New insights into the cellular immunology of the intestine in relation to the pathophysiology of inflammatory bowel diseases

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Abstract

The authors review advances about altered immunological cellular mechanisms in inflammatory bowel diseases (IBD). The innate immune response might play a role in the inductive phase : epithelial barrier defect, production of inflammatory cytokines and defective neutrophil function. Dendritic cells have a pivotal role, since they sense the nature of the micro-organisms in the intestine in order to drive either adaptive immune responses through IL-12 or IL-4 and co-stimulatory molecules, or immunotolerance through regulatory T cells (Tr). T helper(Th)1 cytokines (IFNγ, TNF-α, IL-12) are secreted in excess in Crohn's disease (CD) whereas in ulcerative colitis an atypical Th2 immune response (IL-4, TGFβ) has been reported. However, activation of Th can only lead to effective immune response if co-stimulatory molecules expressed on activated T cells bind to their specific ligands on the antigen-presenting-cells, mesenchymal and endothelial cells. This binding is necessary to generate an effective immune response, to enhance expression of adhesion molecules and T cell recruitment, promoting chronic inflammation in IBD. A defective function of Tr might contribute to excessive T cell response. Innate CD4 + CD25 + Tr derived from the thymus represent 5-10% of T cells in peripheral lymphoid organs. Acquired peripheral Tr downregulate the immune response through IL-10 and TGF-β production. In IBD effector T cells might downregulate the development of Tr cells in the thymus. Another defective mechanism in CD is T cell resistance to apoptosis, leading to inappropriate immune homeostasis and accumulation of T cells in the tissues. New therapeutic agents have been proposed for correcting deficiencies of innate immunity or reducing excessive immune responses, with promising results confirmed by randomized controlled trials. (Acta gastroenterol. belg., **2006**, 69, **393-405**).

Key words : Inflammatory bowel disease, Crohn's disease, ulcerative colitis, immune system, regulatory T cells, immunology, therapeutics.

Inflammatory bowel disease (IBD) is thought to result from environmental and genetic factors associated with an upregulated aggressive immune response and chronic inflammation. Numerous human and animal studies aimed at unravelling the pathogenic mechanisms of these diseases, ie Crohn's disease (CD) and ulcerative colitis (UC), have lead to evidence of complex cells-cytokines interactions within the gut. The aim of this review, far from being exhaustive, is to describe and discuss recent insights into the cellular immunology of the intestine and their implications on the pathophysiology of IBD.

The gut innate immune system

The immune system responsible of our defence against foreign antigens plays its role through two ways : first immediately and repeatedly if necessary, through the innate immune system and then, after a first contact with the antigen, through amplified reactions at further contacts with the antigen, i.e. adaptive immunity. Table 1 summarizes the functions of the different components of the innate immune system and their alterations in IBD, based on the review of Delves and Roitt (1) and recent literature.

Mechanical components

They include the anatomic barrier of the gut constituted by the monolayer of epithelial cells bound by tight junctions and the mucus secreted by goblet cells. Several proteins : occludins, claudins, etc, are involved in the structure and functions of tight junctions (2). Many studies about alterations of intestinal permeability, recently reviewed by Arrieta *et al.* (2), have demonstrated an increase of intestinal permeability in CD patients and also in about 20% of their relatives (3). Epithelial cells, like other mucosal immune cells, express pattern recognition receptors (PRR) in order to sense dangerous microbiota in the gut and to react in contact with their ligands (4). The extra-cellular PRR belong to the family of transmembrane toll-like receptors (TLRs), the main human TLRs being TLR1, TLR2, TLR4, TLR5 (4). Intestinal epithelial cells produce proinflammatory cytokines (IL-1α, IL-1β, TNF-α, etc) in response to invasive pathogens, such as *Salmonella*, *Shigella*, *Yersinia* and *Listeria* (5). Besides the toll- like receptors (TLR, epithelial cells express also a characteristic profile of chemotactic peptides, adhesion molecules, class II MHC. A very high expression of the peroxysome proliferator activated receptor (PPARγ), a nuclear hormone receptor, has been found in colonic eptithelial cells (6). Micro-organisms are enhancing its expression through several mechanisms such as recognition of lipopolysaccharide (LPS) by TLR4. Its role is to inhibit signalling pathways in order to reduce inflammation (7). This receptor has been found reduced in experimental colitis

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Table 1. — **The gut innate system**

Components	Functions	Alterations in IBD (reference)
Normal epithelium with tight junctions	Barrier against luminal & exogenous agents (NSAIDs) Cytokine production against invasive pathogens	↑ permeability in CD (2) ↓ PPARγ in UC (7)
Mucus (<goblet cells) with trefoil peptides	Barrier against luminal & exogenous agents	↓ Muc2 in UC (10)
Intestinal peristalsis	Drainage of pathogens	
Cells		
Phagocytic cells	Discriminate self/non self	
Monocytes(macrophages)	Receptors for Ab and C for germ phagocytosis PRR : NOD2/CARD15	↓ phagocytosis (24) Mutations NOD2/CARD15 in 10-30% CD (14)
Neutrophils	Migration Superoxide generation Phagocytic function and bacterial killing	↓ CD (17,18) ↓ inactive & active CD (19,20) ↓ for Candida in CD (21) ↓ in CD & UC (22)
Dendritic cells	Endocytosis of extracellular Ag PRR : NOD2/CARD15 Activated in APC in presence of PAMP or endogenous signals (INF-α, etc) Priming of the acquired immune response	Mutations NOD2/CARD15 In 10-30% CD (14) ↑ activated DC in blood (37) ↑ TLR2-TLR4 in LPMC (27)
Cells releasing inflammatory mediators		
Eosinophils	Weakly phagocytic Release of reactive oxygen metabolites, leukotrienes, etc	
Basophils and mast cells	High affinity receptors for IgE, release of inflammatory mediators	
Natural killer cells	Destruction of infected and malignant cells by injecting cytotoxic granzymes (1)	
Paneth cells	Secretion of antimicrobial peptides & proteins (lysozyme,etc) PRR : NOD2/CARD15	Paneth cell colonic metaplasia in colonic CD & UC (32) ↓ α-defensin ileal CD (33) Mutations NOD2/CARD15 in 20% CD (30,31)
Soluble factors		
Complement components	↑ phagocytosis of microbes after activation	Complement dysfunction in IBD (35)
Acute-phase proteins	↑ resistance to infection ↑ repair of damaged tissue	
Cytokines	Proinflammatory cytokines against invasive pathogens Messengers for adaptive immune responses : IL-12, IL-4 : see text	

IBD = inflammatory bowel diseases ; CD = Crohn's disease ; UC = ulcerative colitis ; Ab = antibodies ; C = Complement ; PPR = pattern-recognition-receptor ; IF-α = Interferon-α ; PAMP = pathogen-associated molecular patterns ; APC = Antigen-presenting cell ; LPMC = lamina propria mononuclear cells ;TLR = toll-like receptor.

and in UC (7). Recent studies of Sugawara *et al.* (8) in mice and in patients with CD revealed that PPARγ was a susceptibility gene for human CD.

