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# IMMUNOLOGICAL ACTIVITIES OF MILK

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## 1. INTRODUCTION

The mammary gland, that organ characteristic of the class mammalia, sustains the young animal during the early weeks of life. In addition to their vital nutritive properties, mammary secretions also contain components of the immune system which provide the young with protection during the period before its own immune system has responded to the pathogenic organisms of the environment. This review describes these components and discusses ways in which this protection affects the young animal and strategies that can be adopted to increase it.

## 2. IMMUNOGLOBULINS OF MAMMARY SECRETIONS

In describing the immunoglobulins of mammary secretions, an

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TABLE I

Immunoglobulin concentrations of milk and colostrum of several species and their relationship to placental transfer

| Species | Transfer of passive serum antibody | | Immunoglobulin concentration mg/ml | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Colostrum | | | Milk | | | |
| | Placenta | Colostrum | IgG | IgA | IgM | IgG | IgA | IgM | Reference |
| Man | +++ | - | 0.3 | 120 | 1.2 | 0.1 | 1.5 | 0.01 | McClelland et al. (1978) |
| Pig | - | +++ | 62.0 | 10 | 3.2 | 1.4 | 3.0 | 1.9 | Bourne (1973) |
| Cow | - | +++ | 75.0 | 4.4 | 4.9 | 0.35 | 0.05 | 0.04 | Mach & Pahud (1971) |
| Sheep | - | +++ | 101.0 | 6.2 | 2.9 | 0.9 | 0.09 | 0.04 | Smith et al. (1975) |
| Horse | - | +++ | 80.0 | 9.0 | 4.0 | 0.35 | 0.8 | 0.04 | Pahud & Mach (1972) |
| Dog | ± | ++ | 2.0 | 13.5 | 0.3 | 0.01 | 3.6 | 0.6 | Tizard (1977) |

immediate distinction must be made between species in which antibody is transferred from mother to offspring wholly or mainly via the placenta, (man, other primates and rodents), and, those species in which no significant transfer is made in utero and in which colostrum is the vehicle by which the neonatal animal acquires its passive serum antibody. This latter group includes all the common domesticated species.

These species differences are reflected in the immunoglobulin content of colostrum (Table I). In most domesticated species IgG is the major immunoglobulin and is present in higher concentrations than in serum. In contrast, in man and rodents, IgA predominates in colostrum with much smaller amounts of IgG. Not only are there differences in immunoglobulin profile between these two groups but the origin of colostral immunoglobulin also differs. In man and rodents the IgA of colostrum is almost certainly locally synthesised (Ahlstedt et al., 1975; Drife et al., 1976) while the IgG of colostrum in domesticated animals is derived from serum by a selective concentration of IgG by the mammary gland (Larson & Kendall, 1957; Dixon et al., 1961; Bourne & Curtis, 1973).

The much smaller amounts of IgA and IgM found in the colostrum of this latter group is derived partly from serum and partly by local synthesis within the gland (Bourne & Curtis, 1973; Newby & Bourne, 1977). Colostrum in domesticated species is, therefore, a transudation from serum rather than a true secretion, at least in so far as the immunoglobulins are concerned. Apparent exceptions to this rule are the dog and the cat, where IgA is the major immunoglobulin of colostrum. In these species, although much of the serum transfer of immunoglobulin occurs after birth, a significant proportion (5-10%) is transferred across the placenta and these species seem to occupy a transitional position between man and most domestic animals.

Since much of the protein of colostrum is absorbed undegraded by the intestine of the neonate (for review see Simpson-Morgan & Smeaton, 1972), this selective concentration of serum IgG into colostrum provides a mechanism whereby serum antibody from the dam is transferred into the circulation of the neonate.

The mechanism by which this IgG is concentrated in the mammary gland is not fully understood. Bezkorovainy (1971) suggested that bovine $IgG_1$ contained an extra moiety which mediated its transfer into the colostrum. More recently Hammer and Mossman (1978) showed that this transfer was mediated by receptors present upon the $IgG_1$

heavy chains which binds to epithelial cells prepared from the mammary glands. Interestingly, this binding could be inhibited by other mammalian, but not non-mammalian, IgG. This binding could be demonstrated in vitro in acinar epithelial cells prepared from the mammary gland during colostrum formation but not in those obtained later in lactation.

It appears that the mechanism of colostrum formation is under hormonal control, since Smith (1971) showed that changes in the mammary gland of the cow, similar to those seen during colostrum formation, could be achieved by treatment with oestrogens and progesterones, while Weisz-Carrington et al. (1978) showed that in mice a colostrum-like secretion could be produced by a regime of oestrogen, progesterone and prolactin - these hormones being the ones that are active during late pregnancy.

