Intestinal epithelial cells as a source of inflammatory cytokines and chemokines

Andrew W Stadnyk PhD

AW Stadnyk. Intestinal epithelial cells as a source of inflammatory cytokines and chemokines. Can J Gastroenterol 2002;16(4):241-246.

The intestinal epithelium has long been known to provide non-specific defences such as mucus, lysozyme and transport of secretory immunoglobulin via the polyimmunoglobulin receptor. In the past decade, the realization emerged that enterocytes secrete molecules (cytokines) that regulate inflammation. As the focus tightened on this new role as sentinel, so has the interest in enterocyte production of cytokines with chemoattractant properties for leukocytes – the chemokines. Neutrophils are a prominent feature of the cellular infiltrate in various inflammatory diseases, and early reports indicated that epithelial cells secrete neutrophil chemoattractants. More recently, it has been shown that the cells also secrete chemokines for monocytes and lymphocytes. Some of these chemokines appear to be important in the uninflamed intestine but become increased during disease. While a great deal of knowledge has been gained regarding the circumstances leading to chemokine production by epithelial cells, the application of this understanding to the treatment of human intestinal diseases is lacking. Closing this gap is necessary to take advantage of emerging therapies aimed at blocking chemokine function.

Key Words: *Chemokines; Cytokines; Intestinal epithelial cells*

Cellules épithéliale intestinales : source de cytokines inflammatoires et de chimiokines

RÉSUMÉ : C'est connu depuis longtemps, l'épithélium intestinal est source de défenses non spécifiques comme le mucus, le lysozyme et le transport des immunoglobulines sécrétoires par la voie des récepteurs des polyimmunoglobulines. Au cours de la dernière décennie, on a découvert que les entérocytes sécrètent des molécules (cytokines) qui régulent l'inflammation. L'attention s'étant portée sur ce nouveau rôle de sentinelle, la recherche s'est vivement intéressée à la production de cytokines par les entérocytes, dotées de propriétés chimiotactiques à l'égard des leucocytes; ce sont les chimiokines. Les polynucléaires neutrophiles sont des constituants importants de l'infiltrat cellulaire dans diverses maladies inflammatoires et, selon des rapports préliminaires, les cellules épithéliales sécréteraient des facteurs chimiotactiques neutrophiles. Plus récemment, il a été démontré que les cellules sécrètent également des chimiokines à l'égard des monocytes et des lymphocytes. Certaines de ces chimiokines semblent jouer un rôle important dans l'intestin sain, mais leur activité s'intensifie au cours du processus morbide. Tandis que le bagage de connaissances sur la production de chimiokines par les cellules épithéliales s'est considérablement accru, l'application de ces nouvelles connaissances aux maladies intestinales chez l'homme a trouvé peu d'écho. Il est nécessaire de combler le fossé entre les deux si l'on veut tirer profit des nouvelles thérapies visant à bloquer le fonctionnement des chimiokines.

The intestinal epithelium is a remarkably complex structure, with constant renewal from stem cells deep in the crypt. These cells differentiate while rising up and away from the deep crypt, interact with a particular population of lymphocytes, then die at the villus tip or the crypt surface in the small and large intestine, respectively. While there are plenty of unexplored aspects of this cycle, the science of intestinal epithelial cell (IEC) biology has been preoccupied for the past decade with the new awareness that these cells play an important role in the inflammatory response.

Departments of Pediatrics, and Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia
Correspondence and reprints: Dr A Stadnyk, Mucosal Immunology Research Laboratory, IWK Health Centre, 5850 University Avenue, Halifax, Nova Scotia B3J 3G9. Telephone 902-470-8491, fax 902-470-7217, e-mail Andrew.Stadnyk@Dal.Ca
Received for publication February 11, 2002. Accepted February 14, 2002

The understanding is that IECs secrete a constellation of cytokines, which might even be the apex of the inflammatory cascade. The term 'cytokine' is a generous reference to many families of soluble regulating agents of inflammation and immunity, and includes the interleukins (ILs), tumour necrosis factors (TNFs), interferons (IFNs), transforming growth factors (TGFs) and chemokines, to mention a few. The present article touches on the history behind the search for IEC cytokines, reviews the recent work on chemokines and comments on where the science needs to be applied to make greater gains in understanding human disease.

