Transfection of IL-10 expression vectors into endothelial cultures attenuates $\alpha 4\beta 7$-dependent lymphocyte adhesion mediated by MAdCAM-1

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Abstract

**Background:** Enhanced expression of MAdCAM-1 (mucosal addressin cell adhesion molecule-1) is associated with the onset and progression of inflammatory bowel disease. The clinical significance of elevated MAdCAM-1 expression is supported by studies showing that immunoneutralization of MAdCAM-1, or its ligands reduce inflammation and mucosal damage in models of colitis. Interleukin-10 (IL-10) is an endogenous anti-inflammatory and immunomodulatory cytokine that has been shown to prevent inflammation and injury in several animal studies, however clinical IL-10 treatment remains insufficient because of difficulties in the route of IL-10 administration and its biological half-life. Here, we examined the ability of introducing an IL-10 expression vector into endothelial cultures to reduce responses to a proinflammatory cytokine, TNF-$\alpha$.

**Methods:** A human IL-10 expression vector was transfected into high endothelial venular ('HEV') cells (SVEC4-10); we then examined TNF-$\alpha$ induced lymphocyte adhesion to lymphatic endothelial cells and TNF-$\alpha$ induced expression of MAdCAM-1 and compared these responses to control monolayers.

**Results:** Transfection of the IL-10 vector into endothelial cultures significantly reduced TNF-$\alpha$ induced, MAdCAM-1 dependent lymphocyte adhesion (compared to non-transfected cells). IL-10 transfected endothelial cells expressed less than half ($46 \pm 6.6\%$) of the MAdCAM-1 induced by TNF-$\alpha$ (set as 100%) in non-transfected (control) cells.

**Conclusion:** Our results suggest that gene therapy of the gut microvasculature with IL-10 vectors may be useful in the clinical treatment of IBD.

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Background

Endothelial cell adhesion molecules (‘ECAMs’) play essential roles in the development of chronic inflammation by recruiting leukocytes, especially lymphocytes to tissues. ECAMs support several forms of leukocyte adhesion including rolling, firm adhesion and extravasation [1]. Infiltration of tissues by leukocytes is a common hallmark of many chronic inflammatory states that include the inflammatory bowel diseases (IBD), ulcerative colitis (UC), and Crohn’s disease (CD). In the setting of IBD, the expression of ECAMs like ICAM-1, VCAM-1, and MadCAM-1 (mucosal addressin cell adhesion molecule-1) is observed in experimental models of colitis, [2–5] and also within the inflamed human colon in Crohn’s disease and ulcerative colitis [6,7].

Among the adhesion molecules up-regulated in IBD, MadCAM-1, the mucosal cell adhesion molecule, is thought to be preeminent in the development of chronic gut inflammation. MadCAM-1 is normally expressed in the gut, and its expression is dramatically amplified during inflammation [2,3]. The functional significance of increased appearance of MadCAM-1 in IBD is supported by several reports which show that immunononneutralization of either MadCAM-1 or its ligand, the α4β7 integrin, attenuates inflammation and mucosal damage in animal models of colitis [8–10]. However, since monoclonal antibodies directed against other ECAMs, particularly VCAM-1, can as well reduce disease activity in colitis models [11–14], the literature suggests that MadCAM-1 is probably necessary, but insufficient for the maximal penetration of experimental and probably also clinical IBD.

Based on these findings, it is apparent that a better understanding of the mechanisms regulating ECAM expression, especially that of MadCAM-1, might help to devise improved therapies for colitis.

It has been suggested that pathologic activation of the mucosal immune system in response to antigens is a key factor in the pathogenesis of IBD. Furthermore, changes in leukocyte migration and cytokine production appear to contribute to the perpetuation of IBD [15]. Based on modern advances, recombinant anti-inflammatory cytokines (i.e. IL-10) treatment is now being developed for experimental colitis and human IBD. IL-10 produced by macrophages and monocytes appears to limit chronic inflammation [16–18], through a decreased release of inflammatory factors (IL-1, IL-6, IL-12, TNF-α, GM-CSF, GCSF), suppression of adhesive determinants (MHC class II molecule, β7), and by blocking ICAM-1 induction [19–24].

