LAYERS OF MUTUALISM WITH COMMENSAL BACTERIA PROTECT US FROM INTESTINAL INFLAMMATION

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Mammals are highly adapted to their very high densities of non-pathogenic bacteria which inhabit the lower small intestine and colon. This is achieved by a series of functional and cellular layers that normally avoid the inflammatory consequences of immune activation by bacterial molecules. In this review we set out the layers of mucosal immune defence to show the many ways in which mutualism with the commensal flora can break down to trigger chronic intestinal inflammation in animal models and inflammatory bowel disease (IBD) in humans.

SPECIAL PROBLEMS FACED BY INTESTINAL MUCOSAL IMMUNITY—LAYERS OF PROTECTION

The immune system of the intestine is phylogenetically ancient, and its development appears to have preceded most other lymphoid structures, including the thymus.¹ The intestinal mucosal immune system is also of immense size. Approximately 70% of all lymphocytes in the body are within the mucosal immune system and most of the antibody produced in healthy individuals is IgA, which is secreted across mucous membranes. Intestinal immunity must be finely balanced between the capacity for mounting protective immune responses to infectious agents and yet tolerate the enormous load of antigens and immunostimulatory molecules that constitute the commensal intestinal bacteria.

Commensal bacteria are present in vast numbers.² The colon is colonised with approximately 10^{12} organisms/g intestinal contents, making us walking culture media because the bacteria outnumber our own cells! The benefits of commensal bacteria are well known: they help us digest cellulose and salvage energy, synthesise vitamin K, and fill up the microbiological niche that might otherwise be exploited by less benign microbes. Of course, commensals can become pathogens in the right setting, such as debilitated or immunocompromised patients, but on the whole it is impressive how well we coexist with our microbial passengers.

Our bodies have adapted in many ways to achieve this harmonious mutualism with our commensals. The evidence for this comes from comparing animals that are kept in entirely sterile conditions (without an intestinal flora) with genetically identical animals kept in a clean animal unit (containing a simple intestinal flora but no pathogens³). The technology for germ free animal husbandry is cumbersome but the principles are straightforward. The initial animals must be delivered aseptically by Caesarean section and hand reared in a sterile environment but afterwards rodents can be interbred within their sterile plastic bubble and always given autoclaved food and water. There are many differences between germ free animals and those colonised with intestinal bacteria. The epithelial differentiation programme is altered, including the production of antibacterial molecules.^{3–6} Germ free mice have hardly any IgA producing plasma cells in the lamina propria,^{7–8} a reduced content of lamina propria CD4⁺ cells, reductions in some subclasses of intraepithelial lymphocytes (IEL),^{9–11} and hypoplastic Peyer's patches with few germinal centres.¹² Even the immune system outside the intestine is altered by increased levels of serum antibodies and expansion of the structures of the spleen and lymph nodes.^{13–16} All of the germ free alterations occur within a few weeks of introducing commensal bacteria to a previously germ free animal.

A major problem in understanding intestinal immunity is that the molecular composition of the commensal bacteria overlaps with bacterial pathogens. These microbial molecules trigger pattern recognition receptors, including Toll-like receptors (TLRs) and NODs, which are recognised as extremely bad immunological news by most of the immune system but they must be to some extent tolerated in the intestinal mucosa (reviewed by Sansonetti¹⁷), or the vigorous inflammatory responses will disturb the intestinal structure and function.

The evolutionary layered nature of the immune system is reflected in defence at the intestinal mucosal surface. After all, vertebrates have co-evolved with their intestinal bacteria. The paradox that comes from assuming that inappropriate responses to TLRs will always be inflammatory has even been turned on its head by a demonstration that TLR signalling is required for adaptation to

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intestinal bacteria.¹⁸ This may also be true for NOD2, which is necessary for the production of antibacterial defensin molecules by the epithelial Paneth cells, although this is one of many effects of the protein.¹⁹ In subsequent sections we will describe the different protective layers and the many ways in which inflammation can occur when they are defective. There are two lines of evidence that IBD results from loss of this mutualism that evolution with our commensal flora has endowed us. Firstly, almost all rodent models of intestinal inflammation are dependent on the presence of intestinal bacteria because they do not occur under germ free conditions (see table 2). Secondly, diversion of the faecal stream away from segments of active Crohn's disease by surgery is an effective treatment.²⁰ In this review we therefore examine the current status of the different layers of the intestinal mucosa and their relevance for protection from the luminal antigens, resulting in diverse ways in which the exquisite mutualism can be disrupted, culminating in intestinal pathology.

THE EPITHELIAL SURFACE

The intestinal epithelium is an important barrier that restricts the penetration of luminal antigens and microbes. Goblet cells secrete mucus composed primarily of complex mucin glycoproteins that form a viscous gel-like film at the epithelial surface. One of the adaptations to the presence of intestinal bacteria is an increase in the number of goblet cells in the large intestine.²¹ The mucins add to the physical barrier of the intestinal epithelium and prevent firm adhesion of luminal microbes to epithelial cells—this helps contain commensal bacteria in the gut lumen.