IECs AS SOURCES OF CYTOKINES

One of the earliest published reports of IECs producing proinflammatory cytokines was from the laboratory of Serge Jothy, who used immunohistochemistry to show that scattered cells in the human colon and the small intestine stained for IL-6 and the IL-6 receptor (1). This observation was followed by a series of papers describing IL-6 production by the well known nontumorigenic rat small intestinal cell line IEC-6 (2,3). In a peculiar contrast, tumorigenic human IEC lines, including T84, HT-29 and SW620, do not produce IL-6 (4), with Caco-2 being the exception (5,6). Nevertheless, these seminal reports were followed by the observations that IECs produce IL-7, stem cell factor, IL-10, IL-15, IL-18 and, as discussed below, a formidable number of chemoattractants (reviewed in 7-9). Over the past decade, the laboratories of Podolsky and Kagnoff made considerable contributions to the growing list of IEC cytokines, using combinations of IEC lines, freshly isolated cells and in situ detection. Reinecker and Podolsky (10), and Reinecker et al (11) also identified an abundance of cytokine receptors on IECs, inferring that many have the potential for autocrine activity. Eckmann et al (12) focused on cytokines elicited by infection with bacteria, including TNF-α, granulocyte monocyte colony stimulating factor and various chemokines (4,12,13).

The idea that IECs produce cytokines certainly predates 1990. For example, Kurokowa et al (14), and Koyama and Podolsky (15) reported that IECs produce TGF-α and TGF-β, and that both of these molecules affect the normal biology of epithelial cells. The threshold of 1990 merely marks the heralding of proinflammatory cytokine expression by IEC.

My colleagues' and my interest in the intestinal sources of proinflammatory cytokines began with the observation that two different intestinal helminth infections in rats led to strikingly different acute phase responses (16). IECs were subsequently discovered to produce IL-1β early in the course of *Trichinella spiralis* infection (17). Indomethacin-induced small intestinal injury in rats has also been discovered to cause IECs to express IL-1β (18). While these findings complemented those of other reports showing that rat (19) and mouse (20) colonocytes produce IL-1β, this is only observed infrequently in human biopsies (21,22) and freshly isolated cells (23,24), and some investigators have declared that human cells do not produce IL-1β (25,26). The difference may be in the stage of human disease at presentation, because IL-1β expression in all the rodent examples was quite early and transient. It is noteworthy that the related molecule IL-18 (originally described as 'IFN-inducing factor'), which undergoes similar post-translational processing as IL-1β to become biologically active, is detectable in human IECs (27,28).

The rat IEC-18 line was used to model IL-1β expression in vitro, and cell detachment was discovered to be the only treatment capable of inducing this cytokine (29). While confirming that the IL-1β protein was translated in the IEC-18 cultures, the 'decoy' form of the IL-1 receptor, the IL-1RII, was also found to be expressed following detachment (29). The IL-1RII, first described on neutrophils and monocytes, binds IL-1β with greater affinity than does the IL-1RI (the signalling receptor) but does not impart an intracellular signal. The IL-1RII also may be shed by cleavage of the extracellular domain, releasing a soluble fragment that retains the capacity to bind IL-1β. This novel observation of IEC expression of the IL-1RII was validated in the helminth (30) and indomethacin rodent models (18). Whether the IL-1RII is produced by IEC during human disease has yet to be determined, but the T84 and HT-29 cell lines do express and shed this receptor (unpublished observations).

Detaching anchorage-dependent cells such as IECs leads to apoptosis. (Cancer can result from a process by which detached cells overcome apoptosis.) In view of the fact that detachment induces the production of IL-1 and the IL-1RII, whether IL-1 plays any role in the fate of detached cells was investigated. Tipping the balance between the endogenous molecules, either by blocking the IL-1RII using antibodies or by adding IL-1, determined that IL-1 was antiapoptotic (31). This result may suggest a local role for IL-1 in preserving the integrity of the epithelial monolayers during disease. IL-1 is a potent stimulus for the expression of other cytokines, in particular chemokines, and we also explored chemokine expression in the rodent models (18,30).

CHEMOKINE BIOLOGY

The inflammatory responses in various intestinal illnesses such as inflammatory bowel disease (IBD), hypersensitivities, graft-versus-host disease, infection and necrotizing enterocolitis, among others, are all characterized by the presence of leukocytic infiltrates. It is widely appreciated that the inflammation would be tempered if the infiltrate, particularly that of neutrophils, were blocked. The two elements critical to infiltration are the chemoattractants that direct the recruitment, and specific adhesion molecules that tether the leukocyte, first to the endothelium, then to the matrix and possibly to the epithelial cells. While there are a number of different classes of chemoattractants, such as split components of complement and some leukotrienes, knowledge of the IEC-derived chemokines has expanded rapidly in the past few years. These chemokines have chemoattractant properties for leukocytes.