In domestic species other than ruminants, the character of the immunoglobulins of the mammary gland changes rapidly after colostrum formation is over. The IgG concentration falls dramatically and becomes only a minor component of milk, while IgA, although falling in absolute concentration compared to colostrum, now assumes the role of major immunoglobulin class. In addition the nature of the immunoglobulin present undergoes a change, since it is now almost entirely synthesised within the gland. Thus, Bourne and Curtis (1973) have shown that 90% of the IgA and IgM, and 70% of the IgG, in milk of sows is locally derived. In women, although the absolute concentration of immunoglobulin declines during early lactation, no such qualitative alteration occurs since IgA synthesised within the breast remains the major immunoglobulin (Drife et al., 1976).

In most species, therefore, the immunoglobulins of milk are those of a classical secretory immune system, with IgA predominating and the great majority being synthesised beneath the epithelial surface at which they are secreted. This has been confirmed histologically in the mammary gland of the sow by Porter et al. (1970) and Brown et al. (1975), who showed that IgA-producing plasma cells were the predominant type present in the lactating mammary gland. These started to accumulate during the last few weeks of gestation and increased in number during lactation. This accumulation of plasma cells is also probably under hormonal control, since Drife et al. (1976) have shown that oestrogens can alter the plasma cell distribution in the resting breast. Following weaning, the number of plasma cells present in the sow's mammary gland declines rapidly.

Ruminants differ markedly from both patterns so far described. As can be seen from Table I, like other domesticated species, passive antibody passes from mother to offspring via colostrum and IgG is the major immunoglobulin class. However, colostral IgG is restricted almost entirely to one subclass, $IgG_1$, whereas the other major class, $IgG_2$, is present in only trivial amounts. This $IgG_1$ is derived entirely from serum and is concentrated many times above serum levels (Dixon et al., 1961; Pierce & Feinstein, 1965). Smaller amounts of IgA and IgM are also present in colostrum and a proportion of this is locally synthesised within the gland (Newby & Bourne, 1977). During early lactation the immunoglobulin concentration falls rapidly but $IgG_1$ remains the major immunoglobulin throughout lactation and it is entirely serum derived (Newby & Bourne, 1977). Very small amounts of $IgG_2$, IgA and IgM are also present in milk. Since over 80% of the milk immunoglobulin is serum derived, it is clear that in contrast to other species there is no significant local immune system in the lactating ruminant mammary gland. This is confirmed by histological investigation, which reported few, if any, plasma cells in the normal lactating udder (Dixon et al., 1961).

However, Campbell et al. (1950) did find evidence of plasma cells during the period of colostrum formation when, as already described, some local synthesis of IgA and IgM can be detected. Furthermore, Butler et al. (1972), using an in vitro method, could demonstrate synthesis of all three major immunoglobulin classes by mammary tissue, although this was less than that seen in other tissues.

The presence of reagenic antibody in mammary secretion has received relatively little attention. IgE antibody is present in breast milk in low concentrations (Turner et al., 1977) and Petzoldt and Von Beaten (1978) demonstrated PCA activity in the serum and colostrum of cows. The antibody was shown on the basis of heat lability and persistence in the skin to be IgE-like.

## 3. CELLS OF MAMMARY SECRETIONS

Although cells were first identified in human colostrum more than a hundred years ago (Donné, 1844) in recent years they have received less attention than have the immunoglobulins and controversy still exists concerning their significance, function and indeed their very nature. Several cell types have been identified in colostrum and milk, including both T and B lymphocytes, macro-

phages and neutrophils (Smith & Goldman, 1968). In addition, other cells, such as eosinophils, are present in small numbers and also cells, frequently binucleate with foaming cytoplasm, which have not yet been positively identified but which are probably of epithelial origin (Hollman, 1974).

The relative proportion of these types of cells and their absolute numbers have been studied in a number of species and show considerable variation. In women, Ogra and Ogra (1978) showed a decrease in cell numbers during the first few days of lactation but great variation exists between individuals with figures ranging from $1 \times 10^6$ to $1 \times 10^7$ cells/ml (Ho & Lawton, 1978; Ogra & Ogra, 1978). In addition to this variation in total cell numbers considerable variation can be seen in the differential count both between species and between individuals. Although the macrophage is considered to be the major cell type, comprising up to 90% of the total cell count in human milk (Diaz-Jouanen & Williams, 1974), rat milk (Head & Beer, 1978) and cow milk (Jensen & Eberhart, 1975), other studies have indicated that neutrophils can constitute a significant proportion of the cells of human breast milk ranging from 20-80% of the total count (Robinson et al., 1978). Although, particularly in cattle, this increased neutrophil population has been associated with infection of the mammary gland (Jensen & Eberhart, 1975), large numbers can be present in apparently normal glands.

