Old Herborn University Seminar Monograph 1:
Microbial ecology of the human digestive tract.
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II. INTERACTION OF FLORA, IMMUNE SYSTEM AND MUCOSAL CELLS

1. Structure and Maturation Cycles of Digestive Tract Tissue

Lymphoid cells of different kinds are to be found in the mucosa along the whole length of the digestive tract. Some lymphocytes can be found in the loose connective tissue, the lamina propria, others between the epithelial cells as well as at various sites where organised lymphoid tissues are located; e.g. tonsils and Peyer's patches.

The intestinal lining formed by a sheet of enterocytes along the villi has the unique function of absorption of nutrients, water and electrolytes. In addition, it forms a tight boundary between microflora and underlying tissues. This boundary function may explain the presence of large numbers of cells of the immune system in the intestinal mucosa. These cells are dispersed singly or in small groups among the epithelial and connective tissue components of the intestines. The cells comprise T and B lymphocytes as well as NK cells together with monocytes, mast cells and eosinophils. Thus the gut has the (immune) cellular capacity for truly protective local immunity. Epithelial, stromal and lymphoid cells are continuously renewed, either because of their short life span or because they migrate away from the mucosa (*Parrott*, 1987). The crypts of Lieberkühn are the sites where enterocytes are produced. The degree and speed of cell extrusion at the tip of the villi on the other hand, regulates the length of the villi.

It is known that autochthonous flora influence the length of intestinal villi from experiments on germfree mice (*Abrams*, *Bauer* and *Sprinz*, 1963). It is also known that bacteria appear to adhere more readily to immature enterocytes (near the crypts) than mature enterocytes (*Stokes*, *Miller* and *Bourne*, 1987). It is tempting to postulate that the rate of crypt cell division is the consequence of dynamic interactions between autochthonous flora and the local immune system, but this postulate presupposes: a. that the local immune system can influence that rate of crypt cell division and b. that immune responses are mounted against autochthonous flora. This brief review summarises evidence that the rate of crypt cell division is influenced by local cell mediated immune responses.

Vital early experiments were carried out by Drs. Anne Ferguson and Tom MacDonald (*Ferguson* and *Parrott*, 1973; *MacDonald* and *Ferguson*, 1977, 1978): When grafts of foetal mouse intestine - which are empty and have no antigens within the lumen - were implanted heterotopically into adult mice of the same inbred strain, they grew and retained their morphology for several months. If donor and host animals were from different strains, grafts grew only for a few days. Then the grafts got infiltrated by lymphocytes and were rejected. Rejection of foetal mouse allografts is an apparent thymus dependent phenomenon, as it did not occur in thymectomised recipients. The most striking histological effect of rejection was seen before the mucosa was completely destroyed. Grafts became infiltrated by lymphocytes. Villi had become short or absent but the crypts of Lieberkühn appeared normal. These intestinal changes are very similar to those seen in intestinal biopsies in human coeliac disease and parasitic infection.

Because foetal intestinal allografts provided only small pieces of tissue for

examination, subsequently graft-versus-host disease (GvHD) was used to investigate more closely the time course changes in crypts and villi. GvHD was induced in five days old CBAxBalb/C F1 hybrid neonatal mice by intraperitoneally (i.p.) injection of parental spleen cells. A detailed analysis was then made of mucosal cytokinetics upon contact with bacteria. Comparisons between villi, crypts and crypt-mitosis in GvHD mice and untreated littermates used as controls, revealed striking crypt hyperplasia in mice aged 10 days. However, the villi were about the same height in both groups although there was some shortening in GvHD mice. Both studies could be regarded as providing evidence that crypt hyperplasia may be a compensatory response to loss of part of the mature enterocyte population. However, in the GvHD studies a 3 to 4 fold increase in crypt mitosis preceded shortening of the villi by several days. It can therefore be concluded that the crypt stem cell mitosis resulted from implanted (donor) lymphocytes. Later investigations with *Giardia* infected euthymic and athymic mice, indicated that these lymphocytes were presumably T cells. In *Giardia* infected animals, increased numbers of intra-epithelial lymphocytes were found and these cells appeared to be T cells. Like in GvHD mice, in these infected animals with increased (doubled) crypt mitosis rates

Table 1: Immunologically mediated enteropathy after injecting CBA spleen cells into CBAxBALB/c F1 mice*

