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INNATE AND SPECIFIC MUCOSAL IMMUNITY

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SUMMARY

A large mucosal immune system controlled by unique, organ-specific regulations operates alongside and separate from the peripheral or systemic immune system. This local immune system is seen as a major interface between the innate and the specific immune system. We have been interested in the immunopathogenesis of a chronic inflammatory bowel disease (IBD) developing in $CD4^+$ T cell-transplanted, immunodeficient (SCID) mice. Microbial-derived factors drive (directly or indirectly) the activation, expansion and/or Th1 differentiation of adoptively transferred TCR$\alpha\beta$ $CD4^+$ T cells in the colonic lamina propria of transplanted, diseased SCID mice with colitis (the particular manifestation of IBD observed in this model). A TCR-independent, polyclonal stimulus seems to be the major stimulus for this cellular immune response in the colon. This suggests that T cells of the specific peripheral immune system that migrate into compartments of the mucosal micro-environment change their activation requirements and acquire responsiveness to stimuli that usually drive the innate immune system.

INTRODUCTION

The mucosal surface area of the airways and the intestine is more than two orders of magnitude larger than the surface area of the skin comprising an estimated 400 m^2. It is continuously and extensively exposed to antigens derived from either (resident or invading) microorganisms, or ingested or inhaled foreign material. This antigenic challenge operates in an environment in which bacterial products with potent adjuvant activity are continuously and abundantly present. Furthermore, the mucosal immune system is the portal of entry of most pathogenic micro-organisms that makes it the focus of interest of research on infectious disease immunity and vaccine designs.

Abbreviations used:
IBD: inflammatory bowel disease; SCID: severe combined immunodeficiency; IEL: intraepithelial lymphocyte; LPL: lamina propria lymphocyte; mLN: mesenteric lymph node; NK: natural killer; CTL: cytotoxic T lymphocyte; Th: T helper cell; PP: Peyer's patch; GALT: gut-associated lymphoid tissue; CP: cryptopatch; IEC: intestinal epithelial cell; APC: antigen-presenting cell; DC: dendritic cell; UC: ulcerative colitis; CD: Crohn's disease; TNBS: tri-nitro-benzene-sulfonic acid; TNP: tri-nitro-phenyl-; SPF: standard pathogen-free; GF: germ-free; AICD: activation-induced cell death; R: receptor; IL: interleukin; LT: lymphotoxin; f: formylated; pIgA: polymeric IgA; PSGL-1: P selectin-glycoprotein ligand-1; KO: knock-out; TCR: T cell receptor for antigen; FCM: flow cytometry; ELISA: enzyme-linked immunoabsorbant assay; Tr1: regulatory T cell subset 1; IFNγ: interferon-γ; TNFα: tumor necrosis factor-α.

To cope with this massive and continuous challenge with potent antigens and adjuvants of a very heterogeneous nature, the mucosa has evolved a large organ-specific immune system. This finely regulated mucosal immune system exists alongside and separate from the peripheral or systemic immune system. It is very large containing about 80% of all T cells of the organism. Mucosal immunity is made up of heterogeneous components that mediate innate and adaptive (specific), humoral and cellular immune reactivities. Priming this system results in local immunity, in generalised immunity on many different mucous surfaces, in systemic immunity, and in immunity in solid organs (as e.g. liver and pancreas). In addition to inducing immunity, a predominant mode of reaction of the mucosal immune system is the induction of tolerance.

Although the mucosal immune system is highly integrated and finely regulated, dysregulation of the induction and regulation of mucosal responses are not unusual. The system can be either deficient to react, or prone to overreact. Many pathogens overcome the mucosal immune barrier and establish themselves transiently or permanently in the host. Furthermore, mucosal tissues are very susceptible to damage by dysregulated immune responses resulting in chronic inflammatory processes.

Hence, we are looking at a defence system that is continuously challenged by a high load of antigens and adjuvants, that has evolved many (humoral and cellular) components to react, but is prone to dysregulation of its responsiveness.

FEATURES OF THE MUCOSAL IMMUNE SYSTEM

The mucosal immune system displays many unique features. Only some general characteristics are mentioned below.