While this variation in total and differential cell counts may reflect real differences between species and between individuals, the technical difficulties associated with isolating cells from the viscous medium of colostrum and milk, and difficulties in distinguishing between cells, particularly macrophages and epithelial cells, may account for much of the wide variation seen in the literature.

## 3.1 Macrophages

Macrophages, defined morphologically by their ability to adhere to glass and to phagocytose particles, have been identified in the secretions of the mammary glands of many species (Smith & Goldman, 1968; Jensen & Eberhart, 1975; Head & Beer, 1978). Milk macrophages can be distinguished from those isolated from the blood or lung alveoli morphologically by the presence of large fat globules in their cytoplasm (Ho & Lawton, 1978) and functionally by a decreased phagocytic and bactericidal activity (Lascelles et al.,

1969; Ho & Lawton, 1978; Robinson et al., 1978) and it has been suggested that the presence of lipid in the macrophage (presumably absorbed from the milk) reduces their subsequent phagocytic activity This is supported by the observations of Ho and Lawton (1978) that pre-incubation of peripheral blood neutrophils with cell free colostrum reduces their phagocytic activity and of Lascelles et al. (1969) that pre-incubation of human colostral cells with medium for 24 hours increased their phagocytic capacity. This is supported by the observations of Ho and Lawton (1978) that pre-incubation of peripheral blood neutrophils with cell free colostrum reduces their phagocytic activity and of Lascelles et al. (1969) that pre-incubation of human colostral cells with medium for 24 hours increased their phagocytic capacity.

As mentioned earlier, there is still controversy over the nature of the large cells with foaming cytoplasm first identified by Donné in 1844. It is still not clear whether these are true macrophages or whether they are derived from epithelial cells (Hollman, 1974; Lawton & Shortbridge, 1977). The picture is further confused by the observation of Papanicolaou and Maddie (1959) that epithelial cells can transform into phagocytic cells. Recently, Evans (1980) has been able to distinguish between large adherent phagocytic cells and non-adherent non-phagocytic cells on the basis of staining for esterase activity, which may provide a more reliable method for discrimination.

## 3.2 Lymphocytes

Lymphocytes represent approximately 10% of the total cell population of mammary secretions in women (Ho & Lawton, 1978; Ogra & Ogra, 1978) in the rat (Head & Beer, 1978) and in the sow (Evans, 1980).

Both B and T lymphocytes have been demonstrated in breast milk and approximately 50% have been reported to be T cells, 30% B cells and 20% "null" cells (Diaz-Jouanen & Williams, 1974; Ogra & Ogra, 1978).

IgG, IgA and IgM have all been demonstrated on the surface of B cells but only IgA synthesis has been demonstrated in vitro (Murello & Goldman, 1970; Ahlstedt et al., 1975) and IgA producing cells are the major type found in colostrum and milk (Lee et al., 1978).

The viability of these lymphocytes has been demonstrated by their ability to be transformed in vitro with mitogens (Parmley et al., 1976) but comparison of their in vitro proliferative responses to specific antigens with those of peripheral blood lymphocytes indicate differences which suggest that milk lymphocytes represent a selected population of cells (Smith & Schultze, 1977; Head & Beer, 1978).

The origin of colostral and milk lymphocytes will be dealt with in the next section.

## 4. STIMULATION OF ANTIBODY IN MAMMARY SECRETIONS

Many attempts have been made to increase by various immunisation regimes the level of antibody in mammary secretions. This has been done both as a prophylactic measure to combat disease in the suckling animal or in the mammary gland itself, and also as a model with which to study the effect of immunisation upon a local immune system. A variety of routes have been used to achieve antibody responses in mammary secretions.

The simplest method has been to use parenteral immunisation. From the discussion of the origin of colostrum and milk immunoglobulins, it is apparent that serum antibody makes a contribution to the antibody of mammary secretions. In most domestic species this contribution is much greater for colostrum than for milk, although in ruminants the majority of both colostral and milk antibody is serum derived. From this it is clear that immunisation regimes which produce high levels of circulatory antibody also result in the appearance of antibody within colostrum and milk.

The conventional method of achieving these antibody levels is by a regime of subcutaneous or intramuscular injections. This results in a high concentration of IgG antibody in serum and in its appearance in colostrum and milk. Bohl et al. (1975) demonstrated IgG antibody in sow's colostrum following intramuscular injection with killed TGE virus. This antibody declined very rapidly during the first few days of lactation and was present in milk at very low levels. Studies have also been performed in sheep (Wells et al., 1978) with very similar results. A slightly different approach has been made by Chidlow and Porter (1978) who showed that parenteral immunisation could, by using appropriate immunisation regimes, lead to high serum levels of IgG or IgM antibody. This appeared in high concentrations in colostrum but again fell to much lower levels in milk.

