

THE CD40/CD40L COSTIMULATORY PATHWAY IN INFLAMMATORY BOWEL DISEASE

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The complexity of inflammatory bowel disease (IBD) is well recognised at both the clinical and therapeutic levels. Even more complex however appears to be the unravelling of its aetiology and pathogenesis where environmental, genetic, bacterial, and immune factors come together to generate the clinical entities we recognise as Crohn's disease (CD) and ulcerative colitis (UC).^{1,2} When facing a complex problem, a wise approach is to select first its most obvious part, break it down into its various components, and then try to understand the contribution of each component to the problem. IBD is a chronic inflammatory process and therefore inflammation is the fundamental issue, the mechanism responsible for the tissue damage in CD and UC. If chronic inflammation is the central mechanism, what are its components? To keep the answers simple, one can envision a triggering event that initiates IBD, and a number of subsequent events that maintain inflammation and perpetuate IBD. The initial trigger is probably far more dependent on genetics and environmental factors than the events that perpetuate IBD.^{3,4} These are more likely to be tissue dependent events and there is little doubt that dysregulation of the local immune response is what underlies gut inflammation and allows it to persist unabated year after year.⁵ Thus until genetic and environmental factors are fully identified, it seems worthy to investigate the various biological systems that contribute to the chronicity of gut inflammation. The purpose of this review is to discuss one key contributor, the CD40/CD40 ligand (L) system, and present evidence that this system plays a major role in immunity and inflammation, can foster chronic inflammation, is directly relevant to IBD, and its modulation may offer therapeutic benefits to IBD patients. To achieve these aims, it is vital to first understand where the CD40/CD40L system fits in the general scheme of an immune response.

Initiation and progression of an immune response depends on careful regulation of lymphocyte activation. According to the classical "two signal" model, T cells require input from two sources to become fully activated. The first signal, which gives specificity to the immune response, is provided by the interaction of major histocompatibility class II antigens complexed to the antigen recognised by the T cell receptor (TCR). The second signal is delivered by antigen presenting cells (APCs) and promotes T cell clonal expansion, cytokine secretion, and effector function. In the absence of the second signal, lymphocytes fail to respond effectively and become functionally deactivated and incapable of responding to subsequent antigen exposure (anergy). Thus the one-two costimulatory punch is fundamentally important in determining whether a T cell is enabled or not to competently respond to a specific antigen and ultimately mediate an appropriate immune response.

This model is useful but oversimplifies the contribution of signals one and two. Firstly, the strength of the TCR signal influences the degree of T cell activation and differentiation, and T cells may become activated even in the absence of a second signal, like in the case of direct stimulation by superantigens (for instance, staphylococcus enterotoxin A). In addition, and centrally important to the topic of this review, second signals delivered to T cells can be both positive and negative. Some negative second signals are necessary for inducing T cell tolerance (antigen specific unresponsiveness) whereas some positive second signals amplify T cell activation.⁶

There is a large number of T cell costimulatory molecules, many of which display both distinct and overlapping functions, and one of the current challenges in this area is to understand the functions of each of these T cell costimulatory pathways (fig 1). Costimulation is provided by three major families: the B7:CD28 superfamily, the tumour necrosis factor (TNF):TNF receptor subfamily that lack death domains, and the CD2 superfamily, as well as some integrins (a class of cell adhesion molecules). The focus of this review, the CD40/CD40L costimulatory system, belongs to the TNF-TNF receptor superfamily, and will be discussed in the context of IBD pathogenesis. Comprehensive reviews on the overall biology of the CD40/CD40L system can be found in the recent literature,^{7–9} as well as reviews on other costimulatory pathways and their possible relevance to IBD.¹⁰

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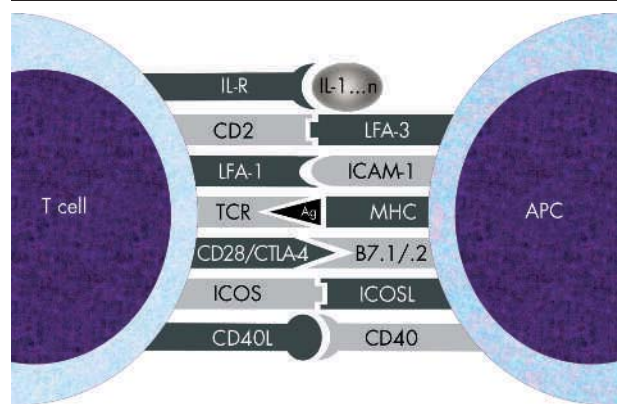


Figure 1 Major molecular receptor-ligand pairs involved in T cell activation, downregulation, costimulation, and amplification of immune responses. Ag, antigen; APC, antigen presenting cell; CTLA, cytolytic T lymphocyte associated antigen; ICAM, intercellular adhesion molecule; ICOS, inducible costimulator; IL-1...n, interleukin 1 to n; IL-R, interleukin receptor; LFA, lymphocyte function associated antigen; MHC, major histocompatibility complex; TCR, T cell receptor.

