RESUMEN

El reclutamiento de leucocitos hacia las áreas de inflamación es un proceso finamente regulado que tiene importantes implicaciones en la patogenia, diagnóstico y tratamiento de la enfermedad inflamatoria intestinal. La expresión de moléculas de adhesión, incluyendo selectinas y miembros de la superfamilia de las inmunoglobulinas, se halla aumentada en la microcirculación de los pacientes con enfermedad inflamatoria intestinal activa. La detección de este aumento en la expresión de moléculas de adhesión endoteliales puede utilizarse para localizar áreas que presentan inflamación activa mediante técnicas de imagen. Los fármacos que modulan la expresión o función de las moléculas de adhesión implicadas en el reclutamiento de leucocitos hacia el intestino inflamado son eficaces para el tratamiento de la enfermedad inflamatoria intestinal. Estudios experimentales han demostrado que inmunoneutralización de VLA-4, VCAM-1 o P-selectina produce una mejoría de las lesiones inflamatorias en diversos modelos de colitis. La inmunoneutralización de VLA-4 resulta muy eficaz en el tratamiento de la enfermedad de Crohn humana, logrando unas tasas de respuesta y remisión similares a las obtenidas con la utilización de anticuerpos anti-factor de necrosis tumoral alfa.


ABSTRACT

The localisation of leucocytes to inflammatory areas is a finely regulated event that has key implications in the pathogenesis, diagnosis and treatment of IBD. Expression of endothelial adhesion molecules, including selectins and members of the immunoglobulin superfamily, is increased in intestinal microvessels in active IBD. Detection of up-regulated adhesion molecules can be used to define areas of inflammation by imaging techniques. Drugs that specifically target adhesion molecules involved in leucocyte recruitment are effective in the treatment of intestinal inflammation. Experimental studies have shown that blockade of VLA-4, VCAM-1, and P-selectin afford significant amelioration of intestinal inflammation. VLA-4 immunoneutralisation has been shown to be effective in the treatment of human Crohn's disease.

KEY WORDS: Adhesion molecules / ICAM-1 / VCAM-1 / P-selectin / E-selectin / VLA-4 / Leucocyte adhesion / Endothelium.
LEUKOCYTE-ENDOTHELIAL CELL ADHESION

The recruitment of circulating leukocytes from the blood into intestinal tissues, as elsewhere, begins with interaction with the blood vascular endothelium, principally within specialised postcapillary venules. In lymph nodes and in mucosal lymphoid organs (Peyer's patches and appendix) the venules involved are lined by «high» endothelium. In the intestinal submucosa and lamina propria, venules supporting lymphocyte extravasation are less distinctive histologically, but here too the endothelial cells are highly specialised for their role in recruiting leukocytes from the blood.

Recruitment of circulating leukocytes comprises a multi-step process in which specialised adhesion and signalling molecules participate to mediate each of a series of sequential steps. In the first step, leukocytes margination from central venular blood flow contact the endothelium and initiate rolling along the vascular lumen. Rolling delays the transit of leukocytes, allowing «sampling» of the local microenvironment for activating factors that act primarily through serpine receptors. In the second step, these activating factors trigger rapid intracellular signalling in the leukocyte, leading to functional activation of cell surface adhesion molecules, which then mediate firm arrest of the cell on the vessel wall. Finally, transendothelial leukocyte migration can occur if a chemotactic signal is generated in the cell on the vessel wall. Finally, transendothelial leukocyte migration occurs if a chemotactic signal is generated in the cell on the vessel wall. Finally, transendothelial leukocyte migration occurs if a chemotactic signal is generated in the cell on the vessel wall. Finally, transendothelial leukocyte migration occurs if a chemotactic signal is generated in the cell on the vessel wall.

Selectins

The selectins, designated as L-, P-, and E-selectins, represent a family of adhesive receptors expressed on leukocytes (L), platelets and endothelial cells (P) or endothelial cells alone (E). In contrast to integrins and immunoglobulin superfamily members that mediate an array of cell-cell interactions throughout the body, selectin function is uniquely restricted to the vascular system.

L-selectin is expressed by most circulating neutrophils, monocytes and eosinophils, in the majority of B cells and virgin T cells, and in a major fraction of memory T cells. L-selectin-mediated binding does not require activation of leukocytes. Indeed, activation of leukocyte with a variety of inflammatory mediators or cytokines results in downregulation (shedding) of L-selectin expression on the plasma membrane.