Although in general systemic immunisation leads to systemic rather than local antibody, under certain circumstances secretory antibody may also be stimulated. The most interesting instance of this is the observation by Svennerholm et al. (1977) that in women already primed by previous exposure to vibrio cholera infection, parenteral immunisation boosted secretory antibody titres. However, most investigations have concluded that systemic antibody is an inefficient way of stimulating milk antibody (Bourne et al., 1975; Chidlow & Porter, 1977) and, even in ruminants, milk antibody levels are probably too low to be effective (Woode et al., 1975). Attempts have been made, therefore, to stimulate the local immune system by direct immunisation of the gland.

Intramammary immunisation by direct injection into the substance of the gland has been investigated in a number of species, including rabbits (Genco & Taubman, 1969; Hurlimann & Lichaa, 1976) guinea pigs (McDowell, 1973) and pigs (Bohl et al., 1972; Bourne et al., 1975; Chidlow & Porter, 1977). Results have been variable with some reports indicating predominantly an IgA response (Genco & Taubman, 1969; McDowell, 1973; Bourne et al., 1975) while other workers have found the response to be mainly IgG (Bohl et al., 1972; Hurliman & Lichaa, 1976; Chidlow & Porter, 1977). The variation seen in the response is probably because this route does not represent true stimulation of the mucosal surface (Bourne et al., 1978). The data from all these studies does, however, suggest that much of the milk antibody, be it IgA or IgG, is synthesised within the mammary gland (Genco & Taubman, 1969; McDowell, 1973; Bourne et al., 1975) and it appears to be a more efficient way of inducing milk antibody than systemic immunisation (Bourne et al., 1975), although the variability of response and the trauma associated with the procedure makes it impractical as anything other than an experimental procedure.

In ruminants, because of the anatomical structure of the teat canal, it is easy to introduce antigen into the lumen of the udder, thereby producing true mucosal immunisation and many studies have shown that this is an effective and non-traumatic method of immunisation. Kerr et al. (1959) demonstrated local antibody following infusion of Trichomonas foetus into the mammary gland. They found that antibody production was greater in glands infused during late pregnancy rather than lactation, presumably since the antigen is not rapidly removed. Similar results have been obtained in the sheep (Lascelles & McDowell, 1970).

There are a number of interesting features to the immune response to intramammary infusion. Unlike injection into the gland, infusion appears to stimulate reproducibly an IgA response (Lascelles & McDowell, 1970; Erno & Aalund, 1972; Newby & Bourne, 1977), although other classes of immunoglobulin are also involved.

In addition to the response in the vaccinated gland, locally synthesised antibody can also be detected in non-immunised glands and in serum (Sarwar et al., 1964; Jenness & Anderson, 1971; Erno & Aalund, 1972; Newby & Bourne, 1977), although the response is strongest and appears most rapidly in the immunised gland (Newby & Bourne, 1977). The speed of the local response to intramammary infusion is remarkable, Sawar et al. (1964) finding agglutinins present in milk only 24h after primary infusion.

A feature of the local response of the cow's udder is the increase in total immunoglobulin that coincides with the antibody response (Wilson et al., 1972). This is due to the very high proportion of total immunoglobulin that is involved in the specific antibody response (Newby & Bourne, 1977) and indicates that in the absence of local immunisation the local immune system of the bovine udder is relatively inactive.

Intramammary infusion in ruminants represents a useful method of immunising the local immune system to produce a consistent IgA response and is a potentially useful model for studying the effect of immunisation on a secretory immune system. In addition this approach could have important prophylactic uses in controlling disease in calves, and also, through the use of immune milk as a basis for formula baby feeds, in man.

Although the immunisation regimes described so far can result in the appearance of antibody in mammary secretions, it is clear that they do not represent the normal method of antigenic stimulation. The first investigation to shed light upon this subject was that by Bohl et al. (1972) who showed that IgA antibody to TGE virus appeared in milk following the infection of the gut of the sow with the virus. They postulated that there was a link between the gut and the mammary gland in the sow such that antigenic stimulation within the intestine lead to an IgA response in the mammary gland, and since this IgA is locally synthesised it must be mediated by the traffic of primed lymphocytes from the gut to the mammary gland. These early observations have since been confirmed and extended in the sow (Bohl & Saif, 1975; Hess et al., 1978; Evans et al., 1980) man (Goldblum et al., 1975) rats

(Michalek et al., 1976) and mice (Roux et al., 1977) and these last authors demonstrated that the link between the gut and the mammary gland is indeed mediated by lymphocytes moving from the gut and lodging in mammary tissue. It is these lymphocytes originating in the gut which are seen to accumulate within the mammary gland in the final stages of gestation. This process continues throughout lactation since Evans et al. (1980) induced IgA antibody in milk by immunising sows after parturition, and although the response in milk coincides with the presence of antibody within the gut, it appears to last longer than does the intestinal response (Evans et al., 1980).