Intestinal epithelial cells also contribute to the innate host defence by secreting antimicrobial peptides called defensins and cathelicidins. These small (2–4 kDa) cationic peptides are produced by Paneth cells which work by disrupting the anionic bacterial cell membranes to limit bacterial growth in the crypts. Gram positive and Gram negative bacteria directly stimulate Paneth cells to secrete α defensins (also termed cryptidins) which are converted into potent microbicidal peptides after being processed by matrilysin, a metalloprotease (MMP-7) also produced by Paneth cells.^{22–23} The NOD2 protein is involved in defensin induction.¹⁹

These defensive features are only part of the way in which the epithelium responds to the presence of the commensal intestinal flora. It is possible to cut epithelial cells from histological sections using a laser driven by microscope software. The RNA from these “cut out” epithelial cells can be used to work out the levels of expression of thousands of different genes from probes immobilised on a chip. This makes it possible to compare gene expression in epithelial cells from mice that are germ free with animals colonised by the single anaerobe *Bacteroides thetaiotaomicron*. It is clear that this very simple flora causes significant changes in the epithelial gene expression profile of the small intestinal villous epithelium.^{3–6–24} For example, the gene “small proline-rich protein2a” (*sprp2a*), which appears to be involved in maintaining the barrier function of the villous epithelium, is upregulated more than 200-fold after *B. theta* colonisation.⁴

The intestinal paracellular permeability barrier is made up of highly adapted intestinal epithelial cells interconnected with tight junctions and overlain with mucus. This only permits very low numbers of bacteria to penetrate the intestinal epithelium. Secretion of bacteriocidal peptides, and probably IgA, reduce the numbers of bacteria that can

get close up to the epithelial surface. Yet there are large numbers of physical and pharmacological agents (for example, non-steroidal anti-inflammatory drugs and alcohol) that can damage the permeability barrier, so it is clear that the barrier is not perfect.²⁵ Moreover, when appropriately stimulated, epithelial intestinal epithelial cells can secrete a wide array of chemotactic mediators such as the neutrophil recruiting chemokines interleukin 8 (IL-8), CXCL8,^{26–27} and ENA-78 (CXCL5),^{28–29} and the CXCR3 ligands IP10 and MIG.^{30–32} Why then do commensal bacterial molecules not cause damage by engaging TLRs? Part of the answer is the low numbers of bacteria that can reach the epithelial layer but these cells also seem rather myopic (see the detailed discussion and references in Sansonetti¹⁷). For example, TLR4 may be expressed intracellularly rather than on the surface of intestinal epithelial cells. Other parts of the lipopolysaccharide (LPS) recognition machinery, including the proteins CD14 and MD2, are deficient in this cell type. TLR proteins may also be compartmentalised, for instance with expression of TLR5 on the basolateral surface of epithelial cells, avoiding responses to flagellin on luminal bacteria.

Primary defects in the epithelial barrier certainly can trigger chronic intestinal inflammation. For example, chimeric mice expressing a dominant negative mutant of N-cadherin will develop colitis as a consequence of the disruption of the adherens junctions for which this protein is responsible.³³ This demonstrates the primary importance of the intestinal barrier. Although there is no evidence that primary genetic defects in barrier proteins commonly underlie Crohn's disease or ulcerative colitis, agents that disrupt the permeability barrier can certainly lead to disease relapse.²⁵

INTESTINAL EPITHELIAL LYMPHOCYTES

Intestinal epithelial lymphocyte subsets

Very large numbers of differentiated T cells that are associated with the epithelial layer contribute to the immense size of the mucosal immune system. These IEL are thought to contribute to local immunosurveillance. Although intestinal IEL are the best studied, similar T cell populations exist in other epithelia, including the skin, urogenital tract, and respiratory tract. It has been estimated that in the human gut, there are 10–20 IEL per 100 enterocytes.³⁴

The character and function of IEL subsets differ from T cells in the systemic immune system. Almost all systemic T cells express the $\alpha\beta$ T cell receptor (TCR), whereas 5–15% IEL in the human small intestine and approximately 40% of colonic IEL express the alternative $\gamma\delta$ TCR, which is encoded by different genes and much more limited in its variability.^{35–36} The remaining IEL are mostly CD8 $\alpha\beta$ TCR T cells that express an $\alpha E\beta 7$ integrin which holds them in the epithelium bound to the E-cadherin epithelial junctional molecule. Whereas almost all CD8 T cells in the immune system away from mucosal surfaces express the $\alpha\beta$ TCR and have a CD8 molecule composed of different α and β chains (CD8 $\alpha\beta$), the IEL are more complex. About half the IEL population have CD8 molecules composed of a single α chain dimer (CD8 $\alpha\alpha$), and these may express $\alpha\beta$ TCR or $\gamma\delta$ TCR. There are smaller intraepithelial populations of CD4⁺, CD4⁺CD8⁺, and CD4⁺CD8[−] IEL.³⁷

