quantities at the cell surface. Within the cell it accumulates together with DR, CD63 and Lamp-1 in characteristic multilaminar compartments. These copartments may be the site at which class II peptide association takes place. Pertinent to this, data will be presented on the interaction between DM, DR and the invariant chain and on maturation of the iindividual DM protein chains.

C1-332 SUPPRESSOR T CELL-ACTIVATING MACROPHAGES IN UV-IRRADIATED HUMAN SKIN INDUCE A NOVEL FORM OF T CELL ACTIVATION CHARACTERIZED BY REDUCED EARLY ACTIVATION GENE IL-2Rα EXPRESSION,

Seth R. Stevens, Akihiko Shibaki, Laurent Meunier, and Kevin D. Cooper, Immunodermatology Unit, Dep.'t of Dermatology., University of Michigan., Ann Arbor, MI 48109

Because UV-induced macrophages (UV-Mph) preferentially activate $CD4^+$ T suppressor-inducer cells, we hypothesized that they induce different patterns of T cell early activation genes than do Langerhans cells (LC), which may, in turn, account for altered immune responses after UV-irradiation. We used epidermal cells from UV-exposed (UV-EC) and control (C-EC) human skin to stimulate resting allogeneic $CD4^+$ T lymphocytes. Semiquantitative PCR showed that both C-EC (LC as dominant APC) and UV-EC (UV-Mph as dominant APC) induced a 10^3 - 10^6-fold increase in IL-2 mRNA in each subject. No IL-2Rα mRNA was detectable 48 hours after stimulation with UV-EC (4/5 subjects, with an only 10^2-fold induction in the fifth subject). By contrast, C-EC-stimulated T cells showed a greater than 10^3-fold increase in IL-2Rα mRNA (5/5 subjects, p=0.004). Flow cytometry verified differential cell surface IL-2Rα protein. Whereas C-EC stimulated $CD4^+$ cell surface IL-2Rα upregulation in a dose dependent fashion from 4.1±2.3% of unstimulated T cells to 15.7±1.8%, UV-EC failed to do so (3/3 subjects). Costimulatory signals were provided by UV-Mph as evidenced by the ability of CTLA4-Ig and LFA3-Ig fusion proteins and antibodies to CD2, LFA3, LFA1 or ICAM-1 to inhibit T cell proliferation induced by UV-Mph. These data demonstrate that $CD4^+$ T cell activation by UV-Mph is distinct from activation by the dendritic Langerhans cell, and from previous models of suppression such as Th2 activation and Th1 anergy. Also, different APC types are capable of differentially upregulating $CD4^+$ T lymphocyte early activation genes; furthermore, UV-Mph do so by a novel mechanism. The initiation of different *in vivo* immune states (i.e. sensitization vs. tolerance) may be linked to differences in APC-induced genes important for T cell differentiation and function.

C1-333 A DENDRITIC-LIKE CELL MAY FUNCTION AS AN APC FOR *CRYPTOCOCCUS NEOFORMANS* AN ENCAPSULATED YEAST.

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C. neoformans is an encapsulated yeast that requires T cell immunity for host defense. Since some conditions that predispose to *C. neoformans* infections have defective antigen presenting cell (APC) function, we are interested in antigen processing of whole yeast, which is likely to be robust. T cells (T) were stimulated with *C. neoformans* in the presence or absence of various APC and ^{3}H-TdR incorporation was studied as a measure of proliferation. We found that T-lymphocyte proliferation was dependent upon APC (cpm±semx10^3; T 0.5±0.1; T +crypto 0.5±0.1; T+APC+crypto 25.1±6.5). To study the requirement for cytolytic cells, leucine methyl ester (LME) depletion was used. Depletion with LME did not eliminate APC function (T+PBMC+crypto 11.5±1.6; T+LME+crypto 9.5±1.8). As cytolytic cells were not necessary APC, we investigated B cells. B cell-depleted cells were still capable of operating as APC (T+PBMC+crypto 8.0±1.9; T+CD19 negative+crypto 19.4±3.1). Since neither of these cell types are necessary, we enriched for dendritic cell by isolating loosely adherent PBMC. A large percentage of loosely adherent cells were CD14 negative and CD19 negative but highly DR positive suggestive of a dendritic cell. Cells that were loosely adherent to plastic (25.1±6.5) were a better source of APC than adherent cells (6.0±1.1) or PBMC (2.0±0.6). These data suggest that the robust APC requirement for processing a whole yeast may be fulfilled by a dendritic-like cell.

