

The mammary gland and neonate mucosal immunity

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Accepted 22 September 1999

Abstract

The passive mucosal protection of neonate mammals is dependent on the continuous supply until weaning of maternally dimeric IgA (monogastric) and IgG1 (ruminants). This lactogenic (humoral) immunity is linked to the gut, the so-called entero-mammary link, because of the translocation of Ig (IgA and IgG1) or the emigration of IgA lymphoblasts from the gut into the mammary gland (MG); on the other hand, studies on the lymphocyte subsets in the MG of artiodactyls sustained the view of a true local immune response, depending on the MG stage development. Accordingly, the increase of the lactogenic immunity may focus on (1) inductor sites (gut and, possibly, the MG), (2) increase in cell traffic from the gut into the MG, and (3) enhancement at the effector site of the Ig production and excretion in milk.

A specific mucosal environment (interleukins and hormones) is responsible for IgM/IgA switch, the induction of mucosal homing receptor onto lymphoblasts and mucosal vascular addressins; very few data are available for the mechanism of lymphoblasts recruitment, either IgA or IgG1, although lactogenic hormones have been implicated in the IgA-blasts homing into the mice MG.

After weaning, the neonate is able to mount a gut immune response, but the shortage of the suckling period did not seem to be detrimental for its onset. In soyabean allergy, both piglet and calf exhibited gut villus atrophy, gut accumulation of IgA (swine) and IgG1 (cattle) immunocytes, sustaining the view that a specific environment in ruminant is responsible for both IgA and IgG1 production. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Mammary gland; Neonate; Gut-lymphocyte homing; Soyabean allergy

1. Introduction

Except for humans, lagomorphs and rodents that do benefit from a transplacental passage of maternal serum antibodies during embryonic development, other eutherian mammals (ungulates), who have a placenta impermeable to immunoglobulins (Ig), are born hypo- or agammaglobulinemic (Salmon, 1984). Although immunocompetent, ungulates can elaborate only a primary immune response, since being devoid of antigenic

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stimuli during fetal life: this response requires a lag period that is too long in relation to the rapid proliferation of many infectious agents, such as colibacillosis and salmonellosis septicemia, thus precluding any active immunisation of the piglet. During the first few hours of life, their survival depends upon the ingestion of colostrum, which provides the newborn with the maternal serum antibodies that arose from antigenic stimulation of the mother's systemic immune system. This antiseptic immunity results in the destruction of pathogens.

Due to the lack of previous antigenic sensitization, the young mammal is unable to develop its own local immune responses that will protect its intestine and respiratory mucosa, the first sites to be invaded and challenged by environmental antigens. The protection against local pathogens proliferating either in contact (such as *E. coli*) or into the gut epithelial cells (such as the transmissible gastroenteritis virus, TGEV) is transmitted mainly by milk in ungulates until weaning. It has been shown originally in swine that this lactogenic immunity (Hooper and Haelterman, 1966) is associated especially in the form of secretory IgA (S(IgA)₂) (Bohl and Saif, 1975) and more generally exhibit a specificity for the antigens and micro-organisms present in the maternal digestive tract, the so-called entero-mammary link.

In adult ruminants, IgG1 predominate over IgA in intestinal and mammary secretions, but IgA predominate in nasal, bronchial, tears and saliva. (Butler, 1998). Although these findings may suggest that IgG1 is efficient in mucosal protection in polygastric, it does not rule out the major role of IgA in mucosal protection and especially in monogastrics where IgA is the predominant immunoglobulin found in secretions of adult (whilst in young IgM predominate).

On the other hand, studies on the lymphocytes subsets in the mammary gland (MG) of artiodactyles sustained the view of a true local immune response depending on the MG stage development; accordingly, the increase of the lactogenic immunity may focus on (1) inductor sites (gut and, possibly, the MG), (2) increase in cell traffic from the gut into the MG, and (3) enhancement at the effector site of the Ig production and excretion in milk.