There are important differences between CD8 $\alpha\beta$ and CD8 $\alpha\alpha$ IEL. CD8 $\alpha\alpha$ cells appear more primitive³⁸ with very limited variability, even of $\alpha\beta$ TCR expression; they do not recirculate in the body and, unlike CD8 $\alpha\beta$, they do not depend on MHC class I restricted antigen recognition in the

thymus for positive selection.^{39–42} CD8 $\alpha\beta$ IEL are more conventional, and they appear similar to CD8 cells in the lamina propria and thoracic duct, suggesting that they can recirculate.⁴³

Intestinal epithelial lymphocyte function

Sadly, our knowledge of IEL function is in almost inverse proportion to their frequency. Away from mucosal sites, CD8⁺ lymphocytes function primarily as cytotoxic cells—for example, with the capacity to kill cells that have become infected with virus. Cytotoxic activity of CD8 intraepithelial T cells can be detected in some reports^{44–45} but not others.^{46–47} As CD4 and CD8 TCR $\alpha\beta$ T cells that are activated in non-mucosal lymph nodes migrate to the intestinal wall and transiently accumulate in the intraepithelial compartment, the observed differences in IEL cytotoxicity may be caused by differences in the activation of the immune system as a whole.^{48–50} For example, during a systemic viral infection, T cells with potent cytotoxic activity reside transiently in the IEL compartment but in the absence of their cognate MHC restricted antigen, cytotoxic effects are not apparent in the epithelium.

In areas of active IBD, patients with both ulcerative colitis and Crohn's disease have elevated frequencies of cytotoxic T cells in the lamina propria and epithelium.⁴⁷ However, in experimental mouse models, intestinal inflammation can also be induced when cytotoxic T cells are absent—for example, following transfer of CD4 T cell subsets into lymphopenic recipients.^{51–52} This suggests that the enhanced cytotoxic activity of T cells in the epithelium and lamina propria of IBD patients contributes to the disease but is not an essential disease inducing factor.

In contrast with CD8 $\alpha\beta$ TCR $\alpha\beta$ IEL T cells, mouse CD8 $\alpha\alpha$ TCR $\alpha\beta$ IEL—even after potent antigen specific activation *in vivo*—remain non-cytotoxic and even appear to express transforming growth factor β (TGF- β), a cytokine with known anti-inflammatory properties.⁵³ Experimental transfer of a polyclonal population of CD8 $\alpha\alpha$ TCR $\alpha\beta$ IEL before giving colitogenic CD4 CD45RB^{hi} T cells to SCID recipients successfully prevented the subsequent induction of intestinal inflammation in this standard mouse IBD model.⁵⁴ In humans there is also evidence that the CD8 T cell subset (CD28[−] CD101⁺ CD103⁺), which recognises the gp180 epithelial cell derived antigen, can exert regulatory functions *in vitro*.⁵⁵

EFFECTOR CELLS OF THE INNATE IMMUNE SYSTEM IN THE SUBEPITHELIAL LAMINA PROPRIA

Despite the elaborate mechanisms aimed at preserving an intact intestinal epithelial layer, it is only a very thin layer separating the large bacterial antigenic load present in the intestinal lumen from immune effector cells in the intestinal lamina propria. This means that the immune responses of the lamina propria must be tightly regulated and coordinated. We will discuss three cellular components of the innate immune system of the intestine: macrophages,⁵⁶ dendritic cells,⁵⁷ and myofibroblasts⁵⁸ that are vital for tissue homeostasis.

Intestinal macrophages

Macrophages are strategically located in the subepithelial area of the lamina propria and can efficiently phagocytose luminal microorganisms that have crossed the epithelial barrier. Despite the proximity of intestinal luminal bacteria and bacterial LPS to these subepithelial macrophages, only

Table 1 List of Toll-like receptors and their ligands (after Backhed and colleagues⁵⁹)

Toll-like receptor	Ligand	Source
TLR1	Triacyl lipopeptides	Bacteria and mycobacteria
TLR2	Lipoprotein Peptidoglycan Lipoteichoic acid	Bacteria Gram positive bacteria Gram negative bacteria
TLR3	Lipoarabinomannan	Mycobacteria
TLR4	Double stranded RNA Lipopolysaccharide Taxol	Viruses Gram negative bacteria Plants
TLR5	Heat shock proteins	Host protein
TLR6	Flagellin Diacyl lipopeptides Lipoteichoic acid	Bacteria Mycoplasmata Gram positive bacteria
TLR7	Zymosan	Fungi
TLR8	Single stranded RNA	Viruses
TLR9	Single stranded RNA	Viruses
TLR10	CpG containing DNA	Bacteria and viruses
TLR11	Unknown	Unknown
	Unknown	Uropathogenic bacteria

low levels of inflammatory reactions are generally observed.⁵⁹ This may be because macrophages are specially adapted, for instance with reduced expression of receptor molecules for LPS (CD14), IgA (CD89),⁶⁰ TLR4 and TLR2 (table 1).⁶¹ TREM-1 is another molecule shown to amplify proinflammatory reactions in myeloid cells⁶² which is absent on most intestinal macrophages.⁶³

