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IS THERE A TIME WINDOW OF THE IMMUNE SYSTEM AS A LEARNING SYSTEM?

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SUMMARY

In rodents there is evidence that during the first week of life orally administered antigens induce immune responses rather than tolerance. Thereafter tolerance also appears and this can be due to the seeding of late thymic migrant T cells proven to have profound regulatory potential and are crucial for the development of tolerance to certain self-structures. These T cells express CD25 and CTLA-4 and may produce suppressive cytokines like IL-10 and TGF-ß.

Maternal anti-idiotypic antibodies can induce responses in the foetus but also in the neonate via the milk. Idiotypic priming directed against one antigen on a microbe seems to enhance the response to other antigens on the same microbe as well.

In man there is no apparent evidence for any early window in the neonates' immune system indicating that an analogous development of regulatory T-cells must happen during foetal life. The human neonate is capable of responding quite well to vaccines directly, but due to the delay in appearance of a broad repertoire of protective antibodies and T cells the maternal transplacental IgG antibodies and the passive protection provided via the milk are important. The milk also contains a number of components and signals, which can activate various systems resulting in a long-term enhancement of the immune and neuro-endocrine systems.

INTRODUCTION

The neonate is in a very special situation when leaving its sterile, protected environment in utero to be delivered into a milieu heavily contaminated with microbes. The new-born is coming out next to the mother's anus which normally render them colonised with the mother's intestinal flora. This is often prevented today in industrialised countries due to misplaced hygienic measures the unnatural positioning of the woman. This has resulted in late occurrence of i.a Gram-negative bacteria in infants stool and a striking early appearance and frequent persistence for months of toxin-producing *Staphylococcus aureus* (*Lindberg* et al., 2000). In a natural birth situation Gram-negative bacteria appeared very soon after the delivery (*Adlerberth* et al., 1999).

The immune system of the new-born may be only a few percent or less of that in an adult (*Adkins*, 1999) The major stimulus to its rapid growth after birth is the exposure of the extensive mucosal membranes of the intestinal tract to the colonising microbes, finally resulting in that 2/3 of the whole immune system is localised to the intestinal tract. Against this background it is reasonable to assume that the changed intestinal flora of infants today in industrialised countries may have consequences for the development of the immune system in early life, with possible consequences in later life. Especially it seems that intestinal colonisation with Gram-negative bacteria may be necessary for the development of an adequate capacity to respond with immunological tolerance to environmental antigens.

It is clear that several components of the innate and adaptive immune systems are deficient at birth. This is illustrated by a reduced and/or different functional capacity for instance of neutrophils (*Levy* et al., 1999). Since experimental animals often used for studies of the early capabilities of host defence, like mice and rats, are less developed at birth than man the subsequent presentation will first discuss such animals and then man.

At this stage, however, it should be mentioned that deficient functions of specific T cells in mice, as well as man, may only partly be a matter of number of cells rather that their functional capacity (*Adkins*, 1999). If the relative deficiency of T cell-produced cytokines in the neonate is compensated for adequate responsiveness is claimed to be seen. With adequate stimuli, like the BCG vaccine, the often expected Th2 response of the human neonate can be transformed into a Th1 response (*Marchant* et al., 1999; *Vekemans* et al., 2001). With certain other vaccines or infectious agents *like Plasmodium falciparum* variably reduced Th1 and also Th2 responses are seen in infants (*Clerici* et al., 1993; *Prescott* et al., 1998). In addition to deficient Th1 function and T cell cytotoxicity in both human and murine T cells, the latter are more focused towards Th2 functions (*Adkins*, 1999).

