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INTERACTIONS BETWEEN DIGESTIVE TRACT MICROFLORA AND IMMUNE SYSTEM

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INTRODUCTION

The digestive tract is a very rich source of living (microbial) and dead (food and microbial) antigens. It is therefore conceivable that, where the gastro-intestinal tract is loaded with antigenically foreign material, much if not most of the activity of the local gut associated lymphoid tissue (GALT) is directed to antigens which actively or passively (by pinocytosis; *Walker* et al., 1974a) cross the intestinal epithelium. In the submucosa and the regional lymphatic tissues these antigens "meet" different cells of the immune system. The likelihood of this event is well illustrated by the difference between the state of activity of the gut associated lymphoid tissue (GALT) in germfree and in conventional animals (*Horowitz* et al., 1964; *Bosman* et al., 1964, *Freter* and *Abrams*, 1972). In this paper the fate of living and dead antigens inside the intestinal lumen will be discussed in conjunction with their importance for the host immune system and pathology. The distribution of (bacterial) antigens in the alimentary canal is largely followed by the quantity of lymphoid tissues of the GALT. This suggests at least (re-)activity of the GALT regarding the microbial distribution in the digestive tract. In this respect it is important to mention at this point already that in the presence of a functioning thymus certain microorganisms may exert either enhancing (T-helper) or tolerogenic (T-suppressor) activity. For the latter, microorganisms may have antigens in common with various organs. The antigenic similarity between microorganisms and their host organism could be a clue in the answer to the question how several bacteria can escape from the T-cell control mechanism(s) which normally prevents them from sensitising the host's immune system. In the absence of T-cells, bacteria belonging to the indigenous microflora may ensue hypersensitivity and/or autoimmune disorders.

THE ROLE OF THE THYMUS IN GUT ECOLOGY

The intestinal flora and the thymus

The thymic cortex is a well-known site of intensive (immune) activity. Early studies have indicated already that the majority of the newly formed or instructed thymocytes migrate to other organs (*Joel* et al., 1971, 1974). The magnitude of migration of cells matured in the thymus (T cells) into the GALT has been studied by *Laissue* and coworkers (1976). They found that the

radioactively labelled T-cells were rapidly catabolised in the small intestine. Furthermore, in early periods of postnatal life of mice, the vast majority of the lymphocytes that enter the GALT appeared is of thymic origin (*Joel*, 1972). *Cottier* and co-workers (1975) have made likely that the growth of GALT-cells and therewith the thymic activity, is largely antigen driven in mammals. In germfree mice, Peyer's patches and mesenteric lymphnodes are poorly developed, but increase in size when stimulated via intestinal route. No such reaction was seen when the antigens were administered parenterally (*Pollard* and *Sharon* 1970). These observations indicate that the microflora may play a crucial role in thymus and GALT activity. Conversely, as will be discussed later on, the immune system may also exert bet strong influence on the bacterial colonisation pattern of the digestive tract. Comparison of the relative thymus weight in germfree mice and conventional mice of different ages showed that at all ages (up to l000 days) the relative thymus weight in conventional mice was higher than in the germfree counterpart. The histology of the thymus correlated well with the age of the animals. Oral treatment of conventional mice with broad-spectrum non-absorbable antibiotics, which causes bacteria-freeness of the animal's digestive tract, has been found to cause a decrease of relative thymus weight to what is seen in germfree mice of the same strain and age (*van der Waaij*, 1986). Not only the thymus but also the spleen appears to decrease in size during total gut decontamination in these animals to values normal in germfree control animals (*van der Waaij*, 1969).

Oral induction of tolerance

Andre et al. (1975) reported an important observation which may help to explain the development of tolerance for intestinal indigenous flora components. They found that prolonged oral administration of sheep erythrocytes to mice decreased the level of plaque forming cells in the spleen after systemic challenge with this antigen. A few years later, *Kagnoff* (1978) showed that this relative hyporesponsiveness was due to the induction of suppressor T-cells. These observations have been confirmed since then by several groups and appear to apply to a number of different antigens.

Immunologic tolerance in gut associated lymphoid tissue (GALT)

The great majority of the intestinal microflora comprising of numerous different anaerobic species and some viridans group streptococci does not elicit an immune response in the digestive tract. On microscopic examination of freshly voided faecal flora of mice (*van der Waaij* and *Heidt*, 1978) and humans (*van Saene* and *van der Waaij*, 1979) with fluorescent anti-IgA antibodies, it has been found that the anaerobic bacteria of mice and humans are not covered with IgA. *Enterobacteriaceae* species present in the faeces, on the other hand, appeared frequently to have an "lgA-paint". In this respect it is of importance that parenteral inoculation of anaerobes isolated from an animals own faecal microflora appears to evoke only a very poor immunologic response (*Foo* and *Lee*, 1971).