C1-334 A SUBSET OF MACROPHAGES EFFICIENTLY PROCESS AND PRESENT NATIVE ANTIGEN TO NAIVE, ANTIGEN-SPECIFIC CD4$^+$ T CELLS FROM MICE TRANSGENIC FOR AN $\alpha\beta$ T-CELL RECEPTOR, William S. Walker, David Askew, Caterina Havenith, Janet Gatewood and Elvia Armendariz, Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105. In an earlier study, we showed that certain cloned macrophages derived from individual precursors residing in the mouse spleen can present alloantigens to naive CD8$^+$ T cells. In experiments reported here, we tested the ability of progeny of individual macrophage precursors to process and present intact, native pigeon cytochrome c (PCC), and a peptide of PCC (p88-104), to naive CD4$^+$ T cells from mice transgenic for a Vα11/Vβ3 T-cell receptor recognizing an epitope in the p88-104 fragment of PCC. Our results show that approximately 20% of splenic macrophage precursors give rise to progeny that constitutively process and present native PCC to naive CD4$^+$ T cells, as measured by IL-2 secretion and CD4$^+$ T-cell proliferation. The response was inhibited with anti-MHC class II antibodies and was a stable characteristic of the macrophage clones. Moreover, the ability to present p88-104 to naive CD4$^+$ T cells and alloantigens to naive CD8$^+$ T cells was restricted to the same macrophage subset that presented native PCC. Since in our previous study, differences in the presentation of alloantigens to naive CD8$^+$ T cells reflected the ability of some macrophages to elicit IFNγ from CD8$^+$ T cells and to respond to this cytokine by producing the required costimulator IL-1α, we are testing whether a similar explanation applies to the differences in antigen presenting activity for naive CD4$^+$ T cells. Whatever the mechanism, we have shown that a subset of macrophage precursors residing in the mouse spleen can give rise to cells that efficiently process and present native antigen to naive CD4$^+$ T cells. (Supported by AI-17979 and NS-32630 from the NIH, and by ALSAC).

C1-335 EFFECTS OF INHALED AND SUBCUTANEOUS SENSITIZATION WITH OVALBUMIN ON PULMONARY FUNCTION AND DENDRITIC CELL DISTRIBUTION IN GUINEA PIG. T.E. Warner, L.L. Millecchia, D.G. Frazer, and J.S. Fedan. Dept. of Pharmacol. & Toxicol., West Virginia Univ., and Physiol. Sect., NIOSH, Morgantown, WV 26505.

In recent years, the relationship between airway hyperreactivity in asthma and airway inflammation has been of interest. In this study of antigen-induced airway inflammation, we compared basal specific airway resistance (SRaw) and breathing frequency response with the distribution and prevalence of dendritic cells (DC) present within guinea pig airways following antigen sensitization and challenge with ovalbumin (OVA). Male Dunkin Hartley guinea pigs were sensitized and challenged with OVA by one of four protocols. Group A and B animals were sensitized with 1% OVA aerosol for 3 min on Day 0 and Day 7. Group A animals were challenged with 2% OVA aerosol on Day 14 for 3 min, whereas Group B animals were challenged until dyspnea occurred. Group C and D animals were sensitized by subcutaneous injection containing 10 µg OVA + 1 mg $Al(OH)_3$ on Day 0 and Day 14. Group C was challenged on Day 21 with 2% OVA aerosol for 3 min, whereas Group D was challenged until dyspnea appeared. Controls (Group E) received saline aerosol for 3 min on Day 21. Guinea pigs were sacrificed 18 hr post-challenge. Trachea and lungs were infused with Histocon, removed and snap-frozen. Consecutive tangential sections (6µ) were incubated with monoclonal antibodies to MHC class II (Cl.13.1) or tissue macrophages (MR-1) (APAAP procedure). DC were identified by positive staining with Cl.13.1, negative staining with MR-1, and morphology.

Immediately following OVA challenge increases in breathing frequency were seen in Groups C and D. Increases in basal SRaw were observed in Groups B and D. Groups A and E showed no significant changes in breathing frequency and SRaw.

Inflammation was observed in all OVA-treated animals. DC were distributed within the lamina propria and submucosa of the trachea and in the lamina propria and adventitia of the upper bronchial regions, particularly clustering around blood vessels. No DC were found within the epithelium or in the alveolar regions. Regardless of protocol, OVA-treatment increased DC density in OVA-challenged animals.