P-selectin is expressed on the surface of activated endothelial cells and platelets. It is stored in Weibel-Palade bodies in endothelial cells and in alpha-granules in platelets. P-selectin is mobilized to the surface of activated endothelial cells within minutes, after which it is either recycled back inside the cell membrane or shed into the plasma. During inflammation, endothelial P-selectin acts to recruit leukocytes into postcapillary venules, while platelet-associated P-selectin promotes the aggregation of leukocytes with platelets to form white thrombi. Endothelial cells can also synthesise and express P-selectin after stimulation with endotoxin or cytokines, resulting in a second peak of P-selectin expression 4-5 hours after stimulation.

Unlike P-selectin, the expression of E-selectin on endothelial cells is entirely under transcriptional control. While E-selectin is not constitutively expressed on endothelial cells, its synthesis (and expression) can be induced by cytokines such as interleukin-1 (IL-1) and TNF-α or by endotoxin. After transient exposure to these stimuli, E-selectin expression is detected as early as two hours after stimulation and returns to baseline values by 8 hours.

Selectin ligands

This recently described family of adhesion molecules is of sialomucin type. Sialomucins are sialic- and threonine-rich proteins that are heavily O-linked glycosylated, with a significant percentage of the molecular mass attributable to O-linked, sulphated, carbohydrate side chains. The regions
of O-linked glycosylation are thought to provide a scaffold for presentation of the polyvalent carbohydrates that have been demonstrated to be involved in ligand binding(3). These highly glycosylated proteins bear the tetrasaccharides sLex, sLea, or their sulphated forms, which have ligand activity for all three selectins.

Of the sialomucins identified, those acting as selectin ligands include: GlyCAM-1, CD34, CD24, and E-selectin ligand-1 (ESL-1). In addition, mucosal addressin cell adhesion molecule (MadCAM)-1, exhibits a dual function since it binds to α4 integrins and L-selectin. Sialylated L-selectin may also have binding affinity for E-selectin.

GlyCAM-1 is expressed on high endothelial venules of peripheral lymph nodes, and acts as a specific vascular adhesion molecule for lymphocyte homing to lymph nodes. In contrast, CD34 is expressed in a wide range of blood vessels, and serves as an L-selectin ligand for lymphocytes and neutrophils in lymphoid organs and at sites in the periphery. PCLP1 is present on vascular endothelial cells and high endothelial venules in peripheral lymph nodes, and acts as a ligand for L-selectin which acts cooperatively with CD34. PSGL-1 is expressed in myeloid cells, platelets and normal vascular endothelia. It can bind the three selectins, but the affinity for each selectin varies depending on the sialylated and fucosylated residues the molecule is decorated with. Interestingly, PSGL-1 may also function as a signalling receptor, for example by inducing Mac-1 expression or enhancing β2 integrin functional activation.

### Integrins
Integrins are heterodimeric proteins consisting of noncovalently associated α and β subunits. At present, 17 α- and 8 β- chains are known. Leukocytes can express 13 different integrins from the existing repertoire with 6 of

