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QUALITATIVE AND QUANTITATIVE DIFFERENCES BETWEEN SPECIES IN THE GUT IMMUNE SYSTEM: CONTROVERSIAL AND UNSOLVED ASPECTS

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SUMMARY

Many parameters of the gut immune system, such as the development of tolerance or protective immune responses, can only be studied in detail in experimental animals. Species differences have to be considered when such results are extrapolated to humans with their mixed genetic background, non-standardised eating conditions and variable exposure to microbial stimulation. In this review some of the major species differences in the gut immune system are discussed: the development, structure and function of Peyer´s patches show enormous differences, and the M-cells differ in number and reagents which label them. Recently discovered lymphoid structures in the gut wall, i.e. cryptopatches and lymphocyte-filled villi, have so far only been described in mice and humans & rats, respectively. Why are there huge species differences in the number of $\gamma\delta^+$ T-cell receptor intraepithelial lymphocytes? What is the function of the different antigen presenting cells in the gut wall, e.g. dendritic cells? The functional relevance of the unique cellular composition of the gut wall in early childhood for tolerance development or vaccination protocols has to be studied in more detail with respect to the role of cytokines, chemokines and the enteric nervous system. Despite the great advances in recent studies on the gut immune system there still seem to be many unsolved questions for future research in laboratory animals and even more in humans.

INTRODUCTION

The gut immune system has two diametrical functions: on the one hand oral tolerance to enormous numbers of nutritional and microbial antigens must be induced early in life (*Weiner*, 1997) and on the other hand specific immune reactions against bacteria, viruses and parasites must protect the body in future contact with these microbial aggressors. The basic concepts of these functions are more complex than previously thought (*McGhee* et al., 1992) and are of major relevance for the development of oral vaccines (*Shalaby*, 1995). Mice with all their well-characterised genetic parameters, transgenic and knockout techniques and wealth of reagents are most often used in characterising the basic mechanisms of tolerance induction and protective immune reactions. However, the species differ greatly, not only in eating habits (omnivora, carnivora and ruminants) but also in the size of the different sections of the gastrointes

