the positive reaction on the squamous epithelium obtained from the esophagus of a monkey. The antigenic substance is in all epithelial layers with the exception of the basal layer, which is always negative, as well as in human tissues. In figure 2, the attached indicator erythrocytes show the presence of an antigen in parenchymal cells of a pancreas, while the island of Langerhans is negative, again repeating the identical reactivity of a human pancreas. Figure 3 shows the reaction in the transitional epithelium of the urinary bladder. Similarly, in all available tissues from Cynomolgus monkey, the red cell adherence test showed almost identical localization of A, B and H antigens when compared with human tissues, with 1 exception; the fixed human erythrocytes, contained in lumina of vessels are always positive, while in the monkey this reactivity was never observed, confirming the results of previous studies that red cells of Old-World non-human primates do not contain A, B or H specific antigens.

The distribution of antigens is presented in the table. In all the organs listed, only the epithelial cells contained the antigenic material while smooth muscle and connective tissues were always negative.

Also, as in the human, the squamous epithelium of the skin contained the antigen only on the surface keratin layer.

An interesting phenomenon occurred in the Brunner's glands where all animals exhibited the presence of A antigen in addition to their own specific antigen (figure 4). Again, this shows certain similarity to human tissues, except that human Brunner's glands in persons of all blood types contain the H antigen[8]. Another unusual reaction was observed in salivary glands and sweat glands which contained in all animals the H antigen. (The weak or ± reaction with H antigen seen in the oral epithelium and stomach mucosa of monkeys Nos 1 and 3, and in the skin of monkey No. 3 could be possibly due to the passive absorption of an H antigenic material produced by salivary or sweat glands, respectively.)

Although not all the tissues were always available for our examination, the table shows clearly that monkey No. 1 was of A type, monkeys Nos 3, 5 and 6 of B type, and monkeys Nos 2 and 4 of 0 type. The lack of positive reaction in the intestinal mucosa of monkey No. 2 in repeated tests could not be explained.

Discussion. This work has attempted to demonstrate the presence of human A, B and H antigens in tissues of Cynomolgus monkey *(Macaca fascicularis),* and to compare the distribution and localization of these antigens with the results obtained on human material. The majority of tissue specimens from each individual animal possessed only 1 human type isoantigen with 2 exceptions. The salivary and sweat glands of all animals showed the presence of H antigen in addition to other specificity. This phenomenon could possibly support the hypothesis established in human tissues, that the production of H antigen is a necessary prerequisite for a subsequent transformation into A or B specificities by the action of genetically controlled enzymes which add the terminal sugars N-acetyl-galactosamine or D-galactose, thus converting the precursor to A or B active substances[8]. However, this explanation does not support the other exception in our study, i.e. the presence of A antigen in Brunner's glands of all examined animals.

The demonstration of the presence of A, B and H antigens in Cynomolgus monkey tissues can serve as a useful model system for the future studies about basic functions and localizations of tissue-associated polysaccharide materials such as blood group substances, and the basic differences in activity or function between the glycolipid and glycoprotein counterparts. Furthermore, the mechanism of the loss of isoantigens during malignant transformation of human cells[9] could be established.

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The effect of *Listeria monocytogenes* lipids on immune response to T-dependent and T-independent antigens

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Summary. The administration of *Listeria monocytogenes* lipids augmented the humoral immune response to ovalbumin (OVA), polyvinyl pyrrolidone and *E. coli* lipopolysaccharide, but failed to support the induction of delayed hypersensitivity to OVA.

Our previous studies have indicated that injection of *Listeria monocytogenes* lipids (LML) results in a significant increase of the humoral immune response to sheep erythrocytes and in an enhancement of resistance to some bacterial and fungal infections[2,3]. In the present work the effect of LML on the immune response to some T-dependent, ovalbumin (OVA)[4], and T-independent polyvinyl pyrrolidone (PVP) and lipopolysaccharide (LPS)[5] antigens was studied.

Materials and methods. Male Swiss albino mice weighing 16–18 g were immunized by i.v. injection of 250 µg OVA or 0.25 µg PVP, or 50 µg LPS in 0.2 ml of saline. Other groups of mice received injections of a mixture of antigen and 100 µg LML. The method of Cunningham and Szenberg was applied for detecting plaque-forming cells (PFC) in spleen[7]. Sheep erythrocytes (SE) coated with OVA[8], PVP[5,9] and LPS[10] were used both for hemolytic plaque assay and for estimation of specific antibodies in heat-inactivated and SE-absorbed sera. A method of passive microhemagglutination was used for determination of the specific antibody titer in native and 2-mercaptoethanol (2-ME)-treated sera. The selective removal of IgG, IgM and IgA was achieved

Table 1. *Listeria monocytogenes* lipids potentiate the humoral immune response of mice to 3 antigens

Immunization	IgM (log_2)	Adjuvant index	IgG (log_2)	Adjuvant index	IgA
Ovalbumin	1.6		1.1		ND
Ovalbumin + lipids	3.8	2.37	1.7	1.54	ND
Polyvinyl pyrrolidone	1.5		1.2		ND
Polyvinyl pyrrolidone + lipids	3.1	2.06	1.8	1.50	ND
Lipopolysaccharide	4.8		ND		ND
Lipopolysaccharide + lipids	8.1	1.69	ND		ND

Specific antibody titers were determined in serum samples pooled from 4 animals 7 days after immunization. Values represent mean values of 3 separate experiments. ND, not detected.

