Regulation of mucosal immune responses in effector sites

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In human disease and rodent models, immune responses in the intestinal mucosa can be damaging. Damage is characterised by villus atrophy, crypt hyperplasia and reduced ability to digest and absorb nutrients. In normal individuals active responses to harmless environmental antigens associated with food and commensal bacteria are controlled by the development of immunological tolerance. Similar pathological changes occur in piglets weaned early from their mothers. Active immune responses to food antigens are observed in these piglets, and we and others have hypothesised that the changes occur as a result of transient allergic immune responses to novel food or bacteria antigens. The normal mechanism for producing tolerance to food antigens may operate at induction (Peyer's patches and mesenteric lymph nodes) or at the effector stage (intestinal lamina propria). In our piglet studies immunological tolerance occurs despite the initial active response. Together with evidence from rodents, this observation suggests that active responses are likely to be controlled at the effector stage, within the intestinal lamina propria. Support for this mechanism comes from the observation that human and pig intestinal T-cells are susceptible to apoptosis, and that this process is accelerated by antigen. We suggest that the role of the normal mature intestinal lamina propria is a balance between immunological effector and regulatory function. In neonatal animals this balance develops slowly and is dependant on contact with antigen. Immunological insults such as weaning may tip the balance of the developing mucosal immune system into excessive effector or regulatory function resulting in transient or chronic allergy or disease susceptibility.

Weaning: Immunity: Tolerance: Development

Expression of mucosal tolerance at the effector stage

The need for the mucosal immune system to control local immune responses to harmless antigens is apparent from the severe pathology which can be induced in individuals allergic to food antigens. Experimentally, expression of this control is demonstrated by the classical 'oral tolerance' protocol, in which feeding a novel protein antigen to a group of animals results in a decreased immune response when they are subsequently challenged with the homologous antigen (Challacombe & Tomasi, 1980). This experimental protocol has been widely used to analyse the mechanisms by which mucosal immune responses are controlled. Considerable progress has been made in understanding these mechanisms, and it is now clear that T-cell anergy, apoptosis and active regulation can all contribute under different conditions. However, these studies have not clarified two major issues. First, the sites and specific cellular and molecular interactions which generate the different mechanisms of tolerance have not been fully identified. Second, we do not fully understand how the mucosal immune system can generate tolerance against 'harmless' antigens and active responses against 'dangerous' antigens, and how, in normal individuals, these broad classes of antigens can be distinguished with a high extent of accuracy.

Post-weaning diarrhoea in piglets

The model system with which we have worked extensively is the introduction of novel food antigens to young piglets at weaning. The pig is a large single-stomached omnivore with a gastrointestinal physiology comparable with that of man and an immune system which is more accessible than the mouse. The litter size (ten to fourteen piglets) and the

availability of lines inbred to a greater or lesser extent makes experimental manipulation easier than other larger domestic species. Intensive work by several groups over the last decade has advanced the situation with regard to immunological reagents and the pig is now a useful immunological model (Pescovitz, 1998; Haverson *et al.* 2001).

In true wild groups of pigs young piglets will continue to suckle until they are many months old, gradually increasing their intake of other food components as milk intake decreases. This situation, which presumably has provided evolutionary selection pressure under which the mammalian mucosal immune system has developed, allows gradual exposure to novel food antigens and gradual change in intestinal microflora, while under at least some protection from milk-derived, maternal immunoglobulin (Ig) A antibody. Under modern farming conditions, economics have dictated that piglets should be weaned from their sows much earlier than would occur in the wild. At present, weaning commonly occurs between 3 and 5 weeks old, at which point the piglets' voluntary intake of dry food is often limited. In some conditions, weaning can occur as early as 7–10 d old.