The task of stimulating the local immune system of the mammary gland, therefore reduces to one of stimulating the local immune system of the gut and a large number of studies have now been performed which indicate that this can be done most effectively by colonising the intestinal tract with a live organism, whereas the use of inactivated or inert antigen is relatively ineffective (Chidlow & Porter, 1978; Evans et al., 1980).

It has been suggested that this link between the gut and the mammary gland is an example of a universal linkage between all mucosal immune systems and the term 'common mucosal system' has been coined to suggest this interrelationship (Bienenstock, 1974). From this hypothesis one would predict that infection of the upper respiratory tract with a virus would result in the appearance of IgA antibody in colostrum and milk. Saif and Bohl (1977) investigated this by infecting the upper respiratory tract of sows during late gestation with pseudorabies virus. They found that the antibody response to the virus in milk was confined to the IgG class. Although the migration of sensitised lymphocytes from the gut to many mucosal sites has been demonstrated (Weisz-Carrington et al., 1979), until the immunisation of a distant site can be shown to lead to an IgA response in milk, the gut-mammary link must be considered as in some way unique.

How far this gut-mammary lymphocyte traffic operates in ruminants is far from clear. In the absence of intramammary infusion there is little evidence of local antibody synthesis in bovine milk (Newby & Bourne, 1977) but plasma cells do accumulate in late gestation and some colostral IgA and IgM is locally produced. It is probable, therefore, that some movement of lymphocytes from the gut to the mammary gland does occur in cattle but that this ceases soon after parturition.

All the evidence cited above is concerned with the appearance of antibody producing cells which are principally IgA. However, evidence is also accumulating that T cells present in the mammary gland are derived from the gut since they respond to antigen specific blast transformation assays with gut antigens while peripheral blood lymphocytes are not reactive (Parmley et al., 1976).

## 5. PROTECTIVE EFFECTS OF THE IMMUNE COMPONENTS OF MAMMARY SECRETIONS

In domestic animals there are two quite distinct protective functions provided by the immune components of mammary secretions. Firstly, colostrum absorbed into the circulation of the neonatal animal provides passive circulating antibody which prevents the invasion of micro-organisms into the circulation, secondly, milk, which is not absorbed and hence acts only within the alimentary tract, provides a passive local immunity which can protect against enteric disease.

This distinction between the two functions is demonstrated very clearly in the effect of colostrum on the susceptibility of calves to the enteric and septicaemic forms of neonatal E. coli infection. Many studies have shown that calves lacking adequate passive serum antibody have an increased susceptibility to the septicaemic form of coli bacillosis (Gay et al., 1965; Smith et al., 1967; McEwan et al., 1970) but passive serum antibody concentrations which protect against septicaemia do not reduce the susceptibility of weaned calves to the enteric form of the infection (Penhale et al., 1970). Similarly, Logan and Penhale (1970a,b) and Penhale and Logan (1971) could induce immunity to the septicaemic form of coli-bacillosis by the intravenous administration of colostral antibody, but this did not protect the calf from the enteric form. Following gut closure in the calf, when colostral antibody can no longer be absorbed, however, the continual feeding of colostrum can protect against the enteric form of the disease (Logan et al., 1974) and against rotoviral infection (Woode et al., 1975).

The majority of the absorbed colostral immunoglobulins is, of course, IgG and this must be the most significant class in protection. However, Logan and Penhale (1971a) have shown that IgM can also be important, particularly in the protection of calves against colisepticaemia. Since both of these immunoglobulins are derived from the serum of the dam, they might be expected to posses

specificities appropriate to the protection of the neonatal circulation.

Although colostral antibody absorbed into the circulation has no discernible effect upon enteric infections, it has been suggested that it may play a part in mucosal defence. The absorption of colostral protein is apparently non-selective (Klaus et al., 1969; Brandon & Lascelles, 1971) and significant amounts of secretory IgA are absorbed in the pig (Curtis & Bourne, 1971) and the calf (Porter, 1972). These levels decline very rapidly and some of this secretory IgA is re-secreted on to mucosal surfaces and can be found on the surface of the respiratory mucosa (Bradley et al., 1976), where it may be important in early mucosal protection.

Milk antibody is not absorbed from the intestine since gut closure has occurred and it can only act locally within the lumen protecting against enteric infections. There is much evidence from a number of species that milk antibody can prevent the appearance of enteric disease in sucking animals. Hanson and Winberg (1972) have provided such evidence in man. In domestic species Haelterman (1965) showed that immune milk protected sucking pigs from TGE infection, while Wilson and Jutila (1975) demonstrated protection of calves from E. coli diarrhoea with immune milk and Nagy et al. (1976b) showed similar protection in piglets against E. coli diarrhoea.