	EL increase	Crypt depth increase	CCPR increase	Villus atrophy	Reference
1. Unirradiated	+++	++	++	-	*Mowat* and *Ferguson*, 1981
2. Lyt-2	++	+	+	-	*Mowat* et al.,
Lyt-1	-	-	-	-	1986
3. nu/+	++	++	++	-	*Mowat* et al.,
nu/nu recipients	++	+++	+++	++	1987
4. Lethally irradiated recipients		*Before Day 3*			*Mowat* et al.,
	++	++	++	-	1988
		After Day 3			
	-	-	-	++	
5. Unirradiat. recipients	++	++	++	-	*Mowat* and *Felstein*,1987
NK cell depleted recipients	+	-	-	-	

*All mice received untreated spleen cells from CBA donors except (2) when Lyt-2 depleted or Lyt-1 depleted spleen cells were used.

more rapid transit of enterocytes along the sides of the villi was seen, but no villus atrophy.

More recently, the GvHD model has been used to examine the relative contribution of helper/inducer, cytotoxic T cells or NK cells to the phenomenon of immunologically mediated enteropathy (including numbers of intra-epithelial lymphocytes (IEL), crypt depth, villous atrophy and crypt cell production rate) see Table 1 (*Mowat* and *Ferguson*, 1981; *Mowat* et al., 1986, 1987, 1988; *Mowat* and *Felstein*, 1987).

In summary, these experiments showed that the helper induced T cells (Lyt2-cell) is primarily responsible for the proliferative enteropathy consisting of crypt hyperplasa and increased numbers of intra-epithelial lymphocytes though natural killer (NK) cells also have a contributory role. Nude athymic and irradiated mice show the additional feature of villus atrophy, which is probably the consequence of (injected parent) cytotoxic T cell activity. The studies indicated that pre-T cells, T cells and NK cells are involved in controlling crypt cell proliferation rate (CCPR) and villus height in a complex way probably by the release of growth factors or interleukins. The GvHD model has therefore been a very useful model of pathological changes in the intestine. Further studies presently in progress using untreated athymic mice indicate that nude mice have a lower crypt renewal cell rate than normal mice. Mucosal T cells therefore may also regulate enterocyte growth under normal conditions, and normal conditions include autochthonous flora.

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2. Interrelationship of Digestive Tract Bacteria with Host Tissues and Immune Systems

The role of the immune system is commonly perceived as protector against infections and toxins and therewith maintains self-integrity. In this concept, autoimmune reactions are considered aberrant and harmful for the individual. It is generally accepted that the immune apparatus has a good recognition mechanism, which prevents it to respond with cellular or humoral immunity to many ‘self’ antigens to which it, comes frequently in contact. This specific unresponsiveness is related to the phenomenon of immunologic tolerance.

It has been known for many years that auto-immunisation with antibody formation is readily achieved with certain special tissues, in particular brain, lens, uvea, thyroid, testes, adrenals and pancreas. Also following a severe trauma with massive tissue (muscle) damage, (auto-) antibody production may be evoked transiently (*DeVay* and *Adler*, 1976; *Schattner*, 1986). These organs were said to contain 'organ-specific' antigens. No unusual manipulations of organ material were required to render the tissue constituents antigenic upon injection; immune responses are invariably transient in these cases. It is a tenet of immunology that reactions are determined in character by the category of the immunologic phenomenon and not by the identity of the antigen. This consideration applies to 'autoimmune' phenomena, all of which must be manifested as cytotoxic, humoral or combinations of these two. The degree to which immune responses become manifest depends on the antigen but also on the condition of the immune apparatus of the host. If an infectious agent lowers

the activity or turns off regulating cells an aberrant immune response may occur to 'self', to altered 'self', and to exogenous antigens. Within these categories, they give rise to lesions, which are indistinguishable from lesions produced by a response to exogenous antigens or to a complex of exogenous allergens with a tissue constituent.

Non-lnfectious Exogenous Antigens

Erythrocytes may be agglutinated or lysed by the action of antibody directed against antigens, which are normal constituents of the red cell blood groups. Such a reaction, of course, is the basis for cell damage in transfusion reaction, haemolytic disease of the new-born but also in some cases of 'acquired haemolytic anaemia'. The antigen involved can be either bacterial, e.g. pneumococcal polysaccharide which is capable of adhering to red cells and cause *in vivo* agglutination and/or lysis, or can be a certain drug (*Schattner*, 1986; *Springer*, 1971). This shows that bacterial antigens can cause an unfavourable immune response in the host by binding to host cells.