The importance of macrophages in maintaining local tissue homeostasis in the intestine is illustrated by the spontaneous occurrence of colitis in mice with selective disruption of Stat3 signalling in macrophages and neutrophils but not in dendritic cells or lymphocytes. In this case, Th₁ T cell mediated colitis is probably the consequence of severely impaired macrophage production of anti-inflammatory IL-10 because of the Stat3 signalling deficiency.⁶⁴

Importance of NOD2/CARD15

As NOD2/CARD15 is a susceptibility locus for Crohn's disease^{65–67} and is expressed particularly in cells of the macrophage/monocyte lineage, dendritic cells, and (especially during inflammation) in epithelial cells,⁶⁸ it is important to understand the consequences of mutated NOD2/CARD15 on intestinal immune reactions. NOD2/CARD15 is a cytoplasmic protein that interacts with muramyl dipeptides (MDP) of peptidoglycans derived from both Gram positive and Gram negative bacteria. NOD2/CARD15 can therefore be considered an intracellular pathogen recognition receptor that senses all bacteria except mycobacteria and chlamydia.^{69–71}

Intracellular triggering of NOD2/CARD15 leads to activation of the coordinator of immune responses nuclear factor κ B (NF κ B). Most NOD2/CARD15 mutants found in patients with Crohn's disease D fail to activate NF κ B in transient transfection experiments,^{69–70} leading to the apparent paradox that absence of NOD2/CARD15 mediated NF κ B activation may trigger the massive inflammatory tissue destruction seen in patients with active Crohn's disease. It is possible that deficient NOD2/CARD15 mediated NF κ B activation leads to impaired control of translocated bacteria in the lamina propria by resident macrophages, and increased transport of bacteria and bacteria laden dendritic cells to the draining lymph nodes where a more potent immune response is

initiated. There is no experimental evidence for this (yet). In fact, there is evidence that the effect of NOD2 mutation may be more complex: the normal readout for NOD2/CARD15 in experiments has been *activation* of NFκB by intracellular MDPs, but another effect of NOD2/CARD 15 is that it can *downregulate* activation of NFκB by ligands for TLR2 at the cell surface.⁷² TLR2 is a receptor for peptidoglycan (table 1), of which a MDP polymer forms the backbone. The intracellular pathways for this downregulatory effect are not known in detail but deficient attenuation of TLR2 signalling in NOD2/CARD15 mutant macrophages can enhance IL-12 production and lead to excessive Th₁ CD4 T cell responses at sites of bacterial infection.

Another group has generated mice that carry a NOD2 gene containing the common human 3020insC Crohn's susceptibility mutation and found a different phenotype. These mice have elevated NFκB activation in response to MDP, with increased processing and secretion of the proinflammatory cytokine IL-1β,⁷³ although this was not as a result of defective regulation of TLR2 signalling in this strain. The 3020insC knockin mice were also more susceptible to induction of colitis with dextran sodium sulphate.

Thus at present we are left with different mouse model experimental systems giving different results for the phenotype for NOD2 mutation (loss of function, gain of function, and loss of negative regulation). To complicate matters still further, mice that carry the human NOD2/CARD15 mutation are much less susceptible to challenge with LPS (a ligand for the TLR4 receptor) *in vivo*, showing that this molecule has other poorly characterised effects on innate immune activation.⁷⁴

Although the presence of functional mutations in the NOD2/CARD15 locus on both alleles increases the risk of

developing human Crohn's disease more than 30-fold,^{75–76} it is clear that homozygous mutations of the NOD2/CARD15 gene locus are insufficient to trigger ileal Crohn's disease alone in patients. Similarly, mice with an inactivated NOD2/CARD15 gene do not spontaneously develop chronic intestinal inflammation.⁷⁴ It remains to be determined how different genetic backgrounds or differences in microbial colonisation of the gut may affect intestinal macrophage and epithelial cell functions in NOD2 deficient mice.

There is abundant clinical and experimental evidence that commensal intestinal bacteria drive intestinal immunopathology both in patients with Crohn's disease and in animal models of chronic intestinal inflammation. The presence of activated Th₁ CD4 cells in patients with Crohn's disease⁷⁷ and the many animal models in which intestinal inflammation is triggered by conventional CD4 cells (table 2) attests to the importance of adaptive immunity in the breakdown of mutualism between host and commensal intestinal bacteria. What is known about NOD2/CARD15 makes it highly likely that these changes are triggered by abnormal handling of whole bacteria or their constituent molecules that penetrate the intestinal epithelium through innate immune pathways. We do not yet know the cell type involved (although the macrophage is a very promising candidate) nor whether induction of adaptive immune responses takes place locally in the mucosa or more distantly in the non-mucosal immune system where activated lymphocytes later home to mucosal sites.