Conventional husbandry of piglets, therefore, provides a model of the ability of single-stomached mammals to cope with abrupt, early weaning, which is also of economic importance in its own right. At 3 weeks old, these piglets suddenly undergo massive challenge with antigens associated with food and with the establishment of a novel commensal intestinal bacterial flora. In addition, facultative pathogens, previously controlled but not eliminated by maternal IgA antibody, can expand and colonise. Thus, the mucosal immune system of the newly-weaned piglet must discriminate rapidly between an enormous range of 'harmless' and 'dangerous' antigens, determining which, if any, might be associated with pathogens and mounting appropriate responses. That these decisions are not always made appropriately is indicated by the observation that early weaning in piglets is always associated with a check in growth and frequently with diarrhoea of varying severity (Hampson, 1986). This outcome occurs as a consequence of atrophy of intestinal villi, hypertrophy of intestinal crypts and accelerated turnover of crypt cells resulting in shorter lifespan and altered state of differentiation (Miller *et al.* 1986). While toxic effects of overgrowth of intestinal bacteria may cause these changes, this type of pathology also occurs in a number of clean experimental models of active immune responses in the intestine, specifically in rodents sensitised to express active mucosal responses to ovalbumin or undergoing graft *v.* host responses (Mowat & Ferguson, 1981, 1982). It is also a feature of a number of multifactorial conditions involving mucosal immune responses, such as infection with gastrointestinal nematodes or rejection crises in transplanted intestines in rodents and man (MacDonald & Ferguson, 1976).

The occurrence of immune responses to fed proteins in weaned piglets has been well documented by several groups. Weaning at 3 weeks old onto soyabean-based diets results in rapid appearance of serum IgG antibody directed against glycinin and β-conglycinin, major storage components of the soyabean (Wilson *et al.* 1989; Dreau *et al.* 1994). The link between immune responses to soyabean and the intestinal pathology is suggested by a study in which a high proportion of the variation in weight gain after weaning could be accounted for by variation in indirect indices of mucosal immune responses (villus atrophy, crypt hyperplasia) and direct measures of systemic immune responses to soyabean (serum antibody, skin delayed-type hypersensitivity reactions; Li *et al.* 1991). Interestingly, serum antibody was, in fact, positively associated with weight gain, while delayed-type hypersensitivity reactions to intradermal soyabean were negatively associated; i.e. cellular or T-helper (Th) 1-type responses were most damaging, while antibody responses might be protective. This observation is consistent with studies on inflammatory bowel disease in rodents and man, where Th1 responses in the intestine are mostly associated with pathology.

In piglets weaned at 3 weeks old the level of the response to fed soyabean was comparable with that produced in response to injection of soyabean together with adjuvant (Bailey *et al.* 1994*b*). In contrast, when soyabean was introduced into the diet of older piglets, only limited antibody responses were observed (Wilson *et al.* 1989). Despite the occurrence of an initial strong active immune response to the fed protein, detected by systemic antibody, the response was still essentially mucosal. This factor was demonstrated first by dosing piglets with soyabean antigen at birth, which decreased the level of antibody produced at weaning in response to fed soyabean, but not injected soyabean (Bailey *et al.* 1994*b*). Second, the initial immune response was followed by tolerance rather than by priming; injection with soyabean at 9 weeks old, after the level of antibody had declined, triggered essentially no antibody compared with systemic primary or secondary responses in control groups (Bailey *et al.* 1993). Studies in piglets kept under conditions approaching the wild, in which weaning occurs gradually up to 12 weeks old, have shown that tolerance to soyabean proteins in these animals is not complete until after 9 weeks old (Miller *et al.* 1994).

The data from studies on the immune response to fed proteins in piglets weaned at 3 weeks old suggests that these animals do mount inappropriate immune responses to antigens in the weaning diet, but that these responses are subsequently controlled by classical 'oral tolerance' mechanisms. This observation has two important implications. First, that tolerance to mucosal antigen is likely to be mediated at the effector stage, rather than the inductive stage. Second, that neonatal infants and animals suddenly exposed to high levels of mucosal antigen are likely to respond inappropriately, generating allergy or failing to respond to pathogens.

Sensitisation precedes tolerance in rodents

The idea that mucosal tolerance is regulated at the effector stage is consistent with a number of observations in rodent models. In the early demonstrations of oral tolerance, it was shown that systemic tolerance, measured as reduced antibody responses to injected antigen, could be accompanied by secretory IgA responses to antigen within the intestine (Challacombe & Tomasi, 1980). Since isotype switching by B-cells requires T-cell cooperation, this response implies the presence of mucosal T-cells capable

