Immunoglobulin mechanisms in health and nutrition from birth to weaning

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Immunological requirements of the neonate

Throughout its development, the foetus is sheltered from maternal antigens by a selective immunological sieve provided by the placenta. Thus only those factors which are of nutritional benefit are permitted to pass and neither maternal cell proteins nor infectious agents may invade the sanctity of the uterus. In consequence the newborn emerges from its protective shelter equipped with a rudimentary immune system which has not hitherto been primed to function, and has the urgent requirement to mount a resistance to the intensive microbial challenge of the outside world. The elements of resistance are cells of the lymphoid and reticuloendothelial system together with immunoglobulins and certain ancillary factors such as complement, lysozyme (*EC* 3.2.1.17) and lactoferrin. With full immunological competence, antibodies and cells extravasate into every tissue of the body, providing local resistance at the seat of any microbial challenge. Under most circumstances, the extent of the protection will be determined by the quantitative capability of the host to provide a satisfactory response to an infective challenge.

In the neonate this capability is negligible and time is of the essence when one considers that the generation time of enterobacteriaceae may be as short as 20 min; thus, within a matter of hours the alimentary tract may, for example, be populated by hundreds of millions of bacteria. All mammals rely extensively on passively derived maternal antibody to cope with the immediate problem of proliferation of enteropathogens in neonatal life.

Many species have the benefit of transfer of maternal immunoglobulin during foetal life; but in certain others, epitheliochorial placentation interposes several layers of epithelium between foetal and maternal circulations, sufficient to bar the transport of antibodies. In such species, and for the present purposes, pigs and calves will be the main subjects for comparison, maternal antibodies are derived entirely by way of the colostrum. The intestines of such species are equipped initially with epithelial cells which are capable of pinocytotic uptake of macromolecules, and immunoglobulins are thereby passed on to the blood circulation of the neonate. In contrast the human foetus acquires maternal

antibodies across the placenta and normally there is no further augmentation post partum; the antibodies in colostrum and milk are not absorbed to any extent.

The phenomenon of intestinal absorption of maternal immunoglobulins has been extensively examined by nutritionists and immunologists alike as one of the most relevant areas bridging the two disciplines. The relatively short duration of the phenomenon is inversely matched by the lengthy literature on the subject which encompasses such topics as mode of absorption, period of permeability, mechanisms of closure, cell surface receptors in transport, specificity factors, anti-proteolytic factors and numerous others. However, the main theme of this paper is to examine the role of lactation in bridging an immunological gap and thereby maintaining the functional integrity of the neonate's alimentary tract against a potentially hostile microbial environment. In this context it is advantageous to neglect the niceties of intestinal transport, which bear obvious relevance to nutrition, and examine the additional purpose in lactation of continuing to supply antibody to the suckling neonate when intestinal closure has occurred.

Transudation and secretion of immunoglobulins in the mammary gland

Considerable quantities of immunoglobulin A (IgA) are secreted in human milk; it is the predominant immunoglobulin (Chodirker & Tomasi, 1963) and there is relatively little change in proportion of individual immunoglobulin classes throughout lactation although the level of immunoglobulin diminishes substantially. The failure of IgA to predominate in the colostrum of the porcine and bovine species as it does in the human (Fig. 1) is understandable in terms of the universal role of immunoglobulin G (IgG) for passive immunity and the physiological requirement for intestinal transport of maternal immunoglobulin in the offspring. However, there are significantly contrasting features apparent in the changing immunoglobulin profile in the mammary secretion as lactation progresses. In both species the total concentration of immunoglobulin falls steeply during the first few days of lactation. It is a rapid decline in the level of IgG during this period which contributes most to the decline in total immunoglobulin content of the milk. This is clearly a necessary economy in a maternal immunoglobulin which is transferred almost exclusively from the blood circulation by a transudative process across the mammary acinar epithelium. The period of mammary transudation is probably very little longer than the period of intestinal permeability in the neonate.