Mucus containing trefoil peptides and mucin glycoproteins contribute also to the barrier against luminal agents and exogenous agents such as non-steroidal anti-inflammatory drugs (NSAIDs) (9). The main mucin secreted by goblet cells, MUC2, has been found reduced in experimental colitis in mice (10) and in patients with UC (11, 12).

Both environmental and pathogenic bacteria are largely killed in the proximal GI tract by salivary lysozyme, gastric acid, pancreatic digestive enzymes and bile acids and if they resist they are removed by intestinal secretion and peristalsis.

Cellular components

Cellular components of the innate immune system are classified into phagocytic cells and cells releasing inflammatory mediators (1).

The *macrophages* deriving from blood-borne *monocytes* can discriminate between self and foreign molecules and, with receptors for antibodies and complement, are able to phagocytose micro-organisms. A reduction of the phagocytic activity of peripheral monocytes has been observed in CD (13). Like Paneth cells, monocytes and macrophages have an intracellular protein, nucleotide oligomerization domain (NOD)2 recently called caspase recruitment domain (CARD)15, with high affinity for lipopolysaccharide (LPS), a component of the peptidoglycan bacterial cell wall. NOD2/CARD15 is a intra-cellular pattern-recognition-receptor (PPR), able to recognize specific pathogen-associated molecular patterns (PAMP), like LPS. The binding provokes an activation of nuclear factor-ϰB (NF-ϰB) which causes an inflammatory response through pro-inflammatory cytokines. As mentioned in the review of Colombel (14), 3 independent mutations within the NOD2/CARD15 gene have been found in 10-30% of Western CD patients, but none in Japanese CD patients.

It was very difficult to explain the harmful effect of these mutations. Recently Watanabe *et al.* (15) demonstrated that wild NOD2/CARD15 inhibited Th1 cytokine secretion after toll-like receptor 2 (TLR2) pathway activation. Therefore Hugot has concluded that NOD2/CARD15, like PPARγ, has normally an immunosuppressive role, which is lost in case of mutation (16).

NF-κB is a key transcriptional factor involved in initiation of immuno-inflammatory responses. It provides a central pathway of macrophage activation through engagement of a variety of membrane receptors for bacterial polymers and pro-inflammatory mediators. Activation of the protean transcriptional regulatory factor NF-κB is a common pathway central to T cell activation and the production of diverse inflammatory mediators (cytokines, chemokines). It also modulates resistance to apoptosis. Several inflammatory factors implicated in intestinal inflammatory bowel disease (IBD) activate NF-κB by eventually stimulating an intermediate kinase NIK (NF-κB inducing kinase) or by binding to receptor interacting protein 2. These lead to phosphorylation of the inhibitor of κB kinase (IκBα) and subsequent dissociation of NF-κB.

NF-κB travels to the nucleus where it can affect gene transcription. The spectrum of mediators that activate this pathway includes inflammatory cytokines : IL-1, TNF-α which bind to their respective surface receptors as well as microbial products such as LPS binding to members of the toll-like receptors family, as already mentioned (Fig. 1).

The *neutrophil* plays a central role in immunity because it can reduce or eliminate by phagocytosis microbial antigens before recruitment of chronic inflammatory cells. Defects have been described in CD in four areas : migration or chemotaxis (17,18), superoxide generation (19, 20), phagocytosis of *Candida* (21) and bacterial killing (22), and in UC in two areas : superoxide generation (19) and bacterial killing (22). Recently, Marks *et al.* (23) confirmed defects of neutrophil functions in CD patients in comparison with controls : lower accumulation of neutrophils after instestinal mucosa or skin traumas with lower production of proinflammatory IL-8 and IL-1β. Following these studies and the association of CD with diseases due to deficiencies in neutrophil functions, such as chronic granulomatous disease, Korzenick and Diergraeve (24) proposed the hypothesis of CD being an immunodeficiency, possibly related to a change of the gut flora.

A key cellular component of innate immunity (1) is *the dendritic cell* (DC). DCs are originating from the bone marrrow and the monocytes. In the gastro-intestinal tract, the different subsets or populations of DC are located in the Peyer's patches, in the lamina propria along the intestinal epithelium with extending dendrites through epithelial cells for sampling the luminal contents (Fig. 1), and in the mesenteric lymph nodes (25). Antigens within the enteric microenvironment are sampled by uptake across the specialised epithelial M cells of the Peyer's patches and presented to the immune system through DCs. Whereas DCs continuously endocytose extracellular antigens, they can be activated and transformed in antigen-presenting cells (APC) if pattern-recognition receptors (PRR) on their surface (TLRs) or in their cytoplasm (NOD/CARD15) recognise specific pathogen-associated molecular patterns (PAMP), such as LPS, the mannose receptors and molecules called toll. DCs can also be activated by endogenous signals : interferon-α (INF-α) and heat-shock proteins. DCs sampling the luminal contents can discriminate between commensal bacteria and bacteria with PAMP by utilizing their innate repertoire of toll-like receptors (TLR) and C-type lectins (25). DCs can migrate into the mesenteric lymph nodes (MLN) under the control of chemokines and chemokine receptors.

After activation and migration into the MLN (Fig. 1), DCs transformed into APC can initiate responses of acquired immunity (priming) by presenting the antigen-derived peptides within a major-histocompatibility-complex (MHC) molecule of Class I for autoantigens and Class II for exogenous antigens to the specific T-cell receptor of a naïve T cell (1,25,26). DCs contribute also to immunotolerance, by means of several mechanisms of suppression of T-cell immune responses and generation of regulatory T cells as it will be specified in section 3.

A study on isolated lamina propria mononuclear cells in IBD patients (27) has demonstrated that intestinal DC expression of two toll-like receptors, TLR2 and TLR4 was significantly increased in CD and UC in comparison with healthy controls. DCs from CD and UC inflamed tissue expressed significantly higher levels of the maturation/activation marker CD40, which was decreased after treatment with anti-TNF-α. In CD but not in UC, more colonic DCs produced IL-12 and IL-6 (27). In a large Belgian genetic study, an association of the TRL4 Asp299Gly polymorphism with CD and UC has been demonstrated (28).

Cells releasing inflammatory mediators comprise eosinophils, basophils and mast cells, natural killer cells and Paneth cells (Table 1). *Eosinophils* are weakly phagocytic and on activation release cationic proteins which probably kill parasites, and reactive oxygen metabolites, as well as leukotrienes, prostaglandins and various cytokines (1). *Basophils and mast cells* have similar functions but have also high affinity receptors for IgE antibodies and, after allergen binding, release histamine, prostaglandins and leukotrienes (1). *Natural killer cells* (NK Cells) destroy infected and malignant cells by inserting a pore-forming molecule (perforin) into the membrane of the target cell and then injecting cytotoxic granzymes. Besides Fc receptors which bind IgG-coated target cells, NK Cells possess killing-activating receptors and killer-inhibitory receptors which bind to MHC class I molecule (1).