- ▶ IBD is a complex disease of unknown aetiology and pathogenesis, mediated by multiple immune and inflammatory pathways.
- ▶ A “two signal” model of T cell activation initiates immune responses where the antigen constitutes the first signal and antigen presenting cells provide a second, costimulatory signal.
- ▶ Interactions between CD40 and CD40L represent a major costimulatory system that amplifies the immune response and can promote inflammation.

expressed by monocytic cells, natural killer cells, B cells, CD8+ T cells, mast cells, and basophils. After its expression, CD40L is cleaved and shed from the cell's surface and circulates systemically in a biologically active soluble form (sCD40L).¹³ Similar to what happens with ligation of TNF- α to its receptor, CD40 forms a trimer that binds to CD40L, triggering a complex signalling cascade involving binding to members of the TNF receptor associated protein family, activation of various protein tyrosine kinases such as ERK-1 and ERK-2, p38, and JNK, and eventually leading to activation of various transcription factors including NF κ B, AP-1, and NF-AT.¹⁴ This causes activation of CD40 bearing cells which produce multiple bioactive molecules whose ultimate effects depend on the differentiation state of the cells involved, the expression level of receptor and ligand, and the tissue microenvironment where the binding occurs.¹⁴

- ▶ CD40 is expressed on the surface of numerous immune and non-immune cells whereas CD40L is expressed primarily by activated CD4+ T cells and activated platelets.
- ▶ The CD40/CD40L system allows interactions between immune cells, and between immune and non-immune cells.

BIOLOGICAL EFFECTS OF CD40/CD40L INTERACTIONS

Cognate interactions between CD40 and CD40L generate intracellular signals and production of surface and secreted molecules that ultimately impact on both humoral and cellular immunity, and eventually on inflammation. One of the first reported and most studied biological effect of CD40 ligation is the switch in recombination and synthesis of immunoglobulins by B cells¹⁵ (fig 2). The critical role of CD40/CD40L interaction in this phenomenon became apparent and gained practical clinical relevance when patients suffering from the X linked hyper-IgM syndrome, where the humoral immune response is severely compromised, were found to have a genetic mutation of CD40L.¹⁶ These patients show defective antibody production with lack of circulating IgG and IgA owed to the inability to switch the IgM isotype. Moreover, binding of T cell CD40L is crucial for maturation of B cells to a memory phenotype and inducing their activation and proliferation. All of these *in vitro* events have been elegantly reproduced *in vivo* in mice carrying a genetic disruption of the CD40/CD40L pathway.^{17, 18}

With the progressive understanding of the importance of CD40/CD40L interactions in humoral immunity, evidence began to accumulate indicating that these interactions were also vitally important to cell mediated immunity (fig 2).^{7, 18} Among a multitude of cellular functions, CD40L regulates the costimulatory activity of APC, inducing B cells to upregulate B7.1 and B7.2, and stimulates DCs to increase the cell surface expression of other costimulatory molecules such as CD54 and CD86 (B7.2).^{19, 20} Additionally, ligation of CD40 on DCs leads to production of various cytokines, including interleukin (IL)-8, TNF- α , and macrophage inflammatory protein (MIP)-1 α . Notably, CD40 stimulation induces DCs to produce IL-12, a cytokine that plays a key role in the polarisation of Th1 immune responses.²¹ Additional functions mediated by CD40/CD40L interactions include induction of apoptosis in

THE CD40/CD40L SYSTEM

After its original discovery in the mid 1980s, at first CD40 became known as a B cell surface receptor whose engagement resulted in potent polyclonal activation, while CD40L (CD154) was recognised in the early 1990s as a T cell surface molecule able to induce contact dependent differentiation of B cells.¹¹ Because of the experimental conditions under which CD40 and CD40L were initially identified and studied, these molecules acquired an almost exclusive immunological connotation that lasted for several years until it was realised that both the receptor and its ligand had a much wider range of cellular expression and participated in a significantly broader spectrum of activities than the originally described T and B cell interactions.^{8, 9} This considerably expanded the biological relevance of the CD40/CD40L system, causing this molecular pair to become the focus of intense investigation of its biological, clinical, and therapeutic implications.

CD40 is a 45–50 kDa type I transmembrane protein which belongs to the TNF receptor superfamily because of its homology with the TNF receptor.¹² It is ubiquitously expressed on the surface of immune cells, including B cells, monocytes, macrophages, and dendritic cells (DCs), as well as non-immune cells such as epithelial, endothelial, and mesenchymal (fibroblasts, myofibroblasts, synoviocytes, stellate cells, etc) cells, and platelets.⁹ CD40L is a 39 kDa type II transmembrane protein member of the TNF gene superfamily (which includes TNF- α , lymphotoxin α and lymphotoxin β , FasL, etc), and is expressed preferentially by activated CD4+ T cells and activated platelets, although it can also be variably

