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EARLY ANTIGEN EXPERIENCE SHAPES THE MUCOSAL T CELL REPERTOIRE

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SUMMARY

It is becoming ever more apparent that influences very early in life mould the individual and immune responsiveness is no exception. This short review summarizes the evidence for mechanisms which are likely to have an impact during foetal and neonatal life on the responsiveness of mucosal T cells in the intestine of the adult. These neonatal mechanisms are important because they influence disease susceptibility in later life. It is concluded that the commensal microflora has the major impact on mucosal T cell development, but that there are also influences from food antigens and from developmental aspects of the antigen presenting machinery. Finally – or first – maternal antigen experience probably has a long-lasting influence on the mucosal T cell repertoire.

INTRODUCTION

Despite the obvious importance of the mucosal immune system of the intestine during the first vulnerable days and weeks of life, we understand remarkably little about how the environment – be it the relatively protected foetal environment, or the subsequent exposure to commensals and pathogens after birth – affect our ability to survive this susceptible period or regulate responses to challenges by food and microbial antigens later in life.

If we understand these early mechanisms, we can then design strategies for intervention – either prophylactic through neonatal vaccination, or therapeutic through targeted pharmaceutics. Given the huge interest over recent years in the Hygiene Hypothesis and in possible links between environment and early immune development and the increasing prevalence of immune hypersensitivity diseases – asthma, food allergies – an understanding of cellular and molecular mechanisms during immune development may guide recommendations for social change and dietary habits.

This brief review has three aims: to provide a synthesis of the current state of the art in the area; to stimulate new work in this important area; and to put forward a hypothesis for discussion.

The first statement that must be made is that there are considerable inter-species variations in immune development during foetal and neonatal life, so that although we know some-

thing about relevant parameters in rodents and man, and to some extent in the pig, the strategies for developing an effective mucosal immune system have been driven through evolution by environmental pressures. Assumptions cannot be made across species and models for the human system – and the questions asked of these models – must be appropriate to the system under study. Factors such as placental structure; permeability of the neonatal epithelial barrier; length of gestation; and environmental niche add largely unknown levels of complexity when we start to define factors shaping the immune repertoire.

In our own studies, we are interested primarily in the mechanisms involved in regulating the antigen specificity repertoire of human mucosal T cells. To some extent we are able to make descriptive studies of gut mucosal T cell phenotype and receptor sequence, but even this is difficult because of obvious and necessary ethical constraints. To draw mechanistic conclusions from these observations challenges probabilities because of low numbers of observations. We have therefore chosen the mouse as a model system in which to probe more mechanistic questions about the nature of the environmental antigens which might impact neonatal T cell development and T cell receptor (TCR) repertoire development.

DISCUSSION

Although the gut of the young neonate is protected by exogenous innate factors in the mother's milk and by endogenous innate cells and antimicrobial peptides (*Newburg* and *Walker*, 2007), full protection against microbial challenge depends on the appropriate development of the adaptive immune system, in particular effector, regulatory and memory T cells. In the adult animal, these gut T cells take on a compartmentalization, with some naïve cells found within the B cell follicular structures in both small and large intestine, but with the majority of gut T cells residing in the mucosal lamina propria (LPL) and epithelial (IEL) compartment, with a mostly activated or memory phenotype. While IEL comprise a complex mixture of phenotypes, with both α/β and γ/δ T cell receptors (TCR) represented and with variable proportions of cells bearing α/β and α/α CD8 co-receptors (*Hayday*, 2001), lamina propria T cells are mostly α/β TCR $CD4^+$. While these are mostly of effector/memory phenotype, they also contain significant numbers of regulatory T cells, likely to be involved in suppressing responses to autoantigens and inappropriate responses to dietary and commensal antigens (*Izcue* and *Powrie*, 2008).