Paneth cells contribute much to gut innate immunity (29). These granulated cells are located at the base of small intestinal crypts (Fig. 1). Their granules consist of

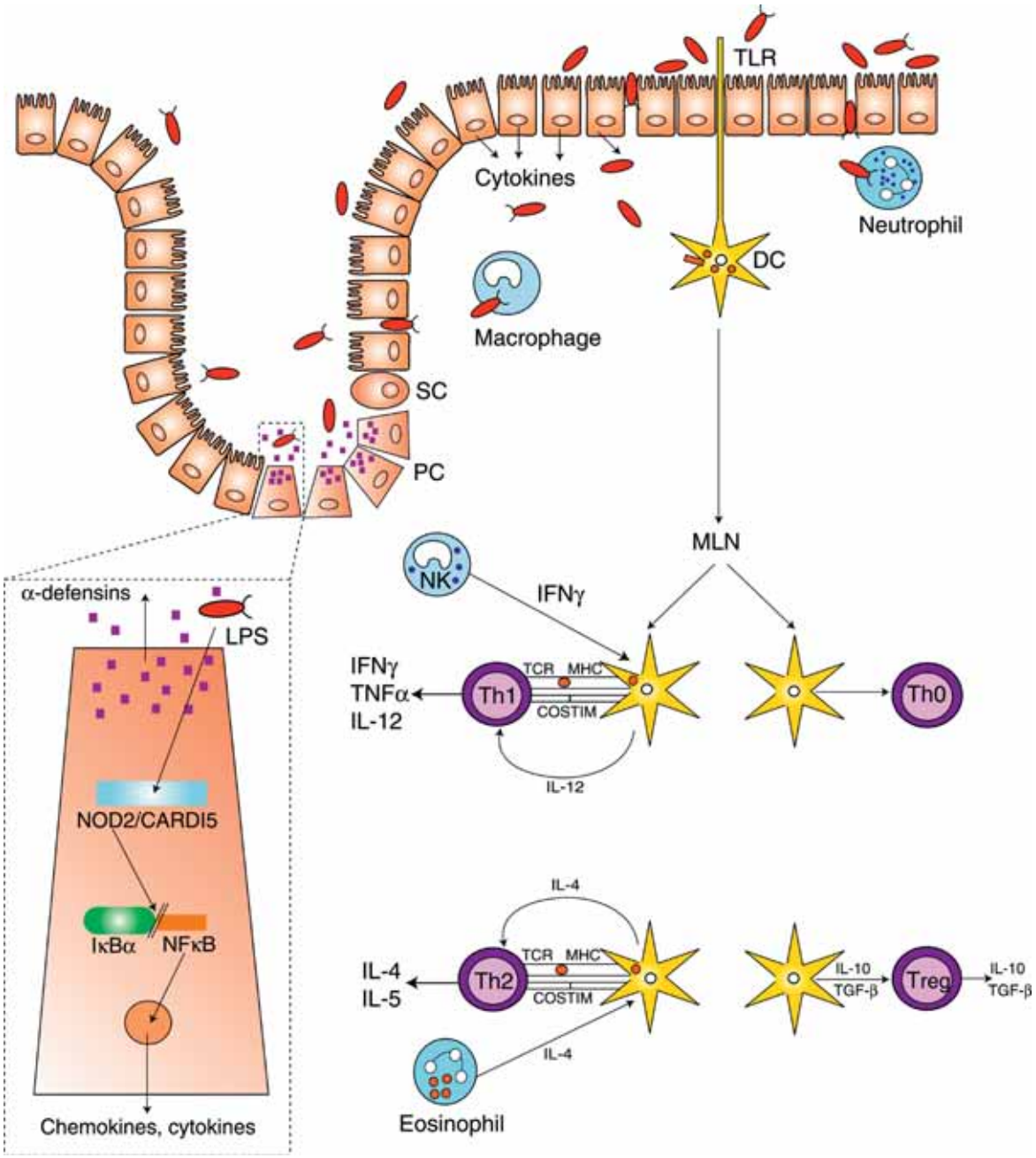

Fig. 1. — Schematic overview of some features of the innate and adaptive immune systems in the small bowel. The first barrier is the monolayer of epithelial cells with tight junctions (not shown), producing inflammatory cytokines. Increased permeability facilitates the passage of bacteria. Other cells from the innate immune system release inflammatory mediators. These cells comprise basophils, eosinophils, macrophages, mast cells, NK cells and Paneth cells. Paneth cells are located at the base of small intestinal crypts, secrete α-defensins and, like macrophages and dendritic cells (DCs) contain in their cytoplasm a pattern-recognition-receptor N02/CARD15, which binds to bacterial components, like lipopolysaccharide (LPS), and thereafter dissociates the complex IκBα-NFκB and allows the transcriptional factor NF-κB to travel to the nucleus for gene transcription and production of proinflammatory cytokines. DCs have extensive dendrites through epithelial cells for sampling luminal contents, as well as toll-like-receptors (TLR) for detection of danger signals (specific pathogen-associated molecular patterns, like LPS), which activate DCs migrating into the mesenteric lymph nodes (MLN). The first signal in the adaptive immune response is the recognition by the T cell receptor (TCR) of antigenic peptides processed by DCs on their MHC (major histocompatibility molecule). The second signal is mediated by interaction between costimulatory molecules and their ligands CD28/CD80/86, CD40/CD40L, etc. The polarization of Th0 cells into Th1, Th2 or regulatory T cells (Tregs) depends on secretion of different cytokines by DC subsets, mainly IL-12, IL-4 and IL-10, respectively. Tregs suppress the immune response through cell contact (CTL4-binding to CD80/86 and inducing negative signaling) or through secretion of IL-10 and TGF-β downregulating the immune response and inducing apoptosis of T effector cells.
TLT = toll-like receptor ; DC = dendritic cell ; SC = stem cell ; PC = Paneth cell ; MLB = mesenteric lymph nodes ; Th = CD4 + T helper cells. Treg = regulatory T cell ; TCR = T cell receptor ; MHC = Major histocompatibility complex ; Costim = costimulatory molecules.

microbicidal peptides and proteins secreted into the crypt lumen after exposure to Gram positive and Gram negative bacteria. Secreted peptides and proteins comprise lysozyme which is an antibacterial protein, secretory phospholipase A2 (sPLA2) which has a bactericidal activity against *Samonella typhimurium* and *Listeria monocytogeneses*, *Escherichia coli*, *Salmonella typhimurium* and *Candida Albicans*. The cytoplasm of Paneth cells contains the pattern-recognition-receptor NOD2/CARD15, like macrophages and DCs (30,31). In UC and CD Paneth cell metaplasia is observed in the colon (32), as well as in diverticulitis and in radiation colitis. In CD ileal biopsies, expression of enteric α-defensin is reduced, with greater reduction in presence of NOD2/CARD15 mutations (33). An increased bacterial adherence to the intestinal mucosa is possibly due to impaired expression of defensins in CD (34).