Lymphopoietic, inductive and effector compartments co-exist within the mucosa

Mucosa-associated lymphopoiesis is evident in clusters of about 10^3 cells in the lamina propria of the small intestine situated just under the crypt epithelium and designated cryptopatches (CP) (*Hurst* et al., 1997; *Saito* et al., 1998; *Page* et al., 1998; *Howie* et al., 1998). These areas contain progenitor cells for T cells of the TCR $\alpha\beta$ and $\gamma\delta$ lineage (but not B cells or myeloid cells) with the phenotype $ckit^+$ Lin^- $IL\text{-}7R^+$ $Thy\text{-}1^+$ $CD44^+$ $RAG\text{-}1/2^+$ that differentiate under the influence of IL-7 and/or oncostatin M (*Rich* and *Leder*, 1995; *Clegg* et al., 1996; *Laky* et al., 1998). Many aspects of this extrathymic T cell developmental pathway are unresolved. It is for example unclear if lymphoid cells (and dendritic cells, DC) of the innate as well as the adaptive mucosal immune system originate at these sites.

Most specific, mucosal immune responses are induced in organised (structured and localised) structures of the small intestine gut-associated lymphoid tissue (GALT) called Peyer's patches (PP). Specific responses primed locally may mature locally in PP, or in regional mesenteric lymph nodes (mLN). Besides these major inductive compartments, innate or specific immune responses may be primed in the epithelial or lamina propria compartment of the mucosa. In these two diffuse compartments, effector cells, such as B- and T-lymphocytes, differentiated plasma cells, macrophages, and other antigen-presenting cells (APC's), as well as eosinophils, basophils, and especially mast cells are predominantly found.

The mucosal tissue represents an important interface between the innate and the adaptive immune system

A large array of different lymphoid cell subsets that are considered part of the innate or the specific (adaptive) immune system are found in the collections of effector cells of the diffuse GALT, i.e. the epithelium and the lamina propria (Table 1). Very little is known about the interaction of these subsets.

Lymphoid and myeloid cell populations in different compartments of the mucosal tissues are strikingly heterogeneous

Intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) of the gut are very heterogeneous in surface phenotype and function. To illustrate this point, Table 2 lists some of the subsets found in the murine small intestine IEL population. It is uncertain to what extent this heterogeneity reflects the presence of distinct lineages, distinct differentiation pathways, or distinct functional activation states that are unique to the mucosa. Peripheral $CD4^+$ $CD8^-$ TCR $\alpha\beta$ T cells that migrate from the intestinal lamina propria into the epithelial layer co-express $CD8\alpha$, and change their integrin and cytokine expression profile (*Reimann* and *Rudolphi*, 1995; *Morrissey* et al., 1995). This illustrates that peripheral T cells undergo distinct changes in phenotype as they traffic through different compartments of the mucosa.

Lymphoid and myeloid cell populations within different compartments of the mucosa display regional specialisation

The effector cells in diffuse compartments of the intestinal mucosa show striking regional specialisation. The small and the large intestine e.g. show a very different composition of effector cell populations in these compartments (*Camerini* et al., 1993; *Beagley* et al., 1995; *Boll* and *Reimann*, 1995a; *Boll* et al., 1995b).

GALT are subjected to tissue modelling (lymphoid neogenesis) dependent on cytokine networks and bacterial stimuli

GALT in germ-free animals are different from those in animals raised under conventional conditions. Data from genetically engineered mice indicate that ligands and receptors of the TNF superfamily are involved in forming the anlage and expanding rudimentary structures of the PP and the mLN. Especially lymphotoxin (LT) α and LTβ, the LTβ receptor (R) and other members of the TNFR receptor family play central roles in the differentiation of these tissues (*Koni* and *Flavell*, 1998). Although many details have not yet been resolved the available evidence indicates that cytokines play a decisive role in controlling the development of inductive sites of the mucosal immune system.

THE INNATE MUCOSAL IMMUNE SYSTEM

Innate immunity is often considered as a separate entity from the adaptive immune response. The current interest in innate immunity seeks to integrate these two distinct types of immune function (*Medzhitov* and *Janeway-CA*, 1997a; *Medzhitov*, *Janeway-CA*, 1997b). Examples for cells of the innate immune system that are found in mucosal tissues are listed in Table 1. The important point that emerges from this (incomplete) list is that the distinction between T cells belonging to either the innate, or the specific immune system is