---

### TABLE I. Adhesion molecules involved in leukocyte-endothelial cell adhesion

<table>
<thead>
<tr>
<th>Adhesion Molecule</th>
<th>Location</th>
<th>Expression</th>
<th>Ligand</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selectin family</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-selectin</td>
<td>All leukocytes</td>
<td>Yes</td>
<td>No (shed on activation)</td>
<td>P-selectin, E-selectin, GlyCAM, CD34, MadCAM-1, PSGL-1, PCLP1</td>
</tr>
<tr>
<td>P-selectin</td>
<td>Endothelial cells</td>
<td>Yes</td>
<td>Yes</td>
<td>L-selectin, PSGL-1, CD24</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Platelets</td>
<td>Yes</td>
<td>Yes</td>
<td>PSGL-1, ESL-1, L-selectin</td>
</tr>
<tr>
<td><strong>Integrin family</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD11a/CD18</td>
<td>All leukocytes</td>
<td>Yes</td>
<td>No</td>
<td>ICAM-1, ICAM-2</td>
</tr>
<tr>
<td>CD11b/CD18</td>
<td>Granulocytes, monocytes</td>
<td>Yes</td>
<td>Yes</td>
<td>ICAM-1</td>
</tr>
<tr>
<td>CD11c/CD18</td>
<td>Granulocytes, monocytes</td>
<td>Yes</td>
<td>Yes</td>
<td>Fibrinogen, C3b</td>
</tr>
<tr>
<td>α4β7 (VLA-4)</td>
<td>Lymphocytes, monocytes, activated granulocytes</td>
<td>Yes</td>
<td>Yes</td>
<td>VCAM-1, fibrinectin</td>
</tr>
<tr>
<td>α4β1 (VLA-4)</td>
<td>Lymphocytes</td>
<td>Yes</td>
<td>No</td>
<td>MadCAM-1, VCAM-1, fibrinectin</td>
</tr>
<tr>
<td><strong>Immunoglobulin superfamily</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICAM-1 (CD54)</td>
<td>Endothelium, monocytes</td>
<td>Yes</td>
<td>Yes</td>
<td>CD11a/CD18, CD11b/CD18</td>
</tr>
<tr>
<td>ICAM-2 (CD102)</td>
<td>Endothelium</td>
<td>Yes</td>
<td>No</td>
<td>CD11a/CD18</td>
</tr>
<tr>
<td>VCAM-1 (CD106)</td>
<td>Endothelium</td>
<td>Yes</td>
<td>Yes</td>
<td>αmβ2</td>
</tr>
<tr>
<td>MadCAM-1</td>
<td>Endothelium (gut)</td>
<td>Yes</td>
<td>Yes</td>
<td>αmβ2, L-selectin</td>
</tr>
<tr>
<td>PECAM-1 (CD31)</td>
<td>Endothelium, leukocytes, Platelets</td>
<td>Yes</td>
<td>No</td>
<td>PECAM-1, αβ2</td>
</tr>
<tr>
<td>VAP-1</td>
<td>Endothelium</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
</tr>
</tbody>
</table>
these glycoproteins that belong to the $\beta_7$, $\beta_1$, and $\beta_2$ subfamilies serving as important modulators of leukocyte-endothelial cell adhesion. Integrins of the $\beta_7$ subfamily involved in leukocyte-endothelial cell interactions consist of a common $\beta$ subunit (CD18) linked to one of three immunologically distinct $\alpha$ subunits designated CD11a, CD11b, and CD11c (Table I). The expression of $\beta_7$ integrins is restricted to lymphocytes and the distribution of $\beta_7$ integrin subclasses differs among the various leukocyte populations. Peripheral blood lymphocytes express primarily CD11a/CD18 whereas neutrophils, monocytes and natural killer cells express all three $\alpha_4$ integrins. CD11a/CD18 is basally expressed on the surface of most leukocytes where it interacts with intercellular adhesion molecule 1 (ICAM-1) and ICAM-2 on endothelial cells to promote leukocyte adhesion(2). Most of the CD11b/CD18 and CD11c/CD18 glycoproteins are stored in granules that can be rapidly (within minutes) mobilised to the surface of activated neutrophils and monocytes by fusion of granule membranes with the cell membrane. CD11b/CD18 interacts with ICAM-1 on endothelial cells. Ligands for CD11c/CD18 include fibrinogen and iC3b; binding of the latter results in cell activation.

A second subfamily of integrins combines the $\beta_2$ (CD29) chain with variable $\alpha$ subunits. The $\alpha_4\beta_1$ integrin (VLA-4) is involved in the adhesion of lymphocytes, monocytes, eosinophils and natural killer cells to cytokine activated endothelial cells. It has also been shown that certain inflammatory conditions, such as sepsis, induce $\alpha_4$ integrin expression in neutrophils, and $\alpha_4$-dependent adhesion of neutrophils to endothelial cells(2). CD11a/CD18 on unstimulated HUVEC, transcription-dependent up-regulation can be elicited by cytokines and LPS in these cells(11,13). Organs with a relatively high constitutive expression of ICAM-1 (e.g., lung) exhibit smaller increments in ICAM-1 expression after cytokine stimulation than those organs with a low constitutive expression (e.g., heart). Organs in the gastrointestinal tract exhibit robust increases in endothelial ICAM-1 expression after LPS or TNF-$\alpha$ stimulation, with peak expression observed at 5 hours and a sustained elevation above basal values at 24 hrs after endothelial cell activation(11,13).

ICAM-2 is a truncated form of ICAM-1 that has a ligand binding site for CD11a/CD18. Like ICAM-1, ICAM-2 is basally expressed on endothelial cells, but ICAM-2 expression is not increased on activated endothelial cells. The affinity of ICAM-2 for CD11a/CD18 seems to be weaker than that of ICAM-1(15).