Our findings indicate that 1) OVA produced a sensitization-route dependent effect on pulmonary function and 2) all OVA treatments increased the prevalence of DC in association with inflammation.

C1-336 SPECIFIC EXPRESSION OF THE Rel/NF-κB FAMILY MEMBER RelB IN DENDRITIC CELLS: ANALYSIS IN *relB*$^{-/-}$ KNOCKOUT MICE

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We have studied the expression of the immediate-early gene *rel*B, a member of the *rel/nfkbi* family of transcription factors, using in situ hybridization and immunocytochemical analysis. The results show that the expression of the *rel*B gene is highly restricted to a subpopulation of cells that colonize the lymphoid tissues and that appear very late during the process of hematopoietic diversification. Low *rel*B expression is observed in the thymus at late stages of embryogenesis but rapidly increases after birth. In adult lymphoid tissues, *rel*B is detected in the medullary region of the thymus, the periarterial lymphatic sheaths of the spleen, and the deep cortex of the lymph nodes, which correspond to the regions where T cells of mature phenotype and interdigitating dendritic cells are present. Using double immunofluorescent labeling of thymic cell suspensions, we have identified the interdigitating dendritic cells as the target of RelB expression. These observations indicate that RelB may play a particular role in the signal transduction pathways that regulate dendritic cell differentiation and its cellular responses.

To further elucidate the function of RelB in vivo, we have generated mice carrying a germline mutation of the *rel*B gene. Mice homozygous for the disrupted *rel*B locus do not have any phenotypic abnormalities at birth. However, as early as 8-10 days after birth, RelB-deficient animals present marked differences from wild-type and heterozygous littermates, including multifocal inflammatory cell infiltration in several organs, myeloid hyperplasia, splenomegaly due to extramedullary hematopoiesis, and a reduced population of dendritic cells in the thymus. In addition, RelB-deficient mice have an impaired cell-mediated immune response as revealed by delayed-type hypersensitivity experiments. Thus, RelB plays a decisive role in the homeostasis of the hematopoietic system and its absence cannot be functionally compensated by any other member of the Rel/NF-κB family. The RelB-deficient mice may be a useful animal model to study dendritic cell function.

C1-337 Phenotype And Functional Properties Of Human Blood Dendritic Cells That Selectively Express CD83, Liang-Ji Zhou and Thomas F. Tedder, Department of Immunology, Duke University Medical Center, Durham, NC 27710

Dendritic cells are potent antigen presenting cells that initiate primary T cell-dependent immune responses. The lack of lineage-associated cell surface antigens for human dendritic cells had made characterization of this cell lineage difficult. In this study, CD83+ cells isolated from human blood were shown to be a phenotypically homogenous and unique population of cells with all of the properties of dendritic cells. CD83+ cells displayed a typical dendritic cell morphology with an irregular nucleus and extensive delicate cytoplasmic projections and veils extending from all aspects of the cell body. CD83 was not expressed by other blood leukocytes. A large panel of monoclonal antibodies that react with important leukocyte cell surface structures were used to phenotypically characterize human blood dendritic cells by two-color immunofluorescent staining in combination with anti-CD83 monoclonal antibodies. In addition to characteristic high expression levels of MHC class I and II molecules, this study demonstrated that CD83+ dendritic cells express some T cell markers like CD1c, CD2, CD5 and CD99; B cell markers like CD40, CDw78, CD80 (B7-1), CD82 and CD86 (B7-2); myeloid and platelet antigens like CD9, CD13, CD15s, CDw17, CD33, CD36, CD41/61 CD42a and CD63; and numerous other leukocyte antigens and adhesion structures. In comparison with other populations of leukocytes, CD83+ cells were the most potent stimulator cells in allogeneic mixed lymphocyte reactions. Blocking experiments revealed that MHC class II, CD11a, CD40 and CD86 (B7-2) played functionally dominant roles in the specialized costimulatory activity of CD83+ cells. Furthermore, cross inhibition assay demonstrated that CD80 and CD86 accounted for the vast majority of CD28/CTLA-4 stimulatory activity for T cell activation and therefore the existence of additional ligands for CD28 and CTLA-4 on CD83+ cells is unlikely. Taken together, these data indicate that CD83 serves as an unique and specific marker for human blood dendritic cells.