Table 1: Cells of the innate and the adaptive immune system found in GALT

Innate immunity			Adaptive immunity		
Cells	Receptors	Ligand	Cells	Receptors	Ligand
CD8α cells	?	?	CD8αβT cells	αβ TCR	MHC-I/P
CD4 CD8αα T cells	αβ TCR	?	CD4 CD8αα T cells	αβ TCR	MHC-I,II/?
CD8αα T cells	γδ TCR	?hsp	CD4 T cells	αβ TCR	MHC-II/P
DN T cells	γδ TCR	?			
			NK1 CD4 T cells	αβ TCR	CD1/?
NK cells	?	?			
B1 cells	Ig	polyreactive	B2 cells	Ig	monoreactive
macrophages*	many	many			
mast cells*, eosinophils, basophils	many	many			
dendritic cells*	many	many			
Intestinal epithelial cells*	many	many			

P: peptide
hsp: heat shock protein
DN: double negative $CD4^-$ $CD8^-$
NK: natural killer
*: many different subsets

Table 2: Heterogeneity of T cell subsets within the murine small intestine IEL population

TCR[1]	CD3[2]	CD4	CD8[3]	Lineage[4]	Function[5]	Proportion[6]
αβ	ζζ	CD4+	CD8-	thymic	?	2-4%
αβ	ζζ	CD4+	CD8αα	thymic	tolerogenic	5-15%
αβ	ζζ	CD4-	CD8αβ	thymic	cytolytic	10-40%
αβ	ζ-FcεRγ	CD4-	CD8αα	extrathymic	epitheliotrophic	20-40%
γ δ	ζ-FcεRγ	CD4-	CD8αα	extrathymic	epitheliotrophic	20-60%
γ δ	ζ-FcεRγ	CD4-	CD8-	extrathymic	B help	5-10%

IEL: intraepithelial lymphocytes

1 the antigen receptor for T cells (TCR) is a heterodimer composed of eithe an α and β, or a γ and δ chain

2 the signal-transducing components of the CD3 complex can contain either the ζζ homodimer, or the ζ-FcεRγ

3 the CD8 coreceptor molecule can be expressed on the cell surface as either a CD8αα homodimer, or a CD8αβ heterodimer

4 T cells develop either in the thymus (thymic lineage), or at another (not well characterized) site (extrathymic)

5 IEL may support tolerance induction (tolerogenic), lyse cells (cytolytic), provide growth factors for epithelia (epitheliotrophic) or B cells (B help)

6 the proportion of IEL subsets listed are representative for the murine small intestine

not clear cut. MHC-II-restricted $CD4^+$ TCRαβ that co-express CD8 after homing to the epithelium, TCRγδ T cells (the recognition specificity of which is still unknown) or CD1-restricted $NK1^+$ $CD4^+$ TCRαβ T cells are examples of subsets that may be classified as cells of the innate or the specific immune system. This raises the question how this distinction has to be defined in the mucosal immune system. We will propose later that one way to view this dichotomy is to acknowledge that lymphoid cells of the immune system can be triggered by either innate, or specific stimuli. Hence the stimulus that drives the response, and not necessarily the distinct subset of lymphoid cells that respond, can classify a response as adaptive or innate. The signal for the adaptive (specific) and/or restricted response goes through the antigen receptor. Less is known about alternative ligand/receptor interactions that trigger innate immunity.

A large group of molecules of very different nature can activate T cells and antigen-presenting cells (APC) resulting in clonal expansion, differentiation and pro-inflammatory cytokine release (Table 3). These molecules are proteins, sugars, glycolipids, formylated peptides, oligodeoxy-nucleotides or lipopolysacchrides. Some of these ligands stimulate T cells similar to conventional MHC-restricted T cell recognition, e.g. the HM3 (MHC class Ib)-restricted presentation of formylated (f) peptides to TCRαβ T cells. The receptor systems and signal transduction cascades involved are often unknown but exciting new insight has been gained in the last years. These substances can be classified as adjuvants, as co-stimulators or as mitogens depending on the nature and state of differentiation/activation of the responding cell and the conditions (cofactors, concentration) of stimulation.

Table 3: Pattern recognition involved in the activation of the innate mucosal immune system

Ligand:	Butyric acid	hsp60	LPS	CpG ODNs	Mannan	?	Super antigen	Glycolipid	f-peptide
Cofactor:	?	?	LBP CD14	?	?	?	-	CD1	MHC-Ib (HM3)
Receptor:	NFκB	?	Toll-like R2 (TLR2)	?	DEC-205	costimulator (e.g. CD2)	TCR Vβ	TCRαβ	TCRαβ
Effect:	IL-1β	IL-6	TNFα IL-12	chemokines	T cell costimulation	T cell stimulation		T cell response	