VCAM-1 is an important modulator of lymphocyte and monocyte trafficking. VCAM-1 is a ligand for the $\alpha_4\beta_1$ integrin (VLA-4) and also binds to $\alpha_6\beta_4$. Although VCAM-1 is absent on unstimulated HUVEC, transcription-dependent up-regulation can be elicited by cytokines and LPS in these cells(12). In murine intestine, the constitutive level of VCAM-1 expression is substantially lower than that of ICAM-1. However, profound increases in endothelial cell surface density of VCAM-1 are noted within 5-9 hrs of cytokine stimulation(10).

The mucosal addressin MadCAM-1 is a ligand for the $\alpha_4\beta_1$ integrin (CD106), ICAM-1 (CD106), the mucosal addressin MadCAM-1, and platelet-endothelial cell adhesion molecule (PECAM-1/CD31). It is likely that additional molecules will prove to participate also in leukocyte recruitment into the intestine. In this regard, vascular adhesion protein-1 (VAP-1) may be of particular interest.

ICAM-1 recognises CD11a/CD18 and CD11b/CD18. ICAM-1 is basally expressed on leukocytes, fibroblasts, epithelial cells, as well as endothelial cells. Endothelial cell activation with cytokines or LPS results in an increased ICAM-1 expression. In vivo studies have shown a remarkable heterogeneity in the intensity of ICAM-1 up-regulation between vascular beds(11,12). Organs with a relatively high constitutive expression of ICAM-1 (e.g., lung) exhibit smaller increments in ICAM-1 expression after cytokine stimulation than those organs with a low constitutive expression (e.g., heart). Organs in the gastrointestinal tract exhibit robust increases in endothelial ICAM-1 expression after LPS or TNF-$\alpha$ stimulation, with peak expression observed at 5 hours and a sustained elevation above basal values at 24 hrs after endothelial cell activation(11,13).

ICAM-1 mediates the adhesion of both leukocytes and platelets to endothelial cells as well as transendothelial leukocyte migration. This adhesion molecule is constitutively expressed on platelets, most leukocytes and endothelial

**Immunoglobulin superfamily**

The immunoglobulin superfamily includes a large number of molecules with multiple immunoglobulin-like domains. Five members of this family have been shown to be involved in leukocyte-endothelial cell interactions:

ICAM-1 (CD56), ICAM-2 (CD102), VCAM-1 (CD106), the mucosal addressin MadCAM-1, and platelet-endothelial cell adhesion molecule (PECAM-1/CD31). It is likely that additional molecules will prove to participate also in leukocyte recruitment into the intestine. In this regard, vascular adhesion protein-1 (VAP-1) may be of particular interest.

ICAM-1 mediates the adhesion of both leukocytes and platelets to endothelial cells as well as transendothelial leukocyte migration. This adhesion molecule is constitutively expressed on platelets, most leukocytes and endothelial
in cultured endothelial cells does not change appreciably after stimulation with TNF-α, IL-1 or INF-γ, but in response to these stimuli PECAM-1 redistributes to the border of endothelial cells and is thought to participate in the endothelial cell-cell interactions that affect leukocyte transmigration and microvascular permeability. PECAM-1 can mediate adhesion through either homophilic and heterophilic interactions(14).

VAP-1 is known to mediate the specific binding of CD8+ T cells and NK cells to peripheral lymph node high endothelial venules independent of L-selectin, PSGL-1, and α4 integrins(15), and is also able to mediate granulocyte recruitment(16). Although VAP-1 does not function as an autonomous lymphocyte adhesive determinant, it cooperatively (with LFA-1, Mac-1, and L-selectin ligands) confers specific binding of CD8+ lymphocytes to lymph nodes and inflamed endothelia. CD4+ cells do not bind VAP-1, but use peripheral node addressins for trafficking(17). Together with peripheral node addressins, VAP-1 seems to be a major determinant of the flux of lymphocytes that occurs in some healthy vascular beds (e.g. lymphoid tissue) and inflamed tissue. This glycoprotein has been implicated in lymphocyte-endothelial cell interactions in inflamed joints, but is also up-regulated in inflamed intestinal lamina propria(18).

REGULATION OF ADHESION MOLECULE EXPRESSION

Inducible gene expression is a key regulatory mechanism that requires transcriptional activator proteins whose DNA binding or transcription activity is induced upon exposure of cells to specific stimuli. Of the many transcription factors that have been described, nuclear factor kappa B (NF-κB) and activation protein (AP-1) appear to be particularly relevant to the regulation of genes involved in the inflammatory cascade. Both factors represent families of polypeptides with related DNA-binding activity but distinct transactivating potential.