Conversely, IL-10 gene-knockout mice develop a chronic colitis that is extremely similar to IBD [25]. IL-10 treatment can reduce inflammation in several models of colitis [26–30] and human IBD [31–35]. However, the clinical efficacy of systemically administered IL-10 for patients mild to moderately active Crohn’s disease has not been as effective as hoped [33–35]. Furthermore the efficacy of IL-10 administration in mouse colitis models is contentious [36].

We have described in vitro that exogenous IL-10 can block the expression of MadCAM-1 in response to TNF-α, and attenuates lymphocyte adhesion to lymphatic node derived endothelium under cytokine stimulating conditions via NF-κB inhibition [5]. The purpose of the current study was to show that induction of endothelial expression of IL-10 through an IL-10 expression vector attenuates MadCAM-1 expression in response to TNF-α and optimistically suggests the possibility of targeted Th2-cytokine gene therapy in IBD.

Methods

Reagents

Recombinant mouse TNF-α was purchased from ENDOGEN (Stoughton, MA) and plasmid containing human IL-10 (phIL-10) was generous gift from Dr. Meng X (Thomas Jefferson University, PA).

Cell culture

The SVEC4-10 line is an endothelial cell line derived by SV40 (strain 4A) transformation of murine small vessel endothelial cells, originally isolated from the axillary lymph node vessels of an adult male C3H/Hej mouse [37,38]. These cell types were all maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal calf serum with 1% antibiotic/antimycotic. Cells were seeded into 24-well tissue culture plates at approximately 20,000 cells/cm², and cultures were used immediately upon reaching confluence (usually 3–4 days after seeding).

Lymphocytes

The mouse CD8+ T cell lymphoma TK-1 cells (that constitutively expresses the α4β7 integrin [39]) were obtained as a generous gift from Dr. Eugene Butcher (Stanford University, CA). These cells were cultured in RPMI-1640 medium supplemented with 10% FCS and 0.05 mM 2-mercaptoethanol (minus antibiotic/antimycotic).

IL-10 gene transfer

The pCYIL-10 vector is an expression vector which was established by Dr. Xianmin Meng based on the pCY4B vector [40]. Cultured SVEC endothelial cells seeded in 24 wells were transfected at 70% confluency with SuperFect (Qiagen). Briefly, 1 μg plasmid DNA was diluted with 60 μl DMEM and then 5 μl Superfect (3 mg/ml) was added. After incubation at room temperature for 10 min, the
complex was mixed with 350 µl medium and added to each well. After 2 h at 37°C, 5% CO2, the complex was aspirated and washed 2 times with medium following replacement by 1 ml of medium. After incubation at 37°C for 24 h cells were stimulated with TNF-α.

**Western analysis of cell lysates**
24 h of IL-10 gene transfer, monolayers were treated with TNF-α (1 ng/ml, 24 h). All cell samples were harvested at 24 hours. Equal quantities of protein (75 µg) from each sample were electrophoretically separated on 7.5% SDS-PAGE gels. Gels were transferred to nitrocellulose membranes (Sigma) and blocked with 5% milk powder in PBS at 4°C (overnight). These membranes were washed twice for 10 min with wash buffer (0.1% milk powder in PBS). Primary rat anti-mouse MAdCAM-1 mAb was added at a concentration of 10 µg/ml and incubated at room temperature for 2 h. These membranes were washed twice with wash buffer. Secondary rabbit anti-rat horseradish peroxidase conjugated secondary antibody (Sigma) was added at a 1:2000 dilution for 2 h. Lastly, membranes were washed 3 times and developed using the enhanced chemiluminescence (ECL) detection system (Amersham, La Jolla, CA). The density of MAdCAM-1 staining was measured by scanning the 60 kD band, using a HP ScanJet™ flatbed scanner. Images were analyzed for density using Image Pro Plus™ image analysis software (Media Cybernetics). The data are expressed as a percentage of TNF-α-induced level of density. In each protocol, treatments were performed at least in triplicate.