Follicular Dendritic Cells; Dendritic Cells in AIDS

C1-400 PROTEIN ANTIGEN PRESENTATION BY EPIDERMAL LANGERHANS CELLS IN NORMAL-APPEARING SKIN OF INDIVIDUALS WITH AIDS IS NORMAL

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Dendritic cell dysfunction has been postulated to play an important role in the immune dysregulation observed in HIV disease. Langerhans cells (LC), epidermal dendritic cells, can be infected with HIV both *in vivo* and *in vitro*, although the functional consequences of LC infection on immune and inflammatory responses have not been thoroughly studied. In a previous study, we found that alloantigen presentation by LC from individuals with AIDS was impaired, but that alloantigen and protein antigen (Ag) presentation by LC in earlier stages of HIV disease was normal. To further investigate LC function in patients with advanced HIV disease, we studied protein Ag presentation by LC in two monozygotic twins with AIDS (CD4+ T cell (TC) counts of 9 and 16). HIV-seronegative twin brothers served as controls. LC were obtained from suction blister tops of normal skin from both patients and controls and were co-cultured with autologous or syngeneic (twin) TC, with and without protein Ags (influenza virus or tetanus toxoid), and with or without IL-12 or anti-IL-10 antibody. The percentage of LC in epidermal cell suspensions and the level of HLA-DR expression by LC was similar between AIDS patients and controls (as determined by flow cytometry). Ag-specific IL-2 production (as detected by ELISA) was comparable in cultures containing control TC and LC derived from either the AIDS patients or controls. IL-2 was not detected in unsupplemented cultures containing TC from the AIDS patients; however, the addition of anti-IL-10 antibody partially reversed IL-2 production in response to influenza in these cultures, whereas IL-12 had no effect. Thus, these studies indicate that protein Ag presentation by LC is normal in advanced HIV disease, and that HIV-associated Ag unresponsiveness as a result of TC dysfunction can be partially reversed *in vitro* by antibody to IL-10.

C1-401 HIV ON FOLLICULAR DENDRITIC CELLS (FDC) IS HIGHLY INFECTIOUS, Gregory F. Burton, Sonya L. Heath, J. Grant Tew and John G. Tew. Dept. of Microbiology & Immunology, Virginia Commonwealth Univ, Richmond, VA. 23298.

HIV is found in the form of immune complexes on FDC in lymphoid follicles. In this study we sought to test the hypothesis that HIV in the FDC associated immune complexes is infectious and plays an important role in viral pathogenesis. To test this, FDC from uninfected tonsils were pulsed with HIV immune complexes, washed to remove unbound virus and cultured with activated CD4+ T cells. PCR analysis was used to detect infection. HIV immune complexes were formed using HIV and serum from a seropositive patient. These were incubated with sorted FDC, washed and cultured with superantigen activated (SEE) autologous T cells. Infection was present in cultures with HIV-bearing FDC and activated T cells but not in controls. To ensure that infection was transferred on FDC and not by contaminating CD4+ cells and that FDC did not need to be infected to transfer the virus, we used xenogeneic (murine) FDC in place of human FDC. Infection was again observed where HIV immune complexes on FDC were cultured with activated T cells but not with controls of FDC plus virus alone. These results indicate that FDC retained HIV immune complexes can infect activated T cells. Since many HIV seropositive individuals make neutralizing antibody, we sought to determine if FDC could convert neutralized HIV into an infectious form. Several doses (pg to mg) of neutralizing antibody were used to form immune complexes with HIV and these were cultured with susceptible T cells ± FDC. While no infection was seen in cultures of HIV immune complexes and activated T cells, infection was clearly observed when FDC were present. These data indicate that HIV complexes on FDC are infective and that FDC can convert neutralized HIV into infectious virus. This may help explain why HIV infected individuals with high levels of neutralizing antibody still have ongoing infection and supports the hypothesis that FDC may play an important role in HIV pathogenesis. Supported by NIH grant AI32406.

C1-402 MECHANISMS UNDERLYING RETROVIRAL VECTOR MEDIATED INDUCTION OF HIV SPECIFIC CTL

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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 intramuscular injection of non-replicating recombinant retroviral vectors containing the genes encoding HIV-1 envelope, ovalbumin or ß-gal. We have also demonstrated that direct intramuscular administration of retroviral vectors encoding HIV-1 env can elicit CTL responses in baboons and macaques. We have demonstrated foreign antigen expression (HIV-1 env and ß-gal) in the muscle fibers of injected mice and have detected provector sequences in draining lymph nodes. These studies are designed to examine the relative roles of muscle cells, dendritic cells and macrophages in antigen presentation and in the induction of systemic immune responses. Results from these studies will be presented.