Third component : soluble factors

They comprise components of the complement, acute-phase proteins and cytokines (1). *Complement* activation generates immunologically active soluble proteins, like C3b molecules which enhance phagocytosis. In IBD complement dysfunction which could impair neutrophil recruitment has been described (35). *Acute-phase proteins*, like C-reactive protein (CRP), amyloid A protein, proteinase inhibitors, coagulation proteins enhance resistance to infection and contribute to the repair of damaged tissue. Some *cytokines* secreted by epithelial cells, NK cells, like the interferons contribute to the innate immunological defence (5). When stimulated with bacterial components, epithelial, bone marrow derived mesnchymal and endothelial cells actively contribute to mucosal protection by secreting antimicrobial proteins and inflammatory mediators or by degrading invasive microbial agents with defensins already mentioned.

The gut adaptive mucosal immune system

The adaptive immune response (Table 2), is triggered following specific antigen recognition by receptors : T-cell receptor (TCR) on T lymphocytes and surface Igs on B cells. T cells developed from primordial stem cells in the fetal liver and bone marrow (1) require a passage inside the thymus in order to become naïve mature T cells ready to encounter an antigen presented by APC on its MHC. This occurs in secondary lymphoid tissues and naïve T cells become effector T cells and memory T cells. Maturation of B cells is done inside the bone marrow and the meeting with the antigen takes place in the secondary lymphoid tissues with the help of dendritic cells. The effector phase of adaptive immune response is mediated by clonal expansion of specific T and B cells with respective effector agents : T cytotoxic cells and immunoglobulins (Igs).

Role of *dendritic cells* (DCs)

As already mentioned, activated DCs transformed into "professional" antigen-presenting cells (APC) into the mesenteric lymph nodes (MLN) are initiating adaptive immune responses through activated T cells. Vukovic *et al.* (37) have observed that in peripheral blood of IBD patients there were more activated DCs than in healthy controls. The modalities of this link between the two immune systems, i.e. the primary reactions towards specific antigens of the gut lumen, have been thoroughly studied in relation with the pathophysiology of IBD. According with recent reviews of numerous studies (26,36,38), subsets of DCs differ in the signals they transmit to naïve CD4 T cells (Th0), which explains the different types of responses : either immune effector reponses of the Th1 or Th2 types, or an induction of immunotolerance through transforming Th0 cells into regulatory T cells.

Corthay (36) has proposed a three-cell model of activation of naïve Th0. As already mentioned, the first signal is the recognition of the TCR by antigenic peptides processed by the DCs, the second signal is the co-stimulation by triggering of CD28 on the T cell by CD80/86. The third signal would be the polarization of T cells into Th1 or Th2 phenotypes through IL-12 and IL-4, respectively, with the help of the innate immune system. It was demonstrated previously that the Th1 polarizing cytokine was IL-12 produced by a subset of DCs and that the Th2 polarizing cytokine was IL-4 produced also by a subset of DCs (39). The secretion of IL12 by DCs depends upon the type of microbial products detected by the toll-like receptors (TLR) but a recent study (40) has demonstrated that interferon-γ (IFN-γ) produced by innate immune cells, like NK cells or already stimulated T cells, could «educate» DCs to secrete IL-12. IL-4, the polarizing cytokine for a Th2 response would be provided by the DCs to other innate immune cells, like mast cells, basophils and eosinophils (36). Danger signals from intracellular bacterial pathogens, such as *Salmonella typhimurium*, as well as fungal and protozoan pathogens stimulate DCs to produce IL-12 (26,41). D'Ostiani *et al.* (42) observed in mice that *Candida albicans* in its yeast stage (non-virulent form) stimulated production of IL-12 by DCs whereas in its hyphal stage (virulent form) it stimulated IL-4 production. The authors concluded that DCs "appear to meet the challenge of Th priming and education in *Candida Albicans* saprophytism and infections". In peripheral blood of patients with IBD, Vukovic *et al.* (37) have found significantly increased number of DCs expressing co-stimulatory molecules compared to healthy controls, confirming the observations already mentioned of an increased activation of DCs in mesenteric lymph nodes of IBD patients (27).

Intestinal epithelial cells, beside their role in innate immunity, can function as APC for the adaptive immune system by activating CD8 suppressor T cells (43). In IBD Mayer *et al.* (44) have observed a lack of induction

Table 2. — **The gut adaptive mucosal immune system**

Components	Functions	Alterations in IBD (references)
Dendritic cells (DCs)	See table I and text	↑ activated DCs in peripheral blood (37)
Intestinal epithelial cells	APC for the mucosal immune system with activation of CD8 + T cells	Stimulation of CD4 + T cells instead of CD8 + T cells (44)
Intra-epithelial lymphocytes	CD8 + T cells ; role not yet determined	
CD4+ helper T cells (Th)	Regulate immune response and immunotolerance	
Th1	mediate cellular immunity against intracellular bacteria and virus by secretion of cytokines (INF-γ, TNF-α, IL-12, etc) with mucosal granuloma formation	↑ INF-γ, TNF-α, IL-2, IL-12 in CD (9,41,50)
Th2	regulate humoral immunity (IgA) and immunity against extracellular parasites by producing IL-4, IL-5, IL-10, IL-13 (45)	↑ IL-5 + TGF-β + Smad7 in UC (9,50)
CD8 + T cells	Cytotoxic effect and subset with suppressor function on CD4 Th1 cells (53)	Lack of stimulation of suppressor CD8 + T cells (44)
Costimulatory molecules	Amplification of T cell activation	↑ expression of CD40 & CD40L in IBD (55)
Adhesion molecules	Receptor-ligand engagement for homing of specialized memory T cells into the GALT (57)	↑ MAD-CAM expression in venules of intestinal mucosa in IBD (55)
B cells	→ plasma cells : ↑ Igs (secretory IgA in gut). Most Ags activate B cells with help of CD4 + Th	

APC : Antigen-presenting cell ; GALT = gut-associated lymphoid tissue ; Igs = immunoglobulins ; Ags = antigens.

of suppressor CD8 + T cells by intestinal epithelial cells whereas CD4 + T cells were stimulated.

The role of *intra-epithelial lymphocytes* (IEL) is not yet determined, particularly in IBD (38,45). They express CD8 and predominantly the uncommon γδ T cell receptor (45). Hoang *et al.* (46) have shown previously that IEL isolated from patients with active UC and control patients could be activated by the human colonic epithelial cell line HT-29. The degree of activation was correlated to the HLA-DR expression by the HT-29 cells related to an IFN-γ like factor secreted by the IEL. There was no significant difference between UC patients and controls.

