

Effect of commensal microbiota on the developing immune system

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Summary:

There are several indications that diseases caused by exaggerated or dysregulated immune responses are connected to the hygienic life-style of affluent Western societies. The commensal microbiota contains the vast majority of all exogenous antigens to which the immune system is exposed, and profoundly influence the development of the immune system after birth. Alterations in intestinal colonization pattern may, thus, underlie the dysregulation of immune responses. We have recently obtained evidence that putative CD4+CD25+ regulatory T cells are expanded in infants colonized by toxin-producing *Staphylococcus aureus* early in life. These toxins function as superantigens activating a broad range of T cells. The results suggest that poor T cell stimulation may underlie immune dysregulation, including allergy.

The incidence of atopic allergy is high and increasing in Europe and other industrialized countries. This is also true of other diseases characterized by excessive and uncontrolled immune responses, such as autoimmune disorders and inflammatory bowel disease (Bach, 2002). According to the hygiene hypothesis, this increasing incidence of immunologically mediated disorders is a consequence of a paucity of microbial stimulation in early childhood, resulting in defective maturation of the capacity to tolerize autoantigens and harmless environmental antigens. This hypothesis is supported by epidemiological studies showing that elder siblings, crowded living conditions, early day care and contact with farm animals and pets from early age protect from foremost allergy, but also Crohn's disease and type-1 diabetes (Bach 2002).

Untoward immune reactions to antigens are controlled by so called regulatory T cells. Naturally occurring CD4+CD25+ regulatory T cells (CD25+ Tregs) are characterized by their constitutively high expression of surface CD25 and intracellular expression of CTLA-4 (Takahashi *et al.* 2000), and transcription of the gene *FOXP3* (Hori *et al.* 2003). Infants with a mutation in this gene develop multiorgan autoimmune disease, colitis, eczema and high IgE levels in serum (Bennett *et al.* 2001).

We suggested some years ago that the increasing incidence of allergy and other immunologically mediated disorders in affluent industrialized societies could relate to an inadequately developed intestinal microflora of infants in these societies, resulting in insufficient immune stimulation (Wold *et al.* 1998). It is well established that the bacteria that colonize the intestine after birth are important stimuli for the developing immune system. For instance, there is a rapid increase in serum

immunoglobulins and secretory IgA production during the first weeks or months after birth (Allansmith *et al.* 1968, Gleeson *et al.* 1982). Studies in animals show that continuous acquisition of new bacterial strains in the microflora are required to keep the immune system in an activated state (Schroff *et al.* 1995). Furthermore, the presence of an intestinal microflora has been shown to be essential for the efficient induction of oral tolerance in animals (Moreau *et al.*, 1988). Experimental studies have suggested that microbes and their products may increase the suppressive potential of CD25+ Tregs (Caramalho *et al.* 2003).

The establishment of the intestinal microflora commences immediately after birth and precedes in a sequential manner until a complete microflora, consisting of more than 400 bacterial species, is obtained at 2-3 years of age. A number of factors may influence this process, including delivery and feeding mode, social contacts and the degree of environmental hygiene (Adlerberth *et al.* 1999). Thus, the intestinal colonization pattern varies considerably between infants in developing and industrialized societies. For instance, Pakistani infants are colonized with enterobacteria much earlier than Swedish infants, and also constantly acquire new enterobacterial strains in the microflora (Adlerberth *et al.* 1998).

In two ongoing studies, the “ALLERGYFLORA ” and the “Immunoflora” studies, we investigate the relation between the intestinal colonization pattern in infancy, the maturation of the immune system after birth (the “Immunoflora” study) and the development of allergy. Swedish infants are followed during their first 18 months of life with regular sampling of their intestinal microflora, and all major groups of aerobic and anerobic bacteria are identified and quantified. In the “Immunoflora” study, blood samples are obtained at birth (cord blood), at 3-5 days and 4 and 18 months of life. Phenotypic analysis of lymphocytes in whole blood is performed by flow cytometry. At 18 months of age, the infants are examined by a paediatric allergologist for signs and symptoms of allergy.

Within the “ALLERGYFLORA” study we have observed that Swedish infants today are colonized relatively late with several typical gut bacteria, especially *E. coli* and *Bacteroides* (Nowrouzian *et al.* 2003, Adlerberth *et al.* in manuscript). Not until six months of age are virtually all infants colonized with *E. coli*, previously regarded as one of the earliest colonizers of the intestine (Adlerberth *et al.* 1999). Bifidobacteria were the anaerobes most commonly isolated, whereas lactobacilli never colonized more than 40% of the infants (Ahrné *et al.* in manuscript). Interestingly, staphylococci, including *Staphylococcus aureus*, have become a common intestinal colonizer in Swedish infants

(Lindberg *et al.* 2000), most likely due to lack of competition from enterobacteria and other “professional” gut bacteria. Staphylococci are skin commensals and the *S. aureus* strains colonizing the infants mostly derive from their parents skin flora (Lindberg *et al.* 2004). Half of the strains produced one or more toxins with superantigen function, i.e. SEA-D (*S. aureus*-enterotoxin A, B, C, D) or TSST-1 (toxic shock syndrome toxin).

In the “Immunoflora” study, infants were followed with analyses of various lymphocyte populations in blood samples. Interestingly, infants who were colonized early by superantigen-producing *S. aureus* strains had higher numbers of putative CD4+CD25+ regulatory T cells in blood at four months of age than other infants (Karlsson *et al.* in manuscript). Superantigens stimulate a high proportion of all T lymphocytes, and we hypothesize that a strain producing superantigen may provide stimulation of the immune system equalling stimulation from a great number of different bacterial strains, which then could result in the expansion of regulatory T cells. This fits well with the hypothesis that a massive stimulation of the immune system by a diverse intestinal microflora is necessary for appropriate maturation of immune functions.

More than hundred of the studied infants have now been examined for allergy development at 18 months of age. The most common allergic symptom by that age was eczema, which was present in 32% of the infants. When analysing the intestinal colonization pattern in relation to eczema by 18 months, we found no clear protective effect of any of the bacterial groups or species studied. However, only one of the 10 infants who were colonized by superantigen-producing *S. aureus* strains by three days of age had eczema by 18 months age, as compared to 33% of other infants (p=0.16). It is possible that the immune stimulation provided by *S. aureus* superantigens induces regulatory T cells, which, in turn, lowers the risk of allergy development in infants otherwise harbouring a microflora providing little stimulation of the immune system.

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