Although in many cases the protection ascribed to milk has been shown to be immune in nature, care must be taken not to attribute all the protective effects of suckling to the immune system. It has been shown, for example, that a major advantage of breast feeding of infants when compared to formula feeding lies in the low buffering capacity of breast milk compared with cow's milk, which lowers the pH of the upper small intestine and so inhibits the multiplication of coliforms (Bullen & Willis, 1971), and milk contains many bacteriostatic agents, such as lysozyme lactoferrins and lactoperoxidase (reviewed by Reiter, 1978) which may also be important in protection.

Since it is clear, from many studies that milk antibody is important in the protection given by milk, how do such antibodies act?

It has been shown that both colostrum and milk may suppress the multiplication of E. coli in the small intestine of the pig (Kohler, 1974) and the guinea pig (Bullen et al., 1972) and in combination with lactoferrin can be shown to be bactericidal (Bullen et al., 1972; Nagy et al., 1976a). Other workers (Brandenburg & Wilson, 1973) have shown that IgG may exert a bacteriostatic action on its own. However, Rutter and Anderson (1972) found no correlation between this bactericidal activity in colostrum and milk and its ability to protect piglets, and other mechanisms of antibody action have been investigated.

The most convincing mechanisms to emerge has been that of anti-adhesive activity, in which antibody is envisaged to act by preventing the sticking of pathogenic organisms to the intestinal surface. This has been elegantly shown in the case of E. coli. Smith and Lingood (1971) demonstrated that the sticking of the organism to the epithelium of the piglet intestine may be mediated by a surface antigen, K88, Rutter and Jones (1973) showed that sows immunised with purified K88 protected their offspring from experimental challenge with K88 bearing pathogenic E. coli. They subsequently showed that colostrum and milk from immunised sows possessed in vitro anti-adhesive activity (Jones & Rutter, 1974). This anti-adhesive effect has also been shown in vivo and has been shown to correlate with protection of the suckled pig (Nagy et al., 1976b). Anti-adhesion as the major protective mechanism of milk antibody is an attractive hypothesis since it will protect the neonatal animal while allowing the multiplication of pathogenic organisms within the lumen of the intestine as reported by Kenney et al. (1974) and Kohler et al. (1967).

Anti-adhesive antibody can be induced in colostrum and milk by a number of immunisation regimes. Rutter and Jones (1973) originally immunised by intramammary injection in the sow, Nagy et al. (1976b) used the intramuscular route, and recently Evans et al. (1980) showed that oral immunisation of sows with K88 bearing E. coli during lactation also produced increased levels of specific anti-adhesins in milk.

However, it is clear that in addition to anti-adhesion, other mechanisms can also be important. Colostral antibody directed against the somatic antigen of E. coli, which is not concerned with the attachment of the organism to the enterocyte, can be protective (Chidlow & Porter, 1979). Furthermore, antibody may also cause alteration in the pathogenicity of micro-organisms.

Thus, Gothefors et al. (1975) have shown that pathogenic micro-organisms in the faeces of breast fed babies are more susceptible to in vitro bactericidal assays than are similar organisms isolated from non-breast fed infants, while Linggood and Porter (1978) and Linggood et al. (1979) have shown that immune colostrum can cause the in vitro loss of virulence determinants from pathogenic E. coli. The significance of these findings in field conditions is not yet fully understood, however.

An important consideration in evaluating the various immunisation regimes is that of deciding which immunoglobulin class is the most efficient in mediating the protection afforded by milk. Studies in which the protective ability of colostral antibody have been assessed have shown that IgG, IgA and IgM are all able to afford protection. (Stone et al., 1977) In addition, evidence has been produced indicating that each class can have advantages under certain circumstances. Chidlow and Porter (1979) showed that IgM antibody directed against the somatic antigen of E. coli is more protective than is IgG antibody, while Nagy et al. (1976b) and Nagy et al. (1978) presented data which demonstrated that protective anti-adhesive antibody appears in milk following intramuscular injection. Although the class of antibody was not determined, there can be little doubt from the work of other groups (Bourne et al., 1975; Chidlow & Porter, 1977) that the antibody is IgG. Finally, Kohler et al. (1975) showed that oral administration of E. coli to sows produced highly protective antibody in colostrum which, as previously described, is IgA.