Another aspect of the role of IgAs in the defense mechanisms of the host is the phenomenon of immune exclusion directed against dietary antigens. The formation of IgA and antigen complexes in the lumen of the intestine prevents the intestinal absorption of these molecules and, therefore, reduces their transfer to the blood circulation. In humans, deficiency of IgAs is often correlated with the incidence of high concentrations of serum antibodies directed against antigens of the alimentary bolus or of enterotropic bacteria. Therefore, secretory IgAs appear to play a major role in the control of allergen absorption and contribute to the protection of the host against the development of allergies of dietary or environmental origin (Welsh and May, 1979).

2. Passive mucosal protection of the neonate: lactogenic immunity

2.1. Humoral

2.1.1. Protective isotypes

There exist some difference in cattle and pig in that the major isotype of pig colostrum is IgG, in comparison to IgG1 in bovine, but IgG1 persisted at high level in bovine

milk, in contrast to pig, where IgA predominate in milk. Thus, the passive mucosal protection of neonate ungulate is dependent on the continuous supply until weaning of maternally dimeric IgA (monogastric) and IgG1 (ruminants). Secretory IgAs have the advantage of being more resistant to proteolysis by secretory enzymes, such as trypsin, chymotrypsin and papain (Welsh and May, 1979). The primary function of these IgAs (and IgMs) present at the surface of mucosa is to block the first phase of enteropathogenic bacteria adherence to enterocytes by masking the surface receptors of these cells. These antibodies also are responsible for neutralization of bacterial enterotoxins and for neutralization of infectious enterotropic viruses. They have an agglutinating action for a great variety of enteropathogenic micro-organisms. This favors their elimination by intestinal peristalsis, but their bactericidal action requires the presence of complement and lysozyme. It is worth noting that the IgAs activate the complement through the alternative pathway and not through the classical pathway. Therefore, they are incapable of inducing lesions on the surface of enterocytes during antigen–antibody reaction (Welsh and May, 1979).

Moreover in pigs, S(IgA)₂ antibodies against enteropathogenic *E. coli* induce an irreversible loss of plasmid coding for an adhesin (Porter and Chidlow, 1979). Lastly, S(IgA)₂ are able to protect (Mazanec et al., 1995) against virus inside the cytoplasm of epithelial cells, as shown in rotavirus infection in mice. In calves, a protection of bronchial tissue and tears was observed by a mechanism of reverse transudation from blood through the epithelial cells (Porter, 1979). Similarly, blood IgG may restrict the replication of virus in gut, as shown in humans with the polio virus, provided that the blood IgG level is high enough (Ogra and Karzon, 1971); furthermore, lacteal IgG protect against TGE disease if they exhibit a very high level of neutralizing activity (Bohl and Saif, 1975). This observation was confirmed later by testing directly (Stone et al., 1977) each isotype from immune colostrum; thus, IgG is able to protect the piglet inasmuch as it is given during sufficient time to compensate for proteolytic degradation. The predominance of IgM in gut of young piglet up to one month old, together with their properties — a lower adherence to the mucous coat of epithelial surfaces than IgA and ability to activate complement — thus make IgM more able than IgA to opsonize the pathogens in gut lumen. This may explain that in swine Colibacillosis, there is a good correlation between the level of specific IgM in the colostrum and the protection of the neonate (Porter and Chidlow, 1979). Presence of polymorphonuclear cells, monocytes or macrophages and lymphocytes in gut as well as in mammary secretions, make feasible a cooperative effect between these cells and the immunoglobulins, including the IgA. However, until now and in contrast to gut cells, colostrum cells (at least in human) showed a rather reduced cellular cytotoxicity against virus-infected target cells and against bacteria (Kohl et al., 1980).

If it cannot be denied that any immunoglobulin isotype may protect the gut, it should be kept in mind that in natural conditions, the content of IgM and IgG high in colostrum, decline in milk; thus, IgA emerges as the dominant immunoglobulin in milk in swine. This explains why investigations are focused on the means of eliciting high level of IgA antibody in milk, specially in diseases where a continuous supply of milk antibody is required for protection.

2.1.2. Origin of immunoglobulins in mammary secretions

2.1.2.1. Transsudation, translocation of blood Igs. The IgG are transferred from the blood to the mammary secretions through the mediation of Fc γ receptors on the surface of epithelial cells. This transport is highly selective for IgG and, in ruminants, specific for IgG1, but not IgG2 (Watson, 1980); the transepithelial transport of IgG1 to the mammary gland is maximal during colostrum synthesis 2–3 weeks before parturition, and is then maintained at a low concentration during lactation (Butler, 1998).

