

Role of CD40 and B7 costimulators in inflammatory bowel diseases

Lino Polese¹, Imerio Angriman¹, Marco Scarpa¹, Lorenzo Norberto¹, Giacomo Carlo Sturniolo², Attilio Cecchetto³, Cesare Ruffolo¹, Davide Francesco D'Amico¹

¹ Dipartimento di Scienze Chirurgiche e Gastroenterologiche P.G.Cèvese, Clinica Chirurgica I; ² Dipartimento di Scienze Chirurgiche e Gastroenterologiche P.G.Cèvese, Divisione di Gastroenterologia; ³ Istituto di Anatomia Patologica, Università di Padova, Italy

Abstract. We analyse the costimulating role of CD40/CD40 ligand and B7/CD28 in inflammatory bowel diseases (IBD) as a potential target of antibody therapy. CD40, expressed by lamina propria B lymphocytes in gut mucosa, interacts with CD40 ligand on T cell. This interaction is implicated in the pathogenesis of IBD. In some animal models of colitis the anti-CD40L therapy demonstrated to be effective. Phase II trials on Crohn's disease are ongoing. B7.1 and B7.2, expressed by macrophages, interact with CD28, on T cell. B7.2 resulted implicated in ulcerative colitis, determining a Th2 pattern, whereas B7.1, a major Th1 stimulator, could be involved in Crohn's disease. In some animal models of colitis anti-B7.1, but not anti-B7.2, was effective. Anti B7 therapy was not yet tested in humans.

Key words: CD40, ulcerative colitis, Crohn's disease

Introduction

The aetiology of Inflammatory Bowel Diseases (IBD), Ulcerative colitis and Crohn's disease, is still unknown. Some evidences suggest the presence of an alteration of the immune response versus self or lumen antigens (1). In fact, IBD are associated with a higher incidence of autoimmune diseases (Sclerosing Cholangitis, Systemic Lupus Erythematosus, Autoimmune Anaemia, Pernicious Anaemia, Insulin-Dependent Diabetes Mellitus, Idiopathic Thrombocytopenic Purpura, Hashimoto's Thyroiditis) (2-6) and with extracolonic events, like arthritis, Erythema Nodosum, uveitis, due to the presence in the blood of immuno-complexes. The response to immunosuppressive drugs (steroids, azathioprine) is another prove that an immunological alteration plays a role in their pathogenesis.

Looking in depth, there is a switch of the intraluminal immunoglobulins from IgA to IgG, with loss

of tolerance versus the luminal antigens (7-9). The association with specific antibodies, like ANCA and ASCA, is also an event that brings the IBD close to the autoimmune diseases (10, 11).

Even if Crohn's disease and ulcerative colitis share some similarities, they are 2 distinct diseases, in the majority of the cases, with not only histological, but also molecular differences.

In Crohn's disease, we find macroscopically: scattered lesions, stenosis, fistulas, deep ulcerations, accompanied by nodular swelling, with cobblestone appearance; microscopically: noncaseating-granulomas, with epithelioid cells and multinucleated giant cells, occurring in all the layers, from the mucosa to the serosa, after the macrophages invasion. Then other inflammatory cells are implicated, including lymphocytes and plasma cells, giving transmural inflammation.

In Ulcerative Colitis the intestinal disease is limited to the colon and the rectum, saving the ileum; ma-

croscopically the lesions are continuous, with hyperaemic, edematous and granular mucosa, ulcerations are frequent, but limited to mucosa, in long-standing disease there are also pseudo-polyps. Fibrosis is uncommon, as stenosis and fistulas, whereas more frequent are bleeding and perforation. Microscopic features are: an inflammation confined to mucosa, with inflammatory infiltrate of neutrophils, lymphocytes, plasma cells and macrophages. The neutrophils usually invade the epithelium and the crypts, determining crypt abscesses. Glands show an altered architecture. Molecularly, both the diseases have a high expression of $\text{IFN}\gamma$, IL-1, IL-6, $\text{TNF}\alpha$ (all cytokines of macrophage origin), but in Crohn's disease there is an increase of IL2 and a reduction of IL-4, whereas in ulcerative colitis there is a higher expression of IL-4 and IL5. Summarized these data suggest the prevalence of Th1 pattern in Crohn's disease and of Th2 lymphocytes in ulcerative colitis (12-14).