Binding sites for NF-κB have been identified in the promoter regions of the genes for E-selectin, VCAM-1 and ICAM-1, while a binding site for AP-1 has been localised on the promoter region of the ICAM-1 gene. Point mutations which decrease NF-κB binding to κB elements result in diminished cytokine-induced E-selectin expression on cultured endothelial cells, suggesting that NF-κB plays an important role in cytokine induction of the E-selectin gene(19). Two closely spaced functional κB elements have also been identified in the MadCAM-1 promoter. It has been shown that inhibitors of the proteasomal degradation pathway for κB lead to decreased nuclear accumulation of NF-κB and the subsequent abrogation of TNF-α induced cell-surface expression of E-selectin, VCAM-1, and ICAM-1 in endothelial cells(20). This response has important functional consequences because proteasome inhibitors also block both the adherence and emigration of leukocytes in HUVEC monolayers.

Activation of NF-κB has been a uniform finding in animal models of IBD including trinitrobenzene sulfonic acid-induced colitis and in TNBS-induced colitis in mice(21), and peptidoglycan/polysaccharide-induced colitis(22). NF-κB activation has also been demonstrated in human IBD(23). Activation of NF-κB is restricted to areas with active inflammation both in Crohn’s disease and in ulcerative colitis(24). In active IBD activation of NF-κB has been shown to occur both in lamina propria mononuclear cells, and in intestinal epithelial cells(25).

ACTIVATION OF ADHESION MOLECULES IN IBD

Animal models of IBD

Increased expression of endothelial selectins has been uniformly detected in all experimental models of colitis studied. P-selectin up-regulation has been observed in acute acid-induced colitis in mice(26) and in TNBS-induced colitis in rats(27) (Fig. 2) and increments in E-selectin expression were observed in the latter model, as well as in dextran sulphate-induced colitis in mice(28). Interestingly, when expression of these two selectins were compared in the same model (TNBS-induced), expression of P-selectin turned out to be 5-fold higher than that of E-selectin, and this difference had a functional correlate, since P-selectin blockade by means of monoclonal antibodies (MoAbs) resulted in a significant reduction in the number of rolling leukocytes in colonic venules, whereas E-selectin immunoneutralisation did not have any significant effect(29).

Remarkable increases in expression of VCAM-1 have been documented in models of TNBS, dextran sulphate- and peptidoglycan/polysaccharide-induced colitis(21,22), as well as in colitis appearing in IL-10 knock-out mice(19). In these animal models, expression of ICAM-1 was not increased or only marginally elevated, whereas a uniform increase in MadCAM-1 expression, of similar magnitude to that of VCAM-1, was observed. An increased expression of MadCAM-1 has also been demonstrated in colonic submucosal and lamina propria venules in severe combined immunodeficient (SCID) mice reconstituted with CD4+ T cells enriched for the CD45RBhigh subpopulation(29), and in IL-2 knockout mice, when these animals develop colitis after 35 days of age(30). Intravital microscopy studies comparing...
the effects of selective immunoneutralisation of each adhesion molecule of the immunoglobulin superfamily has shown that VCAM-1 blockade results in the higher inhibition of leukocyte adhesion in colonic venules of different models of colitis (26).

Human IBD

The contention that vascular endothelial cells are activated in the inflamed intestine of IBD patients is supported by the observation of a profoundly increased capacity of intestinal microvascular endothelial cells isolated from IBD patients to bind leukocytes, relative to endothelial cells derived from control subjects (31). It has also been shown that culture supernatants of colonic mucosal biopsies from patients with ulcerative colitis or Crohn’s disease induce up-regulation of E-selectin and ICAM-1 in cultured human endothelial cells (32).

