

Review

Modulating co-stimulation: a rational strategy in the treatment of rheumatoid arthritis?

Vivianne Malmström, Christina Trollmo and Lars Klareskog

Rheumatology Unit, Department of Medicine, Karolinska Institutet, Stockholm, Sweden

Corresponding author: Vivianne Malmström, Vivianne.malmstrom@cmm.ki.se

Published: 16 March 2005

Arthritis Res Ther 2005, **7(Suppl 2)**:S15-S20 (DOI 10.1186/ar1505)
© 2005 BioMed Central Ltd

Abstract

Rheumatoid arthritis (RA) is a common destructive inflammatory disease that affects 0.5–1% of the population in many countries. Even though several new treatments have been introduced for patients with RA, a considerable proportion of patients do not benefit from these, and the need for alternative treatment strategies is clear. This review explores the potential for a therapy targeting the adaptive immune system by modulating co-stimulation of T cells with a CTLA4-Ig fusion protein (abatacept).

Introduction

A large body of evidence has accumulated during recent years describing the benefit of early control of inflammation in order to avoid long-term disability and to maintain good function in patients with rheumatoid arthritis (RA) [1–3]. The introduction of tumour necrosis factor (TNF)-blocking agents, in particular when they are given together with methotrexate, has further improved the situation in terms of disease activity, joint destruction and function for many patients [4–6]. This success of therapies targeting TNF using monoclonal antibodies or recombinant receptor constructs has set the scene for the introduction of additional 'biological' therapies that target key structures of the immune system. This has also introduced a need to elucidate the roles played by various immune events in the pathogenesis of RA in different groups of patients with this disease, especially those who do not benefit from TNF-blocking agents.

Blocking innate immune responses

Innate immune responses are rapid ways in which the organism may eradicate pathogens. Cells that participate include neutrophils, macrophages and natural killer cells. Common for these cells is the ability to secrete inflammatory mediators upon activation by rather unspecific stimuli from microbial and other agents. In conditions such as RA, which are characterized by chronic inflammation, these cells contribute substantially to the (immune) pathology. Indeed, during the 1990s blocking the proinflammatory cytokine TNF

was demonstrated to be beneficial in experimental arthritis [7] and later also for human disease (see above). Furthermore, blockade of IL-1, IL-6 and IL-15 has been tested in both experimental arthritis in rodents [8,9] and in human RA [10–12], with promising results.

Taken together, these data have led to a general belief that innate immune responses are crucial to the manifestations of RA, and that adaptive immune responses may be less important in the pathogenesis of the disease and more difficult to target. However, that belief over-simplifies this complex disorder, and there are old as well as recent indications that the adaptive immune system is also of major pathophysiological importance in RA, and it may also be an efficient target for RA therapy [13,14].

Blocking adaptive immune responses

Following the rapid immune reactions by cells of the innate immune system, adaptive immune responses are mounted as part of the normal immune response to pathogens. These responses are characterized by their high specificity for the antigen, and under normal conditions they are sequentially upregulated and downregulated. Cells characteristic of the adaptive immune system are B cells, T cells and professional antigen-presenting cells (APCs; i.e. dendritic cells, macrophages and B cells).

B cells

B cells perform important functions as antibody-secreting cells but they can also function as APCs and cytokine producers. In RA, a role for B cells in the pathogenesis of the disease has long been discussed [15–17]. First, rheumatoid factor (i.e. anti-IgG Fc antibodies) is frequently present in sera of patients with RA [18,19] and has even been used as a prognostic marker for the development of an erosive disease course [20]. Second, anti-citrullin antibodies are frequently detected in RA patients [21–23]. These antibodies are very specific for RA; they can appear before the onset of disease

and so can be used as a prognostic marker for disease development [24,25]. Both of these RA-associated antibody responses are initiated with the help of activated T cells.

New therapies for RA are emerging that focus on the adaptive arm of the immune system, one of them being rituximab. Rituximab targets the CD20 molecule, which is selectively expressed on B cells and depletes these cells [26]. This treatment approach has yielded good responses in the majority of rheumatoid factor positive RA patients treated thus far, but more clinical research is necessary before it can be widely applied clinically [17].

