Novel Immunomodulatory Therapies for Rheumatoid Arthritis

Arthur Kavanaugh, M.D.
University of California, San Diego
Professor of Medicine
Director, Center for Innovative Therapy
Division of Rheumatology, Allergy, and Immunology

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease, affecting about 0.5-1 percent of the population worldwide.[1] RA affects women two to three times as often as men and has a peak onset between the ages of 40 and 60. The clinical course is quite variable, ranging from mild joint discomfort to extensive multi-system inflammation. If left untreated, RA can cause significant disability, substantial economic costs, and higher mortality rates.[2, 3]

There has been tremendous progress deciphering the cellular and molecular mechanism of RA, although the etiology remains incompletely defined. This immune driven systemic inflammatory disease is characterized by synovial and vascular proliferation with formation of pannus tissue, which damages articular cartilage and adjacent bone. Activation of specific CD4+ T cells, potentially due to as yet unidentified antigen(s), in an immunogenetically susceptible individual is hypothesized to be an early event in this process.[4, 5] Activated T cells orchestrate a cell-mediated immune response, stimulating monocytes, macrophages, synovial fibroblasts, osteoclasts, and B cells. A cascade of inflammatory mediators is then released that contributes to the sustenance of the ongoing immune activation and also directly causes signs, symptoms, and sequelae of the disease, such as destruction of joints.

University of California, San Diego
9500 Gilman Drive, Mail Code 0943
La Jolla CA 92093-0943

Based on an improved understanding of the pathophysiology of RA, novel therapies have been or are in the process of being developed that have more specific targets. Biological agents, including inhibitors of tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1) have proven to be effective in clinical trials and practice. Currently approved biologics include three TNF-α inhibitors (etanercept, infliximab, and adalimumab) as well as anakinra, a recombinant form of IL-1 receptor antagonist. Part of the impetus to develop novel biologic agents was the growing dissatisfaction with previously available therapies. Traditional therapies such as non-steroidal anti-inflammatory drugs, corticosteroids, and the so-called "disease-modifying anti-rheumatic drugs" (DMARDs) helped ameliorate the symptoms of RA for some patients, but rarely induced sustained remission and can have toxicities that prevent their long-term use. Biologic agents, particularly the TNF inhibitors, have changed the treatment paradigm for RA. The goal now is to adopt an early, proactive approach to treatment as to prevent the damage from chronic synovial inflammation. Studies have shown that biologics can slow disease progression, and when used in combination with DMARDs can potentiate treatment response.

Inhibitors of TNF-α

TNF-α is a soluble 17-kd protein homotrimer that binds to two receptors: type 1 TNF receptor (p55;CD120a) and the type 2 TNF receptor (p75;CD120b).[6] A central cytokine, it has protean effects, including the induction of other inflammatory cytokines such as IL-1, IL-6, and granulocyte-monocyte colony-stimulating factor (GM-CSF), and chemokines such as IL-8. In addition, TNF-α causes tissue destruction by promoting release of matrix

metalloproteinases, up-regulates cell trafficking through adhesion molecules and chemokines, increases the breakdown of proteoglycans in the cartilage, and potentiates osteoclast differentiation and activation.[7, 8] There is now abundant evidence that TNF inhibition dramatically improves patient outcomes in RA, as well as in other autoimmune systemic inflammatory conditions. Three TNF inhibitors are currently available wordwide: etanercept is a recombinant protein that consists of a dimer of the type II (p75) TNF receptor linked to the Fc fragment of the IgG1; infliximab, a chimeric monoclonal anti-TNF-α monoclonal antibody; and adalimumab, a human anti-TNF-α mAb. In both clinical trials as well as clinical experience, all three have been shown highly effective in reducing signs and symptoms of RA, preserving functional status, and inhibiting the progression of joint damage.[9-16] It has been shown that TNF inhitors work synergistically with methotrexate, and that combination has come to represent the "gold standard" for the treatment of RA. Hopwever, not al patients respond. Approximately one third of patients in clinical trials failed to achieve an ACR 20 response criteria. The so-called "60-40-20" rule refers to a response pattern with about 60% of patients reaching ACR20, 40% ACR50, and 20% ACR70. The lack of response by some patients and reactivation of disease activity in others suggests other critical pathways at work.