CD4+ helper T cells (Th) can be activated into effector T cells of the Th1 or Th2 types in normal immunity and activated in excess of the Th1, Th2 or atypical types in IBD and various auto-immune diseases. Since CD4 T cells are the target of human immunodeficiency virus (HIV), an important reduction of the CD4 T cells in peripheral blood is often associated with symptoms of the acquired immunodeficiency syndrome (AIDS). It is interesting to mention that in a patient with an 18-year history of Crohn's disease a complete remission has been observed after an infection with HIV (47). In another study of 4 patients with CD with HIV infection a stable remission of CD was observed over a follow-up period of 5 -8 years but 3 patients died of AIDS (48).

Th1 vs Th2 lymphocytes : excessive adaptive immune responses through effector cells and soluble molecules.

Th1 and Th2 cells form two distinct lineages of CD4 T cells, secreting different cytokines (Table 2). Th1 and Th2 responses are self regulatory, one type of response down-regulating the other type of response (49). In vivo, as already mentioned, IL-4 released from mast cells, NK T cells, γδ T cells basophils, eosinophils is the main driver for a Th2 response through the intermediary of DCs whereas IL-12 produced by DCs with the help of IFNγ provided by NK cells (36) is the main driver for a Th1 response (Fig. 1).

In Crohn's disease (CD) the cytokine profile reflects a Th1 response with increased production of INFγ, TNF-α, IL-2, IL-12 (9,41,50). In contrast ulcerative colitis (UC) does not fit clearly but more closely resembles a Th2 pattern (9,41,50). IL-4 is indeed not produced whereas IL-5 and TGF-β are secreted in excess (9). But increased production of TGF-β in UC has no anti-inflammatory nor immunosuppressive effects since it is associated with high levels of Smad7, an intracellular protein which interferes with TGF-β signal transduction in mucosal mononuclear cells (50). Blockade of Smad7 restores TGF-β normal signaling (51).When the mucosal immune system in patients predisposed to the development of CD is first exposed to an initiating antigenic stimulus, an overly aggressive cytokine mediated T cell response is mounted. Cytokines involved in innate immune responses such as TNF-α, IL-1, IL-6 and possibly IL-12 and IL-18 may play a key role in this phase (52). Activation of CD4 + T cells triggers the production of the effector cytokines involved in the adaptive immune response, already mentioned (Table 2). Novel cytokines such as TL1A and IL-18, IL-23, IL-27 and IL-31 may also contribute to the effector phase (52).

CD8 + T cells

CD8 + T cells are mainly cytotoxic against cells infected by viruses and provoke their apoptosis (1). But

there is a subpopulation of CD8 + T cells with suppressor activity, resembling to regulatory T cells and playing a role on suppression of autoimmunity in the periphery (53,54).

Costimulatory molecules

Major molecular receptor-ligand pairs are involved in T cell activation, downregulation, costimulation and amplification of immune responses. A two signal model of T cell activation initiates immune responses where the antigen constitutes the first signal and APC provides a second costimulatory signal. There are several costimulatory systems with the following molecules expressed on activated T cells : CD40L, ICOS, CD28, …which bind to their respective ligands provided by DCs : CD40, B7h, CD80/86, …(55,56). Interactions between CD40 and CD40L represent a major costimulatory system that amplifies the immune response and promotes inflammation. CD40 is expressed on the surface of immune and non immune cells ; CD40L is expressed on activated T cells and platelets (55). Interactions between CD40 and CD40L expressing cells have an impact on multiple biological phenomena directly relevant to intestinal inflammation : antibody production, activation of macrophages and dendritic cells, production of proinflammatory cytokines, chemokines, prostaglandins, proteolytic enzymes and upregulation of adhesion molecules.

There is objective evidence that the CD40/CD40L costimulatory pathway is activated in IBD tissues as demonstrated by a high expression of CD40 on DCs in the intestinal lamina propria (27) and a marked increase of cells expressing high levels of CD40L and sCD40L in the circulation (55). The contact of CD40L on T cells and platelets with CD40 on mesenchymal and endothelial cells enhances the production of chemokines and the expression of adhesion molecules that lead to recruitment of more T cells that become activated in the inflamed mucosa. This provokes a vicious cycle of cell interaction that promotes chronic intestinal inflammation (55).

Adhesion molecules

Multiple adhesion molecules belonging to different families mediate lymphocyte endothelial interactions. In order to allow different types of lymphocytes, including memory T cells, to migrate to specific sites in the gut-associated lymphoid tissue (GALT), lymphocyte adhesion to endothelial cells occurs in a series of stages regulated by sequential ligand-receptor interactions (57). There are many adhesion molecules (> 200) for T cells, B cells and other leukocytes.

Address codes are provided for gut homing of lymphocytes. Naïve T cells are captured in the postcapillary venules on the Peyers's patch by mucosal addressin cell adhesion molecule (MAdCAM-1) which binds the receptor L Selectin on the lymphocyte. This allows a chemokine, called secondary lymphoid chemokine (SLC) on the endothelium to activate the integrin $\alpha 4\beta 7$ leading to arrest of rolling via binding to MadCAM1 or ICAM1 (intercellular adhesion molecule) (57). Effector memory T cells use the integrin $\alpha 4\beta 7$ and MadCAM-1 for capture on mucosal endothelium in the venules of the small bowel and large bowel (Fig. 2). The chemokine signal for activation is the chemokine receptor CCR9 in the small bowel and CCR10 in the colon for the respective ligands : chemokine TECK (CCL25) and chemokine MEC (CCL28) The next step is firm adhesion (arrest) of the lymphocyte to the endothelial cell, then migration into the endothelial monolayer with the help of integrin $\alpha 4\beta 1$ ligated by vascular cell (57). In IBD, MAdCAM-1 is strongly expressed in the venules of the intestinal mucosa, which enhances inflammation (55).

B cells

B cells activated by contact of the antigens with their B-cell receptor (surface Ig) are transforming into plasma cells which secrete great amounts of Igs, particularly IgA in the gut secretions. IgA is secreted under the form of a dimer, bound together by a J chain and coated with a specialized glycoprotein (secretory component) which renders the complex resistant to intraluminal proteases and acid. Since IgA does not activate complement, it does not induce inflammatory reactions and it prevents infection by agglutinating microbial surface molecules (45). Some antigens such as polysaccharides and polymerized flagellin can stimulate directly B cells but most of the antigens need the help of CD4 T cells (1). This is made through internalisation of the antigen by the B cell and presenting the corresponding peptide with a MHC class II molecule to a CD4 T cell, which expresses a costimulatory molecule, CD 40L ligating CD40 on B-cell, which stimulates also the switching of immunoglobulin class (IgM transformed into IgA).

Innate and adaptive immune interaction

Gut bacterial antigens cross the epithelium. The bacterial antigens induce lamina propria antigen presenting cells (APC) to release IL12 that drives a Th1 immune response producing IFN-γ and TNF-α. The immunopathology is not due to direct action of cytokines on the gut but to activation of residual mucosal fibroblasts by cytokines and increased expression of matrix degrading enzymes (metalloproteinases) that digest the stromal tissue. Cytokine action also changes epithelial barrier function and leads to recruitement of neutrophils from the circulation which amplify tissue damage and migrate transepithelially, further increasing permeability and uptake of bacteria from the lumen (25).