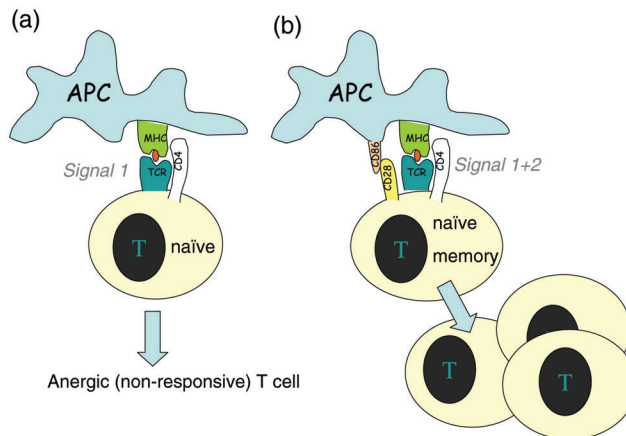
CD4+ T cells

T cells can be divided into CD4+ and CD8+ T cells; the former are the classic helper cells and are crucial, for example, for antibody production and activation of cytotoxic immune responses. The CD4+ cells are also the dominant T cells in inflammatory infiltrates in the synovia of RA patients [27,28]. The impact of this is further substantiated by the fact that MHC class II is also abundantly expressed in the rheumatoid synovium [28]; thus, T cells have the potential to become reactivated locally in the joint. The importance of T cells in arthritis was further validated in mice, in which disease can be transferred to a naïve host by injecting T cells from an affected animal [29]. Also, experimental disease can be controlled by T cell depletion before initiation [30]. Thus, therapeutic interventions targeting T cells have for some time remained an attractive option in RA. However, further studies along this line have been disappointing; in a first trial [31] targeting of CD4+ T cells with monoclonal antibodies did not affect disease development. A different monoclonal antibody caused more profound depletion of T cells [32], but this therapeutic approach was associated with significant adverse effects and mortality. In addition, using other available agents that interfere specifically with T cell functions, such as cyclosporin, has been only moderately successful [33], suggesting that we must find more efficient agents that interfere with T cell activation and investigate their benefit in clinical settings.

CD4+ T cells and co-stimulation

Both naïve and activated/memory T cells traffic in the circulation. However, at the site of inflammation (e.g. the rheumatic joint) only activated/memory T cells are found. This is because of restriction of surface molecules that are needed for homing to tissue, and these are not expressed on naïve T cells [34]. A naïve T cell becomes activated after it encounters antigen. The antigen is presented on HLA molecules of APCs, but just recognition of antigen and MHC by the T cell receptor is not sufficient for a naïve T cell to become activated (Fig. 1a). Co-stimulation is also needed; this is an interaction that sustains APC–T cell contact and amplifies signals in the T cell [35]. The best characterized co-stimulatory signal is that provided by CD28 expressed on T cells ligating to CD80/86 (B7-1 and B7-2 molecules) on APCs (Fig. 1b). CD28 ligation of naïve T cells has been

Figure 1



Activation of naïve T cells requires (a) T cell receptor (TCR)–peptide–MHC interaction (signal 1) and (b) co-stimulation (signal 2) for full activation. This can be provided by so-called professional antigen-presenting cells (APCs; i.e. dendritic cells, macrophages and B cells). In the absence of co-stimulation the T cells will become anergic.