of engaging in effector mechanisms. This early study was followed by a series of experiments using an oral tolerance protocol in mice requiring five consecutive daily feeds with ovalbumin. Using this protocol, it was possible to demonstrate that the initial feeds resulted in immunological priming rather than tolerance, and that this process was associated with delayed-type hypersensitivity reactivity and mild intestinal pathology (Stokes *et al.* 1983). Again, the implication is that antigen-feeding results in the appearance of primed effector T-cells which are regulated at the effector stage, rather than progressing directly from naive to regulated cells. Very recently, studies in which presentation of food-derived antigens to T-cells in the intestine is altered have suggested that the phenomenon of orally-induced systemic tolerance may be completely unlinked to the control of immune-mediated damage in the intestinal mucosa. One hypothesis for maintenance of mucosal tolerance has been that presentation of peptide epitopes to T-cells in the context of major histocompatibility complex class II on non-professional presenting cells (enterocytes in man and rodents, endothelial cells in pigs) may result in tolerance due to failure to engage appropriate co-stimulatory molecules (Bland & Warren, 1986; Mayer & Shlien, 1987; Wilson *et al.* 1996). To test this hypothesis, mice were made transgenic for the co-stimulatory molecule CD80 under the control of the enterocyte-specific promoter for the fatty acid-binding protein (Iqbal *et al.* 1999). This transgene resulted in selective expression of CD80 on intestinal enterocytes and, consistent with the hypothesis, the transgenic mice could not be orally tolerised using standard protocols. If orally-induced systemic tolerance reflects the ability to control mucosal immune responses, then these mice should be expressing active immune responses against normal antigens in their diet. However, the intestines of these mice appear normal; i.e. expression of damaging mucosal immune responses in these mice is being controlled by mechanisms independent of systemic 'oral tolerance' and presumably in the intestinal mucosa.

Organisation of mucosal effector sites

The mucosal immune system in mammals consists of two main compartments. Organised lymphoid tissues are present both close to the mucosal surface, e.g. the Peyer's patches and palatine tonsils, and at sites of lymphatic drainage, such as the mesenteric lymph nodes. However, in addition, there is a large immunological component diffusely scattered through the mucosal epithelium and lamina propria. The size of this component is frequently underestimated; probably half the immunologically-relevant cells in the intestine are present in the villi and around the crypts, even in normal individuals. The organised lymphoid tissues, equipped with antigen-sampling cells (M-cells in the Peyer's patches or migrating dendritic cells in the draining lymph nodes), T-cell zones and B-cell follicles are clearly potential sites for induction of immune responses to novel mucosal antigens. In contrast, the intestinal mucosa has all the hallmarks of an effector site. The phenotype of T-cells normally resident in the mucosa is consistent with antigen-experienced T-cells. In man they express predominantly the low-molecular-weight CD45RO isoform of the leucocyte common antigen, although a proportion co-express CD45RB at variable level (Halstensen *et al.* 1990); in pigs, the majority similarly do not express the high-molecular-weight CD45RC isoform (Bailey *et al.* 1998). In both species there appears to be low level expression of CD25 on the surface, again consistent with antigen recognition in the relatively recent past (Zeitz *et al.* 1988; Haverson *et al.* 1999). Freshly isolated human lamina propria T-cells secrete interferon-γ and can be induced to secrete high levels of interleukin (IL)-2 by polyclonal activation (James *et al.* 1990; Hauer *et al.* 1998).

However, a number of observations suggest that lamina propria leucocytes are not simply effector cells, engaged in surveillance and in provision of recall responses to potential pathogens. In the intestines of mature pigs there is a high extent of spatial organisation inconsistent with simple infiltration as part of a process of 'controlled inflammation' (Vega-Lopez *et al.* 1993). T-cells are largely confined to the villi and do not appear around the crypts, while plasma cells are localised in the crypts rather than villi. Within the villi, CD8+ T-cells are present within and only immediately beneath the epithelium, while CD4+ T-cells are present deep into the core of the villus (Wilson *et al.* 1996). Dendritic cells lie immediately underneath the epithelium, projecting dendrites into the epithelial layer and deep into the villus core (Haverson *et al.* 2000). Although the level of spatial organisation of the mucosa in man and rodents is not as marked as the pig, it does suggest that local immunological cells are carrying out specific controlled functions.

Activation of murine or pig lamina propria cells does not result in a high level production of IL-2: these cells preferentially produce IL-4 (Harriman *et al.* 1992; Bailey *et al.* 1994*a*). Production of IL-2 by human lamina propria cells requires activation through the CD2 surface molecule rather than through CD3; when CD3 is ligated, IL-4 is preferentially secreted, apparently due to alterations in intracellular signalling pathways (Targan *et al.* 1995; Boirivant *et al.* 1996*a*).