These effector/memory T cells protect the adult gut from pathogens. They are activated by interaction with antigen-bearing dendritic cells in the draining mesenteric lymph nodes. This causes a change in membrane phenotype from the CD62L (L-selectin)$^+$, $CCR7^+$, LFA-1^+, $CD45RA^+$ naïve phenotype to the $CD62L^{lo\ /\ neg}$, $CCR9^+$, $\alpha4\beta7^+$ or $\alpha E\beta7^+$, $CD45RO^+$ activated/memory phenotype. These preferentially home to mucosal sites through $\alpha4\beta7$ integrin-MadCAM-1 interactions and, within the intestine, are likely to seed the initial site of antigen challenge through receptors for tissue-specific chemokines (CCR9 inducing homing to CCL25 in the small intestine and CCL10 to CCL28 in the large intestine [*Johansson-Lindbom*

and *Agace*, 2007]). The intestine-homing properties of these effector/memory cells are imprinted by the gut-derived dendritic cells, through mechanisms involving IL-4 and vitamin A-derived retinoic acid (*Iwata* et al., 2004; *Elgueta* et al., 2008).

The diversity of antigenic epitopes, which can be recognised by the antigen receptors of the T cell pool within an individual, is generated through a combination of developmental processes that occur predominantly (but see below) in the thymus. Thus, the germline-encoded V, D and J segments of the TCR genes recombine randomly during development to yield highly diverse mRNA sequences encoding the three complementarily determining regions (CDR) of the V domain of α, β, γ or δ TCR chains. The diversity is particularly focussed on the central CDR3 regions of β and δ chains through recombination with D gene segments (which do not exist within α or γ chain genes) and through the addition of N and P nucleotides at the VD and DJ junctional regions. Through these random events there is a high probability that the antigen specificity of T cell clones within the numerically stable adult pool will be highly diverse and that the pool will, therefore, be polyclonal in specificity. In the peripheral adult naïve T cell pool this is, in fact, the case and this permits responsiveness by an individual to a wide range of antigens. During adult life, however, it is likely that, through antigen exposure, the tissue-seeded segments of the T cell pool will undergo clonal expansion through generation of memory cells to useful, pathogenic, specificities, and possible clonal deletion through induced apoptosis of self-reacting clones. The circulating, naïve segment, however, will remain polyclonal, retaining the ability to respond to a wide range of pathogenic epitopes. With age, then, one would predict that the T cell pool in tissues experiencing environmental antigen would become biased and oligoclonal.

Before the detailed mechanisms of activation and homing of T cells to the gut were defined, it was predicted that the TCR repertoire of gut mucosal T cells would be polyclonal in response to the wide array of antigens within the gut environment. However, numerous studies over the past few years have demonstrated in rodents and in man that the adult gut T cell repertoire is, in fact, remarkably restricted (*Blumberg* et al., 1993; *Holtmeier* et al., 1997; *Regnault* et al., 1996) reviewed by *Probert* et al. (2007a). Localized homing of particular clones seems unlikely as clones with shared specificity have been described in different regions of the intestine (*Gross* et al., 1994), so it is probable that equivalent seeding of clones takes place after activation in the nodes, followed by local expansion in the tissues. Thus, it is clear that at least from puberty onwards human gut intraepithelial and lamina propria, αβ and γδ T cells are oligoclonal.

The question is: How early is this oligoclonality established and what drives the restriction of the TCR repertoire?

In the human foetus, T cells develop in the epithelium and lamina propria of the gut during the second trimester of gestation and increase between weeks 11 and 19 of gestation (*Spencer* et al., 1986). They express an essentially polyclonal Vβ TCR repertoire (*Thomas* et al., 1996; *Koningsberger* et al., 1997). These cells have an activated phenotype, but do not express α4β7 integrin (*Howie* et al., 1998), so it seems unlikely that they are activated in the periphery and home to the intestine, as in the adult, although they could be activated in situ in response to maternally-derived antigens

(but see dendritic cells, below). More recently, a population of $CD3^{-}7^{+}$ cells has been described as early as seven weeks gestation in human foetal gut, which could represent precursors of T cells and, moreover, these cells have the potential to develop ex vivo into $CD3^{+}$ cells (*Gunther* et al., 2005). Thus, human foetal gut T cells could be generated in situ and potentially provide a polyclonal effector population intact to deal with postnatal challenge, although the mechanisms of their in situ activation remains unknown and distinction between these T cell precursors and immature NK cells was not made. This does suggest, however, that the postnatal human gut is already primed with effector T cells with polyclonal receptors, but that these have not been driven by bacterial antigens other than those experienced by and processed by the mother. The observation of pre-Tα chain (pTα) expression in the foetal gut (*Howie* et al., 1998) suggests that these precursors may be rearranging their receptors extrathymically.