**TK-1 lymphocyte adhesion assay**
Briefly, TK-1 cells were suspended in culture medium and fluorescence labeled by incubating TK-1 cells at 2 × 10^6 cells/ml with 0.02 mg fluorescein diacetate (FDA) (Sigma) at 37°C for 30 min. The cells were then washed twice with ice-cold HBSS, spun at 250 g for 5 min to remove unincorporated fluorescence and suspended in HBSS. The TK-1 lymphocyte cell line used in this assay expresses high levels of the α4β7 integrin, [39,41] which can interact with multiple ligands including mucosal addressin-1 (MAdCAM-1), as well as VCAM-1, L-selectin and fibronectin [42]. In this system, TNF-α stimulated TK-1 adhesion to SVEC4-10 endothelial cells is at least 50% MAdCAM-1 dependent [41]. SVEC monolayers were grown in 48-well plates as described, and to activate endothelium, the monolayers were incubated with TNF-α (1 ng/ml) for 24 h. Cytokine treated endothelial cells were washed three times with media. Labeled TK-1 cells were then added to the endothelium at a 5:1 lymphocyte to endothelial cell ratio [43] and allowed to bind for 30 min under static conditions. At the end of the incubation period, the supernatant was removed and the monolayers were washed twice with HBSS. Plates were read on a Fluoroskan Ascent (Labsystems, Helsinki, Finland) set for excitation at 485 nm, and emission at 515 nm. Blank wells (0% TK-1 adhesion) were run as controls that did not contain labeled TK-1 cells. The data are expressed as a percentage of TNF-α-induced level of fluorescence. In each protocol, treatments were performed at least in triplicate.

**Statistical analysis**
All values are expressed as mean ± SD. Data were analyzed using multiple comparisons. Probability (P) values of < 0.05 were considered significant.

**Results**

**Secretion of human IL-10 concentration by transfected endothelial cells**
To screen for the efficacy of IL-10 transfected SVEC, we initially measured the IL-10 concentration in the medium prior to gene transfection. There was no detectable human IL-10 signal in the non-transfected cell medium (control), nor any mouse IL-10 (data not shown). However, the medium from the IL-10 transfected SVEC medium showed a large and significant increase in the IL-10 concentration (1209 ± 2 pg/ml) at 48 h after IL-10 gene transfection (Figure 1). Importantly, SVEC cells transfected with this vector showed no evidence of toxicity, altered proliferation and morphologically were indistinguishable from mock-transfected and untreated controls.

![Figure 1](http://www.biomedcentral.com/1471-230X/3/3)

**Figure 1**
Secretion of human IL-10 concentration by transfected endothelial cells. There was no detectable human IL-10 signal in the non-transfected cell medium (control). However, the medium from the IL-10 transfected SVEC medium showed a large and significant increase in the IL-10 concentration (1209 ± 2 pg/ml) at 48 h after IL-10 gene transfection (n = 5). (# p < 0.05 from control). One-way ANOVA with Fisher’s PLSD test.
MAdCAM-1 expression in IL-10 gene transfected endothelial cells

The anti-inflammatory effect of IL-10 gene transfection in endothelial cells was measured by the reduction in the expression of the endothelial cell adhesion molecule MAdCAM-1 induced by TNF-α (1 ng/ml, 24 h). TNF-α strongly induced expression of MAdCAM-1. This effect was significantly reduced by IL-10 gene transfection (Fig. 2). Alone, the IL-10 gene transfection had no effect on MAdCAM-1 expression.