Hsp: heat shock protein
LPS: lipopolysaccharide
f: formylated
LBP: lipopolysaccharide-binding protein
NF: nuclear factor

THE SPECIFIC MUCOSAL IMMUNE SYSTEM

Specific T cell-mediated mucosal immunity includes $CD4^+$ Th cell subsets, $CD8^+$ cytotoxic T lymphocytes (CTL), and specialised T-cell subsets for the induction of mucosal tolerance. Th cells control commitment of B cells to IgA production, their clonal expansion and their differentiation into plasma cells producing polymeric IgA (pIgA). T cells of the $CD4^+$ or $CD8^+$ phenotype are either naïve (have not yet encountered antigen), or activated (effector cells), or memory cells. The GALT contains a large reservoirs of precursor T cells for different subsets that allows the system to generate *in situ* potent $CD4^+$ Th cell and $CD8^+$ CTL responses after an encounter with bacterial or viral pathogens.

MODELS OF DYSREGULATED MUCOSAL $CD4^+$ T CELL RESPONSES LEADING TO CHRONIC INFLAMMATION

Aberrant immune responses can lead to mucosal inflammation, the chronic form of which is known as inflammatory bowel diseases (IBD). In man, IBD is manifest clinically as either ulcerative colitis (UC), or Crohn's disease (CD). Some animal models reproduce features of human IBD, and are thus of major interest to study the immunopathogenesis of this disease with the aim of developing novel therapeutic approaches. The models in mice and rats for the study of IBD that have emerged either through purposeful design of experimental protocols, or as unexpected 'by-products' of genetically engineered mouse lines, have been recently reviewed (*Conner* et al., 1994; *Bhan* et al., 1994; *Sartor*, 1994; *Reimann* et al., 1995a). Some of these models are listed in Table 4 where they are classified into three groups.

Models relying on local challenge with chemical irritants, immunostimulants or bacterial antigens

The local exposure of the colonic mucosa of mice and rats to the contact-sensitising agent trinitrobenzene sulphonic acid (TNBS) induces chronic colitis. Reactive TNBS modifies many proteins, a reaction that stimulates DTH responses to hapten (TNP)-modified self antigens. $CD4^+$ T cells producing the Th1 cytokines IL-2 and IFNγ stimulate the development of IBD in this model. Some inbred mouse strains exhibit greater susceptibility to disease induction, suggesting a genetic control in the development of IBD.

Genetic models.

Heritable models of colitis have been described in mice and monkeys. For example, the C3H/HeJBir substrain of C3H/HeJ mice develops a heritable (presumably multigenic) colitis early in life that spontaneously resolves with advancing age, and in which Th1 $CD4^+$ T cells apparently play a pathogenically relevant role. Chronic enteric inflammation is induced in transgenic or 'knock out' mice by manipulating T cells or cytokines by gene targeting. The most studied models include the chronic intestinal inflammation that develops in IL-2 knock-out mice, and the severe focal inflammation in both small and large intestines that is manifest in IL-10 knock-out mice. Both diseases are associated with an elevated local production of Th1 cytokines.

Adoptive transfer of immunocompetent $CD4^+$ T cells into syngeneic, immunodeficient hosts

Following the adoptive transfer of

Table 4: Animal models of inflammatory bowel disease (IBD)

I. administration of exogenous agents	
Chemical irritants:	acetic acid ethanol trinitrobenzene sulfonic acid (TNBS) oxazolone indomethacin s.c.
Immunostimulants	Freund's adjuvants immune complexes
Bacterial/synthetic antigens	peptidoglycan-polysaccharides (PG-PS) carrageenan dextran sulfate (DSS)
II. genetic models	
Spontaneous' disease	spontaneous ulcerative colitis in tamarin monkeys heritable murine colitis in C3H/HeJBir mice
Transgene expression	human HLA-B27/β2m transgene expression in SPF rats
Transgenic 'knock-out' models, deleted are:	
cytokine genes	IL-2, IL-10
TCR chains	TCR ß-chain, TCR δ-chain
signal transduction	Gαi2
adhesion molecule	E-cadherin
MHC class II	MHC class II α- and β-chain
multi-drug resistance transporter	mdr1a
III. adoptive $CD4^+$ T cell transfer into immunodeficient host (mice)	

$CD4^+$ T cells into severely combined immunodeficient (SCID) mice, the recipients develop a colitis that is characterised by a striking local expansion of Th1 $CD4^+$ T cells. This model has provided direct evidence that dysregulated Th1-type $CD4^+$ T cell responses are associated with murine IBD. We have studied this system, and have reviewed our data (*Reimann* et al., 1994; 1995a; *Reimann*, 1998).