Intestinal dendritic cells

Intestinal dendritic cells are found both in Peyer's patches where they gain access to the particulate antigens transported through the specialised M cells, and dispersed

Table 2 Mouse models of intestinal inflammation: main molecular defects and influence of intestinal colonisation on incidence and severity of disease

Molecular defect	Examples	Extent of intestinal inflammation*			Reference
		Colonised GI tract	Antibiotic treatment	Germ free	
Defective mucus layer Impaired epithelial integrity	Trefoil factor deficiency	+/-†	?	?	Mashimo ¹¹⁷
	<i>Mdr1a</i> deficient mice	‡	?	?	Panwala, ¹¹⁸ Maggio-Price ¹¹⁹
Aberrant antigen presenting cell activities/activation	DN N-cadherin mutant	+	?	?	Hermiston ³³
	DSS administration	+	?	+	Dieleman, ⁸⁹ Kitajima ¹¹⁰
	TNBS administration	+	+/-	?	Neurath, ¹¹² Fiorucci ¹²⁰
	Stat3 deficient macrophages and neutrophils	+	?	?	Takeda ⁴⁴
	Stat4 tg mice	+	?	?	Wirtz ¹²¹
Impaired enteric motility Aberrant cytokine expression/regulation	Ablation of enteric glia cells	+	+	?	Bush ¹²²
	TNF ΔARE mice (TNF over production)	+	?	?	Kontoyannis ¹²³
	TGF-β RII DN tg mice	+/-	?	?	Hahn ¹²⁴
	IL-10 deficiency	+	+/-	?	Madsen ¹²⁵
	IL-2 deficiency	+	?	+/-	Sadlack, ¹²⁶ Schultz ¹²⁷
Aberrant T cell differentiation/regulation	NFκB p50 deficiency	+	?	?	Erdman ¹²⁸
	TCRα chain deficient	+	?	0	Mombaerts ⁹²
	Tg 26e mice (human CD3e tg)	+	?	0	Veltkamp ¹²⁹
	IL-7 Tg mice	+	?	?	Watanabe ¹³⁰
	CD4 CD45RB ^{hi} T cell transfer into lymphopenic host	+	+/-	0	Powrie, ⁵¹ Morrissey, ⁵² Matsuda ¹³¹
	Gi2α deficient mice	+	?	?	Rudolph ¹³²
	Oxazolone administration	+	?	?	Boirivant ¹¹¹
Genetic models with undefined genetic alterations	WASP deficiency	+	?	?	Snapper ¹³³
	C3H/HeJBir mice	+	?	?	Sundberg ¹¹⁶
	Samp1/Yit mice	+	0	0	Kosiewicz, ¹¹⁴ Bamias ¹³⁴

*+, severe intestinal inflammation; +/-, attenuated, mild, or focal intestinal inflammation; 0, no signs of intestinal inflammation.

†Additional stimuli such as administration of dextran sulphate required for colitis induction.

‡Infection with *Helicobacter hepaticus* protects whereas infection with *H. bilis* exacerbates colitis.

GI, gastrointestinal; DSS, dextran sodium sulphate; TNBS, trinitrobenzene sulphonic acid; TNF-α, tumour necrosis factor α; TGF-β, transforming growth factor β; IL, interleukin; NFκB, nuclear factor κB; TCR, T cell receptor.

throughout the entire gastrointestinal tract where they have been reported to modulate junctional protein expression in epithelial cells to allow their dendrites to poke through to the apical side of the epithelium and sample bacteria from the gut lumen.⁷⁸ This mechanism seems to be most important in the ileum, at least in mice.

Under physiological conditions, lamina propria dendritic cells also phagocytose apoptotic cells, including epithelial cells, and subsequently migrate to the draining lymph node where they are believed to induce tolerance against gut antigens derived from apoptotic cells.⁷⁹ The distribution of the phenotypically and functionally distinct dendritic cell subsets is not random in the gastrointestinal tract. For example, in Peyer's patches of mice, CD11b⁺ CD8 α [−] dendritic cells are exclusively located in the subepithelial dome region while CD11b[−] CD8⁺ dendritic cell subsets are preferentially found in extrafollicular areas.⁸⁰ Intriguingly, only the dendritic cell subset found in the dome region appears to produce high levels of IL-10 on activation with bacteria and prime naïve CD4 cells to become potent IL-10 producing regulatory T cells.⁸¹ For a more detailed discussion of the biology of intestinal dendritic cells the recent review article by Bilsborough and Viney is recommended.⁵⁷

Dendritic cell subsets are also distributed selectively along the length of the intestinal lamina propria. Ileal subepithelial dendritic cells of mice preferentially express the p40 subunit of IL-12, which appears to pair with p19 to form the IL-23 cytokine.⁸² These dendritic cells also contain endocytosed luminal bacteria, which may trigger p40 production at this location, as the IL-12 p40 gene is not induced in the intestine of germ free mice. We do not yet know whether these results in mice can be generalised to humans but IL-23 producing dendritic cells may contribute to the susceptibility of the terminal ileum to activated Th-1 driven Crohn's disease.