C1-403 DENDRITIC CELLS, QUINOLINIC ACID, AND THE MURINE MODEL OF AIDS, Michael G. Espey, Yao Tang,* Herbert C. Morse III,* John R. Moffett, and M.A. Aryan Namboodiri, Department of Biology, Georgetown University, Washington, DC, 20057, *Laboratory of Immunopathology, NIAID, NIH, Bethesda, MD, 20892.

The inoculation of C57BL/6 mice with a mixture of LP-BM5 murine leukemia virus results in the development of a syndrome called murine AIDS (MAIDS). In addition to the development of late-stage lymphomas and enhanced susceptibility to infection, the pathogenesis of MAIDS resembles many of the immunodeficiency features of HIV infection such as $CD4^+$ T and NK cell anergy, polyclonal B cell activation, hypergammaglobulinemia, and a progressive TH1 to TH2 cytokine shift. The potent neurotoxin quinolinic acid (Quin), an endogenous metabolite in the kynurenine pathway of tryptophan degradation, has been shown to be significantly increased in the plasma and CSF of HIV infected individuals and is correlated with the severity of dementia. However, the cellular source of Quin has been a matter of dispute. Using MAIDS as a model of retrovirus-induced immunodeficiency, we have tested the hypothesis that the cellular source of Quin was in the systemic immune system. We determined Quin's relationship to the histopathological changes associated with MAIDS, using immunohistochemical techniques. The results demonstrated a dramatic increase in the number of Quin-immunoreactive dendritic cells early in the infection course. These novel findings may contribute significantly to the understanding of the relationship between dendritic cells, retrovirus-induced immunodeficiency, and Quin. The MAIDS model may be useful in developing a focused strategy towards resolving AIDS dementia complex.

C1-404 PRESENTATION OF SOLUBLE HIV-1 NEF PROTEIN OR LONG PEPTIDES *IN VITRO* TO CLASS I MHC-RESTRICTED CTL BY DENDRITIC CELL-ENRICHED POPULATIONS.

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Cytotoxic T lymphocytes (CTL) probably play a major role in controlling early HIV infection and in maintaining the clinically asymptomatic status of infected patients for several years. However, the immune system eventually fails. This work attempted to re-stimulate *in vitro* CTL from HIV infected patients with soluble, non-infectious antigens for a potential immunotherapy.

The method was set up first using spleen cells from HIV^+ patients suffering from idiopathic thrombopenic purpura (CDC stages IV A or E), then using 50 to 100 x 10^6 peripheral blood cells from four HIV^+ patients with CDC stages II or IV. Antigen presenting cell (APC) populations enriched for dendritic cells (typically 5%), macrophages and B lymphocytes were obtained. These APC were used to re-stimulate lymphocytes from the same patients *in vitro*, in the presence or absence of either purified recombinant nef protein, or long peptides (45 to 64 residues) encompassing the immunodominant regions of the protein.

CTL lines were obtained, which recognized specifically nef epitopic peptides (79-94), (80-100) and (190-198), in association with HLA A-11, -B8 or other HLA class I molecules. They also lysed target cells infected with HIV-1 nef recombinant vaccinia virus. CD8-positive lymphocytes were responsible for this cytotoxic lysis.

The restimulation method used to obtain these CTL is relatively simple. Moreover, long peptides (around 60 residues) encompassing immunodominant regions of an HIV protein are non-infectious and easy to obtain in large amounts. In comparison with 9 residue-peptides optimal for binding to a specific HLA molecule, they can re-stimulate cells from many patients with different HLA phenotypes. Therefore, the use of APC and long peptides may be proposed as a specific immunotherapy to induce class I-restricted CTL against HIV itself or against other pathogens (EBV, CMV) threatening HIV-infected patients when their specific cellular immunity fails.

C1-405 STIMULATION OF GC B CELL GROWTH BY FDC-LIKE CELL LINES, Han-Soo Kim, Xinhong Zhang and Yong Sung Choi, Laboratory of Cellular Immunology, Alton Ochsner Medical Foundation, 1516 Jefferson Highway, New Orleans, LA 70121.