INDUCTION OF T CELL-MEDIATED, CHRONIC MUCOSAL INFLAMMATION

Into young H-2^d C.B-17 *scid/scid* mice (SCID) mice, we transferred histocompatible, non-fractionated $CD4^+$ T cells from congenic C.B-17 +/+ or histocompatible BALB/c or BALB/c^{dm2} (dm2) mice. This reconstituted the hosts with gut-seeking $CD49d^{hi}$ $CD4^+$ T cells of the memory $CD44^{hi}$ $CD45RB^{lo}$ $CD62L^{lo}$ phenotype (*Rudolphi* et al., 1992; 1993a; 1996; *Reimann* et al., 1993; 1995b; *Rudolphi* and *Reimann*, 1993b; *Reimann* and *Rudolphi*, 1995; *Bonhagen* et al., 1996). A similar colitis was observed after heterotopic trans-

plantation of gut wall from an immunocompetent, histocompatible donor into the skin of the SCID host. The histopathology showed that $CD4^+$ T cell-repopulated SPF SCID mice developed inflammatory changes confined to the colonic mucosa. The small intestine down to the terminal ileum was usually devoid of inflammation.

$CD4^+$ T cells prepared from primary lymphoid organs (thymus), secondary lymphoid tissues (spleen; inguinal, popliteal or mLN) or peripheral tissues (lamina propria of the small or large intestine) displayed a comparable IBD-inducing potential. Gut lamina propria $CD4^+$ T cells from euthymic and athymic donor mice induced an IBD in the SCID host. Only $CD4^+$ T cells recovered from the gut epithelial layer of transplanted SCID mice showed a poor repopulation efficiency after transfer into secondary SCID hosts. Hence, following its transfer into the histocompatible SPF SCID host, all tested $CD4^+$ T cells repopulated the immunodeficient animal and induced an IBD (*Reimann* et al., 1995b; *Rudolphi* et al., 1996; *Claesson* et al., 1999).

$CD4^+$ T cells from normal rats expressing the $CD45RB^{high}$ phenotype have the potential to induce autoimmune diseases in congenic, immunodeficient hosts (*Fowell* et al., 1991; *Fowell*, Mason, 1993). Such $CD45RB^{hi}$ $CD4^+$ T cells from healthy animals thus seem to express an autoaggressive potential that can be revealed *in vivo*. $CD45RB^{hi}$ $CD4^+$ T cells from immunocompetent mice induced clinical and histopathological signs of colitis after transfer into a histocompatible, immunodeficient hosts (*Powrie* et al., 1993; 1994a; 1994b; 1996; *Morrissey* and *Charrier*, 1994; *Powrie*, 1995; *Leach* et al., 1996; *Aranda* et al., 1997; *Picarella* et al., 1997). We transferred polyclonal, oligoclonal or monoclonal $CD4^+$ TCR$\alpha\beta$ T cell populations into SPF SCID mice; the transfer of all three types of $CD4^+$ T cell populations induced an IBD in histocompatible SCID hosts (*Reimann* et al., 1995b; *Claesson* et al., 1999). The state of activation of the transferred $CD4^+$ T cells is relevant with respect of their IBD-inducing potential. Mitogen- or antigen-stimulated $CD4^+$ T lymphoblasts are more efficient in inducing IBD than the respective resting $CD4^+$ T lymphocytes (*Claesson* et al., 1999). Taken together, we have shown that many tested $CD4^+$ T cell subsets could induce an IBD after adoptive transfer into SCID hosts. There is thus little evidence that a particular $CD4^+$ T cell subset is involved but the relative efficiency with which different $CD4^+$ T cell subsets induced the disease varied.