In the immediate postnatal period, the human infant gut (small and large intestine) is populated by T cells with polyclonal Vβ receptors and there are multiple populations of cells with phenotypes resembling thymic precursors: $CD3^{+}4^{+}$; $CD3^{+}8^{+}$; $CD3^{+}4^{+}8^{+}$; $CD3^{+}4^{-}8^{-}$, together with expression of pTα, TdT and Rag, suggesting that all the machinery for extrathymic rearrangement of TCR genes is present in the mucosa after birth as well as in the foetus (*Williams* et al., 2004). The "thymic precursor" phenotypes persist in some individuals up to nine months of age. The T cell precursor phenotype - $CD3^{-}7^{+}$ - described by *Gunther* et al. (2005) is also present in these postnatal samples and the co-expression of CD2 suggests that these are closer to T cell lineage than NK lineage. At least in the relatively small group that we investigated, our evidence suggests that by 18 months of age the rearrangement machinery becomes diluted and the TCR repertoire (determined by sequencing) becomes more oligoclonal, approaching the adult gut picture. However, although restriction of the repertoire was noted in two individuals between 6-month samples, and 9-month samples, there was variation between samples and some samples at 18 months were still relatively polyclonal (*Williams* et al., 2004). Of further interest, in one six-day-old individual with a sterile duplication cyst parallel to the ileum, but with no luminal continuity, the repertoire was polyclonal in both sections with no shared clones, indicating antigen-independent homing to different regions of the gut. Also, in one child of 56 months who had a non-functioning colon from birth, the repertoire was not different from other individuals' colons, with a significant restriction of clonality, indicating that developing colonic flora per se was not required to restrict the repertoire – although there was no information available on development of small intestinal flora.

The evidence in human foetus and infant, then, suggests that T cell precursors are seeded into the intestine before birth by non-MadCAM-1-dependent mechanisms; that these precursors can develop into functional $CD3^{+}$ T cells with rearranged receptors and a polyclonal repertoire by birth; that in situ rearrangement continues after birth; and that these endogenously-derived T cells with a polyclonal receptor are gradually replaced over the first 18 months of life by $\alpha4\beta7^{+}$ T cells activated in the periphery to the growing intestinal challenge; that these immigrant T cells develop a restricted repertoire by expansion of clones to dominant enteric antigens.

But are these dominant antigens from the commensal flora or from

food? In order to answer this, we must turn to studies in rodent models.

In comparison to humans, T cell development in the foetal mouse gut takes place relatively late in gestation. From approximately day 18 of gestation, $CD3^+$ cells are seen associated with Peyer's patch anlagen (*Adachi* et al., 1997; *Yoshida* et al., 2001), but nothing is known of the foetal intraepithelial and lamina propria T cell compartments. We have recently characterised the postnatal mouse intestine at three time points: pre-weaning; peri-weaning; and post-weaning, in parallel in germfree and SPF mice in an attempt to separate the effects of diet (weaning) and colonization by commensal flora on intestinal T cell development (*Williams* et al., 2006). In summary, we have shown that a few days after birth, the SPF gut is populated by significant numbers of $CD3^+$ T cells, about one-third of which expresses $\alpha4\beta7$ integrin, and as the numbers of these cells increases in small and large intestine, the proportion of $\alpha4\beta7^+$ cells increases also. The germfree gut, however, contains very few T cells at birth and numbers do not increase significantly through weaning, but all germfree gut T cells are $\alpha4\beta7^+$ throughout. Significantly, immediately after birth germfree and SPF guts contain similar numbers of $CD3^+$, $\alpha4\beta7^+$ T cells. These are presumably T cells that have seeded the gut during foetal life. In the absence of flora, this foetus-derived population expresses L-selectin, a marker of naivety, and the population does not change postnatally. However, in SPF mice, the foetus-derived cells do not express L-selectin, suggesting they are antigen-experienced, and they are gradually overlaid by further antigen-experienced T cells. Administration of a commensal flora to germfree mice causes an influx of $CD3^+\beta7$ T cells into the small intestine after weaning, but these cells express L-selectin and so are presumably not activated to commensal antigens. This may indicate that the combination of food and incomplete microflora leads to homing of cells to the mucosa, but that these are not conventionally activated in the absence of an unknown microflora species. Mucosal $CD11c^+$ dendritic cell maturation was defective in the germfree intestine, with development delayed until just before weaning. This could explain the reduced activation status of those T cells infiltrating the colonized germfree intestine.