In the pig, IgA emerges as the dominant immunoglobulin in the milk (Porter, Noakes & Allen, 1970). Antibody activity attributable to this immunoglobulin class in the milk bears little or no relationship to that in the serum; this has been observed in relation to pathogenic *Escherichia coli* (Porter *et al.* 1970) and transmissible gastroenteritis virus (TGE) (Saif, Bohl & Gupta, 1972). Immunohistological studies have demonstrated the presence of cells synthesizing IgA in the glandular tissue (Porter *et al.* 1970; Brown, Bourne & Steele, 1974), supporting the indirect observation derived from an examination of the transport

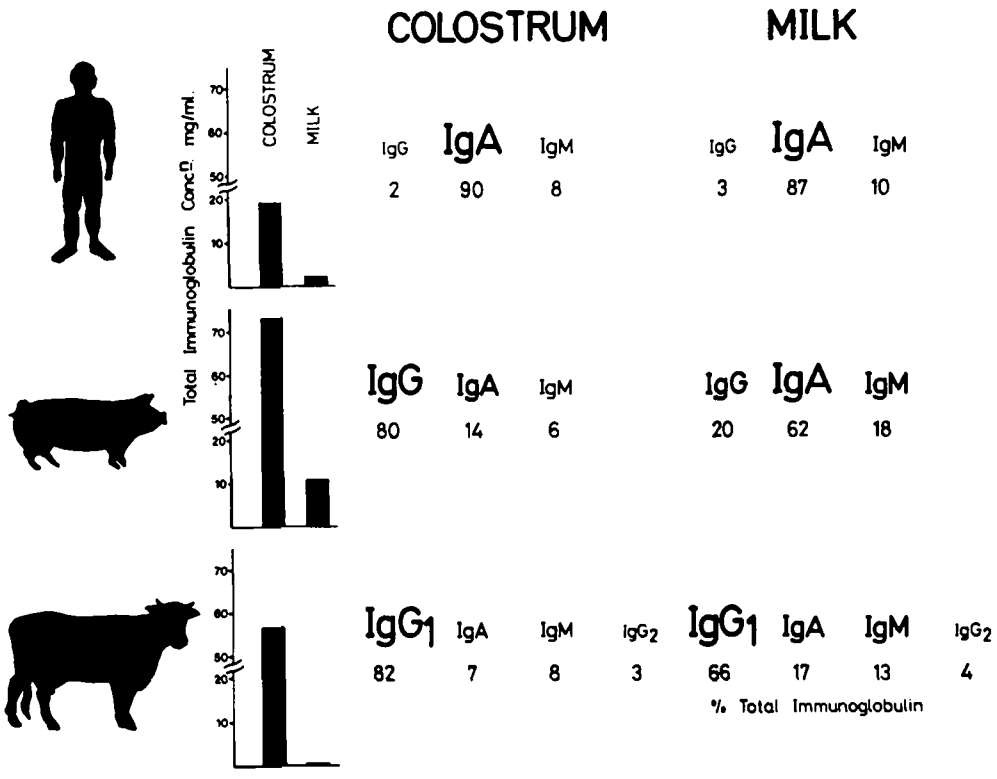

Fig. 1. Comparative levels of immunoglobulin in colostrum and milk of human, porcine and bovine species with its composition in terms of specific classes of immunoglobulin.

of radio-labelled immunoglobulin that more than 90% of milk IgA must be derived by local synthesis (Bourne & Curtis, 1973).

The major source of origin of cells synthesizing IgA in the mammary gland is most likely to be the gut. Antibodies of the IgA class in colostrum and milk of the sow are strongly associated with intestinal colonization; this has been apparent in studies of TGE in particular (Bohl, Gupta, McCloskey & Saif, 1972). The movement of cells from and to the lamina propria of the intestine is a particular feature of lymphoid cell traffic, and it has been suggested that Peyer's patches may act as a source of 'memory' cells which migrate to other lymphoid tissues (Cooper & Turner, 1969). Furthermore, Craig & Cebra (1971) have shown that such cells have the potential to proliferate and differentiate into IgA-producing immunocytes in the lamina propria of the small intestine and it is not inconceivable that the same process would take place in the lactating mammary gland.