Defects in regulatory mechanisms in adaptive immune response. Immunity versus immunotolerance

Cells from the adaptive immunity of the gastrointestinal associated lymphoid tissues (GALT) allow

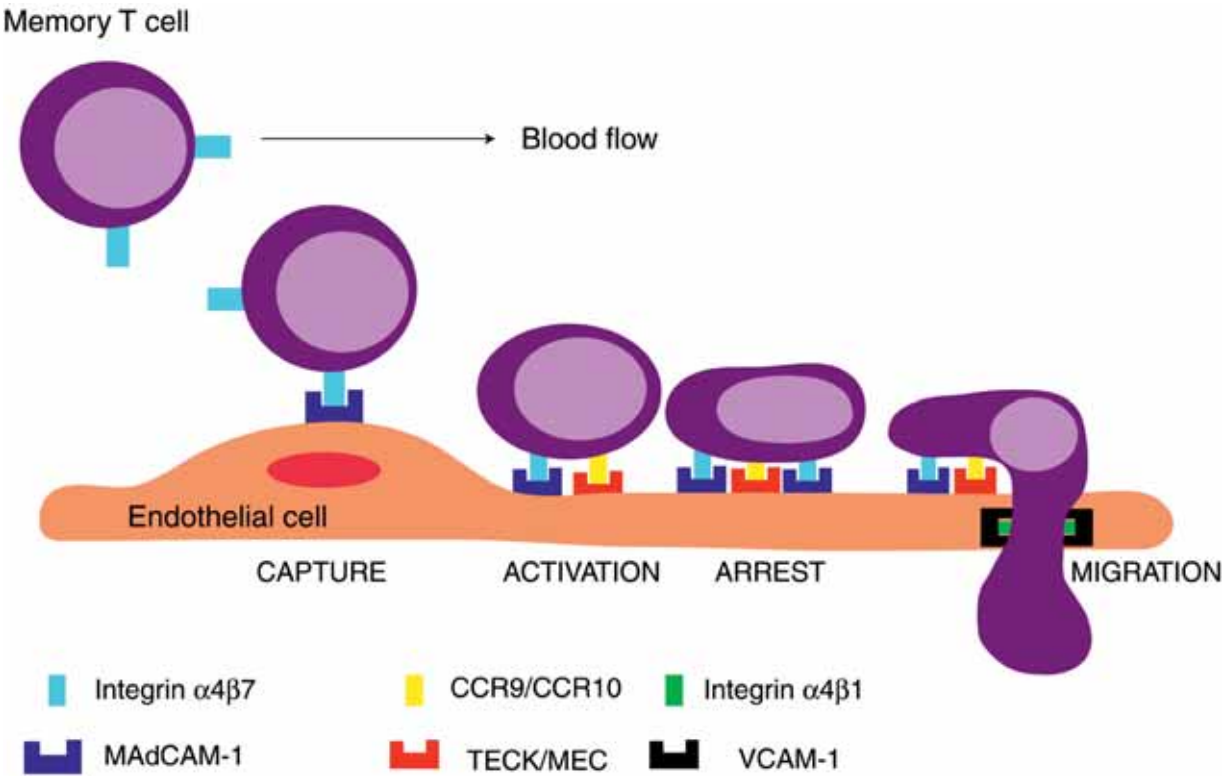

Fig. 2. — Successive steps of memory T cell homing through lymphocyte-endothelial adhesion molecules in the venules of the intestine, after Adams (2003). After free flowing in the bloodstream T cells with expression of the receptor integrin α4β7 are captured on the endothelial cell by the ligand MAdCAM-1 (Mucosal addressin adhesion molecule 1). Thereafter T cells are activated by the interaction between the chemokine receptor CCR9 in the small bowel venules or CCR10 in the large bowel vessels and the ligand chemokine TECK (CCL25) in the small bowel venules or MEC (CCL28) in the large bowel venules . After a change of conformation, with more interactions between adhesion molecules, the lymphocyte becomes arrested . Thereafter, with the help of the integrin α4β1 ligated to VCAM-1 (vascular cell adhesion molecule 1) the lymphocyte migrates into endothelial monolayer and into tissue towards the site of inflammation following a chemotactic gradient of chemokine signal.

oral tolerance and protective responses to invading microbial pathogens and neoplastic cells. The mucosal immune response has the unique characteristic of secretion of antibodies that complex antigen in the lumen without activating complement (secretory IgA) and of induction of tolerogenic T lymphocytes that maintain controlled local response to commensal bacteria. TGF-β, beside numerous anti-inflammatory effects, is necessary for IgA production (45).

Role of dendritic cells (DCs)

The pivotal role of DCs in innate and adaptive immunity has been described in the preceding sections. Reviewing recent studies on mouse and human DCs, Shortman and Liu (26) have proposed that the nature of response of DCs, either toward immunity or toward tolerance, depends more on their stage of activation in presence or not of danger signals from microbial infection or damaged tissue, than on the type of their subset. In the absence of danger signals, quiescent DCs maintain a state of peripheral T-cell tolerance to self antigens or induce the transformation of naïve T cells into regulatory T cells through IL-10 or TGF-β (Fig. 1).

Regulatory T cells

In a normal healthy individual T regulatory cells (Tr) make up approximately 5%-10% of T cells in peripheral lymphoid organs (58). Table 3 gives the classification of the three types of Tr in relation with their origins, specific markers, differentiation factors and suppressive mechanisms.

Thymus derived innate regulatory T cells (CD4+CD25+Tr) are naturally occurring and control autoreactive T cells in vivo by direct contact (53). They express on their surface the molecule CTLA-4 (cytotoxic T-lymphocyte activation antigen) and after contact with CD80/86 (B7) on DCs under transcriptional control of IFN-γ, they induce the production of IDO (indolamine 2,3-dioxygenase) in DCs. IDO is an enzyme that exhibits modulatory activity on T cells (apoptosis) by catabolism of tryptophan, an essential aminoacid for cellular proliferation. IDO is under the regulation of IFN-γ and can be induced in the presence of IFN-α from DCs or other sources. Prostaglandin E2 (PGE2) has been shown to downregulate DC immunostimulatory function through increased production of IL-10. PGE2 produced by DCs also downregulates

Table 3. — **Regulatory T cells with specific markers, differentiation factors and suppressive mechanisms depending on origins**

	Innate	Acquired	Acquired
	Thymic origin	Tr1	Th3
CD4	+++	+++	+++
CD25	+++	–	– → +++
FOXP3	+++	–	– → +++
CTLA-4	+++	+	++
Differentiation factors	?	IL-10	TGFβ, IL4
Suppressive mechanisms	Cellular contact	IL-10	TGFβ

leukotriene B4, IL-12 and MHCII, resulting in down-regulation of immune responses (25).