Discussion

MAdCAM-1 is a 60 kDa endothelial cell surface molecule that is strongly expressed by mucosal endothelial cells, particularly following exposure of these cells to pro-inflammatory cytokines such as TNF-α. Expression of MAdCAM-1 has also been reported in the brain, and in the heart [44,45]; based on these findings, it is now been suggested that MAdCAM-1 might play roles in chronic inflammation of these organs as well.

With respect to inflammatory bowel disease, MAdCAM-1 appears to be essential to the lymphocyte homing to the mucosa associated lymphoid tissue (MALT) [6,37,38,46]. Since MAdCAM-1 is normally expressed mainly within the gut microvasculature, and is dramatically amplified during IBD, it has been suggested that increased MAdCAM-1 expression contributes to the etiology of IBD through its ability to direct homing of lymphocytes to the gut. This notion is well supported by several reports that show that antibodies directed against either MAdCAM-1, or its lymphocyte ligand, the α4β7 integrin will significantly attenuate several indices of gut damage in experimental models of colitis [9,47].
Several studies have indicated that T helper (Th1) immune response has important roles in the development of IBD [48–50]. Moreover, dysregulation of cytokine networks is involved in Th1-dominant immune responses in IBD [48–50]. Among the Th1 cytokines, TNF-α is thought to be perhaps the most important cytokine responsible for driving the onset and evolution of IBD. Because of this prime role of TNF-α in IBD, anti-TNF-α antibody therapy has been very successfully used in IBD to reduce both colonic injury and expression of ECAMs in IBD [51].

IL-10, a cytokine produced by activated macrophages and Th2-type T cells, has crucial inhibitory effect against several Th-1 type immune responses, such as the antigen-presentation functions of monocytes and macrophages [52,53]. IL-10 may play an important role in preventing the induction of IBD, since animals deficient in IL-10 will develop colitis spontaneously, and low levels of IL-10 are positively correlated with recurrences of Crohn's disease [25,54]. However, unlike TNF-α based therapies, the administration of recombinant IL-10 (rIL-10) shows poor clinical efficacy. This may reflect the fact that TNF-α therapies for IBD are aimed at efficiently clearing TNF-α, while IL-10 therapies must increase IL-10 and recombinant IL-10 is likely too rapidly cleared from the circulation after in vivo administration to provide a uniform protection [55]. On the other hand, IL-10 gene transfer technology has been used with some success in models of colitis, however its effectiveness is also variable. One reason for this variability may be that the final serum IL-10 concentration of gene-transfected mice might be below the critical threshold needed for gut protection [55,56]. Therefore targeting of the IL-10 gene to the inflamed colon should ideally exploit tissue (i.e. gut) specific promoters to control selective organ gene transfer technology, endothelial specific promoters and also organ specific intra-arterial injection of vector to activate some genes in specific locations [57].

Administration of IL-10 in vitro prevents TNF-α stimulated expression of MAdCAM-1, and also blocks lymphocyte adhesion to endothelial cells to the same extent as dexamethasone treatment, currently a key therapy in IBD [5]. Interestingly, the low basal levels of MAdCAM-1 expressed by control (cytokine unstimulated) cells were not affected by IL-10 transfection. This suggests that basal expression of MAdCAM-1, is IL-10 independent, while cytokine stimulated MAdCAM-1 is modulated by IL-10. While we previously showed that delivery of IL-10 to the endothelium in vitro is protective against TNF-α [5], in vivo IL-10 has not been as effective. There are at least two regulating pathways in ECAM expression, one is a cytokine-dependent pathway which is widely studied and the other is cytokine-independent [58]. IL-10 apparently prevents cytokine-induced NF-kB binding activity to the ECAM promoter [59] and blocks the excessive expression of ECAMs in cytokine-mediated inflammatory conditions. In this regard, IL-10 has been used as a treatment in inflammatory diseases, like IBD for precisely this reason: because it has few effects at sites remote from inflammation. Similarly, we found that IL-10 transfection did not affect cell morphology, total protein level, or the expression of actin and vimentin in these cells (measured by Ponceau red S staining density on immunoblots). Therefore methods like endothelial gene transfection/transfer in vivo may effectively maintain adequate IL-10 concentrations near the endothelial cells that finally achieve the protection against cytokines that is not uniformly produced by systemic IL-10 administration.