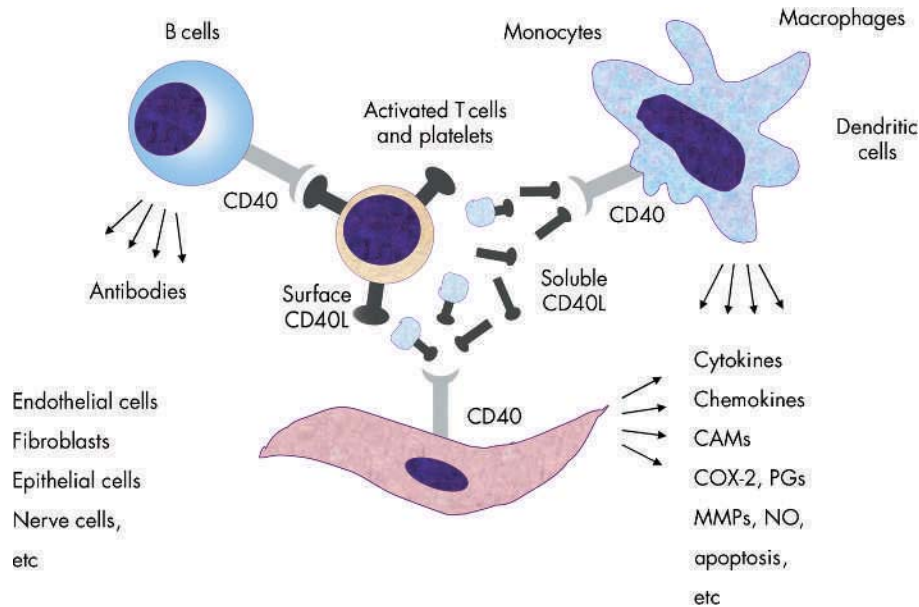


Figure 2 Pleiotropic effects resulting from the interaction of activated CD40L+ T cells, activated CD40L+ platelets, or soluble CD40L with immune and non-immune cells. Engagement of CD40 on B cells leads to antibody production whereas a much wider range of effects are induced in monocytes, macrophages, and dendritic cells activated through the CD40 pathway. Some of these include production of cytokines, chemokines, cyclooxygenases (COX), prostaglandins (PGs), metalloproteinases (MMPs), and nitric oxide (NO), upregulation of cell adhesion molecules (CAMs), and modulation of apoptosis. Activation of non-immune cells through the CD40 pathway induces a spectrum of effects similar to those observed in monocyte cells.

CD4+ T cells²² and generation of CD8+ T cell memory.²³ As might be expected, functional interactions between CD40 and CD40L are bidirectional, as exemplified by the observation that CD40 expressing APCs contribute to T cell activation. This has been demonstrated in CD40L deficient animals whose CD4+ T cells proliferate poorly in response to antigen exposure, produce little IL-4 and interferon (IFN)- γ , and fail to generate antigen specific T cell responses.¹⁸

As mentioned above, in addition to being expressed by classical immune cells, both CD40 and CD40L are found on a whole host of non-immune cells, including epithelial and endothelial cells, fibroblasts, myofibroblasts, synoviocytes, and stellate cells, and platelets. In these cells the CD40/CD40L system serves as an effective way to communicate with immune cells and numerous types of non-immune cells, the usual outcome being amplification of immune and inflammatory responses (fig 2). For instance, ligation of CD40 on endothelial cells or fibroblasts triggers production of numerous chemokines, such as IL-8, MCP-1, MIP-1 α and β , RANTES (regulated on activation normal T expressed and secreted), fractalkine, and cytokines such as IL-1, IL-6, IL-12, and TNF- α , upregulation of cell adhesion molecules, including intercellular adhesion molecule (ICAM)-1, vascular adhesion molecule (VCAM)-1, and E-selectin, secretion of matrix metalloproteinases (MMPs), such as MMP-1, -2, -3 and -9, and tissue factor expression.^{24–28} Engagement of CD40 in fibroblasts upregulates cyclooxygenase 2 expression and prostaglandin E₂ production.²⁹ Moreover, sCD40L is able to activate platelets and cause them to upregulate P-selectin expression and induce release of β -thromboglobulin and 5-hydroxytryptamine³⁰ whereas the membrane bound form of CD40L triggers the release of biologically active RANTES stored in the platelet's granules.³¹ Obviously, the combined effects of all of these mediators can be detrimental to the host by causing inflammation and tissue destruction.

Recently, a novel role of the CD40 pathway has been identified in angiogenesis, a process responsible for vascular remodelling and new vessel formation. Both membrane bound as well as the soluble form of CD40L are proangiogenic, inducing vascular endothelial cell growth factor secretion by endothelial cells and monocytes *in vitro*, and promoting endothelial cell survival and angiogenesis *in vivo*.^{32–33}

- ▶ Cognate interactions between CD40 and CD40L impact on humoral immunity, cell mediated immunity, and inflammation.
- ▶ Activation of the CD40 pathway in immune cells leads to B cell differentiation and antibody isotype switching, and in T cells and macrophages leads to cytokine and chemokine production, upregulation of adhesion molecules, and apoptosis.
- ▶ Activation of the CD40 pathway in non-immune cells (epithelial, endothelial and mesenchymal cells, and platelets) leads to production of proinflammatory cytokines, chemokines, matrix metalloproteinases, prostaglandins, and upregulation of adhesion molecules.

EXPRESSION OF CD40 AND CD40L IN IBD

Given the immune/inflammatory nature of IBD, altered expression of immunoregulatory and proinflammatory molecules is expected in this condition, and this clearly applies to CD40 and CD40L, both of which have been investigated at the tissue and systemic level in CD and UC patients. All of the data currently available in the literature concur that CD40 and CD40L are overexpressed in both forms of IBD, suggesting that this molecular dyad is intimately involved in the pathophysiology of the disease.