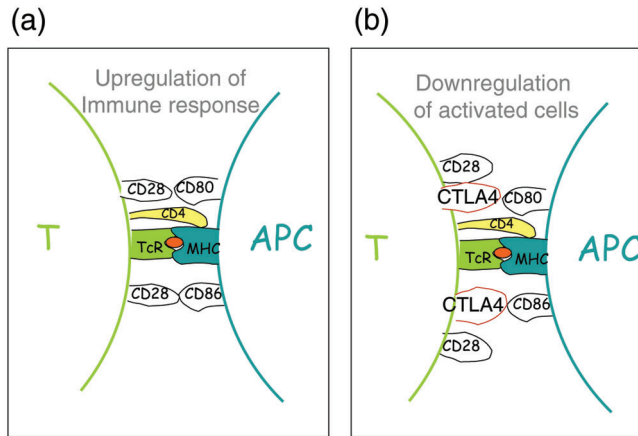
proven to be essential for IL-2 production and cell proliferation [36]. Also, most activated/memory T cells retain their surface expression of CD28, suggesting that this molecule is also involved in reactivating T cells [37].

Since the initial description of CD28 and CD80/86, the list of co-stimulatory molecules has steadily grown and includes ICOS, CD134 (Ox40) and CD27, among others [38]. They do not utilize the CD80 and CD86 molecules, and so the use of a soluble blocking cytotoxic T lymphocyte-associated antigen (CTLA4)–immunoglobulin (Ig) complex only prevents ‘classic’ co-stimulation mediated by CD28.

CTLA4 (CD152) is a molecule that can out-compete CD28 for ligation of the B7 molecules (Fig. 2). Its affinity for the B7 molecules is 10–20 times greater than that of CD28. Biologically, these differences in affinity result in limitation and subsequent downregulation in T cell responses. That this mechanism is needed is clearly demonstrated in CTLA4 knockout mice, which die within 4 weeks of birth from lymphoproliferative disease [39,40].

CTLA4–Ig fusion protein

The use of a CTLA4 fusion protein as a means to block B7 molecules has been well documented in experimental autoimmunity [41–43]. Administration of CTLA4–Ig at the time of immunization prevented collagen-induced arthritis. Interestingly, administration after disease onset also ameliorated disease [44]. Similar effects were obtained when a combination of anti-CD80 and anti-CD86 antibodies were used to block the co-stimulation, indicating the need to block both pathways in the APC. Studies conducted by Tellander

Figure 2

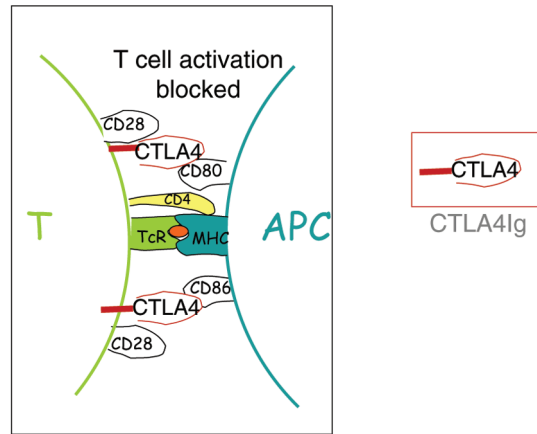
CD28 is constitutively expressed on T cells and CD86 is expressed on antigen-presenting cells (APCs). **(a)** Upon activation CD80 is also expressed on the APC and CD86 is further upregulated. **(b)** Cytotoxic T lymphocyte-associated antigen (CTLA4) expression is induced later during activation, and out-competes CD28 for the interactions, thereby inducing a downregulation in immune response.

and coworkers [45] indicate that antibody titres are also decreased when CD80/CD86 are blocked, indicating the importance of this co-stimulation pathway in B cell help.

These and several other experimental studies have led to the development of the drug abatacept – a recombinant fusion protein comprising the extracellular domain of human CTLA4 fused with a fragment of the Fc portion of human IgG₁ (Fig. 3). A first study was conducted in patients with psoriasis [46], among whom 46% achieved a 50% or greater sustained improvement in clinical disease activity. More recently, abatacept has also been administered to patients with RA, with beneficial effects [47,48] (see also the article by Ruderman and Pope in this supplement).