Role of costimulators in T lymphocyte activation

According to recent studies, the activation of the T lymphocyte against the antigen, requires two factors: the presentation of the antigen by the antigen presenting cell (APC) through MHC, and the contemporary presence, on the surface of the 2 cells, of some molecules, called "costimulators" (Fig 1). If the antigen is presented without the interaction between these receptors, the lymphocyte becomes tolerant. The knowledge of this mechanism has induced to think that these costimulatory molecules could be implicated in the pathogenesis of autoimmune-diseases and that blocking this interaction, we could control the development and the activity of many immune-disorders, included IBD.

Many molecules, expressed by APCs, demonstrated to play a role as costimulators. We analysed the role of 2 of them in the tissue of IBD: CD40 and B7, to hypothesize a new target of antibody therapy.

CD40 in IBD

CD40 is a costimulatory molecule that interacts with CD40 ligand (CD40L or CD154), expressed by

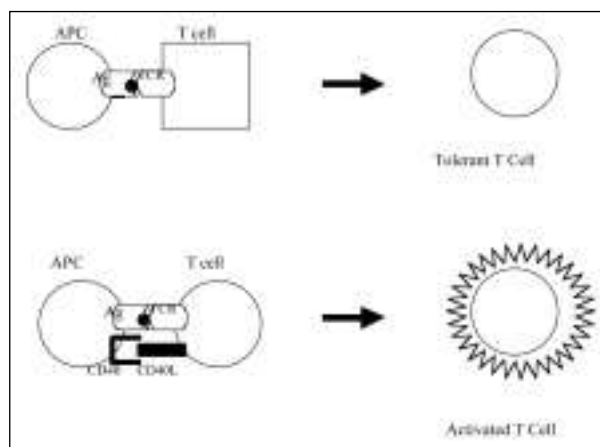


Figure 1. The T cell activity against the antigen requires a second stimulus: the interaction between 2 costimulators (here expressed by CD40-CD40L). APC=Antigen presenting cell; Ag=antigen; TCR=T cell receptor.

T lymphocytes. It is a member of the TNF receptors family, has a molecular weight of 48 Kdalton and is composed by three domains: intracellular, trans-membrane and extra-cellular (15). The CD40 is expressed not only by traditional APCs, but also by other cells, such as fibroblasts, endothelial cells (16) and some epithelia (17, 18). In the normal colonic tissue the expression of CD40 is limited to the leukocytes of the lamina propria and of lymphoid follicles. We found that in non-specific inflammation (follicular proctitis, non-specific colitis), the CD40+ cells were present only immediately below the epithelium, where they respond to stimuli of luminal origin, whereas in UC these were diffusely distributed along the whole mucosal space (19). According to Liu et al. (20) in Crohn's disease CD40+ leukocytes are present also in the submucosa and muscularis propria, and lower numbers also in the subserosal connective tissue. This could be due to a different origin of the stimulus in IBD and other inflammatory events. We analysed by immunohistochemistry the expression of CD40 in the colonic mucosa from 30 patients with ulcerative colitis, 9 with Crohn's disease and 12 with non-specific inflammation (follicular proctitis, non-specific colitis) (19). In our study, CD40 was expressed by $21\pm 11\%$ of the lamina-propria leukocytes in U.C., $24\pm 9\%$ in Crohn's disease and $7\pm 7\%$ in non-specific inflammatory events ($p < 0.005$) (Fig. 2). Its expression in ulce-