With regard to tissue distribution, immunohistochemistry for CD40 in the normal colon reveals staining of few mononuclear cells in the lamina propria, weak staining of microvascular endothelial cells in the submucosa, but no staining of muscularis mucosae cells or submucosa mesenchymal cells.³⁴⁻³⁷ These findings stand in clear contrast with those in actively inflamed CD and UC tissues where there is strong and diffuse CD40 staining of mononuclear, endothelial, and mesenchymal cells in both the mucosa and submucosa.^{36, 37} Of particular interest is one study where, based on a correlation of clinical, endoscopic, and histological criteria, CD40 overexpression was found to be directly proportional to the clinical activity of UC patients.³⁵ By immunostaining and confocal microscopy, only sporadic CD40L+ mononuclear cells are detected in normal colonic mucosa but their number substantially increases in colonic tissue involved by CD or UC.^{37, 38} Costaining reveals that the majority of CD40L+ mononuclear cells are CD4+ T- cells, with a small fraction of CD8+ T cells.³⁴ This was confirmed by studying lamina propria T cells (LPT) freshly isolated from CD and UC mucosa which express higher surface CD40L than T cells from histologically normal mucosa.³⁸ Finally, CD40L is also expressed in microvascular microthrombi found in severely inflamed CD and UC mucosa. Interestingly, costaining for CD42b (a specific platelet marker) reveals that CD40L is upregulated on the surface of activated platelets clustered in microthrombi and stays in direct contact with inflamed endothelial cells.³⁹ This suggests that the physical contact between CD40L+ platelets and endothelium could be responsible for the occurrence of a procoagulant state in IBD, and potentially lead to the thromboembolic events frequently observed in CD and UC patients.⁴⁰

Studies utilising peripheral blood mononuclear cells of IBD patients have confirmed the suspicion that, in addition to occurring in the inflamed gut, activation of the CD40/CD40L system is also detected systemically in CD and UC. CD40 expression is upregulated in patients' circulating lymphocytes and monocytes^{39, 41} but not in platelets (S Danese, unpublished observations). In one report, after stimulation with anti-CD3, peripheral blood T cells (PBT) of IBD subjects were reported to express higher levels of CD40L than PBT from healthy subjects,³⁸ while another study showed no differences when a stronger stimulation with anti-CD3 plus anti-CD28 was used.⁴⁰ Platelets in the circulation of CD and UC patients also express enhanced surface CD40L levels compared with those of normal subjects, a phenomenon likely due to their increased activation state and apparently secondary to their contact with endothelial cells in the inflamed mucosal microvasculature.⁴⁰ Finally, increased plasma levels of sCD40L have been recently reported in the circulation of patients with either form of IBD, and their concentration is directly proportional to the anatomical extent of mucosal inflammation in both forms of IBD.^{40, 42} Thus measurement of sCD40L plasma levels may potentially be of clinical value in estimating disease activity and extension.

As might be expected, activation of the CD40/CD40L system is not a feature exclusive of IBD but one found in numerous other immune mediated, autoimmune, and inflammatory disorders that share inflammatory pathways with CD or UC. Disorders where there is evidence that activation of the CD40 pathway also plays an important pathogenic role include rheumatoid arthritis, systemic lupus erythematosus, autoimmune thyroiditis, dermatomyositis,

multiple sclerosis, acute and chronic graft versus host disease, Alzheimer's disease, febrile transfusion reactions, atherosclerosis, cardiovascular syndromes, and various infectious diseases such as leprosy, leishmaniasis, and tuberculosis.

- ▶ Expression of both CD40 and CD40L is increased in the intestine involved by IBD.
- ▶ Mesenchymal and endothelial cells express markedly elevated levels of CD40 in IBD involved mucosa.
- ▶ The number of CD40L+ T cells and platelets is increased in the inflamed mucosa of IBD patients.
- ▶ Levels of soluble CD40L are increased in the circulation of patients with active CD and UC and may be of clinical value in disease assessment.

MECHANISMS OF CD40 AND CD40L UPREGULATION IN IBD

A number of recent studies performed on cells directly isolated from the intestine and the circulation of IBD patients have shed new light on the mechanisms responsible for the enhanced expression of CD40 and CD40L in the gut and the elevated levels of sCD40L in the circulation. Specifically, induction and modulation of CD40 has been studied in detail using primary cultures of human intestinal fibroblasts (HIF) and intestinal microvascular endothelial cells (HIMEC).

As determined by reverse transcription-polymerase chain reaction, unstimulated HIF or HIMEC contain low spontaneous levels of CD40 mRNA but these are substantially increased when cells are cultured in the presence of IFN- γ , and little by classical proinflammatory cytokines.^{36, 37} These findings are confirmed by flow cytometric analysis showing that unstimulated HIF cultures display low levels of CD40 surface expression which is progressively upregulated by IFN- γ for up to 72 hours when maximal and steady expression is reached. Exposure of HIF to other proinflammatory stimuli, such as IL-1 β , TNF- α , or lipopolysaccharide, does not significantly increase CD40 expression, pointing to the key role that the Th1 cytokine IFN- γ plays in modulation of mucosal CD40. Interestingly, once maximal expression is achieved, HIF continue to express high levels of CD40 for several days.³⁷ Similar to HIF, HIMEC also expressed low spontaneous levels of CD40 which are upregulated by IFN- γ and are also maintained for days on removal of the inducing cytokine. While no differences in CD40 expression level and response to cytokines are found in control and IBD fibroblasts and microvascular cells *in vitro*,³⁷ the kinetics of CD40 induction and expression are of considerable pathophysiological importance. In fact, once CD40 expression is induced, it lasts for a prolonged period of time, allowing non-immune cells to bind to and interact with CD40L on the surface of locally activated cells or its soluble form. From these observations it may be predicted that this lingering interaction may have critical implications for persistence of inflammation, as it will be discussed in the section dealing with functional consequences of activation of the CD40/CD40L system in IBD.