However, although all three classes are capable of protecting the small intestine of the sucking animal, IgA has a number of advantages over the other two. Since it is the major immunoglobulin in milk it does not suffer the great fall in concentration seen in both IgG and IgM during the transition from colostrum to milk and IgA antibody titres will be higher and more persistent (Evans et al., 1980). In addition, IgA is more resistant to the proteolytic activity of enzymes than are IgM and IgG (Underdown & Dorrington, 1974) and will therefore persist longer in the hostile environment of the intestine. It has also been shown that complement fixing antibody can cause damage at mucosal surfaces (Brandtzaeg & Tolo, 1977) and it has been suggested that IgA, which can mediate few biological functions through its Fc region and, in particular, probably cannot fix complement (Colton & Bienenstock, 1974) may not cause such damaging effects (Ferguson, 1977)

Finally, IgA has been shown to be closely associated with the villous and crypt epithelium of the sucking rat (Nagura et al., 1978) and pig (Butler et al., 1981) which would greatly enhance the efficiency with which it could protect the mucosa.

In view of these advantages, it is not surprising that immunisation regimes stimulating high levels of IgA in milk have proved very efficient at protecting the sucking animal. Kohler et al. (1975) have reported almost complete protection against experimental challenge with virulent E. coli when sows were immunised orally with the same organism, and Hess et al. (1978), by orally immunising sows with attenuated TGE virus, produced immunity in 90% of suckled offspring. This protection is dependent upon adequate stimulation of the intestinal tract which currently requires the feeding of virulent or attenuated live organisms that are capable of colonising the intestinal tract.

In addition to protection against infectious disease, there is much evidence in man that breast feeding may protect against the development of allergy. Breast fed infants born to allergic parents have a lower incidence of atopic disease than bottle fed babies born to similar families (Matthew et al., 1977; Saarinen et al., 1979). Since a period of transient IgA deficiency during the first three months of life commonly precedes the development of allergic disease (Taylor et al., 1973) it is possible that increased absorption of cows milk proteins by bottle fed babies as the result of defective immune exclusion (Stokes et al., 1975) may lead to subsequent disease. An alternative explanation may be found in the altered gut flora established in babies fed cows' milk where E. coli predominates (Bullen & Willis, 1971), and it is possible that absorption of adjuventising endotoxin during the IgA deficient phase may lead to sensitisation. Immune exclusion, the blocking of the passage of antigen across mucous membranes by antibody, is not, however, an absolute phenomena (Swarbrick et al., 1979) and the protection from the development of damaging hypersensitivity reaction results at least in part from the development of oral tolerance (Bazin & Platteau, 1977). Factors associated with breast milk feeding that may influence this have not been characterised, but the observation that feeding B-cell mitogen enhances the development of orally induced tolerance to contact sensitising agents (Newby et al., 1980a) and the alteration of immune responsiveness observed during periods of dietary change (Newby et al., 1980b) suggests possible mechanisms.

Although the existence of cells in milk and colostrum has long been recognised, only recently have any functional effects on the suckling offspring been appreciated. The presence of viable macrophages in milk have been shown to protect suckling rats from necrotising enterocolitis (Pitt et al., 1974). The immunologic roles played by T and B lymphocytes in milk has been more controversial. Skin lesions identical to those found in graft-versus-host (GvH) disease have been demonstrated in F1 hybrid suckling rats of females which had rejected F1 hybrid skin allografts (Beer et al., 1976). The authors suggest that these lesions result from immune lymphocytes being absorbed from milk by the F1 hybrid offspring and subsequently mounting a mild GvH reaction. Further suggestive evidence for the absorption of lymphocytes comes from the demonstration of prolonged allograft survival on rats nursed on allogenic foster mothers (Beer et al., 1974). The subsequent wasting syndrome with symptoms typical of GvH that occurred in these suckling rats is evidence that this disease was caused by the absorption of allogenic milk lymphocytes. Considerable evidence has thus been collected to indicate the absorption of viable lymphocytes from milk (for review see Head & Beer, 1978). A physiological role for these absorbed cells has also been suggested.

Mohr (1973) reported positive tuberculin skin tests in children who had been breast fed by tuberculin-positive mothers. Subsequent studies have shown an absence of tuberculin-reactive lymphocytes in cord blood, but their presence in colostrum and early milk of women with tuberculin-reactive peripheral blood T cells (Schlesinger & Covelli, 1977; Ogra et al., 1977). The absence of tuberculin sensitivity in non-breast fed babies of tuberculin positive mothers provides further support that milk is the medium of transfer

In addition to the suckled offspring, milk can also afford protection within the lactating mammary gland itself and in view of the economic importance of mastitis in the dairy industry, much early research was directed to producing antibody in the udder (Derbyshire, 1961; Derbyshire & Smith, 1969; Norcross et al., 1968). However, although most studies have indicated that a degree of protection can be achieved by increasing milk antibody titres, no method of immunisation has produced long lasting protection from disease (reviewed by Norcross & Stark, 1970).