IBD-ASSOCIATED $CD4^+$ T CELLS

Transferred $CD4^+$ T cells repopulate the lamina propria and epithelial layer of the small and large intestine, the mesenteric lymph nodes, the peritoneal cavity and the spleen (but not other peripheral lymph nodes or tissues) of the SCID host. Transfer of even high numbers of $CD4^+$ T lymphocytes or blasts into GF SCID mice did not repopulate the animals; a microbial gut flora is thus an essential prerequisite for successfully repopulating the SCID host with adoptively transferred $CD4^+$ T lymphocytes. The selective reconstitution of the immunodeficient host with gut-seeking $CD4^+$ T cells was confirmed by the surface phenotype of repopulating T cells: all T cells in the SCID host expressed the $\alpha_4\beta_7$ integrin (a homing receptor for mucosa-seeking leukocytes) (*Bonhagen* et al., 1998), and high levels of the P-selectin-binding ligand PSGL-1 (involved in leukocyte-endothelial cell interactions during intestinal inflamma-

tion) (*Thoma* et al., 1998). The repopulating, mucosa-seeking $CD3^+$ $CD4^+$ $CD8^-$ TCRαβ memory/effector cells express high levels of CD44, CD2 and CD28, but low levels of CD45RB and CD62L on the surface, and are $CD95^+$ (susceptible to apoptosis, or 'activation-induced cell death', AICD). A large fraction of these cells in the inflamed colonic lamina propria is *in situ* activated ($CD25^+$, $CD69^+$); the fraction of activated $CD4^+$ T cells increases strikingly with the progression of the disease. The gut lamina propria seems to be the major site of $CD4^+$ T cell proliferation (*Bregenholt* et al., 1998).

Transfers of limiting numbers of cells from polyclonal $CD4^+$ T cell populations into SPF SCID mice repopulate the host with T cell populations with a polyclonal TCRαβ repertoires; we detected no evidence for preferential expansion of oligoclonal populations in the immunodeficient, histocompatible host (*Rudolphi* et al., 1996). Even when 10^5 $CD4^+$ T cells (that were expanded in adoptive hosts for a 3-5 month period) were repeatedly 'passaged' through SCID mice, no reproducible bias in their TCR β-chain repertoire could be detected. We have not been able to select *in vivo* a $CD4^+$ T cell line with an enhanced IBD-inducing phenotype and a restricted TCR β-chain repertoire by repeated passage through different SCID hosts. Oligoclonal and monoclonal $CD4^+$ T cell lines were as efficient as polyclonal $CD4^+$ T cell lines in repopulating the SCID host and in inducing an IBD (*Claesson* et al., 1999). These data suggest that a TCR-independent (mitogen-like) stimulus drives T cell activation and expansion in the colonic lamina propria of transplanted SCID mice. The mitogenic stimulus that drives T cell expansion in the adoptive host is (directly or indirectly) dependent on the microbial flora of the gut.

Almost all $CD4^+$ T cells repopulating the adoptive SCID host produce the Th1 cytokines IFNγ and TNFα detectable either by ELISA (at the population level), or by intracellular staining and FCM analyses (at the single cell level). Very few (or no) IL4- or IL10-producing T cells are detectable. In the ELISA readouts, inducible IL12 levels in mononuclear cell population from repopulated tissues of the adoptive SCID host were high. Hence, the cytokine profile detectable in diseased, transplanted SCID mice displayed a pure Th1 phenotype, similar to that reported in other murine IBD models (*Powrie* et al., 1996; *Stuber* et al., 1996; *Berg* et al., 1996; *Neurath* et al., 1996a; 1996b; *Hörnqvist* et al., 1997; *Ehrhardt* et al., 1997; *Ludviksson* et al., 1997; *Strober* et al., 1997). Evidence has emerged for an IL10-producing immunoregulatory $CD4^+$ (Tr1) T cell subset that prevents Th1 differentiation and may be an important physiological control for preferential Th2 type differentiation in mucosal immune responses (*Groux* et al., 1997). The observation that IL-10 is absent from transplanted SCID mice supports this concept.

The $CD4^+$ T cell-mediated immune effector mechanisms that damage the mucosal tissue are unknown. Th1 $CD4^+$ T cells isolated from the colonic lamina propria of transplanted SCID mice are cytolytic (*Bonhagen* et al., 1996; *Boirivant* et al., 1996; *De* et al., 1996). The TCR-mediated cytolytic effect operates through the CD95(Fas)/CD95L(FasL) pathway. The CD95-expressing targets of this cytolytic attack that are relevant in the pathogenesis of IBD are intestinal epithelial cells (IEC), APC and the $CD4^+$ T cells themselves. A central role of epithelial damage in the pathogenesis of IBD as a result of the immune attack by T cells has been postulated (*Iwamoto* et al., 1996; *Sträter* et al., 1997; *Sakai* et al., 1997). Some $CD95^+$ APC are susceptible to $CD4^+$ T cell attack which may select for APC in the colonic lamina propria that bias the response towards