Levels of co-stimulation molecules in rheumatoid arthritis

In addressing the potential mechanism of action of abatacept in RA patients, it is of interest to examine whether levels of CD28, CTLA4 and CD80/86 vary among cells in the circulation and in different inflammatory compartments. A comparison of CD28 levels on the surface of peripheral blood cells of healthy individuals and RA patients [49] clearly demonstrated the highest levels of CD28 in patients with active disease. Also, CD28 expression is augmented in T cells of the synovial tissue as compared with cells from peripheral blood. This indicates that T cells in patients with active disease have strong potential to interact with and activate APCs, which in turn upregulate their CD80 and CD86 expression and activate more T cells. It is well known that the levels of CD80 and CD86 vary with the degree of activation of the APC.

Figure 3

CTLA4-Ig (cytotoxic T lymphocyte-associated antigen 4-immunoglobulin fusion protein) blocks the T cell-antigen presenting cell (APC) interaction by binding to both CD80 and CD86, thus preventing CD28 interaction. CTLA4 has a higher affinity for the B7 molecules than does CD28. TCR, T cell receptor.

Analysis of CTLA4 levels in peripheral blood [49] indicated a higher baseline level in RA patients than in healthy control individuals. Upon *in vitro* activation the cell surface levels of CTLA4 were similar in the two groups. Among RA patients more cells with surface expression of CTLA4 were found in synovial fluid than in peripheral blood [50].

Patients with co-stimulation deficient T cell populations

There is one subgroup of patients in which CTLA4-Ig and B7 blockade can be assumed to be inefficient, and this is the group with an expanded T cell population consisting of CD28null cells [51]. These cells, lacking CD28 on their cell surface, do not rely on co-stimulation for reactivation [52], and as potent producers of proinflammatory cytokines they may be at an advantage if the rest of the T cell pool, expressing CD28, is suppressed by abatacept. Because these patients are easily identified by flow cytometry, there are two principal options. They can simply be excluded from treatment with abatacept and receive alternative therapy instead, or they may be identified in retrospect in order to investigate whether the heterogeneity in response to CTLA4-Ig among RA patients can be explained by the presence of this unusual cell population. Heterogeneities in treatment response have also been observed for most other antirheumatic drugs including methotrexate and TNF-blocking agents. In all of these cases, there is a need to identify those patients in whom each drug has optimal efficacy and fewest adverse events.

Possible effects of CTLA4-Ig on protective immune responses

Abatacept selectively modulates T cell activation through blocking 'classic' co-stimulation. This selective action allows

other immune pathways to remain largely intact and ensures that T cell activation is modulated rather than completely blocked. The latter scenario would lead to immune suppression and the potential for opportunistic infections. This issue was addressed in the clinical psoriasis study [46], in which immune responses against novel antigens also occurred after initiation of treatment. This selective blockade of CD28-dependent activation pathways was also observed in experimental animal studies [53]; CD28 deficient mice exhibited normal immune function in models of infection both before and after treatment with CTLA4-Ig.

Consequences at the cellular level

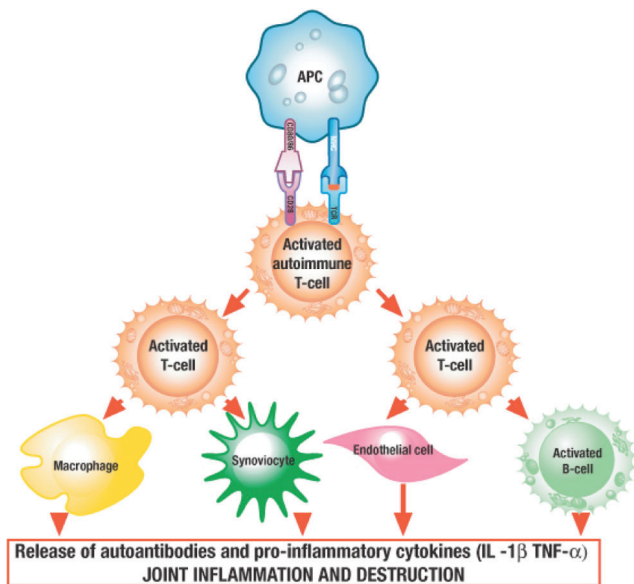
How does CTLA4-Ig work from a cellular point of view? One needs to remember that it does not target T cells directly. Rather, it blocks APCs so that they cannot co-stimulate T cells. Such blockade has direct functional consequences at the APC level; for example, after co-culturing synovial cells in the presence of CTLA4-Ig or anti-B7 antibodies, the amount of proinflammatory cytokines produced by APCs was reduced [54].