The most important index for how well gene mediated recombinant IL-10 delivery might work in IBD appears to be measurement of the inhibition of lymphocyte-endothelium binding mediated by TNF-α induced MAdCAM-1 expression. In this experiment, IL-10 induction in the endothelium efficiently blocked TNF-α induced MAdCAM-1 expression and α4β7-dependent lymphocyte adhesion on SVEC endothelial cells. IL-10 deficiency in colitis increases expression of several ECAMs including ICAM-1, VCAM-1 and MAdCAM-1 [60]. We performed an experiment, where under non-stimulated conditions only 55.7 ± 5.4% of the TK-1 lymphocytes bound to SVEC compared to TNF-α stimulation (100%, or 'maximum' binding). TK-1 were seen to interact with SVEC through ICAM-1 as well MAdCAM-1, but not VCAM-1, since an antibody against MAdCAM-1 reduced adhesion to 27 ± 4.6% of maximum and an anti-ICAM-1 reduced adhesion to 54.6 ± 8.3% of maximum; anti-VCAM-1 reduced adhesion to 91% of maximum (blocks lymphocyte adhesion to endothelial cells (data not shown). Therefore we feel that adhesion in this system is mostly MAdCAM-1 dependent.

IL-10 transfection of endothelial cells reduced MAdCAM-1 expression by 42% (following TNF-α stimulation) and reduced TK-1 adhesion by 50%. Therefore, these data suggest that the reduction of TK-1 adhesion is approximately 84% (42%/50%) MAdCAM-1 dependent, and that the remaining 16% of adhesion depends on other ECAMs, (such as ICAM-1).

Although tissue specific promoters might further increase the organ specificity and transgene delivery of this approach, they have not been evaluated in this report. Our findings suggest that lymphatic or gut endothelial transfection with Th2 cytokines like IL-10 may be an effective method to reduce important clinical symptoms associated with IBD which are mediated by MAdCAM-1 dependent lymphocyte adhesion.
Abbreviations
MAdCAM-1 (mucosal addressin cell adhesion molecule-1), IL-10 (Interleukin-10), HEV (high endothelial venular), ECAMs (endothelial cell adhesion molecules), IBD (inflammatory bowel diseases), UC (ulcerative colitis), CD (Crohn’s disease), ICAM-1 (intracellular adhesion molecule-1), VCAM-1 (vascular adhesion molecule 1), NF-κB (nuclear transfection factor kB), TNF-α (tumor necrosis factor alpha), GM-CSF (granulocyte-macrophage colony-stimulating factor), GCSF (granulocyte colony stimulating factor), ECL (enhanced chemiluminescence)

Competing interests
None declared.