Table 5: Colonic lamina propria T cell activation, expansion and apoptosis *in vivo* in transplanted SCID mice with IBD

T cell activation and expansion
• an increasing fraction of $CD4^+$ T cells is activated *in situ* ($CD69^+$ $CD25^+$) in the colonic lamina propria during progression of the IBD • $CD4^+$ T cells rapidly proliferate mainly in the colonic lamina propria • transferred $CD4^+$ T cells expand >100-fold in number in the adoptive host • freshly explanted colonic lamina propria $CD4^+$ T cells show cytokine-dependent (IL-2/IL-7) proliferation *in vitro*
T cell apoptosis and cytolytic reactivity
• colonic $CD4^+$ T cells are exquisitely susceptible to 'activation-induced cell death' (AICD) *in vivo* and *in vitro* • AICD is triggered *in vitro* by TCR/CD3 ligation (? are $CD4^+$ T cells selected *in vivo* against gut flora-specific reactivities) • CD95/CD95L (Fas/FasL) is involved in AICD of colonic lamina propria $CD4^+$ T cells in IBD
specific *versus* non-specific T cell activation
• the TCR-Vß repertoire of colonic $CD4^+$ T cell populations from diseased SCID mice suggests that they are polyclonal even after repeated transfers through SCID hosts • *in vivo* selection of oligoclonal $CD4^+$ T cell lines with a restricted TCR-Vß repertoire and an enhanced IBD-inducing potential by repeated passages of limiting numbers of $CD4^+$ T cells through SCID hosts was repeatedly unsuccessful • adoptive transfers of oligoclonal $CD4^+$ T cell lines induce colitis

the Th1 direction. We detected massive AICD in 50-70% of the colonic lamina propria $CD4^+$ T cells from transplanted SCID mice *in situ*, in gut-derived $CD4^+$ T cells explanted *in vitro*, and in gut-derived $CD4^+$ T cell populations after *in vitro* TCR-dependent stimulation (*Bonhagen* et al., 1996; 1998; *Bregenholt* et al., 1998). In addition, pro-inflammatory mediators seem to damage stroma cells and the integrity of the mucosal barrier, and to destroy the tissue matrix.

INTERPRETATION OF DATA FROM THE MODEL

The available data represent indirect evidence that a TCR-independent, mitogenic stimulus drives the polyclonal activation, expansion and differentiation of colonic lamina propria $CD4^+$ T cells in the diseased, adoptive host. Because $CD4^+$ T cell repopulation and expansion is only observed in mice with an intact gut flora, a microbial factor has to be a key player that (directly or indirectly) triggers, amplifies and/or directs the T cell response in the colon.

Chronic development of the colitis is associated with a massive infiltration of the colonic lamina propria with donor-type $CD4^+$ T cells. An increasing fraction of these T cells shows evidence of *in situ* activation (i.e. expression of CD69 and CD25) as the disease progesses. We have demonstrated that the transferred $CD4^+$ T cells expand >100-fold in number in the adoptive host. *In vivo* labelling indicates that the colonic lamina propria is the major site of T cell

proliferation in the transplanted SCID mouse. Freshly explanted colonic lamina propria $CD4^+$ T cells from transplanted SCID mice with IBD proliferated *in vitro* in the presence of the cytokines IL-2 and IL-7 (that both are abundantly present in the colonic lamina propria).

Activation and proliferation of colonic $CD4^+$ T cells is accompanied by extensive *in situ* cell death by apoptosis. Histochemical evidence (TUNEL staining) indicates that 50-80% of the lymphoid cells infiltrating colonic lesions are undergoing apoptosis. Lamina propria macrophages contain abundant numbers of tangible bodies indicating their phagocytosis of apoptotic lymphoid cells. All colonic $CD4^+$ T cells are $CD95^+$, and a fraction of 20-30% of these cells express the CD95L. We presented evidence that colonic $CD4^+$ T cells are exquisitely susceptible to 'activation-induced cell death' (AICD) *in vitro*, and that CD95/CD95L (Fas/FasL) interactions are involved in AICD of these T cells in IBD. This AICD is triggered *in vitro* by TCR/CD3 ligation. This makes it unlikely that restricted, antigen-specific stimulation of T cells via the TCR drives this massive T cell expansion. The inverse scenario seems more compatible with the data: a mitogenic stimulus drives the cytokine-dependent, polyclonal proliferation of these $CD4^+$ T cells, and the TCR-dependent, specific and restricted stimulation of the activated T cells by antigens from the gut flora triggers specific deletion of T cells. In this way, the repertoire of the activated and proliferating $CD4^+$ T cell population would be selected *in vivo* against gut flora-specific reactivities.