The molecular nature of the commensal bacteria associated trigger of IL-12 p40 expression in ileal dendritic cells still needs to be defined. Among the many candidate genes are also bacterial flagellins which bind to TLR5. TLR5 is also present on dendritic cells and macrophages. In support of flagellins as a bacterial trigger, a recent study demonstrated the presence of anti-flagellin antibodies in colitis prone mouse strains and in the serum of some patients with Crohn's disease but not those with ulcerative colitis or healthy controls.⁸³

In contrast with these potentially immunopathological effects arising from the sampling of intestinal bacteria by intestinal dendritic cells, small numbers of commensal bacteria can be sampled by dendritic cells in the mouse Peyer's patches to induce a protective response.⁸⁴ These dendritic cells are relatively poor at killing the very small numbers of commensal bacteria that penetrate the overlying epithelium, yet they interact with B and T cells in Peyer's patches to induce IgA⁺ B cells. Following induction, B cells recirculate through the lymph and blood to home back to the intestinal lamina propria as plasma cells. The IgA that they secrete is transported across the epithelial cell layer and binds to commensal bacteria which in turn limits bacterial penetration through the epithelial cell layer as a protective negative feedback mechanism. In contrast with lymphoblasts that recirculate, dendritic cells laden with bacteria cannot penetrate further than the local mesenteric lymph nodes so induction of mucosal immune responses is largely restricted to the mucosal immune system.⁸⁴

Myofibroblasts

Myofibroblasts have also recently emerged as potential players in mucosal innate immunity. Because they express a wide variety of molecular recognition receptors, including TLR1–9, they may respond to luminal microbial antigens that have penetrated the epithelial cell layer by producing proinflammatory mediators such as IL-1, tumour necrosis factor (TNF), and IL-8.⁸⁸ Myofibroblasts also affect local tissue homeostasis by production of cyclooxygenase 1 and 2 metabolites and by TGF- β mediated regulation of epithelial integrity.⁸⁵

Lamina propria CD4 T cells

As described earlier in this review, findings in spontaneous mouse models of colitis and observations in patients with IBD show that intestinal inflammation occurs as a consequence of a dysregulated immune response to normal constituents of the intestinal flora in a genetically predisposed host. Given the increasing number of mutant mouse strains that spontaneously develop a progressive chronic intestinal inflammation (mainly colitis) when colonised with intestinal bacteria, the underlying causes of such a dysbalanced immune response in patients with IBD are likely to be highly diverse (for reviews on animal models of colitis, see Bhan and colleagues,⁸⁶ Mizoguchi and colleagues,⁸⁷ and Bouma and Strober⁸⁸).

In most experimental animal models of colitis induction the onset of disease depends on the presence of T cells although colitis triggered by dextran sodium sulphate administration can be seen in T lymphocyte deficient animals.⁸⁹ In most of these T cell mediated models of colitis, there is evidence that CD4 T cells are responsible. While CD4 T cells are rare in the IEL compartment, they represent the predominant T cell subset in the intestinal lamina propria of both mice and men.

When naïve CD4 T cells are activated in an antigen specific manner, they are functionally pluripotent and secrete a spectrum of cytokines, such as IL-4, to support a potent humoral immune response, and interferon γ (IFN- γ), which is instrumental for an efficient cell mediated immune response. Following repeated activation and cell division, CD4 T cells may become functionally committed effector T cells, by differentiating either into predominant IFN- γ secreting Th₁ CD4 T cells or into IL-4 secreting Th₂ CD4 T cells.⁹⁰ This polarisation is determined by specific transcription factors, T-bet or GATA-3, which become silenced under the influence of cytokines, such as IL-4 or IL-12/IFN- γ , respectively. In turn, the selective production of one of these master transcription factors regulates production of a restricted repertoire of cytokines (T-bet $\rightarrow$ Th₁; GATA-3 $\rightarrow$ Th₂).⁹¹ Intestinal inflammation is not exclusively the result of deviation to a Th₁ or Th₂ phenotype because preferential differentiation of reactive intestinal CD4 T cells towards Th₁ CD4 T cells (for example, the CD4 T cell transfer model of colitis induction in immunodeficient mice) or towards Th₂ T cells (for example, TCR α deficient mice⁹²) can both lead to fulminant colitis. Numerous reports have attempted to identify a dichotomy in patients with IBD. Most experimental data support the notion that Crohn's disease is predominantly a Th₁ T cell mediated disorder⁷⁷ whereas the categorisation for ulcerative colitis is less clear. It should be noted however that in most instances these observations have been made in patients with established disease, and that the disease initiating T cells may functionally differ substantially from those found later in intestinal inflammation.