Table 2. Failure of *Listeria monocytogenes* lipids - ovalbumin mixtures to induce delayed hypersensitivity in guinea-pigs

Treatment	Skin reaction (mm)	Migration index (%)
OVA (1 mg)	2.1 ± 0.8	98.3 ± 11.5
Lipids (LML) + OVA	3.2 ± 1.5	79.8 ± 21.6
Lipids + Freund's incomplete adjuvant + OVA	2.9 ± 1.6	85.5 ± 15.4
Freund's incomplete adjuvant + OVA	3.4 ± 1.3	93.2 ± 8.7
Freund's complete adjuvant + OVA	12.3 ± 3.6*	30.7 ± 9.2*

Values represent the mean ± SD for groups of 6–8 guinea-pigs. * $p < 0.05$ (t-test).

by means of an immunoadsorbent (polyacrylamide gel beads containing rabbit antisera directed against mouse IgG, IgM or IgA)[11]. Radial immunodiffusion was employed for estimation of the total content of IgG, IgM and IgA in the sera studied and calculation of the recovery of Ig after immunoadsorption[12]. The specific rabbit anti-mouse IgG, IgM and IgA sera, and mouse IgG fraction were obtained from Miles Laboratories. In other experiments, guinea-pigs weighing 250–300 g received injections into their hind footpads of a mixture of OVA (1 mg) and LML (0.5 mg) or of a OVA-Freund's incomplete adjuvant-LML mixture. For comparison, two groups of animals received injections of a mixture of OVA in Freund's incomplete or complete adjuvant. Skin tests with 100 µg of OVA and the migration inhibition test[13] were performed 3 weeks after sensitization.

Results. LML administration significantly increased the number of direct PFC in the spleens of mice immunized with the 3 antigens studied (figure 1) and this was seen both after primary and secondary antigenic stimulation. The

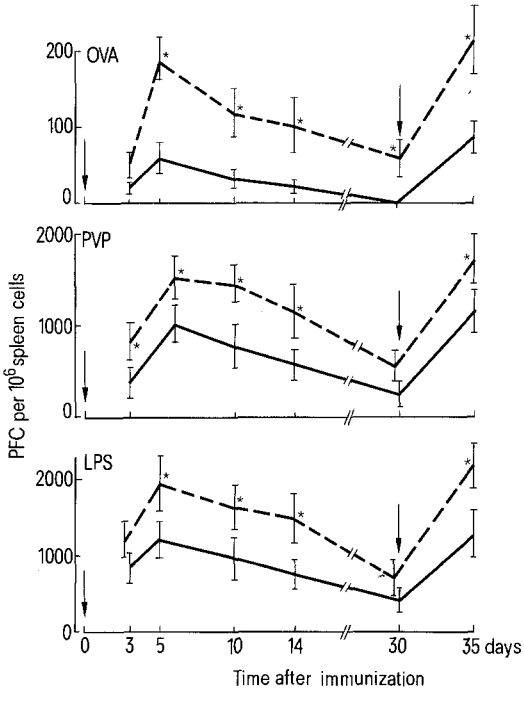

Fig. 1. Effect of *Listeria monocytogenes* lipids on the number of plaque-forming cells (PFC) in the spleens of mice immunized with ovalbumin (OVA), polyvinyl pyrrolidone (PVP) or lipopolysaccharide (LPS). ——, animals treated with antigen only. – – –, animals treated with antigen-lipids mixture. Each symbol represents mean ± SD of 6–8 determinations. Arrows indicate time of the 1st and 2nd antigenic challenge, and asterisks show statistically significant results; $p < 0.05$ (t-test).

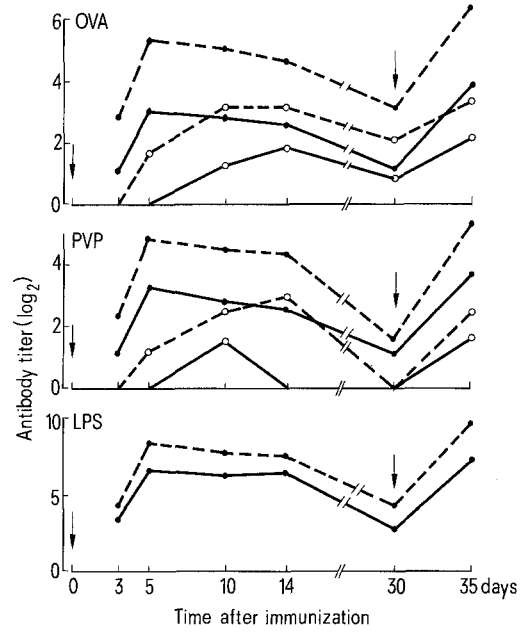

Fig. 2. Effect of *Listeria monocytogenes* lipids on the antibody titer in native (●) and 2-mercaptoethanol-treated (○) sera of mice immunized with OVA, PVP or LPS. Other designations as in figure 1.