Chemokines are grouped into four families based on the pattern of conserved amino terminal cysteines. The cysteines are separated by a variable amino acid in the CXC, or alpha-chemokine family, while the cysteines are juxtaposed in the CC, or beta-chemokine family. The CXC chemokines are further divided into two groups – one group has a glutamic acid-leucine-arginine (ELR) motif and attracts neutrophils. The non-ELR-containing CXC and CC chemokines are mononuclear cell and eosinophil chemoattractants. Together, the CC and CXC families constitute most of the more than 50 chemokines. The remaining two families each have a single member. The CX3C chemokine is 'fractalkine', and this molecule is reportedly produced by IECs (32). The final family is the C chemokine, lymphotactin (among other names).

The receptors for all chemokines are similar in that they weave through the plasma membrane seven times and are associated with intracellular G-protein signalling mechanisms. This mutual signalling strategy makes it difficult to understand how fidelity is achieved because any given chemokine is often able to bind more than one receptor. More complexity became evident when different laboratories simultaneously reported that a single chemokine had different functions. To overcome this confusion, a new nomenclature has been created that acknowledges the cysteine pattern, followed by 'L' for ligand or 'R' for receptor, then a number; for example, IL-8 is 'CXCL8', and fractalkine is 'CX3CL1'. More details of chemokine biology can be found in a recently published review (33).

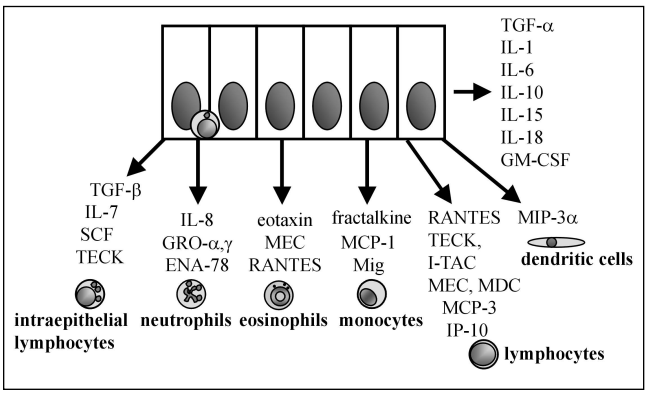

Figure 1) *Summary of the cytokines and chemokines produced by intestinal epithelial cells. The usual target of each chemokine is shown, although in some cases the same chemokine may act on more than one cell type. The figure includes constitutive and induced cytokines. ENA Epithelial-derived neutrophil-activating peptide; IL Interleukin; IP-10 Interferon-inducible protein-10; I-TAC, Interferon-inducible T cell chemoattractant; GM-CSF Granulocyte macrophage-colony stimulating factor; GRO Growth-related oncogene; MDC Macrophage-derived chemokine; MEC Mucosa-associated epithelial chemokine; Mig Monokine induced by interferon-gamma; MCP Monocyte/macrophage chemotactic peptide; MEC Mucosa-associated epithelial chemokine; RANTES Regulated upon activation, normal T cell expressed and secreted; SCF Stem cell factor; TECK, thymus-expressed chemokine; TGF Transforming growth factor*

IECs AS SOURCES OF NEUTROPHIL CHEMOKINES

While it is not incontrovertible, the premise is that IECs provide the signal for the initial wave of leukocyte recruitment during disease, which often includes neutrophils. Accordingly, one of the earliest reported chemokines produced by IECs was the neutrophil chemoattractant IL-8 (12,34) (Figure 1). These reports were followed by observations that human IECs secrete IL-8 directly as a consequence of infection by various bacteria (4) or *T spiralis* (35), following exposure to bacterial flagellin (36) and toxins (37), and following exposure to a number of cytokines such as IL-1 and TNF-α (12,38). One study reported that the epithelial cells in a human fetal small intestinal xenograft transplanted into immunodeficient mice produced IL-8 (and IL-1β) following infection with *Entameba histolytica* (39). The rodent IL-8 homologue macrophage inflammatory peptide-2, was detectable in cells recovered from both *T spiralis*-infected and nonsteroidal anti-inflammatory drug-injured rat small intestinal epithelium (18,30). Despite this persuasive evidence for the production of IL-8 from IELs, it is disputed whether IL-8 is detectable in the epithelium in IBD, and it is still asserted that mononuclear cells are a more important source (40,41). Perhaps IEC expression of IL-8 is unique to patients with early disease (levels are related to histological grade [42]), and such patients are infrequently available for studies. On the other hand, IL-8 is not the only neutrophil chemokine that is present in the inflamed bowel; therefore, other chemoattractants may account for the presence of neutrophils.