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References
1. Granger DN and Kubes P The microrcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion. J Leukoc Biol 1994, 55:662-675
2. Connor EM, Epphimeter MJ, Morise Z, Granger DN and Grisham MB Expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in acute and chronic inflammatory arthritis. J Leukoc Biol 1999, 65:349-355
3. Shigematsu T, Specian RD, Wolf RE, Grisham MB and Granger DN MAdCAM mediates lymphocyte-endothelial cell adhesion in a murine model of chronic colitis. Am J Physiol 1999, 276:G109-G1315
4. Oshima T, Jordan P, Grisham MB, Alexander J, Jennings M and Sasaki M TNF-alpha induced endothelial MAdCAM-1 expression is regulated by exogenous, not endogenous nitric oxide. BMC Gastroenterol 2001, 1:5
5. Oshima T, Pavlick K, Grisham MB, Jordan P, Manas K and Joth T Glucocorticoids and IL-10, but not 6-MP, S-AASA or sulfasalazine block endothelial expression of MAdCAM-1, implications for inflammatory bowel disease therapy. Aliment Pharmacol Ther 2001, 15:1211-1218
6. Briskin M, Winsor-Hines D, Shlyan A, Cochran N, Bloom S and Wilton J Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue. Am J Pathol 1997, 150:197-198
7. Souza HS, Elia CC, Spencer J and MacDonald TT Expression of lymphocyte-endothelial receptor-ligand pairs, alpha4beta7/MAdCAM-1 and OX40/OX40 ligand in the colon and jejunum of patients with inflammatory bowel disease. Gut 1999, 45:856-863
8. Fong S, Jones S, Renz ME, Chiu HH, Ryan AM and Presta LG Mucosal addressin cell adhesion molecule-1 (MAdCAM-1). Its binding motif for alpha 4 beta 7 and role in experimental colitis. Immunol Res 1997, 20:299-311
9. Kato S, Hokari K, Matsuzaki K, Iwai A, Kawaguchi A and Nagao S Amelioration of murine experimental colitis by inhibition of mucosal addressin cell adhesion molecule-1. J Pharmacol Exp Ther 2000, 295:183-189
10. Vainer B and Nielsen OH [The influence of adhesion molecules in inflammatory bowel diseases]. Ugeskr Laeger 1997, 159:7367-7371
11. Bendjelloul F, Malcy P, Mandys V, Jirkovska M, Proksova L and Tuckova L Intercellular adhesion molecule-1 (ICAM-1) deficiency protects mice against severe forms of experimentally induced colitis. Clin Exp Immunol 2000, 119:57-63
12. Hamamoto N, Maemura K, Hirata I, Murano M, Sasaki S and Katsu K Inhibition of dextran sulphate sodium (DSS)-induced colitis in mice by intracolonically administered antibodies against adhesion molecules (endothelial leucocyte adhesion molecule-1 (ELAM-1) or intercellular adhesion molecule-1 (ICAM-1)). Clin Exp Immunol 1999, 117:462-469
13. Sans M, Panes J, Ardidte E, Elizalde J, Arce Y and Elena M VCAM-1 and ICAM-1 mediate leukocyte-endothelial cell adhesion in rat experimental colitis. Gastroenterology 1997, 112:974-984
14. Taniguchi T, Tsukada H, Nakamura H, Kodama M, Fukuda K and Saito T Effects of the anti-ICAM-1 monoclonal antibody on dextran sodium sulphate-induced colitis in rats. J Gastroenterol Hepatol 1998, 13:945-949
15. Kampmann MF and Schummann G [Immunopathogenesis of inflammatory bowel diseases]. Chirurg 2000, 71:30-40
16. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M and O’Garra A IL-10 inhibits cytokine production by activated macrophages. J Immunol 1991, 147:3815-3822
17. Fiorentino DF, Bond M, Mosmann TR. Two types of mouse Th cell helper. IV. Th2 cells secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 1989, 170:2081-2095
18. Del Prete G, De Carli M, Almerigogna F, Giudici MG, Biagiotti R and Romagnani S Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 1993, 150:335-360
19. Bogdan C and Nathan C Modulation of macrophage function by transforming growth factor-beta 1, interleukin-4, and interleukin-10. Ann N Y Acad Sci 1993, 685:713-739
20. Bogdan C, Vodovoz T and Nathan C Macrophage deactivation by interleukin 10. J Exp Med 1991, 174:1549-1555
21. de Waal MR, Abrams J, Bennett B, Figdor CG and de Vries JE Interleukin 10 (IL-10) inhibits cytokine synthesis by human macrophages: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991, 174:1209-1220
22. Wang P, Wu P, Siegel ML, Egan RW and Billah MM IL-10 inhibits transcription of cytokine genes in human peripheral blood mononuclear cells. J Immunol 1994, 153:81-86
23. Wang P, Wu P, Andhes JC, Siegel ML, Egan RW and Billah MM Interleukin-10 inhibits interleukin-8 production in human monocytes. J Immunol 1994, 26:2768-2783
24. Willems F, Marchant A, Daigle JP, Geroni C, Delvaux A, and Velu T Interleukin-10 inhibits B7-1 and intercellular adhesion molecule-1 expression on human monocytes. Eur J Immunol 1994, 24:1007-1009
25. Kuhn R, Lohler J, Rennick D, Rajewsky K and Muller W Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993, 75:263-274
26. Powrie F, Leach MW, Sauze M, Menon S, Cadder LB and Coffman RL Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. Cell 1994, 78:153-162
27. Powrie F, Leach MW, Sauze M, Cadder LB and Coffman RL Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. Int Immunol 1993, 5:461-471
28. Herfarth HH, Mohany M, Rath HC, Tonkonogy S and Sartor RB Interleukin 10 suppresses experimental chronic, granulomatous inflammation induced by bacterial cell wall polymers. Gut 1996, 39:836-845
29. Herfarth HH, Becker O, Janardhanan R and Sartor RB Subtherapeutic corticosteroids potentiate the ability of interleukin 10 to prevent chronic inflammation in rats. Gastroenterology 1998, 115:856-865
30. Tomoyose M, Mitsuyama K, Iwashita H, Toynagawa A and Tankiwa K Role of interleukin-10 in a murine model of dextran sulfate sodium-induced colitis. Scand J Gastroenterol 1998, 33:440-450
31. Schreiber S, Heing T, Thiele HG and Raeder A Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. Gastroenterology 1995, 108:1434-1444
32. Van Deventer SJ, Elson CO and Fedorak RN Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn’s disease. Crohn’s Disease Study Group. Gastroenterology 1997, 113:383-389
33. Fedorak RN, Gangl A, Elson CO, Rutgeerts P, Schreiber S and Wild G Recombinant human interleukin 10 in the treatment of patients with mild to moderately active Crohn’s disease. The Interleukin 10 Inflammatory Bowel Disease Cooperative Study Group. Gastroenterology 2000, 119:1473-1482
34. Schreiber S, Fedorak RN, Nielsen OH, Wild G, Williams CN and Nikolaus S Safety and efficacy of recombinant human inter-
leukin 10 in chronic active Crohn's disease. Crohn's Disease IL-10 Cooperative Study Group. Gastroenterology 2000, 118:1461-1477.