In mice, it appears that the transport of circulating serum dimeric IgA from blood to milk is relatively important during early lactation (Halsey et al., 1982). In ewes, this IgA translocation would depend during lactation upon the availability secretory component molecules at the surface of mammary epithelial cells (Sheldrake and Husband, 1985).

2.1.2.2. Local synthesis of immunoglobulins. The existence of local antibody synthesis superimposed on transsudation is supported (Butler, 1998) by immunohistochemical location of IgA and IgG1 plasma cells in MG of sows and cows (Salmon and Delouis, 1982; Collins et al., 1986; Salmon, 1987; Chabaudie et al., 1993). In sows, the majority of milk antibodies (70% of IgG and >90% of IgM and IgA) result from local synthesis in the mammary gland (Bourne and Curtis, 1973). In cows, IgG1 is mostly derived selectively from serum, although some local synthesis of IgG1 persists in the MG during lactation (Sheldrake and Husband, 1985).

Mammatropic hormones also seem to influence the binding of antibodies to mammary epithelial cells, as well as their transepithelial transport (Weisz-Carrington et al., 1984).

2.1.3. Plasma cells in relation to MG development

2.1.3.1. Kinetics of accumulation in MG. Plasma cells accumulate in developing MG at a rate that differs between the mammals and correlate with the glandular development; IgA plasma cells number peaks after delivery at the end of lactation in sow MG (Chabaudie et al., 1993) as in mice, but in ruminants, plasma cells were more abundant during involution, with a predominance of IgG1 plasma cells over IgA (Collins et al., 1986).

2.1.3.2. Anatomical origin of plasma cells: the entero-mammary link. In natural environment, the mammary gland is not exposed to antigens, in contrast to the gut. In fact, the precursors of IgA plasma cells initiated in the gut, following antigenic stimulation, home not only into the lamina propria of the gut, but also into the other secretory organs (De Buysscher and Dubois, 1978), including the mammary gland (the so-called gut mammary axis), where they mature into plasma cells and secrete dimeric IgA (Roux et al., 1977).

In mice, even the few IgG plasma cells found at secretory sites also derive from homing B blasts originating from the gut to the exclusion of peripheral lymph-node cells (Mc Dermott and Bienenstock, 1979).

In sows, the existence of this gut–mammary link is sustained by the observations of the presence of specific IgA antibodies in milk of individuals whose gut were stimulated by the corresponding antigens (Evans et al., 1980).

2.1.3.3. Domiciliation factors of pre-plasma cells

2.1.3.3.1. Hormones. In mice, shortly before parturition and during lactation, the lymphoblasts (SIgA⁺, C3b⁻) from the mesenteric lymph node, compared to those from peripheral lymph nodes, preferentially migrate to the mammary gland, but their migration to the intestine remains unchanged. This suggests that there may be hormonal control. The treatment of virgin mice, using a combination of progesterone, estrogen and prolactin, induces development of the mammary gland and a simultaneous increase in IgA plasma cells in this organ (Weisz-Carrington et al., 1978). In sows, a correlation was found between density of prolactin receptors in mammary tissue and accumulation of lymphocytes in this organ (Salmon, 1987).

Antigens, although not formally required for the ultimate localisation in secretory sites, play a role in expansion of homed precursors. These cells may multiply further and some may undergo another cycle of homing (Husband et al., 1996).

2.1.3.4. Milk chemoattractant for B cells originating from MLN. Research conducted in mice demonstrated that MLN IgA lymphoblasts migrate into gut lamina propria (Mc Williams et al., 1977; Roux et al., 1981) and are adhesive (Weisz-Carrington et al., 1991) preferentially to the endothelium of blood capillaries in the intestinal lamina propria. The IgA lymphoblast precursor of IgA plasma cells migrate also in the mammary gland (Roux et al., 1977). In vitro adhesion of blood B cells (sIgM) to capillaries of mammary gland is highest in mid and late lactation, as compared to pregnancy, and is inhibited by anti-MadCAM (San Gabriel-Masson, 1992).