To investigate the functional roles of FDC in the germinal center (GC) of lymphoid follicles, we have established a FDC-like cell line, HK. The phenotypic and functional analyses of HK cells suggest that these cells may be derived from FDC. HK cells bound and supported tonsillar B cell proliferation but not resting T cells. There was a preferential binding of $sIgD^-CD38^+$ B cells when B cells were co-cultured with HK cells, resulting in the rescue of these cells from apoptosis, whereas the majority of the unbound B cells underwent apoptosis. We investigated the effects of HK cells on the subpopulations of tonsillar B lymphocytes that are at different stages of maturation and differentiation. The subpopulations of tonsillar B cells were purified by panning and MACS according to the surface expression of IgD, CD38, CD44, and CD77. The data showed that HK cells preferentially bind, rescue and activate GC B cells (IgD^- $CD38^+$) generating $CD38^-CD44^+$ memory B cells in the presence of anti-μ or anti-CD40. The costimulatory activity of HK cells is partly provided in the form of soluble factor(s).

Anti-CD3 antibody activated T cells bound to HK cells and induced the phenotypic changes and growth of HK cells. The T-HK cell interactions involve not only the well-known adhesion ligand/receptor pathways, but also CD40-CD40 ligand as shown by inhibitory effect of soluble CD40 (CD40.Fc).

The cellular interactions between T and HK cells suggest that activated T cells not only stimulate B cells directly but also support B cell maturation indirectly by stimulating the development of FDC. Hence, HK cell line will provide an excellent experimental model for studying B cell growth and differentiation in GC.

C1-406 HLA CLASS I, CLASS II, AND TAP GENES STRONGLY INFLUENCE THE COURSE OF HIV INFECTION, Dean L. Mann, Richard A. Kaslow, Raymond Apple, Mary Carrington, Alvaro Munoz, Lawrence Park, Roger Detels, Charles Rinaldo, John Phair, James Goedert, Alfred Saah for the Multicenter AIDS Cohort Study (MACS) and the DC Gay Cohort (DCG). National Institutes of Health, Frederick, MD 21702, Bethesda, MD 20892.

Objective: To examine associations between HLA gene products and rate of progression of HIV-1 infection.
Methods: We HLA-typed 241 homosexual seroconverters using microcytotoxicity for alleles at class I (A,B) and PCR-based methods both for detecting alleles at class II (DRB1, DQA1, DQB1) and for determining TAP1 and TAP2 variants. AIDS developed in 137 men (100 in <7 yrs), and 104 remained AIDS-free for ~6-13 yrs (99 for ≥ 7 yrs) after conversion. Stratifications and Cox regressions including interactions were used to analyze the data.
Results: Seven low frequency HLA-A/B alleles were identified that were associated with rapid disease progression when the TAP 2.1 or 2.3 variants were present in the same individual. In addition, 4 DRB1-DQA1-DQB1 haplotypes were also associated with rapid progression (<4 yrs) only in association with the TAP 1.2 variant. Six HLA class I alleles were associated with prolonged disease-free interval (> 10 years), three were independent of TAP variants, and three were dependent on the presence of TAP 2.3. These data provide substantive evidence that interacting HLA class I and class II alleles and variation in TAP genes impact heavily on the course of HIV-1 infection. It is also quite apparent that the variants of the TAP genes play an important role in this regard and offer the first evidence, although indirect, that these genes regulate in part the peptides available for HLA class I antigen presentation.

C1-407 CUTANEOUS DENDRITIC CELL-T CELL CONJUGATES AS A SITE FOR ACTIVE INFECTION WITH HIV-1, Melissa Pope[1], Nancy Gallo[2], Lloyd Hoffman[3], Stuart Gezelter[1], and Ralph M. Steinman[1]. [1]Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY 10021, [2]New York Firefighters Skin Bank, New York Hospital, New York, NY 10021, [3]Division of Plastic Surgery, Cornell University Medical College, New York, NY 10021

Both dendritic cells and memory T cells [$CD4^+$ and $CD8^+$] migrate from organ cultures of split thickness skin that has been removed from donors undergoing reductive plastic surgery. The skin cell emigrants consist of free dendritic cells, free T cells, and dendritic cell-T cell conjugates. The three cell subsets can be separated by cell sorting; conjugates continue to form when sorted dendritic cells and T cells are recultured together. The skin cell emigrants are permissive to infection with several HIV isolates. Multinucleated syncytia are the major site of virus production. Infection with $HIV\text{-}1_{mn}$ is dependent on the presence of both dendritic cells and memory T cells, which fuse together to form the syncytia.