A crucial feature of mucosal T-cells suggesting that expression of effector function may not be their only function, and possibly not the main function, is their susceptibility to apoptosis. Studies in man have shown that isolated mucosal leucocytes are extremely sensitive to apoptosis *in vitro*. Intra-epithelial leucocytes expressing the thymus-independent γ and δ T-cell receptors rapidly enter apoptosis pathways without activation (Viney & MacDonald, 1990). Systemic T-cells from orally-tolerised mice also undergo apoptosis and are rescued by the presence of antigen (Garside *et al.* 1996). Susceptibility to apoptosis in the absence of antigen could be a simple mechanism for clearance of excess mucosal effector cells after resolution of antigen challenge, as has been proposed after acute systemic viral infections in man. However, while human and pig lamina propria leucocytes undergo limited apoptosis in resting cultures, apoptosis is markedly increased by activation (Boirivant *et al.* 1996*b*; FJ Plunkett, H-J Rothkötter, R Pabst, CR Stokes and M Bailey, unpublished results). Unlike true effector cells, apoptosis is apparently not due to cytokine deprivation, since exogenous IL-2 does not rescue lamina propria CD4+ T-cells from either man or pigs.

Apoptosis within the villi, induced by antigen-recognition and activation, could be an extremely effective way of eliminating T-cells generated by indiscriminate primary responses to harmless dietary antigens in organised lymphoid tissues.

In addition, studies on the production of regulatory cytokines suggest that immunoregulation may be an important function of T-cells in the intestinal mucosa. Experiments on oral tolerance in mice demonstrated that the organised lymphoid tissues of tolerised mice contained a population of T-cells, termed Th3 cells, capable of secreting high levels of transforming growth factor-β (TGFβ) in response to antigen (Santos *et al*. 1994). In parallel experiments TGFβ was shown to suppress antigen-induced proliferation by primed effector T-cells and to regulate colitis in one particular mouse model of inflammatory bowel disease (Powrie *et al*. 1996). These antigen-specific Th3 cells were, therefore, a potential mechanism for imposing mucosal tolerance, although only identified in organised tissues. A second cell type capable of regulating rodent inflammatory bowel disease was generated by cloning ovalbumin-specific T-cells in the presence of IL-10, and was characterised by its ability to secrete primarily IL-10 (Groux *et al*. 1997). This observation, that IL-10 is the primary driver for differentiation of IL-10-secreting regulatory T-cells, has been confirmed in a number of studies. The presence of regulatory T-cells at sites of antigen-recognition is potentially, therefore, a positive feedback loop, enforcing regulation in an antigen-non-specific manner. The ability of human and pig lamina propria leucocytes to secrete IL-10 at high levels again suggests that regulation may be a major function of the intestinal mucosa (Braunstein *et al*. 1997; FJ Plunkett, H-J Rothkötter, R Pabst, CR Stokes and M Bailey, unpublished results). Despite the demonstration of Th3 cells in organised lymphoid tissues, TGFβ production by lamina propria leucocytes has not been reported. However, TGFβ is produced by a wide range of stromal cells and TGFβ receptors are present on T-cells. Our studies in the pig suggest that TGFβ rescues lamina propria T-cells from apoptosis, and the role of TGFβ in colitic models may, in fact, be due primarily to an effect on T-cells rather than to T-cells secreting TGFβ. This possibility has also been suggested by studies using dominant-negative transgenic mice in which TGFβ signalling is disrupted in T-cells but not in other cell types (Gorelik & Flavell, 2000). These mice still develop the autoimmune pathology, suggesting that regulation requires T-cells responding to, rather than necessarily secreting, TGFβ.