The data on the TCR-Vβ repertoire of colonic $CD4^+$ T cell populations from diseased SCID mice (Table 5) are compatible with this interpretation. IBD-associated colonic lamina propria $CD4^+$ T cell populations are polyclonal. They remain polyclonal even after repeated 3-4 month transfers through SCID hosts in which they repeatedly induced the disease. Although such $CD4^+$ T cell lines that were passaged *in vivo* for 22 months through 6 generations of SCID hosts, went repeatedly through population 'bottlenecks' in the course of these transfers (only 10^5 $CD4^+$ T cells were injected to initiate each new transfer), this *in vivo* selection did not result in the appearance of a reproducible bias in the TCR-Vβ repertoire in the $CD4^+$ T cell populations. We have furthermore demonstrated that adoptive transfers of oligoclonal $CD4^+$ T cell lines of different origin (i.e. from different types of transgenic donor mice) are equally efficient in inducing a colitis. Taken together, these data suggest that a mitogenic (gut flora-dependent) stimulus drives the polyclonal activation and expansion of TCRαβ $CD4^+$ T cells in the colonic lamina propria while the specific and restricted recognition tends to delete the respective T cell clone from the GALT.

THE INNATE/SPECIFIC IMMUNITY INTERFACE

Are there precedents for the TCR/CD3-independent activation of LPL? Evidence is emerging that this is in fact the case. Some ligands bind to surface receptors of lymphocytes and thereby trigger activation. The CD28 molecule expressed by T cells is considered a co-stimulator molecule, i.e. coligation of this determinant with specific antigen recognition triggers T cell activation. Different groups have reported that monoclonal antibodies specific for CD28 trigger T cell proliferation and cytokine gene expression *in vitro* and *in*

Table 6: Alternative (TCR-independent) activation of T cells of the specific (adative) immune system

T cell receptor	ligand
CD2	CD58 (LFA-3), CD48
CD28	CD80 (B7-1), CD86 (B7-2)
integrin $\alpha E\beta 7$	E-cadherin
? surface receptor	Hsp70, hsp96
? intracellular target	butyric acid
? intracellular target	ISS CpG-containing ODN

Hsp: heat shock protein
ISS: immune stimulating sequences
ODN: oligodeoxynucleotides

vivo (*Tacke* et al., 1997; *Flynn* and *Mullbacher*, 1997; *Siefken* et al., 1997; 1998). These data suggest that binding of CD80 or CD86 (expressed by APC) to CD28 (expressed by T cells) can induce T cell proliferation without TCR/CD3 involvement. Interaction of the integrin $\alpha E\beta 7$ on the surface of IEL with E-cadherin on the surface of IEC can activate the former in an antigen receptor-independent manner (*Sarnacki* et al., 1992). The TCR-independent activation *in vivo* and *in vitro* of T cells by antigen-negative heat shock proteins (hsp) of the 70 and 96 kDa class has been demonstrated (*Breloer* et al., 1999). Activation of the CD2 pathway in lamina propria T cells up-regulates IL-2 expression in human LPL (*Gonsky* et al., 1998). Microbial products may mimic some of these ligand/receptor interactions and may thereby activate T cells. Other reagents may readily cross the cell membrane and interfere with signal transduction cascades. E.g. butyric acid listed in Table 3 is known to activate NF-κB. There may thus be a plethora of antigen receptor-independent activation pathways for lymphocytes of the specific immune system, in particular for T cells.

Effector functions (e.g. release of cytokines or cell contact-dependent cellular interactions) do not differ between the innate and the specific immune system. Their difference is generally assumed to be based on the way the system is triggered: specific *versus* non-specific activation. We presented evidence that the specific T cell system seems to be activated non-specifically on an impressively large scale in the mucous membranes of the gut. We may therefore have to modify our view of these systems. Instead of conceiving them as separate, interacting systems, we may have to think of them as the alternative activation pathways of an old (innate) and a new (specific) way to stimulate lymphocytes. The choice for the activation pathway operating may depend on the particular micro-environment it resides in. The mucosal immune system as the primary defence site to foreign challenges may imprint a predominance of innate reactivity. Its strength may be to focus all defences to the quick, limited, short lived responses. This may limit the importance of specific immune responses in the intestinal mucosa.

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