35. Sands BE Therapy of inflammatory bowel disease. Gastroenterology 2000, 118:568-582.

36. Ribbons KA, Thompson JH, Liu X, Pennline K, Clark DA and Miller MJ Anti-inflammatory properties of interleukin-10 administration in hapten-induced colitis. Eur J Pharmacol 1997, 322:245-254.

37. Butcher EC and Picker LJ Lymphocyte homing and homoeostasis. Science 1996, 272:60-66.

38. Butcher EC Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 1991, 67:1033-1036.

39. Ruegg C, Postigo AA, Sikorski EE, Butcher EC, Pytel R and Erle DJ Role of integrin alpha 4 beta 7alpha 4 beta P in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. J Cell Biol 1992, 117:179-189.

40. Meng X, Sawamura D, Tamai K, Hanada K, Ishida H and Hashimoto I Keratinocyte gene therapy for systemic diseases. Circulating interleukin 10 released from gene-transferred keratinocytes inhibits contact hypersensitivity at distant areas of the skin. J Clin Invest 1998, 101:1462-1467.

41. Okuma T, Parleck KP, Laroux FS, Verma SK, Jordan P and Grisham MB Regulation and distribution of MAdCAM-1 in endothelial cells in vitro. Am J Physiol Cell Physiol 2001, 281:C1096-C1105.

42. Hokari R, Misura S, Fujimori H, Tszuku Y, Shigematsu T and Higuchi H Nitric oxide modulates T lymphocyte migration in Peyer's patches and villous submucosa of rat small intestine. Gastroenterology 1998, 115:618-627.