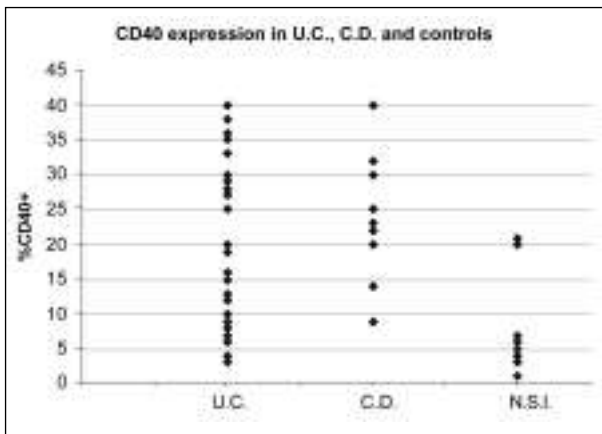


Figure 2. CD40 expression by lamina propria B lymphocytes in ulcerative colitis (mean $21 \pm 11\%$), Crohn's disease (mean $24 \pm 9\%$) and non-specific inflammation (mean $7 \pm 7\%$) ($p < 0.05$). U.C.=ulcerative colitis; C.D.=Crohn's disease; N.S.I.=nonspecific inflammation.

ative colitis, resulted also proportional to the state of activity, according to histological, endoscopic and clinical criteria ($p < 0.05$). The double staining indicated that sub-epithelial lamina propria CD40 cells in ulcerative colitis and Crohn's disease are mostly B lymphocytes (CD20+), typical of a specific immunologic response.

The interaction between CD40 and CD40L, determines a bi-directional stimulatory signal of the T lymphocyte, that becomes activated, and of the B lymphocyte, that expresses B7 family molecules on the surface, becoming APC competent, and IL-1 γ , IL-6, IL-10, TNF- α , leukotriene- α , and GM-CSF (21-23). The block of this interaction should then make the T lymphocyte tolerant and should reduce the expression of inflammatory cytokines by B lymphocyte, like IL-1 and TNF- α , inhibiting the disease activity, especially where and when the inflammation is more severe. The efficacy of anti-CD40L therapy has been studied in some animal models of colitis, induced by CD45Rb(hi), Rag (-/-) (24) or TNBS (trinitrobenzene-sulphonic acid) (25), with encouraging results. Anti-CD154 therapy was effective also in a model of ileitis occurring in C57BL/6 mice after oral infection with *Toxoplasma gondii* (26). From these studies the CD40-CD40L interaction resulted essential to the development of the inflammation and anti-CD154 mAb therapy could control the disease, reducing the

Th1 (IFN γ) and increasing the Th2 response (IL-4). The shift from a Th1 to a Th2 immune response should be mostly effective in Crohn's disease, where the disease is characterized by an increased Th1 response. Also the efficacy of anti-TNF therapy in Crohn's disease, is due to this phenomenon.

The anti-CD154 Antibody therapy has been also tested in humans, on LES patients. A first anti-CD40L antibody, hu5c8, demonstrated to be effective in lupus glomerulonephritis, but unfortunately was burdened by thromboembolic events (2 myocardial infarctions), so the study was interrupted prematurely (27). Another anti-CD154 antibody, IDEC 131, was safe and well tolerated on phase I and phase II trials in LES, but efficacy, compared with placebo, was not demonstrated (28). A II phase trial with anti-CD40L antibodies (IDEC131) on Crohn's patients is ongoing (29).

B7 in IBD

Another co-stimulatory molecule, recently analysed on IBD mucosa, is B7. It is expressed on APC cells and interacts with CD28 or with CTLA-4 on T-Lymphocytes (30). B7 family has many different forms, the most studied being B7-1 (CD80) and B7-2 (CD86). CD80 is constitutive and has ten-fold better affinity for CD28 than CD86 (31). CD28 is a constitutive molecule, the interaction with B7 gives an important co-stimulation signal activating the T cell.

Instead CTLA-4 has an inhibitory effect, preventing T-cell proliferation, especially binding the CD80 (32). For that reason CD86, even with less affinity for CD28, results having more importance for T-cell activation (31). CTLA-4 is not constitutive, but is up-regulated following the T-cell activation, with peak expression between 48 and 72 hours. The majority of this protein remains intracellular.