On the other hand, local recruitment of plasma cell precursors may be accounted for by milk substances acting as a chemoattractant (Czinn and Lamm, 1986). To that view, we have developed a chemoattractant assay comparing MLN and ILN cells (Abda et al., 1998). In sow lactoserum, but not in the serum at the same time, there was a chemotactic activity towards T and B lymphocytes from the MLN to the exclusion of ILN; similarly, a peptide derived from bovine β -casein is chemoattractant for swine lymphoblast as the lactoserum itself (Fronteau et al., 1998), in keeping with a similar activity on mesenteric lymph node B cells IgA and IgG, shown in mice (Czinn et al., 1987).

2.1.4. Immunocytes of the MG

2.1.4.1. Intramammary immunisation. The mammary gland could be a better route of immunization as compared to the gut, needing less amount of antigen since there should be no protein degradation; in addition, this would simulate the natural situation when the piglets may inoculate the MG during suckling.

Earlier investigations in ruminants have shown that the injection of microbial or hapten-conjugated antigens to a suitable carrier into the mammary gland induced S(IgA)₂ in the immunized gland to the exclusion of contra-lateral gland. Infusion of killed bacteria or flagellar antigens into the MG of sheep around 3 weeks before parturition gave rise to local production of antibodies S(IgA)₂, which persisted in the following lactation (Sheldrake and Husband, 1985).

In sows, ferritin used alone led to an IgM response, whilst in presence of complete Freund's adjuvant, which provokes a local granuloma formation and targets the antigen to the secretory site, there was a predominant IgA response (Bourne et al., 1975). The intramammary inoculation of live attenuated TGEV in pregnant sows induced persisting high levels of IgG neutralizing antibodies in milk, whereas in lactation, IgA antibodies were elicited (Saif and Bohl, 1983).

In pregnant sows, after injecting the live virus into the MG, we have got an IgA immune response in the milk of each of the 18 glands (Salmon, 1995). The presence of antibody activity in the milk of non-injected gland (activity lower than that of the injected one) may be explained in several ways: either passage/homing of the virus/plasma cells from one gland to another or the virus may have gained access to the gut (although in this experiment there was no sign of diarrhea) where it might have stimulated gut lymphocytes, which, in turn, have migrated into mucosal tissues — they are thus trapped into each mammary gland with a local restimulation in those previously injected.

These observations raise the possibility of a genuine local immune response. Thus, to ascertain this hypothesis, a search was made of cells necessary to mount an immune response in mammary gland, their kinetics origin and fate in mammary secretions.

2.1.4.2. Lymphocytes subsets in the developing MG. In sows (Salmon and Delouis, 1982), the number of leucocytes and lymphocytes (T, B and null) present in the mammary tissue increases from day 80 of pregnancy, in parallel to the increase of prolactin receptor level onto epithelial cells. All cell types involved in the immune response were present in the mammary gland at the different stages of gestation and lactation, and nearer the alveolar epithelium as gestation proceeded: T lymphocytes, including CD4+ and CD8+, B lymphocytes and class II bearing cells (epithelial cells and macrophages). T lymphocytes, specifically T helper cells, accumulated early in pregnancy; the specific increase of IgA lymphocytes occurring after this phase could suggest a role for these T cells in the induction of IgA response. The local accumulation of immune cells and the increase in CD8+ cells near the epithelium suggests a role in local immune defence (Salmon, 1987; Chabeaudie et al., 1993). Only few functions have been explored; mammary lymphocytes had comparable maximum levels of PHA and conA stimulation as blood lymphocyte, suggesting the presence of virgin lymphocyte at least in sows (Salmon, 1987).

In bovines, studies on the three components involved in production of an immune response, namely antigen-specific T cells, an immune recognition molecule (MHC class II molecule, Fitzpatrick et al., 1992) and antigen itself are present in the MG (see Section 2.2). In the mammary gland of ewes, both pregnant and non-pregnant, the great majority of the lymphocytes in the epithelium were agranulated CD8+ cells; B lymphocytes were present in much lower concentrations and were located mainly in the connective tissues (Lee et al., 1989).