A second IEC-derived CXC chemokine, with consistent in situ evidence, is epithelial-derived neutrophil-activating peptide (ENA)-78 (43,44). Data from human cell lines indicated that ENA-78 is produced relatively late (8 h) compared with IL-8 following stimulation with IL-1 or TNF-α (13). The significance of this order of events in vitro is unclear considering the chronicity of IBD, except to suggest that stimulation may be indirect, ie, IL-1 stimulates a molecule that, in turn, is responsible for the ENA-78 gene expression. Another CXC chemokine, growth-related oncogene-alpha, has been found to be elevated in the serum, mucosa and/or epithelium of IBD patients, and is a product of cell lines (13,45,46).

The redundancy in chemokines is bewildering but presumably has a purpose. Few studies have examined the same biopsy for all of these various CXC chemokines, and patients are not typically studied longitudinally; therefore, it is not entirely clear whether the expression of the different chemokines is mutually exclusive. There may be sequential recruitment of neutrophils, eg, one chemokine or chemoattractant may draw the cells out of the blood while a second chemokine operates to pull the neutrophils across the epithelium into the lumen. Determining whether chemokines are secreted apically or basolaterally by IEC is of paramount importance because presumably only chemoattractants in the lumen can attract cells across the epithelium. Indeed, the chemoattractants that are active in the

inflamed bowel lumen have not been well described. IL-8 and leukotriene B_4 have been detected in colonic dialysates (47,48), and bacterial products are also probably active, but none have been convincingly shown to lead the wave of neutrophils into the lumen.

SECRETION OF MONONUCLEAR CHEMOKINES BY IECs

Not all the CXC chemokines are neutrophil chemoattractants; the molecules lacking the ELR motif act to recruit mononuclear cells. Accordingly, the CXC chemokines 'IFN-γ-inducible protein-10' and 'monokine induced by IFN-γ' are reportedly produced by IECs (49,50) (Figure 1). CXC chemokine expression rarely occurs without concomitant CC chemokine expression, and many of the cytokines that increase CXC chemokine levels also increase CC chemokine levels. IECs secrete a variety of CC chemokines, in uninflamed and inflamed conditions. Soon after the description of IL-8, IECs were reported to secrete monocyte chemoattractant peptide-1 (MCP-1) (51,52), but as with IL-8, there are mixed reports as to whether MCP-1 is expressed in vivo (53,54). Clearly, the human and rat cell lines are potent sources of MCP-1 responding to various stimuli including lipopolysaccharide (in the rat), IL-1, TNF-α and intracellular infection (4,13,55-57). Successive reports have described lymphocyte chemoattractants that are important in the functioning of the normal intestine for recruiting lymphocytes (49); thymus-expressed chemokine is particularly important in the small intestine (58,59). Specific to the colon is a novel memory T lymphocyte and eosinophil chemoattractant called mucosa-associated chemokine (60). Macrophage-derived chemokine is produced constitutively by the epithelium and to a heightened degree in cell lines that are exposed to TNF-α or IL-1 (61). Other studies have shown constitutive or cytokine-mediated upregulation of RANTES (62,63), MCP-3 (64) and IFN-inducible T cell alpha chemokine (50). Eotaxin, an eosinophil chemoattractant, is elevated in the serum of patients with Crohn's disease (65), and the rat IEC line was shown to produce eotaxin when stimulated with IL-4 (66). With this burst of interest and outcomes, issues of redundancy and serial exposures need to be considered when consolidating knowledge obtained from separate studies into a working model applicable to human disease.

Finally, secretion by IECs of chemokines that recruit immature dendritic cells (DCs) may initiate the adaptive immune response. Immature DCs are the only accessory cells capable of effective antigen presentation to naive T lymphocytes, leading to a primary immune response. It was recently reported that DCs migrate into the epithelial layer and form tight junctions with IECs to sample luminal antigens (67). Some human cell lines spontaneously secrete low levels of the DC chemoattractant macrophage inflammatory peptide-3alpha (CCL20), but this secretion can be enhanced by stimulation with IL-1, TNF-α or flagellin (68,69). Flagellin, the principal protein constituent in bacterial flagella, was recently shown to bind the pattern recognition receptor toll-like receptor 5, and thus activate IEC to produce cytokines (70). Therefore, the IEC response to bacteria includes the recruitment of immature DCs that, in turn, process and present antigen.

CHEMOKINE RECEPTORS ON IECs

In an idiosyncratic twist in the biology of chemokines, IECs apparently express a number of chemokine receptors, and oddly, many of these occur on the apical surface. The list presently includes constitutive expression of CXCR4 on normal cells (71), and variable expression of CCR1 through CCR8 in cell lines (72). Other receptors expressed by IECs during inflammation are CXCR1 and, at a low level, CXCR2 (73). The position of the apical receptor immediately suggests two nonmutual possibilities – that IECs secrete the ligands (chemokines) apically and/or that IECs receive signals from leukocytes in the lumen. A third possibility is that chemokines diffuse through ulcerations into the lumen and then act on apical receptors. A clue as to which model is operative might be that leukocytes provide some chemokines, including neutrophils that express IL-8. Neutrophils also cross the epithelium into the lumen and can, therefore, be in a position to secrete chemokines that will act on apical IEC receptors.