Other signalling pathways in the APC are also affected. It was recently suggested that ligation of CTLA4-Ig with CD80/86 on the APC leads to activation of the enzyme IDO (indoleamine-2,3-oxygenase); this enzyme has the potential to modulate the function of the APC similar to that which has been proposed to occur after interaction of APCs with regulatory CD25⁺CD4⁺ T cells [55,56]. Thus, we speculate that one mechanism of action of CTLA4-Ig is that it mimics a function that naturally arising regulatory T cells have on APCs. Over recent years evidence has accumulated that regulatory T cells and the pathways that activate them may be of significance for the development of RA. One such indication is the fact that regulatory T cells are enriched at the site of inflammation in the rheumatic joint, and that these T cells are also functional *in vitro* and so they probably contribute to regulation of local inflammatory reactions [57,58].

A functional consequence of blocking classic CD28-mediated co-stimulation with abatacept is that it also leads to inhibition of the proliferation of both circulating naïve and memory T cells [59]. This may assist in reducing the number of activated autoreactive T-cells available for entry into the synovium.

Interestingly, abatacept appears to have a 'physiological cousin' in mice, in which a splice variant of CTLA4 is expressed that is unable to bind the B7 molecules but nevertheless gives a negative signal to T cells by co-localizing with the T cell receptor and preventing T cell activation [60]. This is an interesting mechanism that adds to the various other means by which peripheral tolerance may decrease the chances of bystander activation of T cells. Only under optimal circumstances, which include co-stimulation, will T cell responses be elicited. Thus far, this phenomenon has not been reported in humans.

Figure 4



Interfering at an early stage in an immune response (i.e. T cell activation) is likely to block subsequent inflammatory events mediated by several different effector cells. APC, antigen-presenting cell; IL, interleukin; TNF, tumour necrosis factor.

Conclusion

T cells are key players in autoimmune diseases such as RA. Once activated, they orchestrate potentially destructive immune responses from other immune cells. Thus, by preventing the initial activation and possibly reactivation of T cells by abatacept, downstream damage mediated by macrophages, fibroblasts and B cells may be controlled (Fig. 4).

Competing interests

The author(s) declare that they have no competing interests.

References

- Gabriel SE: **The epidemiology of rheumatoid arthritis.** *Rheum Dis Clin North Am* 2001, **27**:269-281.
- Lard LR, Boers M, Verhoeven A, Vos K, Visser H, Hazes JM, Zwinderman AH, Schreuder GM, Breedveld FC, De Vries RR, et al.: **Early and aggressive treatment of rheumatoid arthritis patients affects the association of HLA class II antigens with progression of joint damage.** *Arthritis Rheum* 2002, **46**:899-905.
- Furst DE, Breedveld FC, Kalden JR, Smolen JS, Burmester GR, Bijlsma JW, Dougados M, Emery P, Keystone EC, Klareskog L, et al.: **Updated consensus statement on biological agents, specifically tumour necrosis factor alpha (TNFalpha) blocking agents and interleukin-1 receptor antagonist (IL-1ra), for the treatment of rheumatic diseases, 2004.** *Ann Rheum Dis* 2004, **Suppl 2**:ii2-ii12.
- Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, et al.: **Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group.** *Lancet* 1999, **354**:1932-1939.
- Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, et