Thus, there appears a deficiency of endothelial cells membrane receptors preventing B cell traffic through the blood vessels.

Inflammation in the mammary glands of sheep was characterized by the differential recruitment of T and B cells: while T cells seemed to migrate out of existing, flat endothelium-lined blood vessels, resulting in a diffuse distribution at the sites of

inflammation, B cells were characteristically present as clusters of tightly packed cells around capillary vessels lined with plumb endothelial cells. These results indicate differential regulation of adhesion molecules on B and T cells and/or their ligands on endothelium during acute inflammatory reactions (Meeusen et al., 1991). Moreover, 14 days after *St. Aureus* antigen infusion, there was an accumulation of plasma cells, mostly of IgA isotype, suggesting that B cells and helper T cells interaction can take place at the local site of antigen stimulation in the MG (Lee et al., 1992).

2.1.5. Anatomical origin of mammary lymphocytes

The different migration pathways of lymphocytes in the organism, reflected by the compartmentalization of the immune system into systemic and local immune systems, may be determined by the expression of particular structures on the surface of endothelial cells (addressin) and complementary structures on the membranes of lymphocytes (homing receptor) (Springer, 1994). The endothelia of the venules within the lamina propria of the gut and in the lactating mammary gland (Streeter et al., 1988) express a mucosal addressin cell adhesion molecule. Our recent data in mice indicate the presence of MadCAM-1 even on endothelial cells of mammary gland during pregnancy (Tanneau et al., 1999). The binding of MadCAM-1 and the counter-receptor onto lymphocytes, L-selectine or $\alpha 4 \beta 7$, participates in directing leukocytes to mucosal and inflamed vasculature (Salmi et al., 1998).

In sow, when lymphocytes from mesenteric and inguinal lymph node are labelled with fluorescein and rhodamine, respectively *in vivo*, at 90 days of pregnancy 24 h after labelling, we find the same proportion of FITC and RITC cells in the mammary gland, whilst there was a higher proportion of FITC than RITC positive cells in the gut: thus, despite the presence in the sow as in sheep of an intestinal pool of lymphocytes, the mammary lymphocytes were derived from both the MLN and the ILN (Salmon, 1984). Using surface markers, we conclude that T, B and L lymphocytes migrate from the MLN, and that B lymphocytes migrate from the ILN, both types comprising small and medium lymphocytes.

When the lymphocytes were taken from different lymph node and labelled with ^{51}Cr , different results were obtained; recovery of infused mesenteric node cells was lower in lactating than in pubescent pigs (Harp and Moon, 1988).

The findings obtained in cows, when compared with results in sheep and pigs, support the hypothesis that lymphocytes do not migrate efficiently between the gut and mammary gland of ruminants (Harp and Moon, 1987; Harp et al., 1988).

2.2. Transfer of cellular immunity by milk cells

Depending upon the animal species and animals within species, milk contains 2×10^5 to 2×10^7 cells/ml. There are epithelial cells (31% of total cells in sow's milk) and non-nucleated cell fragments, and granulocytes, represented mainly by neutrophils (47%) and a few eosinophils (1%), lymphocytes (12%) and macrophages (9%) (Schollenberger et al., 1986).

T lymphocytes in bovine milk display the phenotype of memory cells; thus, these cells exhibit a weaker reactivity than those obtained from autologous blood lymphocytes for

some mitogens, or in mixed lymphocyte culture, but reveals a strong proliferative response to enteric antigens, whereas no stimulation is observed with peripheral lymphocytes (Taylor et al., 1997). These differential reactivities suggest that lymphocytes accumulated in mammary tissue correspond to a selected population different from the blood. The mean ratio of CD4+ to CD8+ T lymphocytes in the peripheral blood and mammary gland secretions was 1.53 and 0.85, respectively. Activated CD8+ T lymphocytes may play an important role in the regulation and expression of the local immune response to pathogens (Park et al., 1992).