ANTICHEMOKINE THERAPIES AND IECs

The precise role of chemokine receptors on IECs is not known, although there are reports that chemokines are chemotactic (74) and induce the production of other chemokines (72). Clearly, there is much to be learned, yet the finding of chemokine receptors on IEC provides hope that blocking these inflammatory mediators might have a greater impact than preventing leukocyte migration alone.

The idea of reducing inflammation by blocking chemokines was suggested several years ago (75). Clinical trials using chemokine-based therapies in intestinal diseases, however, have only recently begun (76). Knowing that IECs are among the cellular sources of cytokines may mean that therapies can be directed specifically at this cell type, possibly through the lumen. Yet, despite the accumulated knowledge that has been reviewed above, IEC chemokine expression needs to be explored systematically. Understanding issues such as redundancy, serial expression and chemokine-specific patterns in disease will be important in optimizing the blocking therapies as they become available.

ACKNOWLEDGEMENTS: The author's research is funded by the Natural Sciences and Engineering Research Council of Canada, Crohn's and Colitis Foundation of Canada, and the Nova Scotia Health Research Foundation.

REFERENCES

1. Shirota K, LeDuy L, Yuan S, Jothy S. Interleukin-6 and its receptor are expressed in human intestinal epithelial cells. Virchows Arch B Cell Pathol Incl Mol Pathol 1990;58:303-8.
2. McGee DW, Beagley KW, Aicher WK, McGhee JR. Transforming growth factor-β enhances interleukin-6 secretion by intestinal epithelial cells. Immunology 1992;77:7-12.