IMMUNE RESPONSIVENESS IN THE NEONATAL RODENT

In the 1980-ies (*Hanson*, 1981) and (*Strobel* and *Ferguson*, 1984) published the observation that feeding ovalbumin (OVA) within the first week of life resulted in an antibody as well as T-cell response, whereas later feeding induced immunological tolerance reviewed by *Strobel* (1996). Recently it was found that naïve CD8 cells from the thymus were tolerised to a skin-expressed MHC class I antigen reaching the skin only in neonatal and not adult animals. Thus tolerance could be expressed early (*Alferink* et al., 1998). In humans very similar numbers of CD25+ regulatory T cells occurred in the thymus and cord blood as in mice and rats from 5 days of age, presumably cells which can prevent auto-immune diseases (*Wing* et al., 2002). Neonatal colonisation of rats with an OVA-*producing Escherichia coli* strain resulted in immunological tolerance to OVA, the 06 lipopolysaccharide (LPS) of the *E. coli* and its type 1 pili. This was noted when tested at 12 and 13 weeks of age, after immunisations with these antigens at 6 and 12 weeks after the neonatal colonisation (*Karlsson* et al., 1999). In adult rats the colonisation instead increased the immune responses, both the antibody levels and delayed type hypersensitivity to OVA. However, the relative increase of the response to the 06 LPS in the adult

animals was much lower after adult colonisation. The neonatal colonisation also resulted in bystander tolerance against an unrelated antigen, human serum albumin, indicating that at least part of the tolerance was a result of suppression mediated regulatory T-cells. This was confirmed in further studies (*Lundin* et al., 1999). However, it was also noted that oral tolerisation of rats mainly led to active suppression and bystander tolerance in adult rats, whereas anergy was predominant in young rats (*Lundin* et al., 1996).

Neonatal animals responded with antibody production to ng-levels of anti-idiotypic antibodies, whereas higher doses were less effective. This was shown in mice using monoclonal idiotypes and anti-idiotypes against the *E. coli* K13 polysaccharide capsule (*Stein* and *Soderstrom*, 1984). This is especially notable since polysaccharide antigens do not normally induce antibody responses in young animals or children, although polysaccharide-protein conjugate vaccines are well known to do so. Using idiotypic mimicry the K13 polysaccharide was thus transformed into a protein antigen which made it an effective immunogen in the neonate. A maternal transfer of idiotype and anti-idiotype seems to give a better priming for an antibody response than the anti-idiotype alone as shown using poliovirus antibodies in germfree piglets (*Lundin*, 1998).

A remarkable effect on the new-born rat's immune system was noted using monoclonal anti-K13 anti-idiotypic antibodies testing two consecutive generations (*Lundin* et al., 1999). Two days old neonatal female rats were fed mg doses per orally of the anti-idiotype. Six weeks later they were colonised with the OVA-producing *E. coli* 06:K13. No significant effects were noted in these rats on the immune response to the bacterium. However in their offspring a clearly enhanced antibody response was obtained after colonisation with the same strain at 6 weeks of age. The antibody responses to the OVA, as well as the 06 and K13 antigens were increased, more in the group given 10 mg than the one given 1 mg of the anti-idiotype. Concurrently, the proliferative response of spleen cells to OVA and the bacteria was lowered.

Giving new-born mice anti-idiotypes against the *E. coli* K13 and a viral antigen via the mother's milk also resulted in an enhanced serum antibody response in the offspring (*Stein* and *Soderstrom*, 1984; *Okamoto* et al., 1989).

In mice and rats there is a limited placental transfer of antibodies from the mother to offspring, but the milk brings a number of factors from the mother, including antibodies. In a recent study we have investigated the possible effect of the fatty acids in the mother's diet on the offspring's immune response. It was noted that a diet deficient in n-6 and n-3 essential fatty acids (EFA) compared to a control diet enriched in EFA resulted in an increased ratio of saturated/unsaturated fatty acids in the milk of the rat dams (*Korotkova* et al., 2001). The effect of feeding the dams OVA during early lactation on the subsequent immune response of the rat pups was measured. It was found that the pups of the dams on the diet deficient in EFA and exposed to OVA responded significantly less to immunisation with OVA compared to the pups of the dams on the control diet enriched in EFA (*Korotkova* et al., submitted for publication). They produced less delayed type hypersensitivity reactions and less IgG, IgM and IgE antibodies to the OVA. Thus it seems that tolerance to a food protein may be transferred via the milk, but less so by mothers on a diet with a higher n-6 and n-3 fatty acid intake.