The possibility that cells in milk might also be protective may appear at first to be diminished by the reports that milk leukocytes are not as efficient as phagocytosing bacteria as cells prepared from the peripheral circulation (Lascelles et al., 1969; Russell et al., 1977). This defective activity is attributed to phagocytosed caesin, and milk cells washed and resuspended in medium are ten times more active (Lascelles et al., 1969; Hill et al., 1978). The presence or infiltration of leukocytes in the mammary gland does, however, play a significant protective role against coliform pathogens (Schalm & Lasmanis, 1976). Neutrophils isolated from mammary glands stimulated with a staphylococcal culture filtrate have been shown to kill serum resistant strains of E. coli, apparently in the absence of serum derived opsonisation factors (Hill et al., 1978). Infusion of serum resistant E. coli into a single mammary gland led to a rapid increase in the milk leukocyte count and removal from the gland of all viable bacteria. Subsequently these authors have shown that recovery from infection is related not to the number of invading organisms but to the animal's ability to mobilise neutrophils into infected glands and on the ability of those cells to kill the infecting organism. (Hill et al 1979).

## 6. EFFECT OF COLOSTRAL AND MILK ANTIBODY ON THE IMMUNE RESPONSE OF THE OFFSPRING

The immune effects of passively transferred maternal colostral antibody have been variously reported to be either suppressive or enhancing dependent both on the species and experimental system studied.

Antigen specific inhibition of immune responsiveness has been demonstrated in pigs. Hoerlien (1957) showed that whilst the immune responsiveness to a variety of antigens was diminished if colostrum was withheld, feeding colostrum from sows immunised with Brucella abortus suppressed the piglet's primary immune response to that antigen. In contrast, however, Segre and Kaeberle (1962a,b) reported that premixing diphtheria and tetanus toxoids with highly diluted immune sera of porcine or equine origin enabled colostrum deprived baby pigs to make antibody. Subsequent studies, however, indicated that non-immune sera could exert a similar effect (Segre & Myers, 1964). More recent studies with hog cholera virus have shown that whilst colostral

antibody to the virus inhibited the piglet's primary immune response, the response to subsequent challenge with the virulent organism led to a secondary response, indicating that priming of the immune system had occurred (Corthier & Charley, 1978). In these studies IgM antibodies were apparently more suppressive than those of the IgG class. The concentration of passively transferred immunoglobulin have also been reported to be important, concentration being directly related to inhibitory capacity (Rouze et al., 1974).

The possible immunoregulatory role of passively transferred colostral and milk antibody thus remains unclear. The effects of serum antibody following active immunisation are equally complex and dependent on the nature of the antigen, and the class, affinity and concentration of antibody produced (for review see Uhr & Möller, 1968; Playfair, 1974).

Most studies of the regulatory effects of colostral and milk immunoglobulin have involved the use of IgG and IgM antibodies. It has recently been shown that passive transfer of an IgA myeloma with antibody-like binding affinity for DnP can enhance the immune response to DnP conjugates (Stokes et al 1980). Since IgA from colostrum is absorbed intact into the circulation of neonatal calves and pigs (Curtis & Bourne, 1976; Porter, 1972) it is possible that this IgA may have an immunoregulatory role on the immune responses of the young animal.

It has been suggested that milk antibody may suppress the capacity of the local immune system to respond to local immunisation. However, early reports (Plotkin et al., 1966) of the inhibitory effects of breast feeding on the response to oral poliovirus vaccination in the immediate post-natal period have not been substantiated in subsequent studies where a trivalent oral vaccine was used (Deforest et al., 1973).

## 7. HARMFUL EFFECTS OF COLOSTRAL AND MILK ANTIBODY

Although in man the effects of rhesus antibodies in causing haemolytic disease of the newborn are restricted to those that cross the placenta (for review see Beer and Billingham, 1971), members of those species which receive their passive immunity via colostrum and milk are not exempt from such disease. Haemolytic disease of the newborn has been recorded in pigs (Nordstoga, 1965), calves (Dimmock & Bell, 1970) and horses (Franks, 1962). Sensitisation of the dam may occur as a result of repeated pregnancies

(Nordstoga, 1965); following the use of vaccines based on infected calf blood (Dimmock & Bell, 1970) or experimentally after the administration of erythrocytes (Dimmock et al., 1976).

Other harmful effects of colostral and milk antibody have been reported. These include the absorption of potentially harmful anaphylactic antibodies in the calf (Petzoldt & Von Benten, 1978) and the transfer of potentially allergenic substances eaten by the lactating mother to the suckling child (Jakobsson & Lindberg, 1978).

## 8. CONCLUSION

An impressive amount of information has been amassed concerning the immune system in the mammary secretions of domestic species, and this knowledge has been successfully applied in recent years to the control of a number of diseases of young animals, notably neonatal E. coli diarrhoea and TGE in baby pigs. It is to be hoped that as more knowledge is acquired and methods of stimulating high levels of immunity in mammary secretions are refined, that control of other diseases of young animals may be improved and some progress made towards immunoprophylaxis of mastitis.

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