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The Maturation of the Immune System

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Functional Capacity of the Immune Response in the Newborn

More than 95% of the neonate's T cells carry the early T-lymphocyte marker CD38 and the CD45RO marker. These cells have a decreased helper and suppressor function for B cells [1]. Cytotoxic T-cell function is also limited [1]. This, as well as an inefficient activation of macrophages with impaired capacity for antigen presentation, is associated with an immature production of cytokines. Thus, the T cells in the neonate are efficient in producing IL-2 and TNF- β , but they emit only 50% of the TNF- α and only 10% of INF- γ and IL-4 compared to adults [1]. Still there is a capacity of T cells in very young infants to respond to environmental allergens [2].

One of the consequences of a relative T-cell immaturity for the immune response of the neonate is a rapid increase in IgM and IgM antibody responses, presumably in less need of cytokine support. Cytokines required for switch to IgG1 and 3 are clearly functional during the first 2 years of life, whereas those for switching to IgG2 and IgG4 seem inadequate during this time. Serum IgA develops slowly, but secretory IgA antibodies, measured as *Escherichia coli* antibodies in saliva, can reach adult levels in only a few weeks under heavy microbial exposure [3].

The T-cell immaturity with the limited IFN- γ and TNF- α production and hampered development of cytotoxic T cells may be the cause of the increased risk of *Listeria* infections in the fetus and newborn. This is also suggested from studies in rats [4].

Maternal Contributions to the Infant's Immune Response

The placental transfer provides a multitude of maternal IgG antibodies for passive protection of the infant. However, the transfer of idiotypes (id) and anti-idiotypes (a-id) to the fetus may have lasting effects on the immune system of the offspring via the immunological network. That such effects of serum antibodies may occur is supported by animal studies, showing that a-id given neonatally could activate silent B-cell clones [5]. Injection of newborn animals with a-id could prime for a protective immune response [6]. A-id to poliovirus have been shown in human cord blood and milk and antibodies to poliovirus produced by the fetus were found in Swedish newborns [6], although the virus has been eliminated by vaccination by the killed vaccine [7]. How such mechanisms would affect the reported T-cell proliferative responses to allergens is not known.

Human milk provides efficient passive mucosal protection, especially via all the secretory IgA antibodies present. However, there is growing evidence that breastfeeding may actively stimulate the immune system as well. Thus, breastfed infants respond better to vaccines inducing humoral antibodies than not breastfed [8]. One reason could be the presence in the milk of stimulating a-id antibodies [7].

It may be that the T lymphocytes and T-cell products which are abundant in early milk may have an effect on the breastfed infant, as well. Thus, BCG vaccination induces a better response in breastfed than non-breastfed infants [9]. Further effects of milk T cells are suggested by the finding that renal transplants to individuals from their mothers who breastfed them as infants, did better than transplants from mothers who had not been breastfeeding the recipient [10].

The protection against certain infections like *Haemophilus influenzae* type b invasive disease not only during, but also after breastfeeding, again suggests lasting effects on the offspring's immune system [E. Silfverdahl and P. Oleén, pers. commun.].

Early Induction of Oral Tolerance:

A Requirement for the Normally Functioning Immune System

For survival it is fundamental that protective immune responses can be induced from birth on. Equally fundamental may be to establish the natural tolerance against all the antigens to which immune responses would be not only uneconomical as to useless energy expenditure, but also harmful, resulting in inflammatory diseases like allergy.

Autoaggressive T-cell clones are early eliminated centrally in the thymus. From birth on it is likely that oral tolerance plays an important role controlling

the response e.g. to food antigens. Experimental data has been provided to show that oral tolerance depends on different mechanisms, such as active suppression, clonal deletion, or T-cell anergy. Oral tolerance has recently also been demonstrated in man [11].

Oral tolerance has been shown in many experimental systems. One oral antigen dose may be sufficient for its induction, as in the mouse, whereas the guinea pig needs repeated doses. Animals less mature at birth, like rats and mice, may rather be sensitized during the first 7 days of life, later to become tolerized. Guinea pigs and pigs are more mature and get tolerized from birth. The function of such mechanisms in man is not yet clear, but must be determined so that we know when and what antigen dose after birth will sensitize, or tolerize. It is usually easiest to induce long-term tolerance during the neonatal period.

As we will show below, experimental work illustrates the importance of the size and number of doses and the age of the animal for the development of oral tolerance.