The gene Foxp3, a transcriptional regulator (repressor) has been identified as the master control gene for the development of Tr, defining a distinct lineage (58). CD4+CD25+Tr express high levels of Foxp3, which induces their suppressor phenotype and their expansion, following recent studies reviewed by Beissert (53). In a model of colitis induced in adult tge26 mice by transplantation of syngeneic bone marrow, Faubion *et al.* (59) observed a complete destruction of the rudimentary thymus and disappearance of the CD4 + CD25 + Tr. However if colitis was prevented by anti-TNF-α or two other immunological treatments, the thymic architecture was restored as well as the production of CD4 + CD25 + Tr. The negative impact of colitis on Tr development in the thymus was confirmed in a second experimental model of colitis in mice (59).

Peripheral Tr called Tr1, have been discovered by Groux *et al.* (60) in a model of murine colitis. They were generated by repetitive stimulation of CD4 + TCR transgenic T cells with their cognate peptide in the presence of Il-10. They can inhibit the development of colitis by exerting antigen-driven bystander suppression (53). Human Tr1 produce IL-10 with little or no IL-4 (25). They are controlling autoimmune reactions (53).

Peripheral Th3 cells are defined as primarily TGF-β producers with various amounts of IL-10 and IL-4 (25). Like Tr1, they mediate suppression of proliferative T cell responses (25) and suppress autoimmunity (53).

Serrate1, a ligand for Notch1, can transform peripheral naïve CD4 T cells into Tr. Suppression of cellular proliferation through transfer of Serrate1-induced Tr is antigen specific and induces downregulation of IL2 and IFN-γ in responding T cells. IL10, TGF-β and IFN-α have all been implicated in the induction of T cells with regulatory properties (25).

Apoptosis

Various outcomes result from the balance between T cell proliferation and apoptosis in the normal intestinal mucosa or imbalance during chronic intestinal inflammation. In health, mucosal T cell proliferation induced by dietary and enteric flora antigens is counterbalanced by a baseline degree (normal) of apoptosis resulting in the low degree of physiological inflammation found in the normal gut. In CD there is an increased proliferation of T cells induced by still undefined stimuli from bacterial or food antigens and possibly unrecognised pathogens. At the same time, T cells die less because of their resistance to apoptosis. This resistance is due to trans-signalling by IL-6 and contributes to accumulation of activated T cells and chronicity of the disease (41). This defective apoptosis results in inappropriate T cell accumulation that fosters inflammation. If the degree of T cell apoptosis is increased by infliximab a state of controlled inflammation is established (61). An in-depth review of apoptosis in IBD with its therapeutic impact, corresponding also to the meeting of the Belgium IBD Research Group is published in this issue (62).

The pathogenesis of IBD is multifactorial : genetic factors, environmental factors associated to immune and inflammatory responses. Hypotheses

Environmental (smoking in CD, luminal flora, NSAIDs, hygiene ?, pollution ?, stress ? ...) and genetic factors (NOD/CARD15, TLR-polymorphisms, etc) in combination with normal or pathogenic enteric microorganisms induce the activation of the intestinal immune and non immune systems. Both systems interact through release of soluble mediators and expression of cell adhesion molecules. This results in the production of antibodies, cytokines, growth factors, eicosanoids, neuropeptides, reactive oxygen metabolites, nitric oxide and proteolytic enzymes which mediate inflammation and tissue damage (63).

The integrity of the barrier may be compromised by genetic variations in key molecular determinants, a diminished reparative response to injury, or exogenous agents such as NSAIDs, with increased intestinal permeability. Chronic recurrent intestinal inflammation may result from stimulation of the mucosal immune system by products of commensal bacteria in the lumen : penetration of the bacterial products through the mucosal barrier and interaction with activated dendritic cells or other antigen-presenting cells and T cells. Alternatively, bacterial products may stimulate the surface epithelium through receptors of the innate immune system : toll-like-receptors and intracytoplasmic receptors such as NOD2/CARD15 ; the epithelium can in turn produce cytokines and chemokines that recruit and activate immune cells.

The Th1 activation induces a self sustaining cycle of activation with macrophages which produce cytokines promoting the Th1 response as well as costimulatory and adhesion molecules which target a broad variety of cell types including endothelial cells, which facilitates

recruitment of leukocytes, as well as fibroblasts, to the mucosa and the epithelium, modulating their functional properties. These functions may be altered by genetically determined variants (NOD2/CARD15, etc) or by environmental factors.

Th1 and Th2 pathways may be inhibited by regulatory T cells (Tr) and the regulatory cytokines IL10 and TGF-β. In CD counter-regulatory mechanisms and interactions fail when dendritic cells or antigen-producing cells sensing gut bacteria generate mainly IL12 thereby driving a Th1 response. IBD may thus be considered to be induced either by excess T cell effector function or inadequate regulatory Tr function. Following the study of Faubion *et al.* (57) already mentioned, there is a novel concept that the effector T cells which drive colitis might negatively regulate the development of Tr in the thymus. An initial insult in the colon (intestinal infection ?) in a genetically susceptible host destined to develop IBD could initiate a process that starts with colitis and is followed by alterations in thymic function and inhibition of Tr generation leading to perpetuation of colitis with clinical relapses, characteristic of CD or UC (58).

If many pathogenic mechanisms explaining the amplification of the inflammatory cascade have been elucidated, the initial trigger of the reactions is still unknown in CD and UC. The pathogenic role of elements of the intestinal flora is likely, on the basis of many experimental models of colitis. Colitis cannot generally be induced in germ-free animals. However, in a model of spontaneous ileitis resembling CD in the mouse strain SAM-1/YitFC, an increase of epithelial permeability, restricted to the ileum, has preceded the onset of ileitis, which was not related to colonisation with commensal bacteria (64). But in CD patients, many studies reviewed by Shanahan (41) suggest the pathogenic role of the enteric bacteria flora, especially the observations of the diversion of the faecal stream preventing recurrent lesions of CD, which reappear after restoration of the intestinal continuity. Moreover the normal tolerance towards resident intestinal flora is broken in IBD, as demonstrated by Duchmann *et al.* (65).