- al.: **Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group.** *N Engl J Med* 2000, **343**:1594-1602.
6. Klareskog L, van der Heijde D, de Jager JP, Gough A, Kalden J, Malaise M, Martin Mola E, Pavelka K, Sany J, Settas L, et al.: **Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial.** *Lancet* 2004, **363**:675-681.
 7. Williams RO, Feldmann M, Maini RN: **Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis.** *Proc Natl Acad Sci USA* 1992, **89**:9784-9788.
 8. van den Berg WB: **Anti-cytokine therapy in chronic destructive arthritis.** *Arthritis Res* 2001, **3**:18-26.
 9. Ruchatz H, Leung BP, Wei XQ, McInnes IB, Liew FY: **Soluble IL-15 receptor alpha-chain administration prevents murine collagen-induced arthritis: a role for IL-15 in development of antigen-induced immunopathology.** *J Immunol* 1998, **160**:5654-5660.
 10. Bresnihan B, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, Nuki G, Pavelka K, Rau R, Rozman B, et al.: **Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist.** *Arthritis Rheum* 1998, **41**:2196-2204.
 11. Choy EH, Isenberg DA, Garrood T, Farrow S, Ioannou Y, Bird H, Cheung N, Williams B, Hazleman B, Price R, et al.: **Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: a randomized, double-blind, placebo-controlled, dose-escalation trial.** *Arthritis Rheum* 2002, **46**:3143-3150.
 12. McInnes IB, Gracie JA: **Interleukin-15: a new cytokine target for the treatment of inflammatory diseases.** *Curr Opin Pharmacol* 2004, **4**:392-397.
 13. Choy EH, Panayi GS: **Cytokine pathways and joint inflammation in rheumatoid arthritis.** *N Engl J Med* 2001, **344**:907-916.
 14. Malmstrom V, Trollmo C, Klareskog L: **The additive role of innate and adaptive immunity in the development of arthritis.** *Am J Med Sci* 2004, **327**:196-201.
 15. Al-Balaghi S, Strom H, Moller E: **B cell differentiation factor in synovial fluid of patients with rheumatoid arthritis.** *Immunol Rev* 1984, **78**:7-23.
 16. Benoist C, Mathis D: **A revival of the B cell paradigm for rheumatoid arthritis pathogenesis?** *Arthritis Res* 2000, **2**:90-94.
 17. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, Stevens RM, Shaw T: **Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis.** *N Engl J Med* 2004, **350**:2572-2581.
 18. Waaler E: **On the occurrence of a factor in human serum activating the specific agglutination of sheep blood corpuscles.** *Acta Pathol Microbiol Scand* 1940, **17**:172-188.
 19. Rose HM, Ragan C, Pearce E, Lipman MO: **Differential agglutination of normal and sensitized erythrocytes by sera of patients with rheumatoid arthritis.** *Proc Soc Exp Biol Med* 1948, **68**.
 20. Scott DL: **Prognostic factors in early rheumatoid arthritis.** *Rheumatology (Oxford)* 2000, **Suppl 1**:24-29.
 21. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ: **The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide.** *Arthritis Rheum* 2000, **43**:155-163.
 22. van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL, Toes RE, Huizinga TW: **Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study.** *Arthritis Rheum* 2004, **50**:709-715.
 23. Kastbom A, Strandberg G, Lindroos A, Skogh T: **Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project).** *Ann Rheum Dis* 2004, **63**:1085-1089.
 24. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, Sundin U, van Venrooij WJ: **Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis.** *Arthritis Rheum* 2003, **48**:2741-2749.
 25. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, Habibuw MR, Vandenbroucke JP, Dijkmans BA: **Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors.** *Arthritis Rheum* 2004, **50**:380-386.