Mechanisms for maintaining tolerance in the intestinal mucosa

The division of the mucosal immune system into inductive and effector sites provides several distinct possibilities for controlling immune responses to harmless dietary antigens within the intestine. First, discrimination could be made within the organised lymphoid tissue at the inductive stage, resulting in functional deletion of antigen-specific T-cells by anergy or apoptosis and export of T-cells pre-committed to imposing tolerance. Second, the organised lymphoid tissues may simply export primed cells, which re-localise to

the lamina propria and differentiate into effector or regulatory T-cells depending on the microenvironment. This process would have the effect of allowing combined signals from local antigen-presenting cells, from other T-cells, from matrix and from stromal cells to determine the outcome of antigen recognition. Integration of these signals by the T-cell could easily allow recognition of a stable microenvironment, resulting in differentiation into a regulatory T-cell, or recognition of 'damage', resulting in differentiation down classical pathways into effector T-cells.

In this model, in an animal continually exposed to specific dietary antigens, progressive waves of naive T-cells would undergo activation in organised lymphoid tissues, recirculation to the intestinal lamina propria and differentiation to a regulatory T-cell phenotype. Subsequent recognition of antigen would result in activation, secretion of regulatory cytokines and entry into apoptosis (Fig. 1). Local production of IL-4 would contribute to B-cell isotype switching to IgA, while IL-10 would feed back on the next generation of incoming T-cells to promote differentiation into regulatory cells. Apoptosis is a necessary requirement of this system in order to prevent excessive accumulation of T-cells in the mucosa, particularly in a young animal with an active thymus which is continuously renewing the naive T-cell repertoire. In addition, it would provide a potential mechanism whereby tolerance could rapidly be converted to active immune responses as a result of changes in the microenvironment. This process may be more important than has been considered, since studies in rodents and man now suggest that tolerance, in addition to being expressed in response to food antigens, is also expressed to antigens of commensal intestinal bacteria in normal individuals (Duchmann *et al*. 1995*a,b*). Unfortunately, bacteria which exist as harmless commensals at one mucosal surface (the intestine) can be pathogenic in other mucosal sites. Examples are *Escherichia coli* mastitis in dairy cattle and in urinary tract infections in man. It seems likely that efficient immunological handling of commensal bacteria would include the ability to abandon tolerance under some circumstances.

The anti-proliferative effects of TGFβ *in vitro* and the demonstration that neutralisation *in vivo* can promote colitis in CD4^{+} T-cell-transplanted scid mice suggests that TGFβ is important in controlling mucosal inflammation. Our observation that TGFβ rescues lamina propria T-cells from apoptosis provides a mechanism for this control independent of the TGFβ-producing Th3 cell observed in organised lymphoid tissues. The majority of TGFβ in normal mucosa is present as the inactive latent form, which requires processing to become active. In a stable intestinal mucosa CD4^{+} T-cell activation would progress into apoptosis in the presence of latent TGFβ. Mucosal inflammation would result in conversion of latent to active TGFβ which would rescue CD4^{+} T-cells, allowing clonal expansion and increased secretion of IL-10 and IL-4 (Fig. 2). The combination of increased IL-4 and TGFβ would then promote IgA isotype switching, while increased IL-10 and TGFβ would contribute to re-enforcing T-cell tolerance. Importantly, once local inflammation was controlled and conversion to active TGFβ reduced, activated T-cells would once more enter apoptosis pathways. This process therefore provides a
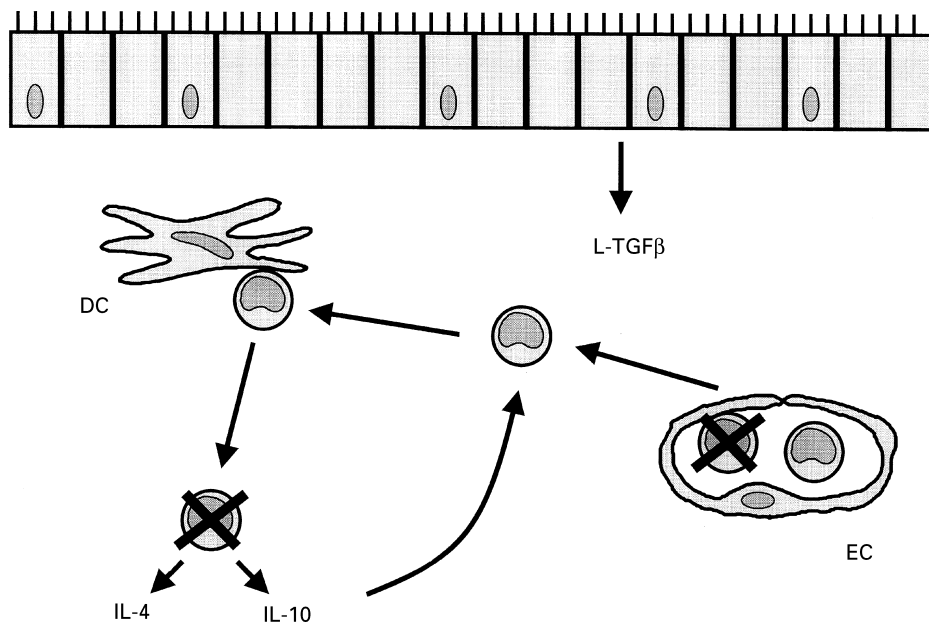
Fig. 1. Maintenance of homeostasis in the normal intestine. The majority of transforming growth factor-β (TGFβ) is present in the inactive latent form; when antigen-specific T-cells recognise antigen in the lamina propria, they activate in the presence of interleukin (IL) 10, becoming 'regulatory' T-cells which secrete further IL-10 and enter apoptosis after activation. EC, endothelial cell; DC, dendritic cell; L-TGFβ, latent TGFβ.