In contrast to these observations, the mammary tissue of the lactating cow is deficient in immunoglobulin synthesis in organ culture studies (Butler, Maxwell, Pierce, Hylton, Asofsky & Kiddy, 1972). This is reflected in very low levels of immunoglobulin in the milk after the first phase of lactation involving colostrum formation. However, the immunoglobulin profile of the colostrum provides some interesting insight into the potential of the bovine udder for local immune

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response, in that the molecular characteristics and antibody activity of immunoglobulin IgA are quite different from that attributable to IgA in the blood serum (Porter, 1972). This IgA in colostrum is present largely as the high-molecular-weight 11S form, containing bound secretory component. Furthermore antibacterial antibody to *E. coli* is associated with it and is not attributable to the 9S dimer and 7S monomer present in the blood plasma.

The uniformly low level of immunoglobulin secretion in milk is a feature of the ruminant species in general. It appears that the properties of local immunosynthesis in the mammary gland are largely dependent upon its physiological state. Early histological studies (Campbell, Porter & Petersen, 1950) provided evidence of plasmacytosis in the dry udder and during colostrum formation, but not during normal lactation. More recent evidence in the ewe shows that the number of interalveolar and intraepithelial lymphocytes decreases rapidly after parturition when suckling begins (Lee & Lascelles, 1970).

There are numerous reports of the induction of antibody activity in bovine milk by local infusion of antigens into the udder. However, this in itself is not satisfactory evidence of local synthesis without details of the class of immunoglobulin involved. Our own studies with *E. coli* antigens show that the main response is a transient high titre of antibody in colostrum and early milk associated predominantly with IgG_1, whereas longer-term, lower antibody titres are associated with IgA. Of interest in this context are the studies of Lascelles and co-workers in the lactating ewe: infusion of antigen into mammary glands a few weeks before parturition stimulates antibody production (Lascelles, Outteridge & Mackenzie, 1966) and this is associated with an increase in numbers of lymphoid cells located in close association with the glandular epithelium (Lee & Lascelles, 1970). Immunofluorescent studies indicated that IgA was the predominant immunoglobulin synthesized by such cells in the antigen-stimulated gland.

The role of maternal immunoglobulin in the neonatal gut

Secretory 11S IgA anti-*E. coli* antibodies of porcine colostrum have been shown to operate entirely in the lumen of the intestine without contributing significantly to antibody in the blood circulation of the piglet (Porter, 1970). Throughout neonatal life the physiological patterns of milk antibody secretion, ingestion and passage through the alimentary tract combine to provide a continuous bathing of the intestinal epithelium with maternal IgA antibody. The effectiveness of the antibody in local defence of the intestine is assisted by its capacity to resist proteolytic degradation by digestive enzymes, a property facilitated by the complexing of secretory component into the 11S IgA structure. Furthermore, during normal suckling patterns the antibody is presented to the alimentary tract with sufficient frequency to discount any short-term ineffectiveness. A further factor facilitating its action is the rate at which milk IgA passes from the stomach after feeding. Studies in fistulated pigs (Porter *et al.* 1970) showed that IgA began to pass into the duodenum within 5 min of a single feed and that the majority of the antibody had passed intact into the intestine within 1 h.

The importance of local protection attributable to milk immunoglobulins in the young pig is best evaluated in relation to the observed decline in passive immunity within its first few weeks of life. The passively acquired immunoglobulins in piglet serum fall in level after 2 d of age when intestinal closure is established. The pattern of decline is almost exponential, with mean half-lives for immunoglobulin M (IgM), IgA and IgG of 2·8, 2·7 and 9·1 d respectively (Curtis & Bourne, 1973). Susceptibility to *E. coli*-associated enteric problems is clearly accentuated by weaning at 2–4 weeks of age, a period of apparent critical antibody deficiency (Miller, Harman, Ullney, Schmidt, Luecke & Hoefer, 1962) and the specific protective function of maternal IgA in maintaining the host–microbial balance healthily in favour of the piglet is beyond dispute in these practical terms. This is particularly emphasized in TGE infections of the neonatal piglet; IgA class antibodies are essential for solid passive protection (Bohl, Gupta, Olquin & Saif, 1972); the high levels of IgG antibody in the colostrum absorbed into the serum of the neonate will not protect against infection with the virus. Furthermore, orally administered milk from immunized sows has been shown to exert a protective effect against enteropathogenic serotypes of *E. coli* in experimentally infected gnotobiotic pigs (Miniats, Mitchell & Barnum, 1970). Comparative studies of oral *v.* parenteral administration of antibodies have been carried out (Owen, Bell, Williams & Oakes, 1961; Kohler, 1967; Miniats *et al.* 1970) and in each instance the oral route provided the most effective control of *E. coli* infection, further emphasizing the value of local antibody in the alimentary tract.