 26. Maloney DG, Grillo-Lopez AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, Janakiraman N, Foon KA, Liles TM, Dallaire BK, et al.: **IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma.** *Blood* 1997, **90**:2188-2195.
 27. Duke O, Panayi GS, Janossy G, Poulter LW: **An immunohistological analysis of lymphocyte subpopulations and their microenvironment in the synovial membranes of patients with rheumatoid arthritis using monoclonal antibodies.** *Clin Exp Immunol* 1982, **49**:22-30.
 28. Klareskog L, Forsum U, Scheynius A, Kabelitz D, Wigzell H: **Evidence in support of a self-perpetuating HLA-DR-dependent delayed-type cell reaction in rheumatoid arthritis.** *Proc Natl Acad Sci USA* 1982, **79**:3632-3636.
 29. Holmdahl R, Klareskog L, Rubin K, Larsson E, Wigzell H: **T lymphocytes in collagen II-induced arthritis in mice. Characterization of arthritogenic collagen II-specific T-cell lines and clones.** *Scand J Immunol* 1985, **22**:295-306.
 30. Ranges GE, Sriram S, Cooper SM: **Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4.** *J Exp Med* 1985, **162**:1105-1110.
 31. Tak PP, van der Lubbe PA, Cauli A, Daha MR, Smeets TJ, Kluijn PM, Meinders AE, Yanni G, Panayi GS, Breedveld FC: **Reduction of synovial inflammation after anti-CD4 monoclonal antibody treatment in early rheumatoid arthritis.** *Arthritis Rheum* 1995, **38**:1457-1465.
 32. Issacs JD, Greer S, Sharma S, Symmons D, Smith M, Johnston J, Waldmann H, Hale G, Hazleman BL: **Morbidity and mortality in rheumatoid arthritis patients with prolonged and profound therapy-induced lymphopenia.** *Arthritis Rheum* 2001, **44**:1998-2008.
 33. Tugwell P, Bombardier C, Gent M, Bennett KJ, Bensen WG, Crette S, Chalmers A, Esdaile JM, Klinkhoff AV, Kraag GR, et al.: **Low-dose cyclosporin versus placebo in patients with rheumatoid arthritis.** *Lancet* 1990, **335**:1051-1055.
 34. Kunkel EJ, Boisvert J, Murphy K, Viera MA, Genovese MC, Wardlaw AJ, Greenberg HB, Hodge MR, Wu L, Butcher EC, et al.: **Expression of the chemokine receptors CCR4, CCR5, and CXCR3 by human tissue-infiltrating lymphocytes.** *Am J Pathol* 2002, **160**:347-355.
 35. Iezzi G, Karjalainen K, Lanzavecchia A: **The duration of antigenic stimulation determines the fate of naive and effector T cells.** *Immunity* 1998, **8**:89-95.
 36. Acuto O, Michel F: **CD28-mediated co-stimulation: a quantitative support for TCR signalling.** *Nat Rev Immunol* 2003, **3**:939-951.
 37. Bitmansour AD, Douek DC, Maino VC, Picker LJ: **Direct ex vivo analysis of human CD4+ memory T cell activation requirements at the single clonotype level.** *J Immunol* 2002, **169**:1207-1218.
 38. Sharpe AH, Freeman GJ: **The B7-CD28 superfamily.** *Nat Rev Immunol* 2002, **2**:116-126.
 39. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH: **Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4.** *Immunity* 1995, **3**:541-547.
 40. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H, Mak TW: **Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4.** *Science* 1995, **270**:985-988.
 41. Finck BK, Linsley PS, Wofsy D: **Treatment of murine lupus with CTLA4Ig.** *Science* 1994, **265**:1225-1227.
 42. Reynolds J, Tam FW, Chandraker A, Smith J, Karkar AM, Cross J, Peach R, Sayegh MH, Pusey CD: **CD28-B7 blockade prevents the development of experimental autoimmune glomerulonephritis.** *J Clin Invest* 2000, **105**:643-651.
 43. Zhou P, Szot GL, Guo Z, Kim O, He G, Wang J, Grusby MJ, Newell KA, Thistlethwaite JR, Bluestone JA, et al.: **Role of STAT4 and STAT6 signaling in allograft rejection and CTLA4-Ig-mediated tolerance.** *J Immunol* 2000, **165**:5580-5587.
 44. Webb LM, Walmsley MJ, Feldmann M: **Prevention and amelioration of collagen-induced arthritis by blockade of the CD28 costimulatory pathway: requirement for both B7-1 and B7-2.** *Eur J Immunol* 1996, **26**:2320-2328.