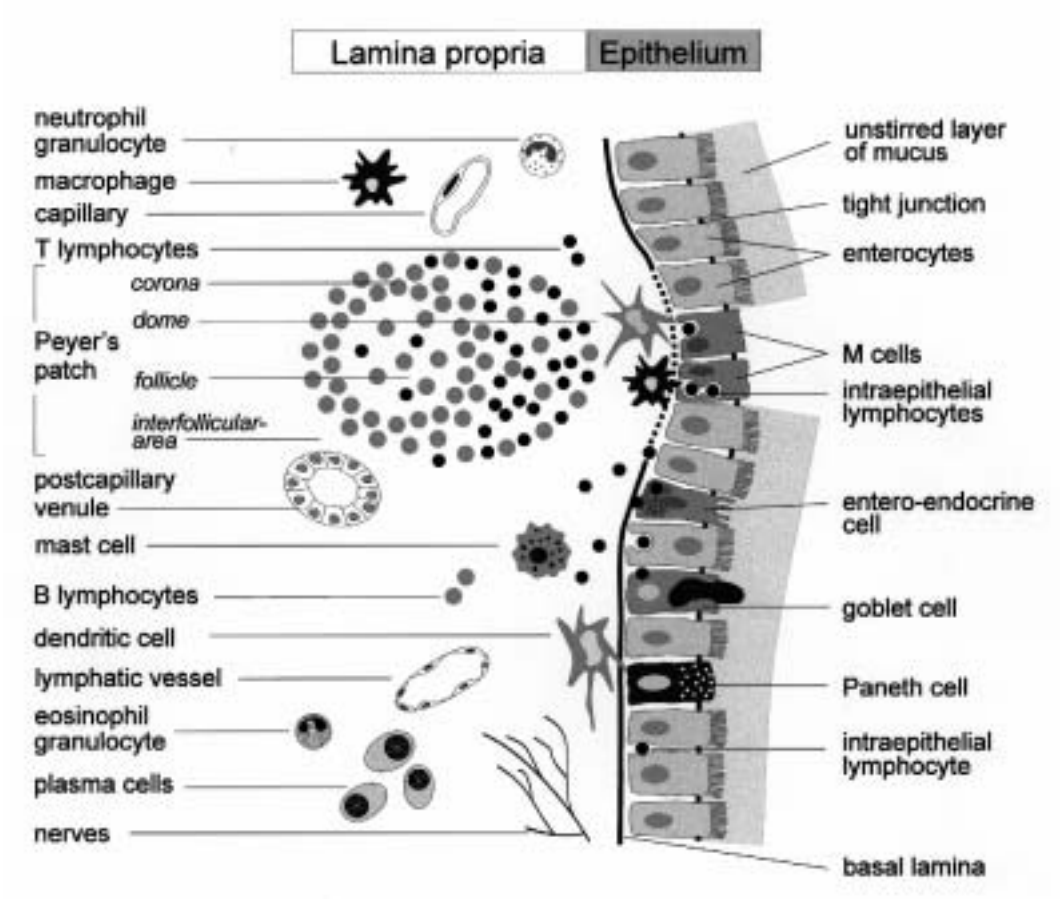

Figure 1: Schematic drawing of the composition of cells of the innate and specific immune system (modified after *Pabst* and *Rothkötter*, 1998).

tinal tract, as well as the amount and composition of bacteria in the gut lumen.

The gut wall consists of different compartments, each with a specific composition of cells of the immune system. The afferent branch of the mucosa-associated immune system is found in isolated follicles and Peyer´s patches, in which antigen is taken up by M-cells and immune reactions initiated. The efferent branch consists of effector T-lymphocytes in the epithelium and the lamina propria and effector cells of the B-lymphocyte family, the plasma cells (Figure 1).

Many aspects of immune reactions in the alimentary canal can only be studied by serial biopsies, local application of antigens and other experimental procedures, which can hardly be performed in humans. Here, we will give examples of species differences of the gut immune system at different ages which stresses the importance of always mentioning the species in studies on the intestinal immune system and the need for caution in extrapolating data from animals to humans.

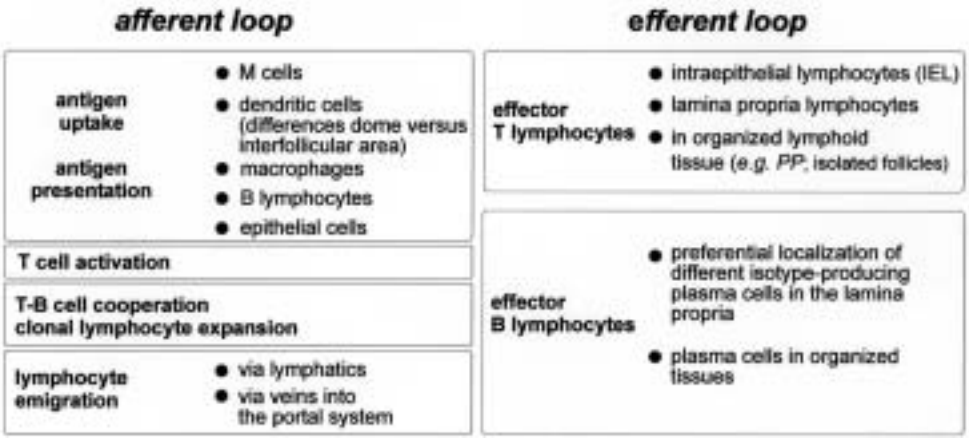

Figure 2: Comparison of the afferent and the efferent branch of the gut immune system

PEYER´S PATCHES DIFFER IN NUMBER, SIZE, AND FUNCTION BETWEEN SPECIES

Peyer´s patches (PP) consist of four well defined compartments: the follicles in several aspects resemble germinal centres in lymph nodes, tonsils or the spleen; the corona or mantle zone surrounds a follicle and consists of more mature B-lymphocytes; the interfollicular area contains mainly T-lymphocytes and can easily be identified by the high endothelial venules in the compartment; and finally the dome area with a mixture of T- and B-lymphocytes, dendritic cells and macrophages is found directly beneath the basal lamina. The most interesting cells in the epithelium of the dome are specialised epithelial cells, the M-cells (Figure 2).