In contrast with most T cell mediated colitis models where disease progression is often associated with selective expansion of distinct CD4 T cell clones, T cell proliferation in the normal intestinal mucosa appears to be tightly regulated and T cells from normal intestinal mucosa display highly reduced proliferative activities on TCR engagement.⁹³ The attenuated CD4 T cell expansion and protection from colitis induction that is observed on co-transfer of CD4 CD45RB^{lo} T cells together with the colitogenic CD4 CD45RB^{hi} T cells in lymphopenic recipient mice^{51, 52} indicated that CD4 T cells with regulatory activities were present among the CD4 CD45RB^{lo} T cell subsets. Most of the immunomodulatory effects of regulatory T cells in the intestinal mucosa have been ascribed to secretion of IL-10 and TGF- β , although other mechanisms, including cognate interactions with antigen presenting cells (for example, CTLA-4-CD80/86 interactions⁹⁴ or LAG-3-MHC class II interactions⁹⁵) may also contribute to overall immunoregulatory activities. Hence these findings imply that lamina propria CD4 T cells normally recognise antigens derived from luminal commensal bacteria and presented by resident antigen presenting cells but that their functional and proliferative activity is tightly controlled by local secretion of IL-10 and TGF- β . This was demonstrated in cocultures where human CD4 T cells isolated from normal intestinal mucosa downregulated the proliferative activity of peripheral blood T cells when autologous intestinal antigen primed APCs were added.⁹⁶ This proliferation attenuating effect was mediated by IL-10 and TGF- β because neutralisation of these cytokines restored proliferation of peripheral blood CD4 cells.⁹⁶ A recent report ascribed most of this regulatory activity to the CD4 CD25^{bright} T cell subset present in the colonic lamina propria. Similar to their counterpart in the mouse, these CD4 CD25^{bright} T cells express the transcription factor FoxP3⁹⁷ and the TNF receptor family member GITR.⁹⁸

The consequences of impaired activity of resident regulatory cells is illustrated by the spontaneous development of progressive intestinal inflammation in mice deficient in IL-10⁹⁹ or TGF- β ,¹⁰⁰ or after adoptive transfer of colitogenic CD4 CD45RB^{hi} T cells without the CD4 CD45RB^{lo} regulatory subset into immunodeficient mice.^{51, 52} Without counter-regulatory T cells, transferred CD45RB^{hi} T cells expand rapidly in draining lymph nodes and colonic mucosa in response to antigens derived from luminal bacteria.¹⁰¹ Transferred colitogenic CD4 T cells subsequently induce excessive activation of colonic macrophages and production of TNF.¹⁰² Intriguingly, the human CD4 CD25^{bright} regulatory T cells are present and functional in the normal colonic mucosa, and in patients with ulcerative colitis and colonic Crohn's disease. It is possible that altered proportions of regulatory and effector CD4 cells in the intestinal lamina propria may offer an explanation for the relapsing-remitting course of clinical IBD which is a milder version of the fulminant colitis seen experimentally in those mouse strains where regulatory T cells are absent or functionally impaired. The plasticity of cell subtypes and their possible function in controlling the disease still needs to be shown in humans, and the natural relapsing remitting course of IBD still needs to be accurately modelled in experimental animals.

TRAFFICKING OF IMMUNE CELLS TO THE INTESTINAL MUCOSA

While cellular layers and functional diversity of the intestinal adaptive and innate immune systems may already appear

highly complex, the cellular composition and distribution of immune cells within the intestinal mucosa is dynamic. For example, T cells expressing the $\alpha 4\beta 7$ integrin are recruited to the intestinal mucosa by $\alpha 4\beta 7$ binding to the mucosal vascular addressin MAdCAM-1, expressed on high endothelial venules of mucosal lymphoid structures and on lamina propria venules.^{103–105} T cells that are activated by antigen laden dendritic cells in mesenteric lymph nodes (MLN) will preferentially express gut homing receptors ($\alpha 4\beta 7$, CCR9). The molecular basis of this differential imprinting of T cells by MLN dendritic cells appears to be—at least in part—local production of retinoic acid (a vitamin A metabolite) by dendritic cells of the MLN and possibly also the Peyer's patches. Subnanomolar concentrations of retinoic acid strongly upregulate $\alpha 4\beta 7$ integrin and chemokine receptor CCR9 expression.¹⁰⁶

Under the influence of TGF- β , some $\alpha 4\beta 7$ expressing T cells may switch to $\alpha E\beta 7$ integrin expression (CD103). This interacts with E-cadherin expressed on intestinal epithelial cells, and the binding may be critical for retaining intra-epithelial T cells within the intestinal epithelium.¹⁰⁷ CCR10, a chemokine receptor which is rarely expressed on mucosal T cells, directs IgA producing plasma cells within mucosal compartments in response to local production of its ligand, the chemokine MEC/CCL28.¹⁰⁸

These recent findings on the mechanisms that control migration of intestinal cell populations are important for our understanding of the regulation of intestinal immune functions. Nevertheless, several relevant aspects remain unclear. These include the mechanisms that regulate the homing of T cells to the colonic, rather than small intestinal, mucosa where, in contrast with small intestinal T cells, most T cells lack CCR9 expression. The mechanisms that are responsible for guiding macrophage and dendritic cell subsets within the intestinal mucosa under either physiological or pathological conditions are also poorly characterised.