Such observations of limited or no immune reactivity to indigenous flora upon parenteral injection suggest that as a rule, the immune system is not stimulated by the bulk of the intestinal bacteria. The intestinal mucosa however, does not completely block invasion of (small) numbers of bacteria. Rather an antigen (including bacterial) uptake through the epithelial layer occurs regularly. The specialised structures of Peyer's patches, solitary lymph follicles, and their associated epithelium allow a controlled uptake of antigenic,

among other bacterial, substances. Therefore, the state of specific immunologic tolerance for the residing indigenous microflora may exist. Evidence for antigen induced hypo- or non-responsiveness of GALT may be related to the presence of suppressor T-cells in the Peyer's patches.

Influence of age on GALT immune reactivity

Kenny et al. (1971) have described a study of oral *Escherichia coli*-O127 infection in mice of different ages. Titres of haemagglutinating antibody to *E. coli*-O127 upon the enteric infection showed significant differences among three age groups. In neonatal and infant mice no antibody was detected until the age of three weeks. Immune response in weaning mice was not consistent. Whereas some mice were able to respond upon oral infection with titres of 160, others demonstrated no response. Adult animals all responded with antibodies from the tenth day after infection.

The colonisation pattern of biotypes of *Enterobacteriaceae* species in thymus-bearing and in congenitally athymic mice

The colonisation pattern of the digestive tract is determined by a mechanism called colonisation resistance (CR). The CR of the digestive tract is caused and maintained by a myriad of factors, which are both of host and microflora origin. To investigate to which extend the genetic composition of the host organism is involved in the CR the following investigation was performed:

Thirty-five individually marked inbred conventional C3H mice were maintained in one large cage inside a germfree isolator during a four week observation period (*van der Waaij*, 1982). Germfree type isolation was applied to guarantee that only sources of Gram-negative (aerobic and facultative anaerobic) bacteria could be cagemates and the foodpellets. The total source was thus known and consisted of twelve biotypes of *Enterobacteriaceae* species. A sample of each mouse's faeces was tested by culture and typing twice weekly. Although the animals were inbred, their Gram-negative (aerobic) flora appeared different. Some animals entered the study with an average of five different biotypes in their faeces; others had two or only one. These differences in number of different biotypes between animals persisted during the four-week study period. Animals that started with one biotype had a low turnover of biotypes with an average of 0.8 biotype per sample, whereas mice with five biotypes at admission had a high turnover with an average of 3.2 biotypes per faecal sample.

There are three intriguing points in these observations:

1. Different mice with similar chances of contamination with the biotypes in common source (twelve biotypes) appeared to make their own selection out of that common environmental source. During the four weeks of study, some animals apparently allowed many different biotypes to pass through their alimentary canal and to appear in the faeces in detectable numbers. Other mice in the same cage appeared to be more selective.
2. This selection occurred in inbred mice, i.e. in genetically identical animals. Whether fewer or more biotypes are permitted to survive during transit through the digestive tract depends on the composition of the indigenous flora. This leads to the conclusion that the latter is apparently phenotypically determined.
3. The mice entered the study with different numbers of biotypes of *Enterobacteriaceae* species in their faeces. Therefore, they had acquired their indigenous flora before the experiment. The experiment started a week after weaning, when the mice

were four weeks of age. It is therefore most plausible that they acquired their intestinal flora from their mothers in the breeding unit. In this experiment we could trace the dams from which the animals used in the experiment originated. The mothers showed differences in numbers of biotypes that corresponded with that of their offspring before and during the four-week observation period. This evidence that mice in different litters had been exposed since birth to different flora with respect to the composition of the indigenous anaerobic bacteria associated with intestinal colonisation resistance.