strongly-regulated system in which inflammation results in expression of local IgA responses while damping down inflammation and maintaining T-cell tolerance, as observed in rodent models of oral tolerance.

Development of the mucosal immune system in the neonate

The model proposed here for local regulation of T-cell responses in the intestine relies on the pre-existing presence of a microenvironment, to which existing regulatory T-cells contribute, which pushes differentiation of incoming antigen-specific T-cells towards a regulatory phenotype. In a normal adult animal the presence of regulatory T-cells specific for the homologous antigen or even for for other unrelated epitopes would provide local IL-10 which could enforce this pathway. However, this system is not present in neonatal animals, where exposure to mucosal antigens has been low. In the pig the mucosal immune system is almost completely absent at birth, although considerable development of the systemic immune tissue has taken place. Peyer's patches consist of primordial follicles surrounded by a small number of T-cells and are difficult to identify. Almost no immunological cells are present in the intestinal villi or between the crypts. Development of the spatially-organised architecture described for the mucosal immune system of the adult takes place over a period of several weeks in a series of defined stages, and is very largely dependent on microbial exposure (Bianchi et al. 1992; Vega-Lopez et al. 1995; Pabst & Rothkotter, 1999). In conventional piglets development can be roughly divided into four distinct stages:

1. the newborn piglet; Peyer's patch follicles and T-cell areas are rudimentary. Almost no T-cells are present in the intestinal villi;

2. the first 2 weeks; follicles and T-cell areas of the Peyer's patches expand rapidly and the organisation present in mature animals is almost complete by 12 d old. Some conventional T-cells enter the intestinal villi, but the predominant cell expresses CD2 in the absence of CD4 or CD8. Currently, the nature of this cell is unclear. Expression of major histocompatibility complex class II antigens in the intestinal villi increases during this time;

3. 2–4 weeks old; CD4+ cells enter the intestinal mucosa and occupy the position in the core of the villi seen in the mature intestine;

4. 4 weeks onwards; CD8+ T-cells enter the epithelium and lamina propria of the intestinal villi. The normal architecture present in mature animals is reached by about 6 weeks old, although absolute numbers continue to increase.