In rats and mice PP develop after birth while PP are already found in the human foetus. In sheep a continuous PP in the ileum, which is up to 2 m in length, is fully mature in the foetus and shows an extremely high rate of lymphocyte production. This ovine ileal PP plays a critical role in B-lymphocytopoiesis: the animal´s B-cell repertoire is expanded by somatic hypermutations (for review see *Griebel* and *Hein*, 1996; *Reynolds* et al., 1999). In this respect the sheep's PP in the jejunum are totally different and more like human PP. *Butler* et al. (1996) proposed a concept of grouping species according to the development of the repertoire of B-lymphocytes: in primates and rodents a so far unknown organ is responsible for an antigen-independent development of the B-lymphocyte repertoire. In chicken, rabbits, ruminants and pigs, a B-cell repertoire develops in the PP in the terminal ileum. A further interesting aspect is the regression of the ileal PP in sheep from about 18 months and a similar disappearance in adult pigs, while the PP in the jejunum alter neither their size nor structure during ageing. Furthermore, the lymphocyte subset composition differs between the continuous ileal PP and the discrete jejunal PP (for review see *Pabst* and *Rothkötter*, 1999). A comparison of the development, lymphocyte subset composition and function between different species has been summarised by *Griebel* and *Hein* (1996).

All too often researchers focus on PP as entry sites for antigens and the isolated follicles in the intestinal lamina

propria are forgotten. In humans a total of 30,000 isolated follicles have been counted. Comparable data on isolated follicles in different species are urgently needed under defined microbial situations to calculate the total area of lympho-epithelium covering PP and isolated follicles. It has to be stressed that there is great variability of antigen uptake between the different dome areas of one PP, between individual PP and between animals bred and kept under comparable conditions (*Rothkötter, Pabst*, unpublished). It would be of great interest to synchronise the activity of M-cells along the small intestines before giving an oral vaccine.

MAJOR DIFFERENCES IN NUMBERS AND MARKERS OF M-CELLS

The relative number of M-cells in proportion to enterocytes reaches 50% in rabbits which is in contrast to about 10% in rats and mice and about 3.5% in humans (for review see *Gebert*, 1997). The uptake of many different bacteria, viruses or particles has been described (for review see *Gebert* et al., 1996). M-cells can hardly be identified by light microscopy and no generally accepted monoclonal antibodies exist for these cells. In addition to electron microscopy some markers have been described which can be used by light microscopy but without knowing why these markers differ between species: in rabbits M-cells are vimentin positive, in pigs cytokeratin 18 positive and in mice cytokeratin 8 identifies M-cells (reviewed by *Gebert*, 1997).

Meanwhile a long list of soluble or particulate tracers, viruses and bacteria has been described as being effectively transported by M-cells through the gut wall (for review see *Gebert* et al., 1996). The vast majority of these have only been taken up by M-cells in one species. It remains to be shown whether one can extrapolate from these specific species to all other mammals including humans.

LYMPHOID STRUCTURES RECENTLY FOUND IN THE GUT WALL

It was very surprising that only recently organised lymphoid structures other than PP and isolated follicles were discovered, since for generations anatomists, immunologists and pathologists have cut the gut wall and missed these structures. For mice, *Kanamori* et al., (1996) described the so-called "cryptopatches". These consist of about 1000 lymphoid cells around epithelial crypts in the small intestine and colon. The most interesting aspect is their subset composition: mostly negative for markers of B, CD3, CD4, CD8 but dependent on IL-7 and c-kit^{+} (*Saito* et al., 1998). They are also found in nude and SCID mice and these structures obviously play a central role in the development of the gut intraepithelial lymphocytes (*Oida* et al., 2000). It has recently been suggested that cryptopatch-like structures might have been conserved in the animal kingdom as a local source of intestinal T-cells during the evolution of vertebrates (*Nanno* et al., 1999).