Bacteria with Antigens Common to Host (Connective) Tissues

There is accumulating evidence that bacteria and human organ specific antigens may have antigenic determinants in common. In other words bacteria may induce an immune response to auto-antigen upon infection. Unlike lesions associated with viruses, which involve a variety of parenchymal tissues, bacterial induced antibodies tend to affect predominantly connective tissue (*Wong*, *Skelton* and *Feeley*, 1985). Particularly in the joint synovialis, in which venous plexus exist in which haematogenous fragments of organisms or soluble immune complexes are readily trapped, immune cross reactivity may come to expression (*Schwab*, 1965). The clinical course of (experimental) post-infectious 'autoimmune' lesions is in general sub-acute, presumably being terminated by the gradual disappearance of bacterial antigen. However, the disease may recur following renewed infections with the organism.

Chronic Remittent Diseases

The chronic, remittent diseases that are presumably associated with bacterial infections are chronic reactive arthritis, ankylosing spondylitis and arthritis associated with psoriasis (*Mielants* et al., 1985). A fourth candidate is possibly chronic inflammatory bowel disease (*Cooper* et al., 1988).

Particularly the reactive arthritis including ankylosing spondylitis have been studied in relation with Gram-negative infections in several centres since the beginning of the present decade (*Ebringer*, 1983). It appears that this disease is associated with the HLA B27 tissue antigen. For example, in Europe this disease has been found to have a 90% relation with HLA B27. In Japan, an association of 66% among B27 positives has been reported versus 1% in a control population. Gram-negative bacilli such as *Klebsiella*, *Yersinia*, *Shigella* and *Salmonella* appear to have proteins in their outer membrane, which closely resemble HLA B27.

Van Bohemen and colleagues have investigated a family with diarrhoea as well as a larger outbreak of infectious diarrhoea in association with HLA B27 and arthritis (*Van Bohemen* et al., 1986a). Some of the family members were B27 positive, others not. All except one family member developed dysentery. Interestingly, only some family members developed arthritis. The two parents remained free but three of four children developed reactive arthritis. Notably, this was not confined to children who were B27 positive, while the

father - who did not develop arthritis - was found B27 positive.

In a larger outbreak of dysentery by *Shigella flexneri* in 62 patients, fortynine B27 negative patients did not develop the disease while seven B27 positive patients experienced also no or only rare transient pains of the joints. In five B27 positive patients with the HLA B27 antigen, reactive arthritis did develop. The *Shigella* appeared to be B27 positive. An interesting observation was that the HLA B27 positive patients who all developed the disease, displayed marked (mostly IgA) serum antibody titres against the *Shigella* strain (*Van Bohemen* et al., 1986b). Antibodies were found in these cases to belong to all major classes of antibody isotypes (IgA, IgM and IgG). Particularly the IgA antibody titre was much higher in the reactive arthritis patients than in those who remained free of the disease (*Van Bohemen* et al., 1986a).

Hypothesis

To explain the observed phenomena, three hypotheses have been postulated:

a. *Ebringer* and colleagues (1977) presume that antigens on the cell surface of these Gram-negative bacteria (*Enterobacteriaceae* species) carry HLA B27 antigen, which would induce an immune response to 'self'.
b. *Geczy* et al. (1983) presume that antigens on the cell surface of Gram-negative bacteria can be associated with receptors on tissues and these receptors are either B27 antigen or structures closely related to them.
c. *Geczy* and co-authors (1987) have recently put forward a complementary theory in which they propose that parts of a plasmid of Gram-negative bacteria, which code for certain antigens, can be transfected into human tissue cells. This would cause expression of the bacterial B27 antigen on the cell surface.
d. *Van Bohemen* and co-workers (1986 a,b) presume that their observations in diarrhoeal patients indicate that reactive arthritis is not only correlated with the presence of HLA B27 antigen on connective tissue cells. They state that if there are antigens on bacteria which resemble B27 antigen, this means that these antigens may not exclusively resemble cross-reactive antigens on synovial cells, as these antigens also exist on, for example, B and T cells. During the process of induction of immunity to the bacteria, both B cells and T helper as well as T suppressor cells are influenced. The latter are turned off by the infection due to enhanced contra-suppressor cell formation, while high antibody titres can be expected as were indeed found in their study.

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