It was also noted that the diet deficient in EFA to the dams reduced the

leptin levels in the serum of their pups (*Korotkova* et al., 2001). The hormone leptin has a structure similar to the IL-1 cytokine and is known to stimulate Th1 reactivity (*Lord* et al., 1998). It is not clear whether this can influence the immune responsiveness of the offspring, although leptin was recently found to stimulate CD4 as well as CD8 lymphocytes via their JAK/STAT pathway (*Sanchez-Margalet* and *Martin-Romero*, 2001).

THE IMMUNE RESPONSE IN THE HUMAN NEONATE

Secretory IgA and IgM antibodies to *E. coli* O antigens and to poliovirus antigen were found in amniotic fluid, meconium, urine and saliva from human neonates (*Mellander* et al., 1986). These antibodies could not have come from the mothers who only transfer IgG antibodies, but presumably appeared as a result of stimulation with anti-idiotypic antibodies from the mother. This was further substantiated in a new-born of a hypogammaglogulinaemia mother lacking IgA and IgM and only given IgG prophylactically (*Mellander* et al., 1986).

The neonate is deficient in a number of ways as to its immune system both as to size and quality (*Schelonka* and *Infante*, 1998). There is a smaller bone marrow reserve, a reduced serum complement function, more immature T lymphocytes and a deficient capacity to respond to bacterial capsular polysaccharide virulence antigens.

The new-born is usually colonised with an intestinal flora where anaerobes slowly take over to become totally predominant (*Adlerberth* et al., 1999). That flora together with the small fraction of facultative anaerobes like *Escherichia coli* provide a colonisation resistance limiting colonisation with other potentially pathogenic microbes (*van der Waaij*, 1999).

The innate immune system is deficient initially in the neonate with e.g. granulocytes having a small storage pool and being only slowly produced after exposure to bacteria or cytokines like G-CSF. The neutrophils show diminished migration, phagocytic capacity and killing. This may relate to a reduced actin polymerisation in response to chemotaxis, a defective defensin production (*Merry* et al., 1998; Salzman et al., 1998) and a lack of bactericidal/permeability increasing protein (*Levy* et al., 1999). Other important characteristics of cells participating in innate immunity, like dendritic cells, monocytes/macrophages, natural killer cells and mast cells, such as presence and functional capacity of Toll-like receptors (TLR) has apparently not been studied in the human neonate. These cells are very important in the initiation of adaptive immune responses especially by enhancing antigen presentation. It needs to be studied when the new-born has cells equipped to recognise and react to all potential pathogens by Pattern Recognising Receptors like TLR's, thus producing the cytokines, which promote microbial uptake by dendritic cells making them mature to efficient antigen presenting cells. The new-born's dendritic cells are less efficient because initially their capacity to produce IL-12 is impaired (*Goriely* et al., 2001; *Liu* et al., 2001).

Initially the great majority of CD4+ cells are CD45RA+ which gradually are replaced by CD45RO+, whereby memory function appears. Development of cytotoxic T cells has been reported to be less efficient than later. B cells are initially producing fewer isotypes, with little following IgM. The T cells pro-

duce IL-2 adequately, but only 50% of GM-CSF, TNF and IL-10, and 10% of IFN-γ and IL-4 compared with adult cells according to some reports (*Schelonka* and *Infante*, 1998). The CD4/CD8 ratio remains high up till 2 years of age. Recent work debates earlier data and suggests that human cord blood lymphocytes can efficiently produce Th1 and Th2 responses after polyclonal activation, although this may nor reflect the natural situation (*Chipeta* et al., 2000).

These still somewhat contradictory studies may be best understood in the light of some quite efficient vaccine responses in the neonate. This is noted for neonatal BCG vaccination even in a problematic African surrounding (*Marchant* et al., 1999) and the same has been found also with a first dose of oral poliovirus vaccine given on the day of birth as recommended by WHO. The latter may, however, become inefficient if the infant is simultaneously breastfed since the milk contains high levels of poliovirus neutralising secretory IgA antibodies (*Zaman* et al., 1993).

The human neonate is able to manage several functions of host defence, but because of the initially less functional innate immune mechanisms and the yet inexperienced and therefore not fully expanded adaptive immune system it is clearly at increased risk of infections.