Rugtveit et al. (33), by immunohistochemistry, analysed the expression of B7-1 and B7-2 from colonic and ileal tissue of Crohn's disease, ulcerative colitis and controls. They found a common expression of B7-2 in the inflamed as well as in the normal tissue, but in the inflamed IBD mucosa B7-2 was increased and

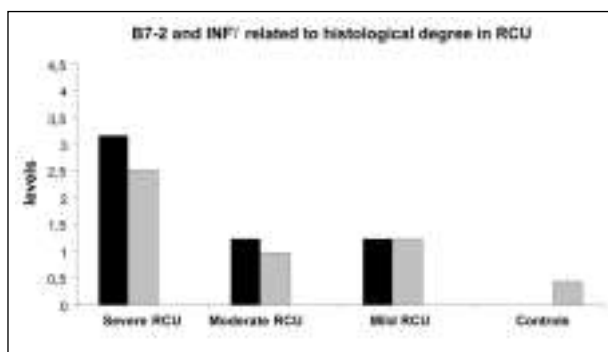


Figure 3. B7-2 (black) and IFN- γ (gray) in Ulcerative colitis (severe, moderate and mild) and in controls (where B7-2 is not expressed).

appeared also the presence of B7-1+cells. The B7 population was constituted overall by macrophages.

We too analysed (34), by Polymerase Chain Reaction (PCR), the expression of B7-2 and B7-1 in the large bowel mucosa of patients affected by IBD. The patients were: 21 affected by UC, 9 by CD, 3 healthy subjects. From our study we found B7-2 expression in the large bowel mucosa of patient affected by ulcerative colitis, higher during the onset of the disease ($p=0.002$) (Fig. 3). Surprisingly we did not find B7-2 expression in Crohn's disease or in controls, whereas the IFN- γ expression resulted similar in UC and CD, increased respect to control. This could be explained considering the B7-2 mostly implicated in the diseases characterized by a prevalent Th2 lymphocyte activity (35).

If we consider anti-B7 therapy in some experimental autoimmune diseases, we see that anti-B7-1 prevents the development of experimental autoimmune encephalomyelitis (EAE) in mice (switching from a Th1 to a Th2 response, with increase of IL4) (36, 37), whereas anti-B7-2 suppresses the onset of diabetes in nonobese diabetic mice (switching from a Th2 to a Th1 response) (38).

The analysis of anti-B7 therapy in Chronic Experimental Colitis, induced in Scid mice, transferring CD45RB^{high} CD4⁺ Tcells from syngeneic mice, was conducted by Liu et al (39). They found that in this model of colitis, the anti-B7-1 but not anti B7-2 therapy prevented the disease, reducing the IL-2 and IFN- γ production by lamina propria CD4⁺ cells. This

colitis, with a major Th1 response, is more similar to Crohn's colitis than ulcerative colitis, even if histological patterns are of the latter.

This induce to think that anti-B7-1 therapy could be useful in the treatment of Crohn's disease and anti-B7-2 therapy in the treatment of ulcerative colitis. The inhibition of CD28 was similarly able to prevent the colitis in murine model, whereas anti-CTLA-4 treatment led to deterioration of the disease. This confirm that CTLA-4 plays a role in the regulation of T response and in maintaining the lymphocyte tolerance.

Therapy with anti-B7 or anti-CD28 antibodies has not yet been tested in humans, whereas anti-CTLA-4 was used in patients affected by Rheumatoid Arthritis.

Conclusions

Costimulatory molecules show to play a major role in the pathogenesis and activity of autoimmune diseases. In IBD the interaction between CD40 and CD40L is certainly implicated and promises to be a valid target for specific antibodies therapy. Less clear is the role played by B7-1 and B7-2, considering also their possible interaction with CD28 or CTLA-4, that have opposite functions on T-cell activation. Antibody-therapy against CD40L has been already safely tested in patients affected by LES and its use in IBD seems to be promising. An association with other antibodies, like anti-TNF, is conceivable.

References

1. Radford-Smith G. Ulcerative colitis: an immunological disease? *Bailliere's Clinical Gastroenterology* 1991; 11: 35-52.
2. Snook JA, de Silva HJ, Jewell DP. The association of autoimmune disorders with inflammatory bowel disease *QJM* 1989; 72: 835-40.
3. Greenstein AJ, Janowitz HD, Sachar DB. The extra-intestinal complications of Crohn's disease and ulcerative colitis: a study of 700 patients. *Medicine Baltimore* 1976; 55: 401-12.
4. Snook J. Are inflammatory bowel diseases autoimmune disorders? *Gut* 1990; 31: 961-3.
5. Stevens C, Peppercorn MA, Grand RJ. Crohn's disease associated with autoimmune neutropenia. *J Clin Gastroenterol* 1992; 15: 2-3.