Immunohistochemistry studies of intestinal mucosal biopsies from patients with IBD have demonstrated an increased expression of various endothelial adhesion molecules. In keeping with findings in animal models of IBD, an increased expression of P-selectin and E-selectin in venules and capillaries has been documented in inflamed areas from biopsies and surgically resected specimens in Crohn’s disease and ulcerative colitis (33). Characterisation of ICAM-1 expression in human IBD has produced discrepant results, with initial studies reporting an increased expression of ICAM-1 (34), and later studies failing to confirm that contention (35). Contradictory results of these studies may be in part related to limitations in quantification of adhesion molecule expression by immunohistochemistry. It has also been observed that the proportion of venular endothelium within the lamina propria that expresses MAdCAM-1 is increased, compared with normal tissues, at inflammatory foci associated with ulcerative colitis and Crohn’s disease (36). VCAM-1 expression in intestinal mucosa from IBD patients has been reported to be similar to that of controls (37), a finding which contrasts with observations in experimental IBD showing a constant increase in VCAM-1 expression in diverse animal models, and with studies of soluble forms of adhesion molecules, which show a marked increase in soluble VCAM-1 in association with active IBD (see below). Probably, significant expression of endothelial VCAM-1 in active human IBD should not be completely excluded until more accurate techniques for assessment of human adhesion molecule expression are developed. Interestingly, a study performed on human intestinal microvascular endothelial cells has demonstrated that ICAM-1 is constitutively expressed and VCAM-1 is not detectable in basal conditions, but a marked increase in both adhesion molecules is observed after challenge of these endothelial cells with IL-1β, TNF-α or LPS (38), and each of these factors has been found in elevated concentrations in human IBD tissues.

Figure 2. Endothelial P-selectin is up-regulated in inflammatory bowel disease. (A) Immunohistochemical staining with an anti-P-selectin antibody in a control non-colitic animal shows absence of P-selectin expression on endothelial cells (arrows). (B, C) Experimental colitis (TNBS-induced) is associated with an intense P-selectin expression on the endothelial cells of capillaries and small vessels (arrows). Original magnification x 128 (A, B); x 510 (C).
As for leukocyte adhesion molecules, an increased expression of CD18 and ICAM-1 has been found on peripheral blood mononuclear cells from patients with Crohn’s disease patients, but not in patients with ulcerative colitis.\(^{(35)}\) Circulating mononuclear cells from patients with Crohn’s disease form granuloma-like aggregates \(\textit{in vitro}\), which mimic \(\textit{in vivo}\) granulomas in size and organisation. The formation of these aggregates, which significantly correlates with clinical activity, appears to be dependent on CD11b/CD18 and ICAM-1.\(^{(40)}\) Immunohistochemical studies of mucosal biopsies have shown marked increases in the expression of \(\beta_2\) integrins\(^{(41)}\), with CD11a/CD18 mainly expressed in mononuclear cells, and CD11b/CD18 in granulocytes. Increased ICAM-1 has also been documented in lymphocytes, with the intensity of the adhesion molecule expression paralleling the degree of inflammation.\(^{(35)}\)

**ADHESION MOLECULES IN THE ASSESSMENT OF IBD ACTIVITY**

Assessment of disease extension and activity is of major importance in patients with IBD, since this information guides the therapeutic strategy and has prognostic implications. Up-regulation of an endothelial adhesion molecule at a site of inflammation can potentially be detected by administration of a radiolabeled MoAb followed by scintigraphy scan. Targeting adhesion molecules whose expression is associated with active inflammation, such as E-selectin or VCAM-1, may be of value in defining the inflamed areas. In that regard, scintigraphy using a \(\text{\textsuperscript{111}}\text{In}\)-labeled anti-E-selectin MoAb proved as useful as standard \(\text{\textsuperscript{99m}}\text{Tc}\)-HMPAO-labeled leukocyte scintigraphy in the assessment of disease extension in patients with active Crohn’s disease.\(^{(42)}\)

In a model of experimental colitis, scintigraphic uptake of a radiolabeled MoAb followed by scintigraphy scan. Targeting this process has the potential to impact the expression of all endothelial CAMs and consequently exert a profound inhibitory effect on leukocyte recruitment. While there are several strategies that can be used to inhibit the biosynthesis of endothelial CAMs, some of these have recently received considerable attention and appear to hold much promise (Fig. 3).