Lymphocyte subpopulations in mammary secretions of dairy cows change during the lactation cycle. In involuting glands (dry gland), 80–90% of lymphocytes were CD2+ T cells that decreased to 50% at the colostrum stage and fluctuated between 50 and 60% in normal (mature) milk. Throughout the lactation stages, less than 5% were B cells. CD4+ T cells constituted 55% of lymphocytes (30–40% CD8) in the dry gland secretion, but decreased drastically at parturition (40–50% CD8) and maintained at the level below 20% (30–40% CD8) throughout normal lactation (Yang et al., 1997).

Therefore, milk contains all the immune components to permit the passive transfer of specific cellular immunity. A number of research studies carried out in humans and rodents demonstrated that there was transmission from mother to offspring, through mammary secretions, of such reactions as cell-mediated hypersensitivity (Mohr, 1973) and skin transplant rejection (Beer et al., 1974). However, the efficiency of the transfer depends upon the ability of the cells to survive in the digestive tract of the young. That is, unless the passive acquisition by the young of the immunity mediated by maternal cells results from the passage of soluble factors produced by lymphocytes, such as transfer factor, rather than from the actual transfer of lymphocytes themselves (Welsh and May, 1979). In newborn pigs, IEL are devoid of NK activity against TGEV infected cells, and transfer from adult pigs of blood MNC increased the resistance to TGEV (Cepica and Derbyshire, 1984).

3. Generation of mucosal immunity

In mammals, the defense of the intestinal, respiratory and urogenital mucosa, as well as that of epithelia of exocrine glands (salivary, lacrimal and mammary) is supported by a 'local' or 'secretory' immune system unrelated to the systemic immune system (Butcher, 1988).

These immune responses are characterized by local production of antibodies, among which the IgA isotype predominates in swine and IgG1 in bovine (Butler, 1998).

Although the mechanisms that determine the preferential expression of the IgA isotype among the B cells of the mucosal associated lymphoid tissue are not elucidated completely, several factors seem to be involved, which begin to be tested in MG, specially in the milk.

Induction of IgA secretion at the intestinal level appears to be related to the particular environment of Peyer's patches and it seems to depend upon the interaction of the B cells with the T cells and with dendritic cells derived from these lymphoid follicles (Spalding et al., 1984; Spalding and Griffin, 1986). The switch from sIgM B cells to sIgA B cells is

under the control of TGF- β inducing mRNA transcripts from non-arranged C α genes (Stavnezer, 1995).

In Peyer's patches, TH2 lymphocytes are involved in the amplification of the B cell pool expressing membrane IgA (mIgA+) via IL-4. This interleukin induces the stimulation of transcription of messenger RNA encoding for the heavy chain of these immunoglobulins (Harriman and Strober, 1990). TH2 lymphocytes also act at a later stage, at the level of the effector sites of immune responses (intestinal mucosa). They control the production of IgA antibodies with the mediation of IL-5 and IL-6 secretions. IL-5 favors production of IgA from mIgA(+) B cells stimulated by an antigen. It increases the number of IgA secreting cells, and IL-6 increase the synthesis rate of antibodies. The fact that IgA+ dividing cells derived from the mesenteric lymph node can migrate to other secretory sites, such as the urogenital tract or the lungs (Mc Dermott and Bienenstock, 1979) suggests the existence of a local immunity generalized to all the mucosa even though the antigen is not essential for the appearance of IgA cells in the mucosa; its presence can attract and retain more of these cells locally and/or can stimulate the proliferation of IgA plasma cell precursors (Husband et al., 1996).

The clarification of cellular immune response to TGEV is important because cell mediated immunity plays a direct role in protection and recovery from infection, and the production of antibodies is regulated by various cytokines derived from activated mononuclear cells during an immune response. Antigen-specific cell-mediated immune mechanism that have been described in pigs infected with TGEV include antibody dependant cell cytotoxicity, T-lymphocyte cytotoxicity and lymphocyte proliferation. A study on the cytotoxic response of TGEV with lymphoblastoid cell lines from histocompatible pigs has shown cytotoxicity of MLN cells from immunized pigs (Murata et al., 1998) to target cells infected by vaccinia recombinant exhibiting M or S protein. Infection of neonatal gnotobiotic lambs with a bovine strain of rotavirus was used to characterize the kinetics of the primary cellular intestinal immune response to this agent (Bruce et al., 1995). Other mechanism of antiviral immunity act in non-specific manner to limit virus spread in a host before replicating virus reaches the antigenic threshold and elicits a specific immune response. These innate mechanisms include the type I IFN that are produced by MNC, epithelial cells and fibroblasts after contact with viruses and NK cells that shows spontaneous cytotoxicity for virus-infected cells (Salmon et al., 1989). TGEV is a potent inducer of IFN alpha (Charley and Laude, 1988).