The property of the bovine mammary gland in sub-class selection between IgG_1 and IgG_2 is a well-known phenomenon, but the significance of this has not been defined. The calf intestine exhibits no selection in the absorption of maternal immunoglobulins and the interesting phenomenon occurs in which high levels of 11S secretory IgA are temporarily present in the blood circulation (Porter, 1972). Under normal circumstances in all mammalian species examined, little or no 11S IgA ever appears intravascularly, and in studies in man in which 11S IgA was administered intravenously, the immunoglobulin disappeared rapidly from the circulation, exhibiting a half-life of less than 2 d (Butler, Rossen & Waldman, 1967). The relevance of this phenomenon in the calf bears examination in relation to enteric infections. Susceptibility to septicaemia caused by *E. coli* in the neonatal calf is attributable to deficiency in absorption of colostral antibody and probably more specifically to antibody with given immunoglobulin class characteristics. For example, Logan & Penhale (1971) have tested the protective efficiency of the IgG and IgM fraction of colostral whey in colostrum-deprived calves and found IgM to be significantly more effective than IgG. However, it was interesting that the activity of the colostrum itself was unaccounted for by proportionate recombination of these two fractions, implying the presence of other undefined factors.

It is in this respect that colostral 11S IgA may play a significant role. Enteric infections in the calf probably commence with the attachment of bacteria to the intestinal epithelium. It is of interest that a common K antigen, designated K99,

occurs in calf and lamb enteropathogenic strains with the specific property of facilitating adhesion to calf and lamb intestine (Smith & Linggood, 1972). A primary role of maternal immunoglobulin in protection of the neonate will therefore be to interfere with the local attachment of enteropathogenic *E. coli*. The most effective immunoglobulin will therefore have the property of functioning locally. Of course the neonatal calf ingests substantial levels of maternal immunoglobulin for only its first 2 or 3 d of life. Thereafter it is frequently weaned on to milk-substitute feeds, or if it does continue to suckle, the level of immunoglobulin in the maternal milk declines to such low levels as to be insignificant. However, the interesting feature of its initial absorption of colostral 11S IgA is the fact that this immunoglobulin is subsequently passed by a transudate process into various external secretions, including those of the alimentary tract. This process continues for a period of approximately 10 d, providing the basis of a short-term, passive barrier to infection.

The mechanism of maternal antibody function in the neonatal intestine has yet to be fully evaluated. Colostral IgA is a potent agglutinin of enteric organisms; furthermore porcine colostral IgA acts with complement and lysozyme to lyse *E. coli* (Hill & Porter, 1974), presumably by activating the complement sequence through the alternate pathway (Gotze & Muller Eberhard, 1971). There are adequate levels of lysozyme in colostrum, milk and intestinal secretions to effect bacteriolysis, but evidence for a functional complement sequence active in the alimentary tract needs to be established before one can discount the most probable mechanism being bacteriostasis. Peristaltic flow is a simple factor working against the establishment of micro-organisms in the anterior regions of the small intestine. Bacteriostasis and agglutination, together with peristalsis, may well be critical host-defence mechanisms. It has been demonstrated that antibody directed specifically against the determinants of bacterial adhesion, K88 in the pig, will protect the neonate from infection with enteropathogenic *E. coli* (Rutter & Jones, 1973). Thus locally active intestinal mechanisms may well be sufficient without having to invoke such second-line mechanisms as opsonization or enterotoxin neutralization. Nevertheless solid immunity may only be attained with the provision of every possible type of host defence. The simplistic attack on bacterial colonization by antibody interference with adhesion may not always succeed, since the enteropathogen is quite capable of camouflaging itself against an aggressive environment mediated through antibodies in the intestine by manipulating its own surface antigens. Thus colonization of the upper small intestine is not necessarily determined in the pig by the adhesion properties of K88 (Miniats & Gyles, 1972; Wilson & Hohmann, 1974), and other effective mechanisms must contribute which will demand other types of resistance in the host.