WITH SOME HELP FROM THE MOTHER

The two well-known modes of support from the mother, as IgG antibodies via the placenta and various defence factors via her milk, are of major importance for the protection of the infant. However, we now recognise that at least the latter of these two modes of transfer of passive immunity also is a system of quite complex active signalling to the infant.

The transfer of IgG via the placenta is assumed to occur via Fcg receptors. This may not be the only mechanism involved because it seems that both specificity and affinity of the antibodies affect their transfer although these are characteristics of the Fab portion of the antibody molecule (*Avanzini* et al., 1998).

The mother's milk is certainly important by providing proper nutrient and efficient passive protection against infections. Actually enhanced breastfeeding by 40% in the poor parts of the world would more than half the mortality in diarrhoea and pneumonia according to WHO. These two diseases are the two major causes of death in young children. Beyond that there is now an increasing interest in the fact that the milk provides a number of components and signals, which actively influence the long-term outcome of the child. One could be anti-idiotypic antibodies which as has been mentioned above have induced antibody responses in breastfed offspring (*Stein* and *Soderstrom*, 1984; *Okamoto* et al., 1989).

Another mechanism seems quite surprising. Several studies have shown that leukocytes, including lymphocytes from human milk are taken up and are capable of transferring immune responsiveness for instance to vaccines (for review see *Hanson* et al., 2001). In fact it seems that the breastfed infant develops tolerance to the mothers HLA, so that the cells can be accepted and taken up. Evidence for that comes from the observation that renal transplantation with a mother donating a kidney to her child (even as an adult), causes significantly less rejection if she has breastfed that child (*Campbell* et al., 1984).

Breast fed individuals also has fewer cytotoxic cells reacting against maternal than paternal cells (*Zhang* et al., 1991).

The major protein in mature milk is lactoferrin. It efficiently kills bacteria, viruses and fungi and prevents urinary tract infection in an animal model (*Haversen* et al., 2000). It also blocks production of inflammatory cytokines like IL-1, TNF, IL-6 (*Mattsby-Baltzer* et al., 1996). This may help the new-born to dampen the effects of the sudden microbial exposure on the intestinal mucosa after birth diminishing production of these inflammatory and catabolic cytokines.

The milk contains numerous cytokines, soluble cytokine receptors, hormones etc. It is likely that they can play many different roles in the infant. Thus breastfed infants respond significantly better than non-breastfed against the *Haemophilus influenzae* type b (Hib) polysaccharide-protein conjugate vaccine with IgG2 antibodies to the Hib polysaccharide. They also respond better to Hib infections (*Silfverdal* et al., 2002). It might be that the IFN-γ required for IgG2 antibody production comes from the milk, which often contains significant amounts.

A better functional immune system of the breastfed child may explain why breastfeeding seems to protect against celiac disease (*Ivarsson* et al., 2002). There are suggestions that protection may also exist against certain auto-immune diseases, as well as Crohn's disease and ulcerous colitis, but this is based on few studies and must await confirmation (*Hanson* et al., 2001).

There is a continuous debate whether of not breastfeeding provides long term protection against asthma and allergy. A recent meta study supports a protective capacity, which is even more pronounced if there is a family history of atopy (*Gdalevich* et al., 2001) and so do a few other recent studies (*Oddy* et al., 1999; *Kull* et al., 2001). There is also some evidence that in a small group of infants the presence of maternal asthma may increase the risk of asthma in the child after long term breastfeeding (*Wright* et al., 2001). However, this was not seen in another study (*Oddy* et al., 2002).

Breastfeeding seems to have a number of further long term effects on the child like promotion of brain development, decreased risk of obesity in school age (*von Kries* et al., 2000) and lower blood pressure in adolescence (*Singhal* et al., 2001). Such effects might result from some of the many signals provided via the maternal milk and illustrate the complexity of mechanisms involved.

In full-term babies there does not seem to be any convincing evidence for any early window in the immune responsiveness. Giving cow's milk proteins during the first week to a breastfed infant did not decrease or increase the risk of cow's milk allergy (*Juvonen* et al., 1996). That may be taken as evidence against an early deficiency in the capacity to develop tolerance.

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