The mechanisms of CD40L induction have also been studied *in vitro* using LPT, PBT, and platelets freshly isolated from IBD mucosa and peripheral blood. Both PBT and LPT upregulate CD40L on stimulation with anti-CD3 or anti-CD3

plus anti-CD28, resulting in high levels of CD40L that last for at least 48 hours.^{37, 38} This *in vitro* event mimicking antigen encounter by T cells must have an *in vivo* counterpart in the inflamed mucosa, as LPT isolated from IBD subjects display higher levels of membrane CD40L compared with those of normal individuals.³⁸ In addition to T cells, induction of CD40L has also been investigated in platelets. Platelets can be activated and induced to express CD40L by a variety of different factors, including thrombin, collagen, platelet activating factor, lipopolysaccharide, IL-1 β , and thrombin receptor activator peptide, most of which are found in the mucosa and circulation during the active stages of IBD. Unstimulated platelets from healthy subjects display no or minimal levels of CD40L which, after thrombin stimulation, rise sharply within a few minutes and fall to baseline within an hour. In contrast, CD and UC platelets freshly isolated from peripheral blood contain a significantly higher percentage of CD40L positive cells and, after thrombin stimulation, CD40L expression increases in platelets from all groups, although significantly more in CD and UC.⁴⁰ Finally, a novel mechanism of CD40L upregulation has been recently described. Inflamed HIMEC have been shown to induce a

contact dependent upregulation and secretion of CD40L by platelets, suggesting a key role of the inflamed intestinal microvasculature in mediating platelet activation in IBD and highlighting the critical importance of CD40/CD40L interactions in IBD pathogenesis.

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- ▶ Exposure to IFN- γ is the likely mechanism responsible for increased expression of CD40 by endothelial and mesenchymal cells in IBD mucosa.
- ▶ Upregulated expression of CD40 by mucosal endothelial and mesenchymal cells lasts for several days, allowing prolonged interaction with CD40L+ T cells that may foster inflammation.
- ▶ Interaction of platelets with the inflamed mucosal microvasculature leads to induction of CD40L expression by platelets and release of soluble CD40L.

FUNCTIONAL CONSEQUENCES OF ACTIVATION OF THE CD40/CD40L SYSTEM IN IBD

Having described the presence and upregulation of CD40 and CD40L in CD and UC, the next logical step is to try to