However it is not excluded that one or several specific micro-organisms normally present or acquired in the gut might be the trigger factor challenging the innate immune system through danger signals provoking a production of IL-12 or IL-4. This could explain that environmental factors play a role, as suspected in twin studies in CD : concordance of CD in only 44% of monozygotic twins (41) and report of conjugal cases of IBD (66). An important proportion of patients with CD have antibodies against mannan, a component of the yeast *Saccharomyces cerevisiae,* responsible of production of anti-*S. Cerevisiae* antibodies (ASCA), which are not related to the activity of the disease. Seibold *et al.* (67) have demonstrated that serum mannan-binding lectin (MBL), a component of innate immunity which binds *S. cerevisiae,* was significantly lower in a group of ASCA-positive CD patients than in ASCA-negative patients. This was associated with mutations of the MBL gene. Israeli *et al.* (68) have analysed sera of members of the Israeli military recruits stored since 1980 and have found that ASCA was present in 10/32 CD patients a long time before the clinical diagnosis of CD and absent in 95 controls and 8 UC patients. These two studies (67,68) point to the association of a genetic factor with an environment factor. Seibold (69) suggested that ASCA might be "a marker of an immune response to an environmental antigen that occurs in the context of an early stage of the disease". It was already mentioned that *Candida albicans* in its yeast stage stimulates production of IL-12 whereas in its hyphal stage it stimulates IL-4 production (42). It is interesting to mention a recent study of Yamaguchi *et al.* (70) reporting that gastrointestinal *Candida* colonisation promotes sensitisation against food antigens by affecting the mucosal barrier in mice. In search of intestinal antigens provoking experimental IBD, it was demonstrated that multiple strains of colitic mice had antibodies against flagellin, a common bacterial antigen present on most motile bacteria in the gut (71). Surprisingly, patients with CD had frequently IgG antibodies against flagellin (Cbir1) but not UC patients The presence of this antibody was associated with complicated CD (72). The role of *mycobacterium paratuberculosis* (MAP), responsible of an epidemic colitis in cattles (Jones'disease) has been hypothesised since a long time but the studies were not conclusive for its aetiologic role, despite the frequent presence of antibodies against this bacterium, like antibodies against other gut bacteria. Despite a study demonstrating by polymerase chain reaction (PCR) the presence of the IS9OO insertion element of MAP in 52% of CD resected tissues versus 2% in UC specimens (73), the controversy persists and more studies are needed to rule out the role of this bacterium, frequently present in the environment (74). Recently a new hypothesis has been proposed : a mimicry between MAP and intestinal proteins (75).

New therapeutic perspectives

Advances in the knowledge of gut innate and adaptive immunity related to IBD have promoted the introduction of new therapeutic agents that will be reviewed briefly without discussing in details their respective indications and side effects Their efficacy in CD and UC has been assessed by randomized controlled trials (RCTs).

Agents related to gut innate immunity

Reinforcing the mucus barrier has been the goal in treating UC patients with *phosphatidylcholine* (PC), the main phospholipid of mucus, in the form of an oral retarded released PC rich preparation. A RCT has demonstrated that the remission rate after 3 months was

significantly higher with PC than after placebo in a series of patients with chronically active UC (76). *Agonists of PPARγ* have reduced inflammation in experimental colitis ; 5-aminosalicylic acid (5-ASA) is a ligand of PPARγ, which explains its anti-inflammatory effect in IBD, particularly in UC (7). Other more potent agonists of PPARγ are in development for trials in IBD. *Sargramostim*, *a granulocyte-macrophage colony-stimulating factor* (GM-CSF) stimulates cells of the intestinal innate immune system (epithelial cells, macrophages, granulocytes). A RCT in patients with moderate-to-severe CD has shown that the drug (6 mg/day) administered subcutaneously during 2 months did not induce more frequently a clinical response characterised by decrease of 70 points in the Crohn's disease Activity Index (CDAI) in comparison with placebo. However there were significantly more remissions in the patients treated by the GM-CSF than by the placebo (77). *Inhibition of NF-ϰB* is a recently discovered mechanism of action of sulfasalazine (78). *Modulation of dendritic cells by helminths* is possibly the mechanism of action of this new type of therapy, as related in this issue (79,80).

Agents related to gut adaptive immunity

Beside immunosuppressive agents, such as azathioprine and its active metabolite 6-mercaptopurine, methotrexate, cyclosporine, used since a long time in order to inhibit T-cell function, *agents neutralizing specific cytokines secreted by Th1 c*ells have been introduced since the nineties. The prototype of the *agents against TNF-α* has been the monoclonal antibody *infliximab* whose efficacy for severe CD has been confirmed by several RCTs (81), as well as its efficacy for severe UC following recent RCTs (82). New anti-TNF antibodies have been introduced and their efficacy confirmed by RCTs : *adalimumab* (83) and *certolizumab pegol* (84). An *anti-interferon γ antibody, fontolizumab* has been found efficient in clinical response of active CD versus placebo after 56 days, if the drug was administered at day 0 and 28, following a recent RCT (85). Another RCT was negative for clinical response although there was a significant decrease of the endoscopic index of severity and serum CRP in the patients treated by fontolizumab versus placebo (86). *An anti-IL-12 monoclonal antibody* has been evaluated by a RCT for active CD and found efficient in clinical response versus placebo after 7 weeks of continuous treatment (1 subcutaneous injection per week). However the difference in response rates was no longer significant at 18 weeks of follow-up (87). *Agents neutralizing specific adhesion molecules or chemokine receptors* and selectively blocking lymphocyte recruitment into the gut are promising. *Natalizumab*, which blocks the integrin α4β7 was evaluated in active CD by a recent RCT that demonstrated a significant clinical response versus placebo after 8 and 12 weeks of treatment (88). An *inhibitor of the chemokine receptor CCR9* expressed by mucosal-homing leukocytes in the intestine has been evaluated by a Phase 2 RCT in active CD with encouraging results, in particular a significantly more rapid reduction of the activity index CDAI in comparison with placebo (89). *Removing proinflammatory cells : granulocytes and monocytes by adsorptive apheresis for severe UC* has been evaluated in a multicentre RCT in comparison with high dose steroid and demonstrated effective (90).

Agents influencing regulatory mechanisms comprised regulatory dendritic cells, already mentioned, and regulatory T cells. Their direct transfer in IBD patients has not yet be attempted at our knowledge but *helminth treatment* is perhaps an indirect way to stimulate the production of both types of regulatory cells (80). The *regulatory cytokine IL-10* under the form of a recombinant human IL-10 (rhuIL10) has been evaluated by two RCT in CD patients, with clinical and endoscopic improvement observed in a minority of patients (23.5%) in one trial (91) and negative results in the other trial (92). *Induction of apoptosis* of activated T cells and macrophages by various drugs (sulfasalazine, 5-ASA, infliximab, etc) is discussed in the article of Verstege *et al.* (62) in this issue. Finally resetting the immune system by *autologous hematopoietic stem cell transplantation* has been proposed in refractory CD Among 12 patients treated by this procedure which was well tolerated, 11 had a sustained remission (CDAI < 150) after a median follow-up of 18.5 months. Only one patient developed a recurrence of CD after 15 months (93).

Conclusions

Numerous studies have indicated that besides multiple susceptible genes recently discovered and environmental factors still unknown except smoking in CD, many precise abnormalities of innate and adaptive immunity may play a role in IBD pathogenesis. The increasing knowledge of these mechanisms allows a pathophysiological-based therapeutic approach (50), with increasing use of target-drugs rather than empiric therapy. However the potential side-effects of some potent drugs and the high placebo response rate revealed by RCTs still require good clinical judgement.

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