3. McGee DW, Beagley KW, Aicher WK, McGhee JR. Transforming growth factor-β and IL-1β act in synergy to enhance IL-6 secretion by the intestinal epithelial cell line, IEC-6. J Immunol 1993;151:970-8.
4. Jung HC, Eckmann L, Yang S-K, et al. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J Clin Invest 1995;95:55-65.
5. Michalsky MP, Deitch EA, Ding JY, Lu Q, Huang QH. Interleukin-6 and tumor necrosis factor production in an enterocyte cell model (Caco-2) during exposure to *Escherichia coli*. Shock 1997;7:139-46.
6. Vitkus SJD, Hanifin SA, McGee DW. Factors affecting Caco-2 intestinal epithelial cell interleukin- 6 secretion. In Vitro Cell Dev Biol Anim 1998;34:660-4.
7. Stadnyk AW, Waterhouse CCM. Epithelial cytokines in intestinal inflammation and mucosal immunity. Curr Opin Gastroenterol 1997;13:510-7.
8. Christ AD, Blumberg RS. The intestinal epithelial cell: immunological aspects. Springer Semin Immunopathol 1997;18:449-61.
9. Kagnoff MF, Eckmann L. Epithelial cells as sensors for microbial infection. J Clin Invest 1997;100:6-10.
10. Reinecker H-C, Podolsky DK. Human intestinal epithelial cells express functional cytokine receptors sharing the common gamma c chain of the interleukin 2 receptor. Proc Natl Acad Sci USA 1995;92:8353-7.
11. Reinecker H-C, MacDermott RP, Mirau S, Dignass A, Podolsky DK. Intestinal epithelial cells both express and respond to interleukin 15. Gastroenterology 1996;111:1706-13.
12. Eckmann L, Jung HC, Schürer-Maly C-C, Panja A, Morzycka-Wroblewska E, Kagnoff MF. Differential cytokine expression by human intestinal epithelial cell lines: regulated expression of interleukin 8. Gastroenterology 1993;105:1689-97.
13. Yang S-K, Eckmann L, Panja A, Kagnoff MF. Differential and regulated expression of C-X-C, C-C, and C-chemokines by human colon epithelial cells. Gastroenterology 1997;113:1214-23.
14. Kurokowa M, Lynch K, Podolsky DK. Effects of growth factors on an intestinal epithelial cell line: transforming growth factor β inhibits proliferation and stimulates differentiation. Biochem Biophys Res Commun 1987;142:775-82.
15. Koyama S-Y, Podolsky DK. Differential expression of transforming growth factors α and β in rat intestinal epithelial cells. J Clin Invest 1989;83:1768-73.
16. Stadnyk AW, Baumann H, Gauldie J. The acute-phase protein response in parasite infection. *Nippostrongylus brasiliensis* and *Trichinella spiralis* in the rat. Immunology 1990;69:588-95.
17. Stadnyk AW, Kearsey JA. Pattern of proinflammatory cytokine mRNA expression during *Trichinella spiralis* infection of the rat. Infect Immun 1996;64:5138-43.
18. Stadnyk AW, Dollard CD, Issekutz TB, Issekutz AC. Neutrophil migration into indomethacin-induced rat small intestinal injury is CD11α/CD18 and CD11β/CD18 co-dependent. Gut. (In press)
19. Radema SA, Van Deventer SJH, Cerami A. Interleukin 1β is expressed predominantly by enterocytes in experimental colitis. Gastroenterology 1991;100:1180-6.
20. Meijssen MAC, Brandwein SL, Reinecker H-C, Bhan AK, Podolsky DK. Alteration of gene expression by intestinal epithelial cells precedes colitis in interleukin-2-deficient mice. Am J Physiol 1998;274:G472-9.
21. Radema SA, Tytgat GNJ, Van Deventer SJH. In situ detection of interleukin-1β and interleukin 8 in biopsy specimens from patients with ulcerative colitis. Adv Exp Med Biol 1995;371B:1297-9.
22. Woywodt A, Ludwig D, Neustock P, et al. Mucosal cytokine expression, cellular markers and adhesion molecules in inflammatory bowel disease. Eur J Gastroenterol Hepatol 2000;11:267-76.
23. Jarry A, Vallette G, Cassagnau E, et al. Interleukin 1 and interleukin 1β converting enzyme (caspase 1) expression in the human colonic epithelial barrier. Caspase 1 downregulation in colon cancer. Gut 1999;45:246-51.
24. Lundqvist C, Melgar S, Yeung MM-W, Hammarström S, Hammarström M-L. Intraepithelial lymphocytes in human gut have lytic potential and a cytokine profile that suggest T helper 1 and cytotoxic functions. J Immunol 1996;157:1926-34.
25. Casini-Raggi V, Kam L, Chong YJT, Fiocchi C, Pizarro TT, Cominelli F. Mucosal imbalance of IL-1 and IL-1 receptor antagonist in inflammatory bowel disease. A novel mechanism of chronic intestinal inflammation. J Immunol 1995;154:2434-40.