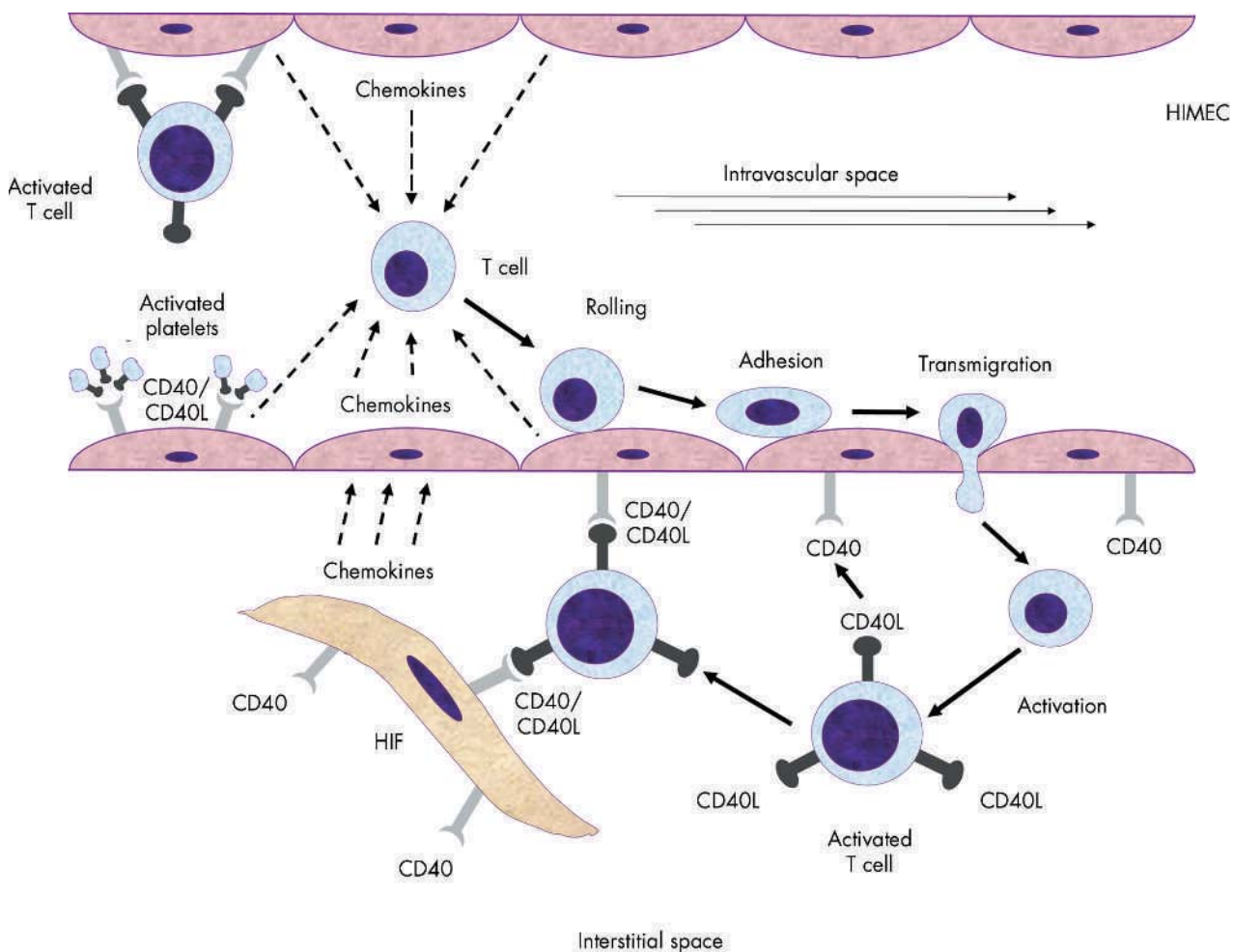


Figure 3 Proposed framework for continuous recruitment of T cells into the gut mucosa through CD40/CD40L dependent mechanisms. In the early stages of mucosal inflammation, local T cells become activated and express CD40L, binding to and activating CD40+ mesenchymal (HIF) and endothelial (HIMEC) cells. HIF and HIMEC start secreting chemokines that attract more T cells that transmigrate into the interstitial space, become activated, and express CD40L, initiating a new cycle of chemokine secretion by non-immune cells which results in additional T cell recruitment. Activated T cells and platelets in the circulation of inflammatory bowel disease (IBD) patients contribute to this process through expression of CD40L on their surface. This framework supports the notion of a non-immune, cell mediated, antigen independent mechanism perpetuating IBD.

understand the significance of these events in IBD. Based on the evidence presented so far, it is clear that activation of the CD40/CD40L system can very effectively amplify immune responses in specific organs and tissues by serving as a biological link between activated T cells and many other cells types, leading to the production of cytokines, chemokines, adhesion molecules, eicosanoids, MMPs, and nitric oxide (NO), in addition to modulating cell survival and apoptosis. In principle, all of these events are relevant to IBD pathogenesis but only some have been investigated so far.

Cocultures with freshly isolated CD40L+ IBD LPT induce peripheral blood monocytes to produce IL-12 and TNF- α , especially after IFN- γ priming, pointing to the functional contribution of the CD40/CD40L interactions to the polarisation of Th1 immune responses in the gut.³⁸ On the other hand, the contribution of CD40L to B cell function and antibody production in the mucosa has yet to be explored. In IBD, to date there has been greater interest in exploring the role of the CD40 pathway in immune-non-immune than immune-immune cell interactions. Specifically, the outcome of CD40L+ T cells and CD40L+ platelets with CD40+ HIF and HIMEC has been explored in detail utilising well established coculture systems.^{36 37 39} Both HIF and HIMEC cocultured with CD40L+ T cells or soluble CD40L produce substantial levels of the chemokines IL-8, IL-6, MCP-1, and RANTES, and their baseline expression of ICAM-1 and VCAM-1 is augmented. In particular, culture supernatants of HIF stimulated with CD40L+ T cells contain chemoattractants able to induce transmigration of T cells through HIMEC monolayers, an event that may recapitulate what occurs in vivo when the intestinal mucosa is chronically involved by IBD.³⁷ This has major pathogenic implications because it suggests that the CD40/CD40L system mediated increase in chemokine and adhesion molecule production results in the attraction and interaction with additional T cells. Once located in the inflamed microenvironment of the IBD mucosa, the newly recruited T cells can become activated and de novo express CD40L which would allow them to bind to local mesenchymal CD40+ cells, activate p38 and NF κ B,^{36 37 39} leading in turn to further cytokine and chemokine production and upregulation of cell adhesion molecules, creating a vicious cycle that fosters perpetuation of inflammation (fig 3).

An additional proinflammatory circuit may be contributed by activated CD40L+ platelets when they enter in physical contact with the local mucosal microvasculature. After adhering to HIMEC, activated platelets induce upregulation of ICAM-1 and VCAM-1 expression as well as IL-8 production by endothelial cells.³⁹ These events occur in a CD40 dependent fashion and are mediated by p38 MAP kinase signalling, as shown by the selective inhibition of phosphorylated p38. Even more significant may be the finding that, on binding to HIMEC, platelets release large amounts of biologically active RANTES, a potent T cell chemokine, which is retained on the nearby microvascular endothelial cell surface where it can facilitate T cell adhesion and thus amplify inflammation (fig 4). The combined results of the above experiments provide a conceptual framework for CD40 dependent but antigen independent mechanism of recruitment of T cells into the inflamed intestinal mucosa, and underscore the importance of the CD40/CD40L system in maintenance of IBD.

Finally, in addition to contributing to the chronicity of IBD, binding of T cell membrane bound CD40L or sCD40L can

induce HIF to downregulate their collagen synthetic capacity, pointing to an unsuspected but potentially beneficial anti-fibrotic effect of the CD40/CD40L system in IBD.⁴³

- ▶ CD40L+ mucosal T cells induce activation of monocytes and production of proinflammatory cytokines.
- ▶ Contact with CD40L+ T cells or exposure to soluble CD40L induces upregulation of adhesion molecules and production of chemokines by microvascular endothelial and mesenchymal cells, resulting in the recruitment of more T cells that become activated and induce further chemokine production that attracts additional T cells, creating a vicious cycle that may perpetuate mucosal inflammation.
- ▶ Contact of activated CD40L+ platelets with microvascular endothelial cells induce the release of RANTES which binds to mucosal endothelial cells and attracts additional T cells which can amplify inflammation.