A further lymphoid structure of the gut wall in humans only recently de-

scribed and characterised are the "lymphocyte-filled villi" (LFV) (*Moghaddami* et al., 1997). These structures in the human small intestine contain MHC-class II^+ dendritic cells, many memory T-lymphocytes, few B-cells and no evidence of immature lymphocytes expressing c-kit or CD1a. Thus, the cellular composition is totally different from that of cryptopatches in mice. In rats LFV have also been described: In new-born rats the lymphoid cells expressed neither surface immunoglobulin nor CD3, but a different more mature lymphocyte composition has been described for 4-5 week old rats (*Mayrhofer* et al., 1999). It has been speculated that in species with a long life span as in humans, LFV might have different functions in different phases of life. In a recent short article *Kanamori* et al. (2000) discussed the similarities and differences between cryptopatches and LFV and indicated the field of future research on these burning issues.

The obvious questions for future research programs are: are there cryptopatch-like structures in species other than mice? Are LFV unique to rats and humans? What are their size, number, and cellular composition at different ages in humans? Which role do these structures play in the gut immune system?

WHY ARE THERE SUCH DIFFERENT NUMBERS OF INTRA-EPITHELIAL LYMPHOCYTES EXPRESSING THE γδ T-CELL RECEPTOR?

The proportion of $\gamma\delta^+$ IEL has been described as about 10% in humans, approx. 50% for bovine gut and a huge variation in mice (20 - 80%). Age, antigen-exposure and the presence of the thymus all influence the number of $\gamma\delta^+$ IEL (for review see *Mowat* and *Viney*, 1997). *Lefrançois* and *Puddington* (1999) have recently summarised the great species differences with respect to number, subset composition and functional role of the IEL. It remains to be studied which immune functions of the gut wall might be influenced by the presence or absence of certain subsets of IEL. It can be predicted that chemokines will play a central role in lymphocyte migration to the different compartments of the gut wall (e.g. *Sallusto* et al., 1999; *Mackay*, 1999). In a recent study nearly all lymphocytes in the lamina propria and the epithelium of the human gut expressed the CXCR3 and CCR5 receptors, which are associated with TH1-lymphocytes. The pattern of chemokine receptors seems to be typical for the gut wall and during intestinal inflammation an upregulation is observed (*Agace* et al., 2000).

The exact function of γδ IEL was long under debate. In respect to the interaction of gut epithelial cells and IEL some recent findings are of interest. *Boismenu* (2000) summarised *in vitro* and *in vivo* data, demonstrating an important role of IEL in stimulating gut epithelial cells to divide and thus repair epithelial damage via keratinocyte growth factor (KGF). These protective effects of γδ IEL by inducing re-epithelialisation in a mouse model of colitis were conclusively shown by in situ hybridisation and PCR of KGF in γδ IEL. Thus, there is growing evidence that not only epithelial cells regulate IEL function by secreting cytokines or processing and presenting antigen to IEL (*Hershberg* and *Mayer*, 2000) but also γδ IEL stimulate epithelial cell growth by recognising self-antigens expressed by damaged epithelial cells.