In addition to furthering our infection studies using the primary patient-derived isolates, we have investigated cadaver skin as a source of dendritic cells. Comparable numbers of dendritic cells and memory T cells [$CD4^+$ and $CD8^+$] migrate from cadaver skin relative to fresh skin. Some of the dendritic cells and T cells also form stable conjugates, and the three cell subsets are able to separated by cell sorting. The dendritic cells and T cells are functionally competent and susceptible to infection with HIV-1. Cadaver skin taken within 24 hours of death has provided access to a much greater number of cells, assisting in our studies of the infectibility of cutaneous dendritic cells with patient-derived isolates.

Infection of skin cell emigrants with either syncytia-inducing or non-syncytia-inducing HIV isolates requires both dendritic cells and $CD4^+$ T cells together. AZT blocks infection with all HIV isolates tested. Cutaneous dendritic cells and memory T cells alone show little if any sensitivity to infection with HIV-1, but together they create a microenvironment that is permissive to productive and cytopathic infection. This cellular milieu may play an important part in the transmission and production of virus in vivo.

C1-408 DIFFERENCES IN ENDOCYTIC PATHWAYS OF DCs AND MACROPHAGES EXPLAIN EFFICIENT HIV-1 CARRIAGE BY DCS.

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Blood dendritic cells (DCs) may play a critical role in the pathogenesis of HIV-1 infection by means other than their direct infection. CD4- blood DCs exposed to HIV-1, although uninfected, can efficiently transfer infection to CD4+ T cells, whereas cultured monocytes are less efficient. The ability of virus-pulsed DCs to transfer virus to T cells is transient and carriage occurs in a trypsin resistant compartment. To examine differences in the endocytic pathway of DCs and macrophages, uptake and endocytosis of latex beads by cells at different stages of maturation was observed using confocal microscopy and FACS analysis.

Freshly isolated (immature) blood DCs were found to take up beads of sizes ranging from 14nm to 2300nm, with a decrease in uptake as the bead size increased. In contrast, cultured (mature) DCs were less efficient at bead uptake than immature DCs. Cells incubated with beads in the presence of HLA-DR-FITC enabled beads within class II and non-class II containing vacuolar compartments to be identified using confocal microscopy. DCs incubated with 100nm beads and DR-FITC for 2 to 4 hours revealed the majority of beads residing in the endocytic and class II containing compartments, with only a small proportion forming aggregates in the non-class II containing compartments. In contrast, the majority of beads formed aggregates in monocytes after 3-4 hours in culture. This suggests the inefficient transfer of HIV-1 from monocytes to T cells is related to their ability to process and degrade virus in acidic compartments, whereas virus remains mainly in the non-acidic compartments of both mature and immature DCs, thereby enabling transfer to T cells. The number of beads within DCs was also found to decrease over 2 to 4 hours which may reflect the cell's ability to internalize and recycle vesicles in the endocytic pathway and, may explain the transient ability of DCs to transfer HIV-1 to T cells.

C1-409 BLOOD MONOCYTE-DERIVED CULTURED DENDRITIC CELLS ARE SUSCEPTIBLE TO HIV-1 INFECTION AND EFFICIENTLY TRANSMIT VIRUS TO RESTING T CELLS IN THE PROCESS OF NOMINAL ANTIGEN PRESENTATION, Yasuko T. Yokota, Kiyoko Akagawa, Hiroshi Kimoto, Sachiko Yasuda, Andreas Meyerhans and Toshitada Takemori, Department of Immunology, National Institute of Health, Tokyo 162, Japan, Department of Virology, Institute of Medical Microbiology and Hygiene, Freiburg, Germany