Development of intestinal immunity in the neonate

The animal kingdom has evolved in constant association with micro-organisms, and it is hardly surprising that an organ so extensively colonized as the intestine should develop mechanisms of defence for its tissues. Immunological investigation

of primitive vertebrates among the fishes suggest that this form of defence probably evolved 250 million years ago (Good, 1967). It is true that certain components of the intestinal flora play an indispensable role in nutrition and development of the host, yet others recognized as pathogens are detrimental to the host. The way in which the host tolerates the proliferation of certain micro-organisms and yet sets up mechanisms of rejection to others remains to be determined. But one central fact remains: for survival to be assured at weaning, the immune mechanisms of the intestine must have developed sufficiently well in neonatal life to compensate for the withdrawal or decline of maternally derived immunity.

The specialized local immune system located at or on epithelial surfaces and mediated mainly by IgA has now been well defined in the majority of mammalian species, and it appears to be a common feature of defence against the external environment. It has particular relevance to alimentary tract infection and especially with regard to weaning when enteropathogenic *E. coli* frequently proliferate among the intestinal flora in response to the removal of the controlling characteristics of maternal milk. In intensive piglet-rearing systems the majority of animals will suffer infectious episodes and a number will die. Svendsen, Larsen & Bille (1974) reported 82% morbidity due to *E. coli* in an extensive field survey of weaned pigs in Denmark.

Intensification contributes to environmental deterioration and the problem is exacerbated by continuous animal throughput. This was particularly well demonstrated by studies of the early weaned calf (Roy, Palmer, Shillam, Ingram & Wood, 1955). Enteropathogenic *E. coli* are not normally invasive at this time and their damaging effects appear to be largely due to toxic interaction with the tissues (Sojka, 1971). Thus in order to contribute to the correct balance in the host–pathogen relationship the local intestinal immune system should be exerting its authority over the microbial flora before weaning.

The enteric flora appear to be mainly responsible for developing immune mechanisms in the alimentary tract (Crabbé, Bazin, Eyssen & Heremans, 1968). In the germ-free state, the pig shows virtually no development of lymphoid tissues even at 5 weeks of age (Kenworthy, 1970) whereas after 10 d of mono-contamination with *E. coli* the intestinal tissues provide essentially the same picture as that of the conventional animal (Kenworthy, 1971). The use of fistulated animals has facilitated investigations of the ontogeny of immunoglobulin synthesis in the intestinal mucosa. It is significant that in both the pig and the calf, lymphocytes infiltrate the intestinal mucosa during the first week of life and are predominantly concerned with the synthesis of IgM, not IgA (Allen & Porter, 1973*a*,*b*, 1975). Evidence for the secretion of IgM across the epithelial lining indicates the primary role that this immunoglobulin plays in local defence. Subsequent development in the neonate leads in time to the increasing role of intestinal immunocytes in IgA synthesis and secretion.

Recent studies by immunoelectronmicroscopy indicate that IgM and IgA are transported across the epithelial cell in the form of membrane-bound vesicles

(Allen, Smith & Porter, 1973, 1976). The process is almost the complete reverse of that used to transport colostral immunoglobulin. Thus the vesicles penetrate the intracellular spaces and are taken into the epithelial cells by pinocytotic mechanisms, passing up through the cytoplasm to accumulate in the supranuclear region before being passed out into the gut lumen (Fig. 2). IgA is readily