6. Govindarajan R., Galpin OP. Coexistence of Addison's disease, ulcerative colitis, hypothyroidism and pernicious anemia. *J Clin Gastroenterol* 1992; 15: 82-3.
7. MacDermott RP, Nash GS et al. Altered patterns of secretion of IgA and IgG subclasses by ulcerative colitis and Crohn's disease intestinal mononuclear cells. *Advances in Experimental Medicine and Biology* 1987; 216A: 335-44.
8. MacDermott R, Beale M, et al. Synthesis and secretion of IgA, IgM and IgG by peripheral blood mononuclear cells in human disease states, by isolated human intestinal mononuclear cells, and by human bone marrow mononuclear cells from ribs. In McGhee JR and Mestecky J eds The secretory Immune System
9. Scott B, Goodall A, et al. Rectal mucosa plasma cells in inflammatory bowel disease. *Gut* 1983; 24: 519-24.
10. Abad E, Tural C, Mirapeix E, Cuxart A. Relationship between ANCA and Clinical Activity in Inflammatory Bowel Disease: Variation in prevalence of ANCA and Evidence of Heterogeneity. *Journal of Autoimmunity* 1997; 10: 175-80.
11. Targan S, Landers C, et al. Perinuclear anti-neutrophil cytoplasmic antibodies are spontaneously produced by mucosal B cells of ulcerative colitis patients. *Journal of Immunology* 1995; 155: 3262-326.
12. Mullin G, Lanzanby AJ, Harris ML, et al. Increased interleukin-2 messenger RNA in the intestinal mucosal lesions of Crohn's disease but not ulcerative colitis. *Gastroenterology* 1992; 102: 1620-27.
13. Mullin G, Vezza F, Sampat A, et al. Abnormal IL10 mRNA production in the intestinal lesions of inflammatory bowel disease. *Gastroenterology* 1993; 104: A751.
14. Mullin G, Maynon Z, Katz R, et al. IL-13 in the mucosal lesions of inflammatory bowel disease. *Gastroenterology* 1994; 106: A740.
15. Bajorath J, Aruffo A. Construction and analysis of a detailed three-dimensional model of the ligand binding domain of the human B cell receptor CD40. *Proteins: Structure, Function and Genetics* 1997; 2:5 9-70.
16. Karmann K, Hughes CCW, Schechner J, Fanslow WC, Pober JS. CD40 on human endothelial cells: inducibility by cytokines and functional regulation of adhesion molecule expression. *Proc Natl Acad Sci USA* 1995; 92: 4342-6.
17. Van Kooten C, Gerritsma JSJ, Paape ME, van Es LA, Banchereau J, Daha MR. Possible role for CD40-CD40L in regulation of interstitial infiltration in the Kidney. *Kidney Int* 1997; 513: 711-21.
18. Fernandez E, Vincente A, Zapata A. Establishment and characterization of cloned human thymic epithelial cell lines. Analysis of adhesion molecule expression and cytokine production. *Blood* 1994; 83: 3245-54.
19. Polese L, Angriman I, Cecchetto A, et al. The role of CD40 in ulcerative colitis: histochemical analysis and clinical correlation. *European Journal of Gastroenterology and Hepatology* 2002; 14: 237-41.
20. Liu Z, Colpaert S, D'Haens GR, et al. Hyperexpression of CD40 Ligand (CD154) in Inflammatory Bowel Disease and its Contribution to Pathogenic Cytokine Production. *J Immunol* 1999; 163: 4049-57.
21. Van Kooten C, Banchereau J. Functional Role of CD40 and its Ligand. *Int Arch Allergy Immunol* 1997; 113: 393-9.
22. Van Gool SW, Vandenberghe P, De Boer M, Ceuppens JL. CD80, CD86 and CD40 Provide Accessory Signals in a Multiple-Step T-Cell Activation Model. *Immunological Reviews* 1996; 153: 47-81.