Importantly, very little of this development occurred when piglets were kept under germ-free conditions, suggesting that exposure to microbial antigen is an important driver for maturation of the immune system (Pabst et al. 1988; Rothkotter et al. 1991; Barman et al. 1997). In the case of T-cell development it is not known whether the effect of microbial flora is antigen-specific or non-specific. However, elegant studies on Ig gene rearrangement have demonstrated that the effect on mucosal B-cell development is almost certainly antigen-non-specific. Unlike man and rodents, but like the majority of other species characterised, the pig Ig heavy-chain gene contains only a single Jh
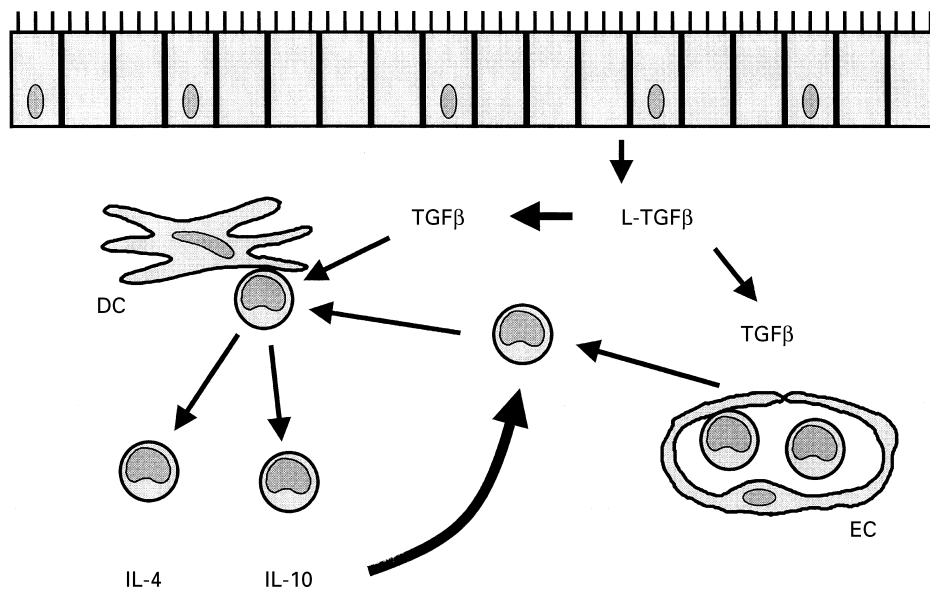
Fig. 2. Maintenance of homeostasis in the mildly-inflamed intestine. Conversion of latent to active transforming growth factor-β (TGFβ) results in clonal expansion of local T-cells. These populations can secrete higher levels of cytokines and the combination of interleukin (IL) 4, IL-10 and TGFβ predisposes to immunoglobulin A secretion and control of inflammatory processes. EC, endothelial cell; DC, dendritic cell; L-TGFβ, latent TGFβ.

segment. In the fetus and newborn piglet limited Vh and Dh segment usage (80% of B-cells using four V segments and two D segments) resulted in a limited B-cell repertoire (Sun *et al.* 1998). In germ-free piglets this limited Vh and Dh segment usage persisted. However, in conventional piglets, or in germ-free piglets colonised with a defined intestinal flora, V segment usage increased, indicating increased repertoire (Butler *et al.* 2000). At the same time point mutations accumulated in IgM transcripts at the same rate as IgG transcripts, suggesting polyclonal activation unrelated to isotype switching.

In order to maintain digestive and absorptive intestinal function in the face of postnatal microbial colonisation, this development of the mucosal immune system must take place in a controlled regulated way. In other words, at all stages it must have the capacity to discriminate between 'harmless' and 'harmful' antigens and respond appropriately. Even at 3 weeks old, when very few CD4+ T-cells were present in the mucosa, the bias of cytokine production was already towards IL-4 rather than IL-2 (Bailey *et al.* 1994*a*). It seems likely, therefore, that development occurs as a balance between effector and regulatory functions, and that intestinal integrity depends on maintaining this balance between certain safe limits (Fig. 3). A similar concept of balanced development of a mucosal immune system in young individuals has been proposed for the respiratory system, as a balance between Th1 and Th2 effector systems (Holt *et al.* 1997). However, it is difficult to see how the balance between a Th1 response and a Th2 response results in failure to develop damaging mucosal immune responses and, given the accumulating evidence for regulatory T-cells in the intestinal mucosa, a balance between effector and regulatory responses seems conceptually more useful. In the domestic pig, and perhaps the mouse, the phenotype of mucosal T-cells suggests that this balance is tilted towards

regulatory function, whereas in man effector function seems to predominate, at least in the developing countries where such studies have been done, and this factor might be relevant to the increasing incidence of atopic disease.