These studies suggest that, although foetal intestinal T cell development is delayed in the mouse compared to the human, mucosal T cells are transferred from the foetus to the neonate in the same way. If these cells are transferred from foetal to neonatal gut to protect the neonate from pathogenic antigens, it seems likely that the repertoire of these early cells is established during foetal life under the influence of maternal antigen experience – in those neonates derived from germfree dams, the starting population of gut T cells has a naïve phenotype. However, the protective nature of this population of early gut T cells is an assumption. Without functional and further phenotypic data, these cells could equally be regulatory cells, helping to shape the neonatal T cell repertoire, based on maternal antigen experience. Adoptive transfer of gut cells from germfree or SPF foetuses into TCR transgenic recipients will address this question.

With regard to the intestinal TCR repertoire, as in humans, the adult gut T cell repertoire of mouse and rats is oligoclonal (*Regnault* et al., 1996; *Edwards* et al., 2008). Interestingly, the recent comprehensive study of TCR V-segment usage in the adult rat by *Edwards* et al. (2008), analyzing clonality in all 22 Vβ families, compares the

mucosal and peripheral repertoires and clearly demonstrates restriction of the mucosal repertoire together co-existing with a polyclonal peripheral repertoire in the same individuals. Both of these studies, on inbred mice and rats, show that although the mucosal T cell repertoire is restricted in all adult individuals, the repertoire differs significantly between different inbred individuals in the same cage fed the same diet. Although this may reflect different stable flora in different individuals, it seems more likely that this represents the end stage of many activation and clonal expansion events during the lifetime development of a stable flora. The effects on the adult repertoire of introduction of a normal flora have been investigated in IEL in rats and show restriction of the germfree polyclonal repertoire after introduction of a flora at birth or weaning (*Helgeland* et al., 1996, 2004).

In the first study of the intestinal T cell TCR repertoire during the neonatal period in germfree and SPF mice (*Probert* et al., 2007b), we have analyzed Vβ clonality by spectratyping and sequencing and have confirmed concordance of the data generated by the two methods. In both the small intestine and colon of SPF mice the data show that the repertoire (total gut T cells, IEL and LPL) is polyclonal before weaning and restricted with expanded clones after weaning. In the germfree mice, there are very few clones at five days of age and thus the repertoire appears oligoclonal. By weaning, the germfree repertoire in both small intestine and colon is restricted, but showed some evidence of polyclonality in the large intestine from weaning onwards. As the SPF repertoire becomes oligoclonal at weaning, it is possibly influenced by diet, developing microflora, or both, but as food at weaning did not appear to have a marked effect on the repertoire, we suggest that the major effect on the repertoire is bacteria. On the other hand, in the absence of bacteria the few examples of polyclonality observed after weaning are presumably driven by food antigens. The contraction of the repertoire in small and large intestine occurred almost simultaneously and we observed identical clones in large and small intestine in post-wean samples, suggesting that effector/memory clones activated to bacterial antigens from one region of the intestine are then seeded throughout the intestine. With regard to our phenotyping studies, one would predict that the small population of neonatal gut $CD3^{+}\beta7^{+}$ cells derived from the foetus is oligoclonal in the germfree mouse, but may be polyclonal or shielded by a larger polyclonal population of cells in the SPF gut.

In summary, we hypothesise that the major influence on the shape of the adult gut T cell repertoire is the development of the commensal flora, either alone, or itself influenced by growing dietary complexity. This will, in turn, be influenced by those aspects of development of the individual that will affect presentation of antigen. However, it seems certain that the final repertoire of effector/memory T cells in the adult gut will, like so many other aspects of biology, be influenced by history – antigenic in this case – of the mother.

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