43. McCafferty DM, Mudgett JS, Swain MG and Kubes P Inducible nitric oxide synthase plays a critical role in resolving intestinal inflammation. Gastroenterology 1997, 112:1022-1027.

44. Schable UE, Vestweber D, Butcher EC, Stehle T and Simon MM Expression of endothelial cell adhesion molecules in joints and heart during Borrelia burgdorferi infection of mice. Cell Ades Commun 1994, 2:465-479.

45. Kanwar JR, Kanwar RK, Wang D and Krissansen GW Prevention of a chronic progressive form of experimental autoimmune encephalomyelitis by an antibody against mucosal addressin cell adhesion molecule-1, given early in the course of disease progression. Immunol Cell Biol 2000, 78:641-645.

46. Springer TA Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell 1994, 76:301-314.

47. Picarella D, Hurlbut P, Rottman J, Shi X, Butcher E and Ringler DJ Monoclonal antibodies specific for beta 7 integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) reduce inflammation in the colon of scid mice reconstituted with CD45RBhigh CD4+ T cells. J Immunol 1997, 158:2099-2106.

48. Fuss LJ, Marth T, Neurath MF, Pearlstein GR, Jain A and Strober W Anti-interleukin 12 treatment regulates apoptosis of Th1 T cells in experimental colitis in mice. Gastroenterology 1999, 115:1759-1772.

49. Sartor RB Pathogenetic and clinical relevance of cytokines in inflammatory bowel disease. Immunol Res 1991, 10:465-471.

50. Simpson SJ, Hollander GA, Mizoguchi E, Allen D, Bhan AK and Wang B Expression of pro-inflammatory cytokines by TCR alpha beta+ and TCR gamma delta+ T cells in an experimental model of colitis. Eur J Immunol 1997, 27:17-25.

51. Baxt FJ, D'Haens GR, Peeters M, Hiele MI, Schable TF and Shealy D Tumor necrosis factor alpha antibody (infliximab) therapy profoundly down-regulates the inflammation in Crohn's ileocolitis. Gastroenterology 1999, 116:22-28.

52. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M and O'Garra A IL-10 inhibits cytokine production by activated macrophages. J Immunol 1991, 147:3815-3822.

53. Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M and Moore KW IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. J Immunol 1991, 146:3444-3451.

54. Mereba B, Rugeerta P, Malchow H, Dubucquoi S, Desaillat JP and Cohard M Low ileal interleukin 10 concentrations are predictive of endoscopic recurrence in patients with Crohn's disease. Gut 2002, 50:25-28.

55. Chernoff AE, Granowitz EV, Shapiro L, Vannier E, Lonnemann G and Angel JB A randomized, controlled trial of IL-10 in humans.

Inhibition of inflammatory cytokine production and immune responses. J Immunol 1995, 154:5492-5499.

56. Barbara G, Xing Z, Hogaboam CM, Gauldie J and Collins SM Interleukin 10 gene transfer prevents experimental colitis in rats. Gut 2000, 46:344-349.

57. Sferra TJ, McNeely D and Johnson PR Gene transfer to the intestinal tract: a new approach using selective injection of the superior mesenteric artery. Hum Gene Ther 1997, 8:681-687.

58. Gao J, Choudhary S, Banerjee AK and De BP Human parainfluenza virus type 3 upregulates ICAM-1 (CD54) expression in a cytokine-independent manner. Gene Expr 2000, 9:115-121.

59. Song S, Ling-Hu H, Roe buck KA, Rabbi MF, Donnelly RP and Finnegan A Interleukin-10 inhibits interferon-gamma-induced intercellular adhesion molecule-1 gene transcription in human monocytes. Blood 1997, 89:4451-4469.

60. Kawachi S, Jennings S, Panes J, Cockrell A, Laroux FS and Gray L Cytokine and endothelial cell adhesion molecule expression in interleukin-10-deficient mice. Am J Physiol Gastrointest Liver Physiol 2000, 278:G734-G743.