45. Tellander AC, Pettersson U, Runstrom A, Andersson M, Michaels-son E: **Interference with CD28, CD80, CD86 or CD152 in collagen-induced arthritis. Limited role of IFN-gamma in anti-B7-mediated suppression of disease.** *J Autoimmun* 2001, **17**:39-50.
46. Abrams JR, Lebowitz MG, Guzzo CA, Jegasothy BV, Goldfarb MT, Goffe BS, Menter A, Lowe NJ, Krueger G, Brown MJ, et al.: **CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris.** *J Clin Invest* 1999, **103**:1243-1252.
47. Moreland LW, Alten R, Van den Bosch F, Appelboom T, Leon M, Emery P, Cohen S, Luggen M, Shergy W, Nuamah I, et al.: **Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4Ig and LEA29Y eighty-five days after the first infusion.** *Arthritis Rheum* 2002, **46**:1470-1479.
48. Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S, Russell A, Dougados M, Emery P, Nuamah IF, et al.: **Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig.** *N Engl J Med* 2003, **349**:1907-1915.
49. Salazar-Fontana LI, Sanz E, Merida I, Zea A, Sanchez-Atrio A, Villa L, Martinez AC, de la Hera A, Alvarez-Mon M: **Cell surface CD28 levels define four CD4+ T cell subsets: abnormal expression in rheumatoid arthritis.** *Clin Immunol* 2001, **99**:253-265.
50. Liu MF, Yang CY, Li JS, Lai KA, Chao SC, Lei HY: **Increased expression of down-regulatory CTLA-4 molecule on T lymphocytes from rheumatoid synovial compartment.** *Scand J Immunol* 1999, **50**:68-72.
51. Weyand CM, Goronzy JJ: **T-cell responses in rheumatoid arthritis: systemic abnormalities-local disease.** *Curr Opin Rheumatol* 1999, **11**:210-217.
52. Fasth AE, Cao D, van Vollenhoven R, Trollmo C, Malmstrom V: **CD28nullCD4+ T cells: characterization of an effector memory T-cell population in patients with rheumatoid arthritis.** *Scand J Immunol* 2004, **60**:199-208.
53. Elloso MM, Scott P: **Expression and contribution of B7-1 (CD80) and B7-2 (CD86) in the early immune response to *Leishmania major* infection.** *J Immunol* 1999, **162**:6708-6715.
54. Shimoyama Y, Nagafuchi H, Suzuki N, Ochi T, Sakane T: **Synovium infiltrating T cells induce excessive synovial cell function through CD28/B7 pathway in patients with rheumatoid arthritis.** *J Rheumatol* 1999, **26**:2094-2101.
55. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinario F, Falorni A, Candeloro P, Belladonna ML, Bianchi R, Fioretti MC, et al.: **CTLA-4-Ig regulates tryptophan catabolism in vivo.** *Nat Immunol* 2002, **3**:1097-1101.
56. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P: **Modulation of tryptophan catabolism by regulatory T cells.** *Nat Immunol* 2003, **4**:1206-1212.
57. Cao D, Malmstrom V, Baecher-Allan C, Hafler D, Klareskog L, Trollmo C: **Isolation and functional characterization of regulatory CD25brightCD4+ T cells from the target organ of patients with rheumatoid arthritis.** *Eur J Immunol* 2003, **33**:215-223.
58. Cao D, van Vollenhoven R, Klareskog L, Trollmo C, Malmstrom V: **CD25brightCD4+ regulatory T cells are enriched in inflamed joints of patients with chronic rheumatic disease.** *Arthritis Res Ther* 2004, **6**:R335-R346.
59. Fontenot AP, Gharavi L, Bennett SR, Canavera SJ, Newman LS, Kotzin BL: **CD28 costimulation independence of target organ versus circulating memory antigen-specific CD4+ T cells.** *J Clin Invest* 2003, **112**:776-784.
60. Vijayakrishnan L, Slavik JM, Illes Z, Greenwald RJ, Rainbow D, Greve B, Peterson LB, Hafler DA, Freeman GJ, Sharpe AH, et al.: **An autoimmune disease-associated CTLA-4 splice variant lacking the B7 binding domain signals negatively in T cells.** *Immunity* 2004, **20**:563-575.