26. Daig R, Rogler G, Aschenbrenner E, et al. Human intestinal epithelial cells secrete interleukin-1 receptor antagonist and interleukin-8 but not interleukin-1 or interleukin-6. Gut 2000;46:350-8.
27. Pizarro TT, Michie MH, Bentz M, et al. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. J Immunol 1999;162:6829-35.
28. Monteleone G, Trapasso F, Parrello T, et al. Bioactive IL-18 expression is up-regulated in Crohn's disease. J Immunol 1999;163:143-7.
29. Waterhouse CCM, Stadnyk AW. Rapid expression of IL-1β by intestinal epithelial cells in vitro. Cell Immunol 1999;193:1-8.
30. Stadnyk AW, Dollard CD, Issekutz AC. Neutrophil migration stimulates rat intestinal epithelial cell cytokine expression during helminth infection. J Leukoc Biol 2000;68:821-7.
31. Waterhouse CCM, Joseph RR, Stadnyk AW. Endogenous IL-1 and type II IL-1 receptor expression modulate anoikis in intestinal epithelial cells. Exp Cell Res 2001;269:109-16.
32. Muehlhoefer A, Saubermann LJ, Gu X, et al. Fractalkine is an epithelial and endothelial cell-derived chemoattractant for intraepithelial lymphocytes in the small intestinal mucosa. J Immunol 2000;164:3368-76.
33. Yoshie O, Imai T, Nomiyama H. Chemokines in immunity. Adv Immunol 2001;78:57-110.
34. Kelly CP, Keates S, Siegenberg D, Linevsky JK, Pothoulakis C, Brady HR. IL-8 secretion and neutrophil activation by HT-29 colonic epithelial cells. Am J Physiol 1994;267:G991-7.
35. Li CKF, Seth R, Gray T, Bayston R, Mahida YR, Wakelin D. Production of proinflammatory cytokines and inflammatory mediators in human intestinal epithelial cells after invasion by *Trichinella spiralis*. Infect Immun 1998;66:2200-6.
36. Steiner TS, Nataro JP, Poteet-Smith CE, Smith JA, Guerrant RL. Enteroaggregative *Escherichia coli* expresses a novel flagellin that causes IL-8 release from intestinal epithelial cells. J Clin Invest 2000;105:1769-77.
37. Thorpe CM, Smith WE, Hurley BP, Acheson DWK. Shiga toxins induce, superinduce, and stabilize a variety of C-X-C chemokine mRNAs in intestinal epithelial cells, resulting in increased chemokine expression. Infect Immun 2001;69:6140-7.
38. Schürer-Maly C-C, Eckmann L, Kagnoff MF, Falco MT, Maly F-E. Colonic epithelial cell lines as a source of interleukin-8: stimulation by inflammatory cytokines and bacterial lipopolysaccharide. Immunology 1994;81:85-91.
39. Seydel KB, Li E, Swanson PE, Stanley SL Jr. Human intestinal epithelial cells produce proinflammatory cytokines in response to infection in a SCID mouse-human intestinal xenograft model of amebiasis. Infect Immun 1997;65:1631-9.
40. Grimm MC, Elsbury SKO, Pavli P, Doe WF. Interleukin 8: cells of origin in inflammatory bowel disease. Gut 1996;38:90-8.
41. Brandt E, Colombel J-F, Ectors N, et al. Enhanced production of IL-8 in chronic but not in early ileal lesions of Crohn's disease (CD). Clin Exp Immunol 2000;122:180-5.
42. Mazzucchelli L, Hauser C, Z'Graggen K, et al. Expression of interleukin-8 gene in inflammatory bowel disease is related to the histological grade of active inflammation. Am J Pathol 1994;144:997-1007.
43. Keates S, Keates AC, Mizoguchi E, Bhan A, Kelly CP. Enterocytes are the primary source of the chemokine ENA-78 in normal colon and ulcerative colitis. Am J Physiol 1997;273:G75-82.
44. Z'Graggen K, Walz A, Mazzucchelli L, Strieter RM, Mueller C. The C-X-C chemokine ENA-78 is preferentially expressed in intestinal epithelium in inflammatory bowel disease. Gastroenterology 1997;113:808-16.
45. Brandt E, Müller-Alouf H, Desreumaux P, Woerly G, Colombel J-F, Capron M. Circulating growth-regulated oncogene α contributes to neutrophil priming and interleukin-8-directed mucosal recruitment into chronic lesions of patients with Crohn's disease. Eur Cytokine Netw 1998;9:647-53.
46. Imada A, Ina K, Shimada M, et al. Coordinate upregulation of interleukin-8 and growth-related gene product-α is present in the colonic mucosa of inflammatory bowel disease. Scand J Gastroenterol 2001;36:854-64.
47. Cole AT, Pilkington BJ, McLaughlan J, Smith C, Balsitis M, Hawkey CJ. Mucosal factors inducing neutrophil movement in ulcerative colitis: the role of interleukin 8 and leukotriene B_4. Gut 1996;39:248-54.