Glucocorticoids and salicylates, two of the most commonly used drugs in IBD treatment, have among their multiple anti-inflammatory effects, the ability to modulate adhesion molecule synthesis. A major step in understanding the anti-inflammatory mechanism of glucocorticoids resulted from the observation that ligand activated glucocorticoid receptor inhibits AP-1 and NF-\(\kappa\)B mediated expression of adhesion molecules ICAM-1, VCAM-1, and E-selectin. Recent evidence shows that in human IBD cessation of the inflammatory process in response to steroid treatment is associated with disappearance of NF-\(\kappa\)B from nuclear extracts of intestinal mucosa, and that failure to induce NF-\(\kappa\)B activation results in persistence of the inflammatory process. Salicylates also exert a potent inhibitory effect on NF-\(\kappa\)B.
activation. These drugs inhibit NF-κB activation by preventing phosphorylation and subsequent degradation of IκB, and this results in blockade of the TNF-induced increase in mRNA levels of ICAM-1, VCAM-1 and E-selectin, and a dose-dependent inhibition of TNF-induced surface expression of these adhesion molecules. Proteasome inhibitors have been tested in experimental models of inflammation with promising results. In a rat model of experimental colitis induced by peptidoglycan/polysaccharide, proteasome inhibition using MG-341 significantly suppressed VCAM-1 and iNOS up-regulation, which is inhibition of adhesion molecule function. This strategy was achieved through immunoneutralisation with MoAbs of these adhesion molecules (48).

Proteasome inhibitors have been tested in experimental models of inflammation with promising results. In a rat model of experimental colitis induced by peptidoglycan/polysaccharide, proteasome inhibition using MG-341 significantly suppressed VCAM-1 and iNOS up-regulation, which is inhibition of adhesion molecule function. This strategy was achieved through immunoneutralisation with MoAbs of these adhesion molecules (48). Proteasome inhibitors have been tested in experimental models of inflammation with promising results. In a rat model of experimental colitis induced by peptidoglycan/polysaccharide, proteasome inhibition using MG-341 significantly suppressed VCAM-1 and iNOS up-regulation, which is inhibition of adhesion molecule function. This strategy was achieved through immunoneutralisation with MoAbs of these adhesion molecules (48).

Thus, chronic administration of anti-P-selectin antibodies to TNBS-induced colitic mice did not show higher effectiveness than placebo (52). A larger controlled study including 299 patients with active Crohn's disease showed that the remission rate in the ISIS 2302-treated and placebo groups were similar. However, the pharmacokinetic analysis of this study indicates that patients with higher exposure to the drug had a better outcome, suggesting a potential benefit of higher doses of ISIS 2302.

**Adhesion molecule function**

The experimental approach for attenuation of leukocyte-endothelial cell adhesion that has received the most attention is inhibition of adhesion molecule function. This strategy has proven to be very effective in limiting both acute and chronic forms of inflammation in animal models and has only received attention in the clinical setting lately. In most of these studies, inhibition of adhesion molecule function was achieved through immunoneutralisation with MoAbs that target specific adhesion molecules.

The therapeutic value of E-selectin immunoblockade has been assessed in the Cotton-top tamarin colitis (53) with negative results. Although P-selectin immunoneutralisation is followed by a marked reduction in leukocyte rolling in colonic venules of colitic animals irrespective of the experimental model tested (24,54), the influence on subsequent leukocyte adhesion and on the clinical course of colitis seems to be highly dependent on the type of intestinal inflammation. Thus, chronic administration of anti-P-selectin antibodies does not afford significant protection against TNBS-induced colitis in the rat (52), whereas in DSS-induced colitis in mouse either treatment with anti-P-selectin antibodies or colitic ablation of P-selectin significantly decreased leukocyte rolling and adhesion in colonic venules and reduced clinical
MAdCAM-1 (27). This is an interesting finding because this
microscopic lesions, than blockade of either ICAM-1 or
clinical improvement, and reduction of macroscopic and
immunoneutralisation resulted in a significantly higher
sulphate-induced colitis showed that VCAM-1
value of selective adhesion molecule blockade in dextran
of an anti-VCAM-1 MoAb (Fig. 4) (26). Comparison of relative
signs of colitis are markedly reduced by chronic administration
increased leukocyte adhesion in colonic venules, and clinical
the severity of colonic inflammatory disease in SCID mice
subpopulation (29). In the rat model of TNBS-induced colitis,
blockade of P-selectin is to be tested in human IBD, it is
more likely to be effective in the treatment of ulcerative
colitis (mimicked by the DSS model) than in Crohn’s disease
(mimicked by the TNBS model).