There are now several reports exploring the cytokine in milk; a recent study conducted in bovine has shown the presence of cytokine mRNA in milk cells; no TNF α , IL2 or IL4 were detected in contrast to IFN γ , IL-6, IL-10, IL-12 (Taylor et al., 1997). These results should be extended onto MG tissue and compared with monogastric, to see if there is a microenvironment specific of the MG.

4. Soyabean allergy in calves and piglets at weaning

The development of local and systemic immune responses to soyabean proteins was investigated in early-weaned pigs. After weaning at Day 21, pigs were fed diets containing the corresponding soyabean products. In the treated group, diarrhea was more

frequent, the size of duodenal villi was reduced by 24–36% and densities of plasma cells in the lamina propria were increased (IgM and IgA, 3 times; IgG1 and IgG2, 6 times). The increase of CD2+ T cells were accounted for by a rise in both CD4+ and CD8+ T cell subsets in the lamina propria as well as in the epithelium of the duodenal mucosa; in addition, there was also an increase in the γ/δ T-cell subset in the epithelium.

Immunoblotting patterns of raw soybean with sera from 28-day-old pigs showed two bands (22 and 36 kDa) recognized by IgA and IgM, respectively. These immune reactions in the duodenal mucosa, involving both B and T lymphocytes may be related to atrophy of the duodenal villi in soyabean piglets (Dreau et al., 1994, 1995, 1995b).

Similarly, calves fed soya proteins may develop severe gastrointestinal disorders. One-month-old calves were fed for 2.5 months a mixture of whey and highly antigenic soyabean products. They developed specific Abs, including IgG1 and IgA isotypes, both systemically and locally (in gut secretions) with similar patterns of antigenic specificity for IgA and IgG1 (Dreau et al., 1995a). Thus, in soyabean allergy, both piglets and calves exhibited gut villus atrophy, gut accumulation of IgA (swine) and IgG1 (cattle) immunocytes sustaining the view of a specific environment in ruminant responsible of both IgA and IgG1 production.

5. Conclusion

Lactogenic immunity is potentially an exciting area of research into the development of vaccines for mucosal/secretory immunity because inactivated or attenuated antigens often fail to retain their immunogenicity for mucosal IgA responses and hence for IgA mediated milk immunity.

The dissemination of antigen-sensitized and IgA committed cells from inductive site to remote effector sites has important implications for the design of vaccines and seem to depend on the extent of local antigenic stimulation and the example of lactogenic immunity is very demonstrative showing dependence on hormonal control; which could be the rule and not the exception in mucosal tissues towards the induction of TH2 cells.

The next step to enhance our understanding would be to investigate further the mechanisms that induce the expression of the IgA isotype or IgG1 by B cell precursors, for instance those localized in Peyer's patches, without overlooking the part played by the auxiliary T cells, antigen-presenting cells and the nature of the antigen itself.

The circulation pathways of the IgA lymphoblasts still are unclear, particularly if we take into account the variation due to the diversity of animal species. Because research has demonstrated existence of organ specific markers in vascular endothelia that determines the homing of immunocompetent cells, it seems important to verify that these findings can be extrapolated to all mammals.

Localized at the mucosal level, IgA lymphoblasts differentiate into plasma cells that locally produce specific secretory antibodies. There are few data available of the possible regulation in situ of this local humoral response by lymphokines and/or by tissue factors released by adjacent cells.

Lastly, with a better insight into what determines the expression of membrane antigens at the level of the endothelial cell, sites that are specifically recognized by homing

receptors present on the surface of lymphocytes, it may be possible to envision the control of lymphocyte circulation in the organism. Understanding this control process may, for example, allow us to induce specific migration of immunocompetent cells to the mammary gland to start or enhance the specific humoral immune response and to improve the immune quality of the milk produced by the mammary gland.

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