23. Grewal IS, Flavell RA. The role of CD40 Ligand in Costimulation and T-Cell Activation. *Immunological Reviews* 1996; 153: 85-105.
24. Liu Z, Geboes K Colpaert S, Overbergh L, et al. Prevention of experimental colitis in SCID mice reconstituted with CD45RBhigh CD4+ T cells by blocking the CD40-CD154 interactions. *J Immunol* 2000; 164 (11): 6005-14.
25. Stuber E, Strober W, Neurath M. Blocking the CD40L-CD40 interaction in vivo specifically prevents the priming of T helper 1 cells through the inhibition of interleukin 12 secretion. *J Exp Med* 1996; 183: 693-8.
26. Li W, Buzoni-Gatel D, Debbabi H, et al. CD40/CD154 ligation is required for the development of acute ileitis following oral infection with an intracellular pathogen in mice. *Gastroenterology* 2002; 122 (3): 762-73.
27. Boumpas DT, Furie R, Manzi S, et al. A short course of BG9588 (Anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis *Arthritis & Rheumatism* 2003; 4: 719-27.
28. Kalunian KC, Davis JC, Merrill JT, et al. Treatment of systemic lupus erythematosus by inhibition of Tcell costimulation with anti-CD154. *Arthritis & Rheumatism* 2002; 46: 3251-8.
29. Dumont FJ. IDEC-131. IDEC/Eisai. *Curr Opin Investig Drugs* 2002; 3 (5): 725-34.
30. Sperling AI, Bluestone J A. The complexities of T-cell costimulation: CD28 and Beyond. *Immunological Reviews* 16; 153: 155-82.
31. Manzotti CN, Tipping H, Perry LCA, et al. Inhibition of Human T cell proliferation by CTLA-4 utilizes CD0 and requires CD25+ regulatory T cells. *Eur J Immunol* 2002; 32: 2888-96
32. Alegre ML, Frauwirth KA, Thompson CB. T-cell regulation by CD28 and CTLA-4. *Nature Reviews Immunology* 2001; 1: 220-8.
33. Rugtveit J, Bakka A, Brandtzaeg P. Differential distribution of B7.1 (CD80) and B7.2 (CD86) costimulatory molecules on mucosal macrophage subsets in human inflammatory bowel disease (IBD). *Clin Exp Immunol* 1997; 110: 104-13.
34. Scarpa M, Angriman I, Behboo R, et al. Costimulation molecules B7 and IFN γ in the pathogenesis of inflammatory bowel disease. *Hepato-Gastroenterology* 2002; 49, Supplement I: XXX-XXXI.
35. Kuchroo VK, Das MP, Brown JA, et al. B7-1, and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell* 1995; 80: 707-18.

36. Racke MK, Scott DE, Quigley L, et al. Distinct roles of B7.1 (CD80) and B7.2 (CD86) in the initiation of experimental allergic encephalomyelitis. *J Clin Invest* 1995; 96: 2195.
37. Miller SD, Vanderlugt CL, Lenschow DJ, et al. Blockade of CD28/B7.1 interaction prevents epitope spreading and clinical relapses of murine EAE. *Immunity* 1995; 3: 739.
38. Lenschow DJ, Ho SC, Sattar H, et al. Differential effects of anti-B7-1 and anti B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J Exp Med* 1995; 181: 1145.
39. Liu Z, Geboes K, Hellings P, et al. B7 interaction with CD28 and CTLA-4 control tolerance or induction of mucosal inflammation in chronic experimental colitis. *The Journal of Immunology* 2001; 167: 1830-8.
40. Moreland LW, Alten R, Van den Bosch F, et al. Costimulatory Blockade in patients with rheumatoid arthritis. *Arthritis & Rheumatism* 2002; 46: 1470-9.

Correspondence: Dr. Lino Polese
Clinica Chirurgica I
Policlinico Universitario
Via Giustiniani 2
35128 Padova (Italy)
E-mail: linopolese@hotmail.com