Old Herborn University Seminar Monograph 13:
Polyspecific immunoglobulins, their possible role in the normal (physiologic) clearance of microorganisms and tissue fragments.
Editors: Peter J. Heidt, Volker Rusch, and Dirk van der Waaij.
Herborn Litterae, Herborn-Dill, Germany: 139-143 (2000).

OLD HERBORN UNIVERSITY SEMINAR ON POLYSPECIFIC IMMUNOGLOBULINS, THEIR POSSIBLE ROLE IN THE NORMAL (PHYSIOLOGICAL) CLEARANCE OF MICROORGANISMS AND TISSUE FRAGMENTS: MINUTES AND REVIEW OF THE DISCUSSION

FRITS SCHUT

Microscreen BV, Groningen, The Netherlands

DISCUSSION PARTICIPANTS (in alphabetical order):

Ingegerd Adlerberth, Nico Bos, Pnina Fishman, Neil S. Greenspan, Peter J. Heidt, Hilmar Lemke, Paul Nieuwenhuis, Jörg Reimann, Volker D. Rusch, Frits Schut, Yehuda Shoenfeld, Petr Síma, Dick Veenendaal, and Dirk van der Waaij

NATURAL IGA: EFFECT ON TRANSLOCATION

When the intestinal tract of a newborn child is colonised by bacteria, the production of IgA's in the gut lumen is profound. Currently, the specificity of these IgA's is unknown. Studies in mice mono-associated with segmented filamentous bacteria (SFB) suggest that, although the IgA response to this bacterium is very high, little or none of this IgA is specific for the SFB (Gram-positive bacteria that do not produce lipopolysaccharides). How IgA production is stimulated is not known. Probably, IgA induction does not occur through stimulation of class II enterocytes and local release of IgA's by T-cells since most are of the γδ-type, not αβ-type, making a link between class II lymphocytes and T-cells unlikely.

Comparison of IgA responses in mice monoassociated with a variety of different bacteria indicate that the production of specific IgA's (measured against bacterial lysates on slides) does not correlate with the 'total' IgA response. Different species of bacteria each result in different responses and the mouse strain does not affect the response pattern. Furthermore, the level of specific IgA's that is produced does not seem to correlate in any way with the translocation rates determined for these bacteria (measured as the number of bacteria that can be cultured from spleen or mesenteric lymph nodes). Against *Oochromobactrum anthropi*, for example, no specific IgA's are produced, and translocation can be demonstrated. A non-invasive mutant of *Listeria monocytogenes*, on the other hand, is confronted with high levels of specific IgA's and still translocates at high rates. The translocation of *Morganella morganii*, however, is completely prevented once the production of specific IgA's is induced. No general conclusions can be drawn at this moment concerning the level of the specific IgA response and the translocation of the bacterium. One should however bear in mind that when measured as described above, translocation over the intestinal lining may in fact occur at high rates, but rapid clearance of the bacteria in the lamina propria can result in low numbers of culturable cells from peripheral tissues as spleen and mesenteric lymph nodes.

Additional experiments may include investigations on the effect of specific IgA's on injected antigen. The induction of oral tolerance could be used to determine the levels of specific IgA directed towards the antigen versus the level of natural IgA in a culture independent way.

A discussion was raised considering the interpretation of experimental data on specific / non-specific / natural or total and polyspecific and the interpretation of cross-reactivity in this. It was suggested that cross reactivity and specificity are blurred by affinity, which is the result of a physico-chemical relationship between two molecules. Description of affinity, and therefor specificity, in terms of absolute values for one molecule alone are not necessarily meaningful.

The point was made that physiologically meaningful translocation may also involve the translocation of nucleic acids or antigens only. Also, intracellular bacteria cannot be reached by IgA. This complicates the issue even further.

The properties of the individual bacteria are likely more determinative to translocation then the level of IgA production in the lumen. Still, specific IgA's are necessary for the prevention of translocation of *M. morgani*, as was demonstrated in an experiment using mice monoassociated with SFB (high non-specific IgA response) which were superinfected with *M. morgani*. As in germ free-mice, the translocation of *M. morgani* was stopped completely upon the production of specific IgA's against this bacterium. Currently, the bacterial surface antigens with which these specific IgA's interact are not known.

Both B1 and B2 cells may be involved in IgA production. Serum IgA's in mice monoassociated with *O. anthropii* were 100% a type and therefor of B1 origin, whereas a superinfection with *M. morgani* resulted in serum IgA levels that were for 61% of the B2 derived b type.

The time it takes for SFB to colonise the caecum of new-born mice is shortened if the mother is immunocompromised. The levels to which colonisation occurs and the prevalence is affected by the fact whether the offspring is immunocompromised or not. It is therefrom inferred that the IgA's of both the mother and the offspring are important for the composition of the flora and its rate of establishment.

Comparative studies on breast fed children of Pakistan and Sweden indicate that colostral IgA's, from which Pakistani children are deprived, may have a dramatic effect on the turnover of different enteric bacterial strains in the intestinal flora. Frequent, but temporary colonisation with different enteric strains is characteristic for intestinal flora's in Pakistani children, which face higher exposure to these bacteria than do Swedish children. In Pakistani children, the translocation is often higher than what the immune system can handle, resulting in relatively high levels of infant mortality as a result of sepsis (1%).

NUTRITION AND GALT EVOLUTION

The question raised was whether there is a relationship between nutritional status of the mother, the properties of the breast milk and the survival of children. At present, there is no knowledge whether the milk of malnourished mothers is of reduced quality. If an analogy exists with the nutritional status of the mother and the quality of the foetus, one might expect there is no such an effect.

