Intestinal epithelial cells as a source of inflammatory cytokines and chemokines

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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

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.
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. Reineker and Podolsky (10), and Reineker 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 CCX 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 CXC3 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).

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 Entamoeba 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 IECs, 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

![Diagram of cytokines and chemokines](image)
inflamed bowel lumen have not been well described. IL-8 and leukotriene B4 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 recog-
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Inflammatory cytokines and chemokines

Can J Gastroenterol Vol 16 No 4 April 2002 245


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