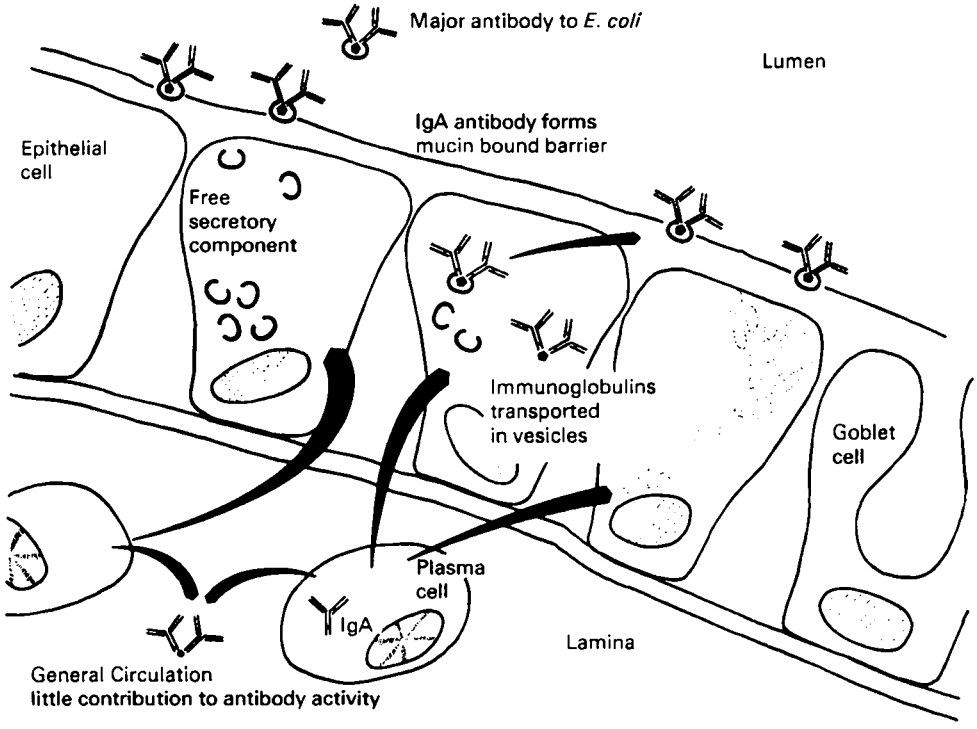

Fig. 2. Schematic representation of the mechanism of immunoglobulin A (IgA) secretion into the intestine of the young pig.

complexed by secretory component, which facilitates the binding of the immunoglobulin into the mucin layer over the external surface, thereby binding antibody in high local concentration and producing a local barrier to infection. IgM, on the other hand, is not complexed by secretory component and is released into the lumen.

Active synthesis and secretion of immunoglobulins in the intestinal tissues is of little significance to the neonate at weaning if antibody specificity is not directed against potential pathogens. In this context, examination has been made of oral immunization with sterile preparations of bacterial antigens extracted from recognized enteropathogens, and the effects in the piglet and calf have been recorded in terms of conventional parameters of performance and health (Porter, Kenworthy, Holme & Horsfield, 1973; Porter, Kenworthy & Thompson, 1975). The bacterial antigens were incorporated into the diet at levels calculated to exceed the minimal required dose at least 10-fold and in this way routine oral dosing of large numbers of animals was conveniently achieved. Significant benefits were

recorded in terms of reduced incidence of diarrhoea and requirement for medication, but additionally there were significant improvements in growth rate and nutritional performance. The implications of this latter observation are that the young weaned animal has successfully made the transition from maternal protection to its own secretory-mediated defence, maintaining functional integrity of the intestine and thereby offsetting a temporary growth depression.

This contribution to animal performance and nutrition is now a consistent finding substantiated in other laboratories (Balger, 1974; Schollenburger & Frymus, 1974; Svendsen, Larsen, Bille & Nielsen, 1974; Balger, Chorherr, Sickel & Gieben, 1975). The long-term benefits of oral immunoprophylaxis against bacterial enteropathogens in improving and maintaining health patterns and environment have yet to be examined thoroughly; but the general indications are that, as well as the more obvious parameters of health, a greater uniformity of performance may be maintained in intensive systems with a continuous throughput of animals. Thus by seeking to maintain the continuance of antibody function throughout neonatal life and weaning, a link is completed which benefits environment, health and nutrition.

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