However, the foetal gut is getting

shape after 6 weeks and is formed by endodermic folding. The gut associated lymphoid tissue (GALT) of the foetus starts developing very soon after that. Functions begin to pick up rapidly. Hormonal influences are measurable at 17-20 weeks of development. It has been determined in epidemiological studies that children may experience diseases later in life (at the age of 40) at higher instances in the case of maternal malnutrition in week 10-20.

It seems that nucleotides play a key role in the further development of newborns. Especially nucleotides, which cannot be synthesised *de novo*, are necessary for proper growth of all proliferating tissues, including gut epithelium, cells of the central nervous system as well as cells of the immune system. Considering that 1 mitoses requires 10^9 nucleotides, and assuming an incorporation efficiency of only 5%, a growing child may need up to 450-700 mg of nucleotides per day. Cow milk contains less than 1 mg of nucleotides per litre. Human breast milk, on the other hand, contains 20-70 mg of nucleotides per litre. Specifically in developing countries, children may suffer serious nucleotide deficiency resulting in retarded growth of the nervous system and the GALT. In fact, nucleotide supplements in milk can decrease the incidence of gut infections in young children.

It is known that nucleotides play various important roles in cellular metabolism. As precursors of nucleosine-phosphates (e.g., AMP, ADP and ATP) they have a metabolic function in signal transduction pathways (cyclic AMP and cGMP). An interesting phenomenon is that enterocytes can exhibit *de novo* synthesis of nucleotides as well as direct uptake. This may be of critical importance to the growing gut.

ORAL TOLERANCE IN RELATION TO AUTOIMMUNE DISEASE

Oral tolerance or immunoparalysis was defined as suppression of a systemic immune response upon oral administration of the antigen involved. In mammals, adults can be tolerised only by administering high doses, while neonatals require only low doses that should be administered continuously. It is long since known that systemic injection of albumin results in a stronger immunoparalysis when it is preceded by its oral administration. This holds generally for nominal (non-proliferating) antigens but is also the case for lysates of bacteria from the individual's indigenous flora. For some bacteria of the indigenous flora however, there is no tolerance. Such bacteria exert a systemic immune response and may play a role in autoimmune diseases such as IBD. One mechanism for IBD could be that in the case of mucosal damage and contact between non-tolerised bacteria in the gut flora and the systemic immune system, the reaction in the lamina propria leads to inflammation and consecutive damage of the lining. In this way a vicious circle develops. Autoimmune diseases of remote organs (such as rheumatoid arthritis) were also discussed in relation to (somewhat disappointing) experiments on oral administration of collagen fibres in comparison to control group. Beta-2 glycoprotein, produced in liver, is present at high concentrations in neonatals and may play an important role in the induction of tolerance to foreign antigens.

Vaccination may also have an effect on the establishment of autoimmune diseases. The relationship between hepatitis B virus vaccination and multiple sclerosis was mentioned, indicating that vaccination may not always be safe.

It was concluded that allergies develop via a different pathway than au-

toimmune diseases. The antibody isotypes involved are obviously different (IgE vs. IgG, respectively). The spectrum of tolerance developed by new-borns in Estonia and Pakistan may be different from that of new-borns in Sweden and may largely be due to the difference in contact with micro-organisms in the environment, i.e., by hygienic circumstances. This may explain the difference in allergies encountered in children raised in the Western world and those raised in developing countries.

IDIOTYPIC NETWORKS: THE INSTRUCTION OF THE IMMUNE SYSTEM DURING THE PERINATAL PERIOD

The meeting avoids the use of the word "idiotype" but accepts that there are antibodies that are directed towards other antibodies. Ab1 is the idiotype; Ab2 is the anti-idiotype.

The idiotypic network, part of the innate defence system, was conserved from ancient host defence pathways from our evolutionary forefathers. The system can be modulated by the thymus-dependent humoral immune system of the mother during pregnancy and during the lactation period. The transplacental and lactational transfer of antibodies from the mother to the new-born forms an important modulator of the foetal immune system and prepares the new-born for its encounter with environmental antigens.

The composition of the gut flora of a normal euthymic mother and the interaction with her T-cell system before and during pregnancy determines the degree of chronic graft vs. host disease (GvHD) following bone marrow transplantation experiments in her offspring (see Heidt, Veenendaal and van der Waaij elsewhere in this volume). Whereas acute GvHD is primarily T-cell mediated, late onset or chronic GvHD is predominantly antibody mediated.

B cell deficiency implies the possibilities of a hole in the antibody repertoire. Such a deficiency can be induced by the administration of an anti-idiotypic antibody to a mouse in a defined period shortly after birth. This throws a hole in the repertoire in the sense that the mouse is sensitive to a challenge of a bacterium for which the idiotypic antibody had affinity. The workings of the idiotypic antibody is thus affected by an anti-idiotypic antibody and there is an obvious consequence for later in life.

This has consequences for bone marrow transplantation experiments. During the period shortly after birth a hole in the antibody repertoire develops depending on the interaction of the immune system of the mother and her own microflora. This determines her IgG spectrum, and - since IgG's cross the placental barrier - also determines the polyspecific antibody repertoire in her young. It seems from literature that the acquisition of a restricted repertoire of antibodies is important during a certain phase early in life in order to have a broader repertoire later on in life and can also be used to protect offspring via immunisation of the mother during pregnancy.

COMMON THERAPEUTIC APPROACHES: INFLAMMATION AND CANCER

If bone marrow transplantation without GvHD would be possible, then this treatment could be used to cure autoimmune diseases like multiple sclerosis.

Currently, the bone marrow transplantation cannot guarantee success as GvHD lesions may be as severe as the autoimmune disease itself. More research on the interaction between the microflora and the immune system of bone marrow donors is urgently needed, specifically in relation to the effect of the graft on the recipient and its microflora.