DISRUPTING THE CD40 PATHWAY FOR IBD THERAPY: EVIDENCE FROM ANIMAL MODELS AND IMPLICATIONS FOR HUMAN DISEASE

Considering the importance of costimulatory signals in the initiation and magnification of immune responses, targeting costimulation becomes therapeutically attractive because it provides new methods to block undesired T or B cell responses mediating tissue damage. This is particularly true for CD40/CD40L interactions because they regulate a large variety of immune and inflammatory processes, and designing specific molecules to disrupt CD40 or CD40L actions may provide novel therapeutic strategies for diseases where CD40 signalling has a pathogenic role. In vivo evidence to support this approach is usually first obtained in animal models, and in fact administration of CD40L antibodies has been shown to benefit mice with several autoimmune and inflammatory diseases, including chronic collagen induced arthritis, lung inflammation and fibrosis, experimental allergic encephalomyelitis (a model for multiple sclerosis), lupus nephritis, atherosclerosis, acute pancreatitis, and oophoritis. Interestingly, although it is generally assumed that costimulation blockade is the underlying mechanism, a recent report suggests that the observed immunosuppression is caused by Fc dependent depletion of activated T cells through CD40L specific antibodies.⁴⁴ Whether the first or second mechanism, or both, are responsible for clinical improvement remains to be determined.

The first evidence that CD40/CD40L interactions are important in gut inflammation was demonstrated by the beneficial effect of CD40L antibodies in the murine model of hapten induced colitis, a typical Th1 immune response.⁴⁵ This was followed by additional evidence in other models of experimental and infectious colitis. In another Th1 model, induced by transfer of CD45RB^{high} CD4+ T cells into SCID mice, the development of colitis was paralleled by increased expression of CD40 and CD40L protein and mRNA.⁴⁶ The associated wasting disease and intestinal inflammation were completely abrogated by an eight week course with a monoclonal CD40L antibody when treatment was started immediately after T cell reconstitution and, when started after colitis was established, CD40L blockade still induced significant clinical and histological improvement. These findings were confirmed by another group of investigators

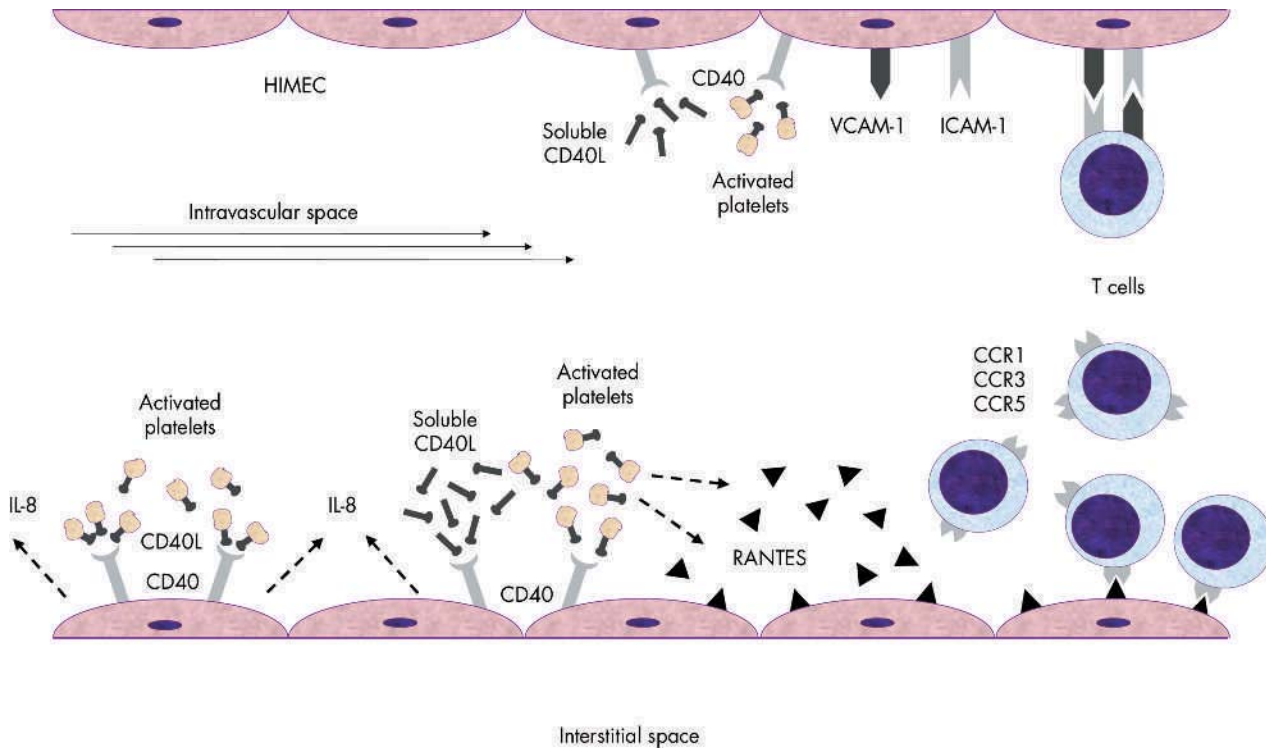


Figure 4 Proposed framework for recruitment of T cells into the gut mucosa by activated CD40L+ platelets. CD40L+ platelets and soluble CD40L bind to CD40+ endothelial cells (HIMEC) that start producing chemokines such as interleukin (IL)-8. On contact with HIMEC, activated platelets release massive amounts of RANTES (regulated on activation normal T expressed and secreted) stored in their granules which is retained on the surface and HIMEC and mediates binding of T cell to the microvascular cells through the receptors CCR1, 3, and 5. In addition, CD40L+ platelets bind to HIMEC where they induce upregulation of intercellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1, both of which contribute to recruitment of additional T cells.