Treatment with a blocking MoAb against ICAM-1 in
rats with acetic acid-induced colitis results in a significant
reduction of signs of inflammation, including macroscopic
and microscopic damage and the generation of reactive
oxygen metabolites (59). Similar protection has been noted
with an anti-CD18 (56) or anti-CD11b (57) MoAb in a rabbit
model of TNBS-induced colitis. A potent effect of MoAbs
directed against the α4 integrin in attenuating the spontaneous
colitis in cotton-top tamarin has been observed (58, 53). It has
also been shown that antibodies to either MAdCAM-1 or
its ligand α4β7 block lymphocyte recruitment and reduce the
severity of colonic inflammatory disease in SCID mice
reconstituted with CD4+ T cells enriched for the CD45RB+b+ subpopulation (58). In the rat model of TNBS-induced colitis,
increased leukocyte adhesion in colonic venules, and clinical
signs of colitis are markedly reduced by chronic administration
of an anti-VCAM-1 MoAb (Fig. 4) (58). Comparison of relative
value of selective adhesion molecule blockade in dextran
sulphate-induced colitis showed that VCAM-1
immunoneutralisation resulted in a significantly higher
clinical improvement, and reduction of macroscopic and
microscopic lesions, than blockade of either ICAM-1 or
MAdCAM-1 (58). This is an interesting finding because this
molecule, in contrast with ICAM-1 or MAdCAM-1, is not
involved in physiological leukocyte recirculation, and
selective blockade of its function might attenuate the
inflammatory response without altering physiological
immune mechanisms.

There is already information from human trials of adhesion
cell molecule blockade by means of MoAbs. The results of a
placebo-controlled study assessing the effects of a MoAb
against α4 integrins (natalizumab) in patients with mild to
moderately active Crohn’s disease demonstrated that the
reduction in Crohn’s disease activity index, and the proportion
of patients achieving remission at week two, was significantly
higher in patients receiving natalizumab than in those treated
with placebo (60). The efficacy of natalizumab in the treatment
of Crohn’s disease has been confirmed in another dose-
finding study that shows that administration of two infusions
of natalizumab four weeks apart achieve response and
remission rates twice as high as the placebo group (60). In a
pilot study, administration of natalizumab resulted in clinical
improvement in 6 out of 10 treated patients with active
ulcerative colitis (58).

Since natalizumab is an anti-α4 antibody, it blocks both
α4β7- and α4β1- integrins, and therefore all VCAM-1 and
MAdCAM-1 mediated leukocyte-endothelial cell interactions.
Results of a pilot study using a more specific anti-α4β7-
antibody in patients with moderately active ulcerative
colitis did not show a beneficial effect (60). A limitation
in interpreting negative findings with MoAbs is the uncertainty
regarding whether blocking doses are achieved in vivo.
Target levels are generally based on the minimum MoAb
concentration required to achieve maximal inhibition of
leukocyte adhesion in vitro. Experience gained from open-
labeled clinical trials indicates that blocking doses are
more difficult to achieve in vivo than those predicted from
in vitro leukocyte binding assays. Another potential limitation
of prolonged MoAb usage, at least in chronic models of
inflammation, is immunogenicity. This limitation has
fostered the search for alternative approaches to block
adhesion molecule function, including administration of
soluble receptors or aptamers, but those therapeutic modalities
have not been applied to treatment of intestinal inflammation
yet.

CONCLUSIONS

Intervention in the initial steps of inflammation such as
the means by which leukocytes adhere to venular endothelium
and migrate into tissues, represents an attractive novel target
for the therapy of IBD. Although much has been learned of
the various endothelial and leukocyte adhesion molecules
involved in adhesive interactions between such cell types
In animal models of IBD, comparatively little information has emerged as to the role of these molecules in actual human disease. Several key steps in the inflammatory cascade that result in leukocyte recruitment appear amenable to pharmacological inhibition, but the challenges posed by the potential for disruption of alternate physiological processes as well as immune suppression are significant. Nevertheless, these limitations may be overcome by research that focuses on the identification and characterisation of chemical pathways that uniquely serve the process of leukocyte-endothelial cell adhesion, either at the level of receptor activation, adhesion molecule biosynthesis, and/or adhesion molecule function. The development of safe and effective drugs that target these molecular components of the inflammatory response may yield novel, improved therapies for IBD.

ACKNOWLEDGEMENTS

Part of the research work included in the present review has been supported by grants QLG1-CT-2000-00562 from the European Commission, and grant 01/0039-01E from Fondo Investigación Santaruta, Instituto Salud Carlos III, Spain. Dr. Mireia Peñalva is recipient of a grant from GETECCU.

CORRESPONDENCE TO:
Julián Panés
Gastroenterology Department
Hospital Clinic
Villarroel 170, 08036 Barcelona, Spain.
Tel: 34-93-2275418 Fax: 34-93-4515272
e-mail: panes@medicina.ub.es

REFERENCES