THE ROLE OF INTESTINAL MICROFLORA IN AUTOIMMUNE PHENOMENA AND WASTING SYNDROME

Microflora associated with wasting syndrome in congenitally athymic mice

Investigations in congenitally thymusless mice with and without an intestinal microflora have indicated that these animals develop an abnormal flora several weeks after weaning. This applies to both aerobic and facultative anaerobic Gram-negative bacteria. The abnormality of the faecal flora in athymics is apparent in the great number of different biotypes of *Enterobacteriaceae* species which these animals appear to acquire from environmental sources when maintained under conventional conditions (*van der Waaij*, 1981). An abnormal intestinal population pattern appears just prior to the development of a clinical syndrome called "wasting disease" (*Jutila*, 1973). The thymusless (nude/nude) mice which are selectively decontaminated (SD) (*van der Waaij*, 1988) with antimicrobial drugs after weaning and are maintained thereafter in an *Enterobacteriaceae*-free condition by continuous SD-treatment, do not develop wasting disease (*van der Waaij*, 1981). Totally decontaminated athymic mice which are mono-associated with an *Escherichia coli* strain under isolation circumstances after weaning, however, also have been found to remain free of symptoms of wasting disease regardless the fact that their intestinal flora consisted of excessive numbers of Gram-negative bacteria (*van der Waaij*, 1981). *Wilson* and *Bealmear* (1965) have presented evidence that in mice the presence of a conventional flora is reflected in their relative thymus weight.

The role of T cells in controlling the intestinal flora for the prevention of wasting disease and comparable syndromes

The absence of functional T cells could be of importance in both the development of respectively the wasting syndrome in thymusless mice and in chronic graft-versus-host disease (GvHD) after allogeneic bone marrow transplantation. The mechanism involved however must be quite complex. As mentioned above in this paper, the presence of potentially pathogenic bacteria in the microflora of the thymusless mouse does not imply that wasting disease necessarily will develop. This applies also to chronic GvHD after allogeneic bone marrow transplantation (*Veenendaal*, this monograph). It is known for example, that the nude/nude mouse can remain without signs of autoimmunity, when it is maintained (bacteriologically) isolated under SPF-conditions (*McIntire* and *Sell*, 1964; *Reed* and *Jutila*, 1976). Association with potentially pathogenic bacteria early in life - as even may occur under the hygienic circumstances in SPF-breeding units - does apparently not condition for

wasting- or autoimmune disease. This could possibly be ascribed to the aspecific immune suppression that has been observed to exist in first weeks of life in mice (*Strobel* and *Ferguson*, 1984).

Endotoxin releasing bacteria (or other T-cell independent antigens) may be responsible for the IgM secretion in the nude/nude mice in the first months before the lymphoid tissues involute (*Pritchar* et al., 1973). Heavy oral and, perhaps more importantly, repeated oral infections, which occur in conventionally maintained mice, initially may lead to lymphoproliferation with immune complex disease, which is however, soon followed by exhaustion of the lymphatic tissues. As long as the immunosuppressive substance is in the circulation this event is perhaps prevented, the more oral infections occur there after the shorter the interval of clinical healthiness. Supportive evidence for the importance of unimpaired proliferation of lymphatic cells (predominantly B-cells) as a result of frequent oral infection with immune stimulating bacteria may come from the fact that chronic GvHD is not enhanced in mice associated before transplantation with only one or two Gram-negative rods (*E. coli* or *Klebsiella*) but maintained isolated after bone marrow (BM) engraftment, i.e. in the period of about three weeks post transplantation during which no or insufficient T-cell control of immune reactions has developed (*van Bekkum* et al., 1974).

In the athymic mice, T-cell control can obviously not develop so that massive B-cell proliferation is unavoidable upon repeated oral infection with bacteria which carry B cell polyclonal stimulating substances in their cell wall. The great majority of bacteria indeed carry T-cell independent antigens such as lipopolysaccharides and peptidoglycan. Therefore, B-cell response to many different bacterial antigens is likely to occur in such animals until lymphatic exhaustion. In case bacteria involved in immune stimulation share antigens with the host organism, autoimmmune phenomena may occur. Otherwise immunecomplex-disease may explain the multi-organ autoaggression in the athymic animals prior to the exhaustion; the "wasting syndrome". The same hypothesis could possibly apply to chronic GvHD where B cell proliferation may remain uncontrolled for a long period once it has started in the absence of a GALT T-cell system. Early studies of *Skopinska* (1972) and *Keast* (1973) may support this hypothesis. When they injected mice several days after BM-transplantation severe chronic GvHD occurred; however, the longer they made the interval between BM-transplantation and endotoxin injection the less signs and symptoms of a subsequent GvHD were.

With working hypothesis, it is understandable why a certain turnover of potentially pathogenic and other immunogenic bacteria that can translocate should be prevented during ageing in the congenitally athymic as to minimise autoimmune phenomena.

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