in the same model transfer but also the tge26 bone marrow transplant model.⁴⁷ Of note, when reconstitution was carried out using T cells from CD40L deficient animals, there was a marked reduction in clinical and histological scores of colitis, and serum levels of IL-12 were significantly lower compared with wild-type T cell recipients, indicating attenuation of the dominant Th1 phenotype. In another study, in which CD4+ T cell lines reactive to commensal bacteria were transferred to immunocompetent mice, CD40/CD40L costimulation was demonstrated to be required for development of colitis.⁴⁸ In this report, cell lines that lacked significant CD40L expression failed to induce colitis. As in the previous reports, administration of an anti-CD40L antibody prevented colitis induced by the transfer of enteric bacteria reactive CD4+ T cells. Finally, the proinflammatory role of the CD40 pathway was more recently confirmed in the development of acute ileitis following oral infection with *T gondii*.⁴⁹

Clinical trials with different monoclonal antibodies have been initiated in humans with various autoimmune and immune mediated conditions. A phase I trial with a chimeric antagonistic monoclonal antibody against CD40 (5D12) is underway in CD patients.¹⁰ Phase I and II trials with the humanised CD40L antibody (IDEC-131) have been initiated in CD as well as in psoriasis, idiopathic thrombocytopenic purpura, multiple sclerosis, and systemic lupus erythematosus.⁵⁰ In a preliminary report, IDEC-131 was safe and well tolerated in patients with systemic lupus erythematosus but, when compared with placebo, no efficacy could be demonstrated.⁵¹ Evidence of efficacy for IDEC-131 in the other

diseases is still not available because some studies have been on hold due to thromboembolic complications. The trial for idiopathic thrombocytopenic purpura has been resumed, and that for multiple sclerosis patients is about to restart. A very recent report describes the outcome of administration of another humanised CD40L monoclonal antibody (BG9588, 5c8) to patients with systemic lupus erythematosus.⁵² Blockade of the CD40 pathway significantly reduced serum autoantibodies, proteinuria, and clinical activity of the disease in a small group of patients. Although the use of this antibody was also associated with side effects, these results are notable because they provide in vivo proof of principle, and demonstrate the considerable potential of anti-CD40L therapy in immune mediated disorders.⁵³

As often is the case, the beneficial effects expected from rationally developed biological therapies, such as blockade of CD40 or CD40L, are not as readily demonstrable and obvious as desired. Nevertheless, as long as the underlying reasoning and experimental evidence are solid, such studies must continue until a safe blocker is identified, the correct dose and appropriate administration regimens are defined, and adopted in carefully designed multicentre protocols that include large numbers of properly selected patients. Only after their completion will it be possible to conclude how effective therapy based on disruption of CD40/CD40L costimulation is, and how well it compares with other biological therapies targeting proinflammatory cytokines, adhesion and homing molecules, or alternate costimulatory pathways.

- ▶ Disruption of CD40/CD40L interactions may offer therapeutic benefits by interrupting immune-immune and immune-non-immune cell interactions that sustain inflammation.
- ▶ Blockade of CD40 signalling with monoclonal antibodies has been shown to prevent or improve colitis in several animal models of IBD.
- ▶ Various monoclonal antibodies against CD40 and CD40L are undergoing clinical trials to test their potential clinical benefits in patients with IBD and other autoimmune diseases.

SUMMARY AND CONCLUSIONS

- ▶ Many different types of immune and non-immune cells are involved in IBD pathogenesis, and they communicate with each other utilising a variety of stimulatory and costimulatory pathways. Among these, the CD40/CD40L costimulatory pathway is of particular interest because of its diffuse distribution, potency, and variety of actions.
- ▶ Interactions between CD40 and CD40L expressing cells impact on multiple biological phenomena directly relevant to intestinal inflammation, including antibody production, activation of macrophages and dendritic cells, production of proinflammatory cytokines, chemokines, prostaglandins, proteolytic enzymes, and NO, and upregulation of adhesion molecules.
- ▶ There is objective evidence that the CD40/CD40L costimulatory pathway is activated in IBD tissue, as demonstrated by a marked increase in the number of cells expressing high levels of CD40 and CD40L, and the presence of elevated levels of sCD40L in the circulation of IBD patients.
- ▶ The contact of CD40L+ T cells and CD40L+ platelets with CD40+ mesenchymal and endothelial cells results in enhanced production of chemokines and expression of adhesion molecules that lead to the recruitment of more T cells which may become activated in the inflamed mucosa, express CD40L de novo, and induce further chemokine production, creating a vicious cycle of cell interactions that promote chronic intestinal inflammation.
- ▶ Based on presently available evidence, blockade of CD40/CD40L interactions may provide therapeutic benefits to IBD patients. This is strongly supported by the clinical and histological improvement observed in various animal models of experimental colitis treated with CD40L antibodies. Monoclonal antibodies against CD40 and CD40L are available for use in humans with IBD and several other autoimmune and chronic inflammatory conditions. The results of ongoing and future clinical trials will define the value of blocking the CD40 pathway within the expanding armamentarium of biological therapies for IBD.

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