MOUSE MODELS OF IBD

The importance of a finely tuned balanced immune response for maintaining intestinal tissue homeostasis was only fully appreciated when spontaneous onset of intestinal inflammation was observed in genetically modified mouse strains with deficiencies in molecular components of the innate or the adaptive immune system. Disturbance of the local immune system by adoptive transfer of T cell subsets lacking regulatory T cells into lymphopenic recipients,^{51, 52} or administration of agents that damage epithelial permeability¹⁰⁹ probably leads to penetration of luminal (bacterial) antigens which activate phagocytic cells and T cells, resulting in an imbalanced local immune response and severe intestinal inflammation. Classical examples of this latter type of murine colitis include administration of dextran sodium sulphate in the drinking water¹¹⁰ or of haptening agents such as oxazolone¹¹¹ or trinitrobenzene sulphonc acid.¹¹²

Several principles can be derived from these numerous animal models of intestinal inflammation.¹⁰⁹ In general, intestinal inflammation may be ascribed to excessive activity of components of the innate or adaptive immune system, such as overproduction of TNF- α such as in Δ ARE TNF mice due to the increased stability of TNF- α mRNA. Alternatively, impaired regulatory activity of the local immune system in the intestinal mucosa can be due experimentally to the complete absence of T cell subsets with regulatory properties (for example, following transfer of CD4 CD45RB^{hi} T cells into

lymphopenic recipients) or in the absence of molecules essential for generating regulatory cells, or regulatory cytokines (for example, IL-10, TGF- β).

These numerous mouse models show that many types of immune imbalance can lead to aberrant immune responses against mucosal antigens with subsequent inflammation in the gastrointestinal tract. These diverse primary defects which result in a final common pathway of inflammation are almost certainly different from the defects in human IBD but nevertheless the broad groupings of Crohn's disease and ulcerative colitis will probably also be reclassified in time into separate conditions depending on the distinct underlying causes. In the majority of animal models of colitis, T cell responses are eventually skewed towards a Th₁-like phenotype, characterised by enhanced production by macrophages and dendritic cells of IL-12, TNF- α , and IFN- γ by T cells. In some of these Th₁ dominated mouse models of intestinal inflammation, histologically granulomatous inflammatory reactions are seen, similar to patients with Crohn's disease. This is particularly true in the SAMPl/Yit mouse that spontaneously develops an ileitis reminiscent of Crohn's disease.^{113 114}

Information obtained with these animal models of intestinal inflammation is relevant for our understanding of the aetiopathogenesis of IBD in humans. In particular, the influence of the composition of the intestinal flora and the genetic background on incidence and severity of disease became very obvious in most experimental models of disease. For example, rectal administration of TNBS in ethanol conditioned SJL mice, but not C57BL/6 mice, leads to the rapid induction of a severe colitis, and in general a more rapid onset of colitis is observed in mice maintained under conventional or specific pathogen free conditions compared with the same strain kept germ free (table 2). Excessive reactivity of the adaptive immune system against luminal antigens has also been defined as the driving force of intestinal inflammation in several models of colitis, including a T cell transfer model of colitis¹¹⁵ and the spontaneous mouse model of colitis, the C3H/HeJBir mouse.¹¹⁶

The diversity of mouse models of intestinal inflammation thus reflects the situation in patients with IBD where NOD2/CARD15 is one of many susceptibility loci. The diversity and complexity of Crohn's disease as a cluster of intestinal inflammatory disorders, possibly leading to comparable histopathological alterations and clinical signs of disease, remains to be demonstrated. Hence the question on the most relevant animal model that best reflects human IBD is probably obsolete as a single mouse model is very unlikely to cover the whole spectrum of human diseases. However, spontaneous models of intestinal inflammation such as the C3H/HeJBir mouse,¹¹⁶ a mouse line derived from the TLR4 deficient C3H/HeJ mouse, and the SAMP/Yit mouse, may respectively reflect human ulcerative colitis or Crohn's disease better than chemically induced or genetic models of colitis. The chemical models nevertheless allow us to study the early steps in the initiation of a local inflammatory reaction. In most of these models the disease progresses in a predictable and reproducible manner. Hence they are still very useful to assess the potential of therapeutic regimens in the treatment of inflammatory disorders. The challenge of understanding the disrupted mutualism between host and environment, especially the intestinal bacterial flora, remains, and we still need integrate genetic factors and

environmental factors, including the composition of the intestinal flora, in unravelling the mechanisms for the disease and its course. The problem is that in trying to work on disrupted mutualism between host and environment, we have to do experiments in vivo to work out the interactions between two complex biological supersystems: the intestinal mucosa and the consortia of the luminal bacterial flora. If we can understand the immunopathogenesis properly in animal models, we should be able to work out the relevant defects in our patients from genetic data and correlation in human disease and finally be able to treat them effectively enough to alter the natural history of their nasty illnesses.

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