Within this model, the disturbances associated with early weaning in piglets would result from transient imbalance between effector and regulatory function (Fig. 4). There is clear evidence for an effect of abrupt weaning on the immune system of neonatal pigs. Within 24 h of withdrawal of maternal milk, expression of major histocompatibility complex class II and CD3 mRNA was increased and by 4 d after weaning the numbers of CD2+ cells in the intestinal mucosa was increased (Dreau *et al.* 1995; Vega-Lopez *et al.* 1995; McCracken *et al.* 1999). During the first week, the
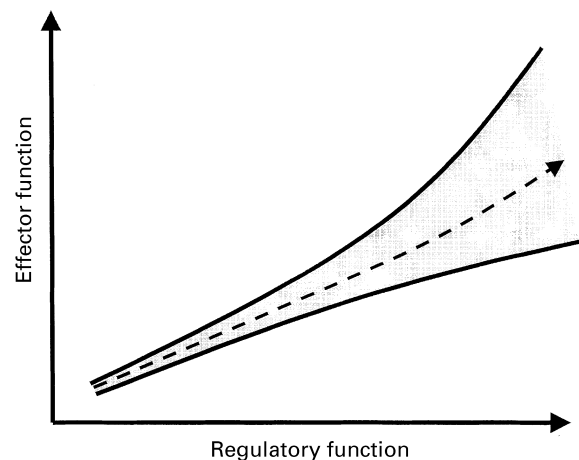
Fig. 3. Proposed model for the development of a mucosal immune system as a balance between regulatory and effector function. (▭), The limits of a 'safe' balance, within which the developing piglet (- →) must remain to maintain mucosal integrity.
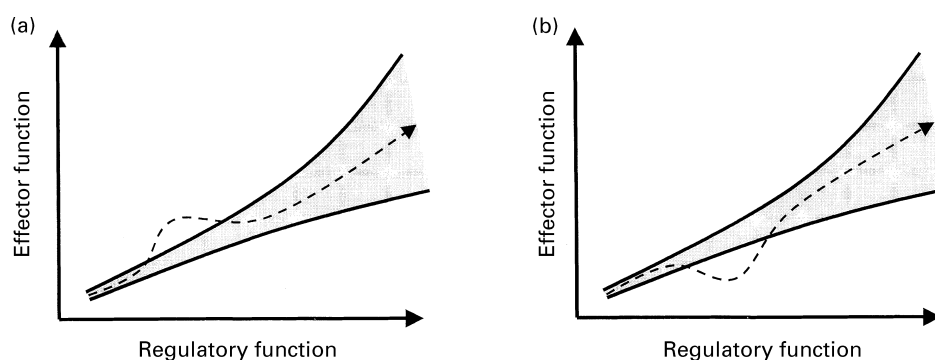
Fig. 4. Transient disturbance of the balance as a result of weaning. (▦), The limits of a 'safe' balance, within which the developing piglet (-➤) must remain to maintain mucosal integrity. (a), The developing piglet mounts inappropriate effector immune responses against 'harmless' antigens, resulting in transient allergic intestinal damage. (b), The piglet excessively regulates responses to potential pathogens, resulting in microbially-mediated damage.

ability of systemic T-cells to produce IL-2 in response to mitogens was decreased (Bailey *et al*. 1992; Wattrang *et al*. 1998). These effects may be a direct result of withdrawal of antibody or growth factors such as TGFβ in milk, of alterations in intestinal flora; or of physiological effects of environmental stress such as mixing in piglets or reduced maternal contact in man. The observed responses to soyabean antigens in young piglets and to cow's milk protein in human infants are consistent with the occurrence of excessive development or expression of effector function; excessive regulatory function is suggested by the overgrowth of largely non-pathogenic bacterial strains frequently associated with post-weaning diarrhoea.

In piglets the immunological and gastrointestinal disturbances associated with weaning are usually transient, and development of the mucosal immune system proceeds normally. However, it seems likely that under certain environmental or genetic conditions, transient disruption of the balance between mucosal effector and regulatory responses may become permanent. Depending on the imbalance, such animals or man would be permanently predisposed to development of allergies (excessive effector function) or to chronic infections (excessive regulatory function).

Conclusions

Maintenance of intestinal integrity and digestive and absorptive function is at least partly dependent on the ability of the mucosal immune system to discriminate between 'harmful' and 'harmless' antigens and mount appropriate responses (tolerance or active immunity). While one level of discrimination may take place within the organised lymphoid tissues during induction of an immune response, several lines of evidence suggest that regulation can occur after induction, implicating effector sites in discrimination. These effector sites are largely absent in the neonate, and develop with age and exposure to antigens. We propose that development occurs as a balance between effector and regulatory function, and that disturbances in this balance result in immediate disturbance of intestinal function, but may also, in some individuals, result in chronic allergic or infectious disease in later life.

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