Firstly, we found that the gut has a remarkable capacity to distinguish between microbial and food antigens. Thus, we noted that germ-free adult rats monocolonized in the gut with an *E. coli* strain responded well to the bacterial antigens, but not to the ovalbumin (OA) given in the food at the same time [12]. Inserting the gene for OA into an *E. coli* strain gave by the bacterial presentation of OA after intestinal colonization also a strong IgG, IgE and T-cell response to the OA antigen. The immune response to OA caused diarrhea in the animals when they were fed OA [unpubl. data]. If the rats were colonized after birth they did not respond to the bacterial antigens, only to the OA. This reactivity to OA could be down-regulated by further feeding of OA [13].

If another antigen like human serum albumin (HSA) was immunized together with OA in OA-tolerant animals the animals could be shown to be tolerant to both the antigens when injected together [14]. This tolerance involved IgE and IgG antibody formation, as well as T-cell reactivity. Presumably this is an effect of OA-specific T-suppressor cells locally producing potentially suppressive cytokines like TGF- β . It is of interest that the draining lymph glands of nontolerant animals became enlarged in response to OA immunization and there were no TGF- β -expressing cells. In contrast, the OA-tolerant animals showed no enlargement of the gland and had scattered TGF- β -expressing cells in the T-lymphocyte region of the lymph node in response both to OA and HSA [15]. It is presently unknown what the bystander tolerance could mean in man. It might be that individuals with suppressor T cells against several allergens may have a decreased risk of reaction to another allergen, if exposed to that together with any of the others.

Our recent work suggests that tolerance to OA in young and adult rats may be due to different mechanisms. Thus, spleen cells from adult tolerized rats transferred suppression. In addition, bystander suppression of the IgE, IgG anti-

body responses and the T-cell-mediated delayed-type hypersensitivity which responds to an unrelated antigen, was obtained in the adult animals. This suggests the presence of suppressive T cells, which might function via cytokines like TGF- β , also explaining the reduced enlargement of the draining lymph nodes after immunization [16]. Others have noted that orally induced tolerance in adult animals involved anergy, peripheral clonal deletion and TGF- β producing suppressor T cells [17]. We and others have shown that oral tolerance may affect T_H1 as well as T_H2 cells [16, 18]. In our system we have seen suppression of T_H1 and T_H2 cells via CD8+ suppressor cells [unpubl. data]. However, there is evidence for the presence of CD4+ suppressor cells as well [19].

We did not induce T-suppressor cells in the young animals, which might relate to the fact that MHC class II antigens only started to appear at the age of 3–4 weeks in the rats [20]. This age is near weaning and the exposure to other foods than maternal milk. This is also the age when we first can induce oral tolerance [21].

Animals tolerized at young age did not show bystander suppression and enlargement of the local lymph glands after coimmunization with an antigen they were not tolerant to. In the young animals there was no tolerance in the IgE and IgG antibody response to OA. Presumably anergy was induced in the young animals. Preliminary data investigating the effect of tolerization at different weeks after birth suggests further variability in relation to age [14].

Maternal sensitization as well as tolerance can be transferred to the offspring. Antibodies from the mother can influence the immunological repertoire as seen in newborn pigs of hyperimmunized, or tolerized pigs [22].

In agreement with the possibility of idiotypic-anti-idiotypic interaction between mother and offspring, we have seen that tolerance can be induced in newborn rats to OA by giving orally minute amounts of monoclonal idiotypic to OA [unpubl. data].

Tolerance is regularly discussed in relation to T and B cells. However, tolerance induction in these cells will change their cytokine patterns, which could have consequences for subsequently appearing cells participating in inflammatory reactions. In accordance with this, we have observed that animals tolerized and then immunized to OA have significantly fewer anti-IgE-stained mast cells in the small intestine than immunized and not tolerized animals, after colonization with the OA-producing *E. coli* strain [14]. Similarly we have noted significantly more IL-2R-carrying goblet cells in the gut of the nontolerized compared to the tolerized animals after colonization with the OA-producing bacteria [22]. In parallel there was a significant increase of T cells in the core of lamina propria of the villi expressing IL-2R in the OA-tolerant rats. Oral tolerance may help reduce mucosal reactivity by lowering numbers of potentially symptom-causing cells like mediator-releasing mast cells and mucus-producing goblet cells.

Conclusions

It is clear that the interplay between mother and offspring via placental passage and breast-feeding, not only provides passive protection but also active modulation of the immune system of the offspring. This has consequences for the appearance of protective immune responses and development of tolerance preventing harmful reactivity, like allergy. It is likely that the natural mechanisms for development of tolerance, oral tolerance, can be utilized to suppress and prevent allergic reactivity.

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