Dendritic cells (DCs) were generated from monocytes in PBMC in the presence of IL-4 and GM-CSF. They showed a unique dendritic morphology and potent presenting activity of alloantigen as well as bacteiral superantigen, SEB. They were positive for MHC class I and II, CD1, CD4, CD40, B7-1, B7-2, CD32, CD54, and CD58 but negative for CD3, CD8, CD14, CD20, CD16, CD56, and CD64. These properties were consistent with those of cultured immature DCs described by Sallusto and Lanzavecchia (J. Exp. Med.,1994). In order to study the susceptibility of these cultured DCs to HIV-1 infection, they were highly purified by a cell sorter and infected with a various strains of HIV-1. PCR analysis revealed the late product of provirus (LTR-gag) in DCs, which was abolished by an anti-CD4 antibody. Despite of complete cDNA synthesis, production of p24 antigen in the supernatant was not detectable by ELISA and the level of HIV-mRNA expression was low. Based on the frequency of HIV-1 infected DCs analyzed by quantitative PCR, these cultured DCs were more susceptible to T-tropic HIV-1_{Lai} than Mϕ-tropic HIV-1_{BaL} infection. Importantly, when HIV-1_{Lai}-infected DCs were co-cultured with autologous resting T cells for 7 days in the presence of nominal antigen, PPD (purified protein derivative) from M. Tuberculosis, p24 production was enhanced and numerous virus buddings were observed from activated T cells. In contrast, this enhancement was not observed in a similar co-culture of HIV-1_{Lai}-infected Mϕs. These data suggest a possibility that HIV-1-infected, monocyte-derived DCs play a role in the dissemination of T-tropic HIV-1 by acting as antigen presenting cells to resting T cells.

Late Abstracts

ROLE OF CYTOKINES IN THE INDUCTION OF TOTAL AND ALLERGEN SPECIFIC *IN VITRO* IGE SYNTHESIS

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Peripheral blood mononuclear cells (PBMC) from grass and tree pollen allergic patients were cultured for 7 days. The influence of IL-4, IL-13 and anti-CD40 antibody on the synthesis of total and allergen specific IgE synthesis was investigated. Total IgE was determined in the culture supernatants by RIA whereas allergen specific IgE synthesis was tested by immunoblotting using natural and recombinant tree and grass pollen allergens and subsequently quantified by densitometry. In contrast to the total IgE production which was significantly increased by addition of IL-4, IL-4+αCD40, IL-13; none of the added cytokines influenced the synthesis of allergen specific IgE.
These results suggest that production of allergen specific IgE *in vitro* is due to long-lived B cells.

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INTERACTION OF HIV-I, HTLV-I AND HTLV-II WITH IN VITRO-GENERATED DENDRITIC CELLS,

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Although it is known that impairment of dendritic cells (DC) plays a role in the pathogenesis and immunosuppression of retrovirus-asociated diseases, it is not clear whether, or to what extent, these antigen-presenting cells themselves become infected. The realization that the cells can be generated in vitro in larger numbers that can be freshly isolated from circulating blood or bone marrow raised the possibility that they could be used for therapeutic purposes. Therefore, we investigated whether DC generated in vitro from CD_{34} precursors are susceptible to infection by these viruses when cocultured with HIV or HTLV-infected cell lines. While there was remarkable affinity of the viruses to the plasma membrane and villous processes of the DC, no infection was observed in 30 experiments carried out over varying time periods. However, when CD_4 cells isolated from the same blood donor were infected with viruses and then subsequently cocultured with DC generated from the autologous stem cells, interiorization and budding of viruses was easily demonstrable. All studies were conducted on the ultrastructural level using the immunogold method to identify relevant epitopes on DC that had interacted with the viruses. The observations suggest that DC generated from heterologous stem cells may be more desirable than autologous specimens in a therapeutic setting e.g. to temporarily boost the afferent arm of the immune response in immunocompromised HIV or HTLV-infected patients.

DENDRITIC CELLS FROM THE CHOROID OF THE RAT PRESENT RETINAL ANTIGENS TO NAIVE T CELLS,

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The intraocular components of the eye are considered sites of 'immune privilege' due to the lack of a lymphatic system and absence of antigen presenting cells. However, our recent studies (Invest. Ophthalmol. Vis Sci. (1994) 35,64-77) have shown that intraocular uveal tissue contains a rich network of dendritic cells and ED1, 2 and 3 positive macrophages. We have now isolated these cells from both human and rat choroid and show that they express the DC pheontype. In addition, videotime lapse experiments identify two morphological cell types, a large cell with veils/ruffles and a smaller highly mobile cell. Using a sensitive cell polarisation assay (Newman & Wilkinson, Immunology (1993) 78,92-98) which permits evaluation of antigen presentation capacity with very small numbers of cells ($<1x10^3$) we have shown that choroidal dendritic cells, harvested from the rat eye, can present retinal auto-antigens to naive CD^{4+} T cells. Choroidal DC's are equally effective in this response as splenic DC's. These results indicate that immune privilege in the eye is not related to lack of immunocompetent APC's and that other mechanisms must be operative.