increase in the number of PFC was accompanied by an increase in the specific antibody titers (figure 2). It should be noted that in response to OVA and PVP both 2-ME-resistant (7S) and 2-ME-sensitive (19S) antibodies appeared while in the mice injected with LPS only 19S antibodies could be detected. The results in table 1 indicated that LML more significantly increased the elaboration of IgM antibodies rather than those of IgG class. However, no differences were found in the total content of IgG, IgM or IgA in the sera of mice which had received antigens only or an antigen-LML mixture. In contrast to Freund's complete adjuvant, LML administered together with OVA to guinea-pigs failed to induce delayed hypersensitivity to this antigen (table 2).

Discussion. Our data indicate that LML could be regarded as a B- rather than T-orientated adjuvant. This conclusion is based on the observation that LML more significantly augmented the production of IgM than IgG. It is believed that IgG production is more dependent on T-cell function[14]. However, the possibility of the influence of LML on helper T-cell activity could not be excluded. The inability of LML to aid OVA in the induction of delayed hypersensitivity also implies a lack of influence on T-lymphocyte activity. The observed increase of the immune response to T-independent antigens also supports this conclusion, because B-orientated adjuvants are very active in potentiating the response to the antigens mentioned above. T-orientated adjuvants are rather ineffective in augmenting the response to T-independent antigens or they even suppress the immune response to them[15–18]. The results obtained suggest that LML may exert their adjuvant action through direct activation of B-cell proliferation. It is interesting to note that many bacterial species are mitogenic for B-lymphocytes[19]. Probably mitogenic activity of LML is associated with phospholipids which constitute about 80–85% of the total lipids of *Listeria monocytogenes.* It has been found that some bacterial phospholipids are mitogenic for B-lymphocytes[20].

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Induction of interferon by levamisole and concanavalin A in HA/ICR and NZB/W mouse spleen cells

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Summary. Levamisole alone and in combination with concanavalin A (Con A) induced interferon in splenic cultures of NZB/W and HA/ICR mice. Levamisole did not potentiate interferon induction by Con A. HA/ICR mice consistently produced greater amounts of interferon than NZB/W.

The antihelminthic drug, levamisole (2,3,5,6-tetrahydro-6-phenyllimidozo-(2,1-b)-thiazole) has been shown to have beneficial effects for the treatment of the spontaneous autoimmune disease of NZB/W mice. Russel[1] demonstrated that this drug significantly decreased the mortality of these mice, and postulated that the mechanism of its therapeutic action involved improved cell-mediated immunity. Since this disease has been associated with a viral etiology as well as an abnormal immune response[2], interferon might be suspected as a possible mediator of levamisole's therapeutic action. Matsubara et al.[3] recently showed that levamisole does induce interferon in DDl mice in vivo. Heremans et al.[4] however, demonstrated that when given exogenously, interferon actually increased the severity of the autoimmune disease in NZB and NZB/W F_1 hybrid mice. In order to gain more insight into this paradox, the interferon-inducing ability of levamisole, alone and in combination with concanavalin A (Con A) was investigated in NZB/W as well as HA/ICR mouse cultures in vitro.

HA/ICR female mice were purchased from Jackson Laboratories (Bar Harbor, Me.). NZB/W F_1 hybrid mice (NZB/W female × NZB male) were bred locally. Mice were housed 5–6 per cage and maintained on commercial rodent chow and tap water ad libitum until 9 months of age.

Concanavalin A (Difco Labs, Detroit, Ml.) was obtained as a sterile powder. Levamisole was obtained through the courtesy of Janssen Pharmaceutica (Beerse, Belgium). RPM1-1640 medium was obtained from Gibco (Grand Island, N.Y.) and used for all splenic cell cultures. The media was supplemented with 10% heat-inactivated fetal calf serum (Sterile Systems, Inc., Logan, Utah), 100 units/ml of penicillin G sodium (E.R. Squibb and Sons, Inc., Princeton, N.J.) and 100 µg/ml of streptomycin sulfate (Pfizer, Inc., New York, N.Y.). In addition, 0.025 M H-2-hydroxyethylpiperazine-N'-Z'-ethanesulfonic acid (HEPES) buffer (Gibco) was added to the media. The pH was adjusted to 7.2 with $NaHCO_3$.

Spleens were removed from mice and were gently teased. The cells obtained were suspended at 5×10^6/ml media. The cell suspensions were added to tissue culture tubes in