48. Nielsen OH, Gionchetti P, Ainsworth M, et al. Rectal dialysate and fecal concentrations of neutrophil gelatinase-associated lipocalin, interleukin-8, and tumor necrosis factor-alpha in ulcerative colitis. Am J Gastroenterol 1999;94:2923-8.
49. Shibahara T, Wilcox JN, Couse T, Madara JL. Characterization of epithelial chemoattractants for human intestinal intraepithelial lymphocytes. Gastroenterology 2001;120:60-70.
50. Dwinell MB, Lügering N, Eckmann L, Kagnoff MF. Regulated production of interferon-inducible T-cell chemoattractants by human intestinal epithelial cells. Gastroenterology 2001;120:49-59.
51. Reinecker H-C, Loh EY, Ringler DJ, Mehta A, Rombeau JL, MacDermott RP. Monocyte-chemoattractant protein 1 gene expression in intestinal epithelial cells and inflammatory bowel disease mucosa. Gastroenterology 1995;108:40-50.
52. Grimm MC, Elsbury SKO, Pavli P, Doe WF. Enhanced expression and production of monocyte chemoattractant protein-1 in inflammatory bowel disease mucosa. J Leukoc Biol 1996;59:804-12.
53. Mazzucchelli L, Hauser C, Zgraggen K, et al. Differential in situ expression of the genes encoding the chemokines MCP-1 and RANTES in human inflammatory bowel disease. J Pathol 1996;178:201-6.
54. Uguccioni M, Gionchetti P, Robbiani DF, et al. Increased expression of IP-10, IL-8, MCP-1, and MCP-3 in ulcerative colitis. Am J Pathol 1999;155:331-6.
55. Kucharzik T, Lügering N, Pauels H-G, Domschke W, Stoll R. IL-4, IL-10 and IL-13 down-regulate monocyte-chemoattracting protein-1 (MCP-1) production in activated intestinal epithelial cells. Clin Exp Immunol 1998;111:152-7.
56. Ohno Y, Lee J, Fusunyan RD, MacDermott RP, Sanderson IR. Macrophage inflammatory protein-2: chromosomal regulation in rat small intestinal epithelial cells. Proc Natl Acad Sci USA 1997;94:10279-84.
57. Waterhouse CCM, Joseph RR, Winsor GL, Lacombe TA, Stadnyk AW. Monocyte chemoattractant protein-1 production by intestinal epithelial cells in vitro: a role for p38 in epithelial chemokine expression. J Interferon Cytokine Res 2001;21:223-30.
58. Papadakis KA, Prehn J, Moreno ST, et al. CCR9-positive lymphocytes and thymus-expressed chemokine distinguish small bowel from colonic Crohn's disease. Gastroenterology 2001;121:246-54.
59. Kunkel EJ, Campbell JJ, Haraldsen G, et al. Lymphocyte CC chemokine receptor 9 and epithelial thymus-expressed chemokine (TECK) expression distinguish the small intestinal immune compartment: epithelial expression of tissue-specific chemokines as an organizing principle in regional immunity. J Exp Med 2000;192:761-7.
60. Pan J, Kunkel EJ, Gosslar U, et al. A novel chemokine ligand for CCR10 and CCR3 expressed by epithelial cells in mucosal tissues. J Immunol 2000;165:2943-9.
61. Berin MC, Dwinell MB, Eckmann L, Kagnoff MF. Production of MDC/CCL22 by human intestinal epithelial cells. Am J Physiol 2001;280:G1217-26.
62. Warhurst AC, Hopkins SJ, Warhurst G. Interferon gamma induces differential upregulation of alpha and beta chemokine secretion in colonic epithelial cell lines. Gut 1998;42:208-13.
63. Kolios G, Wright KL, Jordan NJ, Leithead JB, Robertson DA, Westwick J. C-X-C and C-C chemokine expression and secretion by the human colonic epithelial cell line, HT-29: differential effect of T lymphocyte-derived cytokines. Eur J Immunol 1999;29:530-6.
64. Wedemeyer J, Lorentz A, Göke M, et al. Enhanced production of monocyte chemotactic protein 3 in inflammatory bowel disease mucosa. Gut 1999;44:629-35.
65. Chen W, Paulus B, Shu D, Wilson I, Chadwick V. Increased serum levels of eotaxin in patients with inflammatory bowel disease. Scand J Gastroenterol 2001;36:515-20.
66. Winsor GL, Waterhouse CCM, MacLellan RL, Stadnyk AW. Interleukin-4 and INF-γ differentially stimulate macrophage chemoattractant protein-1 MCP-1 and eotaxin production by intestinal epithelial cells. J Interferon Cytokine Res 2000;20:299-308.
67. Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2001;2:361-7.
68. Izadpanah A, Dwinell MB, Eckmann L, Varki NM, Kagnoff MF. Regulated MIP-3α/CCL20 production by human intestinal epithelium: mechanism for modulating mucosal immunity. Am J Physiol 2001;280:G710-9.
69. Sierro F, Dubois B, Coste A, Kaiserlian D, Kraehenbuhl J-P, Sirard J-C. Flagellin stimulation of intestinal epithelial cells triggers CCL20-mediated migration of dendritic cells. Proc Natl Acad Sci USA 2001;98:13722-7.
70. Hayashi F, Smith KD, Ozinsky A, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 2001;410:1099-103.
71. Jordan NJ, Kolios G, Abbot SE, et al. Expression of functional CXCR4 chemokine receptors on human colonic epithelial cells. J Clin Invest 1999;104:1061-9.
72. Dwinell MB, Eckmann L, Leopard JD, Varki NM, Kagnoff MF. Chemokine receptor expression by human intestinal epithelial cells. Gastroenterology 1999;117:359-67.
73. Williams EJ, Haque S, Banks C, Johnson P, Sarsfield P, Sheron N. Distribution of the interleukin-8 receptors, CXCRI and CXCR2, in inflamed gut tissue. J Pathol 2000;192:533-9.
74. Wilson AJ, Byron K, Gibson PR. Interleukin-8 stimulates the migration of human colonic epithelial cells in vitro. Clin Sci 1999;97:385-90.
75. Van Deventer SJ. Review article: chemokine production by intestinal epithelial cells: a therapeutic target in inflammatory bowel disease? Aliment Pharmacol Ther 1997;11(Suppl 3):116-20.
76. Power CA, Proudfoot AE. The chemokine system: novel broad-spectrum therapeutic targets. Curr Opin Pharmacol 2001;1:417-24.