ANTIGEN PRESENTATION IN THE GUT WALL

The number and function of antigen-presenting cells in the gut wall differs depending on the markers and species studied. Much more is known about dendritic cells, their antigen uptake and transport to regional lymph nodes in the respiratory tract (summarised by *Stumbles* et al., 1999). The number of dendritic cells in the epithelium of the trachea increased several hundred folds within two days after exposure to viruses, live or killed bacteria and to a lesser degree after chronic exposure to dust. These dendritic cells then migrated to the draining bronchial lymph nodes. There are fewer data on dendritic cells in the lamina propria of the gut and Peyer´s patches (*Liu* and *McPherson*, 1995; *Kelsall* and *Strober*, 1996; *Maric* et al., 1996). *Ruedl* and *Hubele* (1997) described the maturation of dendritic cells by cytokines of PP in the rat. A surprising finding was that in pigs endothelial cells of vessels in the lamina propria were strongly MHC-class II^+ (*Bailey* et al., 1996). It can be questioned why these cells express MHC-class II and what functional relevance it might have in this species. The antigen presentation in respect to mucosal pathogens has been summarised by *Reyes* et al. (1997).

IgA-PRODUCING PLASMA CELLS IN THE GUT WALL HAVE DIFFERENT ORIGINS

Undoubtedly IgA is the prevailing antibody class produced by plasma cells in the gut lamina propria. In the human small intestine about 80% and in the colon 90% of all plasma cells are IgA^+ (IgA_2 approx. 66% in contrast to only 7% IgA_2 in the nasal mucosa) (for review see *Brandtzaeg* and *Farstad*, 1999). For years the concept was accepted that the IgA-producing cells all originate from B-cells stimulated in the gut wall, emigrate via lymphatics and return preferentially to the gut lamina to mature to IgA-producing plasma cells. Meanwhile, there are growing numbers of studies mainly in mice, differentiating the IgA precursor B-lymphocytes into B1- and B2-cells. The $CD5^+$ B1-cells are found in the peritoneal cavity and produce a more unspecific IgA in the gut wall (*Su* and *Tarakhovsky*, 2000), while the B2-cells are the precursors of the classical IgA-producing plasma cells (*Kroese* et al., 1994). In a very recent study *Hiroi* and *Kiyono* (2000) summarised their data showing that IL-15 secreted by enterocytes (as shown for mice, rats and humans) induces the differentiation of B1-lymphocytes and only B1-cells have the IL-15 receptor. The differentiation of B2-cells in contrast is dependent on IL-5.

POSTNATAL DEVELOPMENT OF THE GUT IMMUNE SYSTEM

There are major species differences in the early postnatal development of the gut barrier. In species with a multilayered placenta, such as pigs and sheep, the animals are born without antibodies, and these have to be absorbed postnatally from the colostrum. The phenomenon of the so-called gut closure is still ill-defined for many species. Cytokines are critical for the development of PP as shown in knockout mice, e.g. $TNF^{-/-}$ and $lymphotoxin^{-/-}$ animals show obvi-

ous defects (for review see *Mowat* and *Viney*, 1997). Once more details about these regulatory factors are known, it might be possible to stimulate the development of PP with active M-cells before an oral vaccination. In the human foetal gut 2 IEL with the αβ receptor per 100 enterocytes were found. IEL with the γδ receptor are hardly seen before birth but later about 10% of the IEL express this marker (*Cerf-Bensussan* and *Guy-Grand*, 1991). It is still unclear how much the entry of lymphocytes and how much local proliferation contribute to the expansion of IEL after birth and after antigen exposure. In mice and pigs IEL incorporate the DNA analogon bromodeoxyuridine (*Penney* et al., 1995; *Rothkötter* et al., 1999) which seems not to be the case in humans. Further experiments have to clarify the role of age, nutritional and microbial factors which regulate the number of IEL in the many different species.

In all developmental aspects the balance between entry and exit, local proliferation and apoptosis have always to be considered when explaining an increase or decrease of cell numbers in a compartment (*Pabst* and *Rothkötter*, 1999). Further details on the role of adhesion molecules and lymphocyte traffic to the gut can be found in the reviews by *Pabst* and *Westermann* (1997) and *Dunkley* et al. (1995).

In conclusion, the many species differences in the number, structure and function of immune cells in the gut and the effect of nutritional and microbial antigens on the barrier function have to be studied in much more detail. Extrapolation of data from species living under very artificial SPF conditions in animal breeding facilities should only be extrapolated to the situation in humans with great caution.

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