Inhibitors of IL-1

IL-1 also appears to have a prominent role in synovial inflammation and displays many overlapping effects with TNF-α. RA patients have increased levels of IL-1 in the plasma and synovial fluid and its concentration has been correlated with disease activity.[17] The IL-1 family encompasses 3 important cytokines involved in the disease process: IL-1α (a predominantly intracellular agonist), IL-1ß (a secreted agonist), and IL-1Ra (a secreted antagonist).[18] The two agonists, IL-1α and IL-1ß, are different in sequence identity but bind to a common receptor, IL-1 receptor type I (IL-1RI), and have similar biological properties.[19] Like TNF-α, IL-1 can activate a variety of inflammatory cells and mediators through signal transduction pathways. Several approaches toward inhibiting the activity of IL-1 have been explored. One proven strategy is to produce IL-1Ra to compete for binding of IL-1 to the IL-1 receptor. Anakinra is a recombinant form of the human IL-1Ra that acts as a competitive receptor antagonist. Administered via daily subcutaneous injection, anakinra has been shown in several studies to reduce signs and symptoms of RA, although the extent of improvement is less than that seen with TNF antagonists.[20, 21] Treatment with anakinra did demonstrate some slowing of radiographic progression.[22] In general, anakinra was well-tolerated, with injection site reactions as the most frequent adverse event.[23]

Combination therapy involving anakinra offered theoretical benefits by blocking the inflammatory pathway at multiple sites. In animal models of arthritis, the combination of an IL-1 inhibitor and a TNF inhibitor achieved synergistic efficacy in controlling inflammation and in preventing joint damage.[24] But in a controlled study involving 240 RA patients, the combination of anakinra and etanercept had similar ACR responses to the group receiving etanercept alone.[25] There was a 'biologic effect' in that combination therapy was found to increase the risk of adverse events, particularly serious infections (6 seen with combination therapy compared to none with etanercept monotherapy). Therefore, treatment with both anti-IL-1 and anti-TNF is currently contraindicated.

Inhibitors of IL-6

IL-6 is a pleiotropic cytokine produced by a variety of immunocytes and mesenchymal cells. It is involved in many immune activities including the differentiation of B cells into plasma cells and T lymphocytes into cytotoxic T cells. IL-6 can also stimulate the activation of osteoclasts leading to proteoglycan breakdown26 and the production of acute phase reactants by hepatocytes.[27] IL-6 binds to soluble or cell-bound IL-6 receptor (IL-6R) which then associates with cell surface glycoprotein gp130 stimulating cellular activation.[28] The presence of higher levels of IL-6 in the synovial fluid and serum of patients with RA compared with controls suggests a role in the pathogenesis of RA.[29] Serum IL-6 concentrations correlate with disease activity[30] and radiologic joint damage.[31] Decreased levels have been found after treatment with DMARDs. Positive results from inhibition of IL-6 in animal studies led to investigation of IL-6 blockade as a strategy for treating RA. A humanized IL-6R mAb called MRA (tocilizumab) was developed to bind to human IL-6R and be less immunogenic than murine ones. An initial study involved patients with active RA who had failed at least one DMARD. Preliminary results have been promising, and further study is underway.

Inhibitors of IL-15

IL-15 is pro-inflammatory, pleiotropic cytokine involved in the activation of T cells, macrophages, and neutrophils.[32] These targeted cells are of importance to the pathogenesis of RA. Levels of IL-15 have been shown to

increase with RA disease duration in the serum[33] as well as in the synovial membrane.[34] IL-15 has been shown to enhance synovial T cell proliferation and cognate interactions between T cells and macrophages that lead to cytokine (TNF-α, etc) production.[35] Therefore, IL-15 could serve as an attractive therapeutic target. There are several potential approaches to IL-15 blockade including neutralizing antibodies, soluble IL-15 receptor antagonist (IL15Ra), and mutated IL-15 species, for example generated as fusion proteins. In murine CIA models, administration of IL15Ra[36] and fusion protein[37] has been shown to markedly inhibit the incidence and severity of arthritis. A human IgG1 mAb directed against IL-15 (AMG 714) showed encouraging results in early clinical trials.[38] Larger studies are needed in order to validate its therapeutic potential.

Inhibitors of IL-18

IL-18 is a cytokine belonging to the IL-1 family. It induces the synthesis of pro-inflammatory factors such as TNF-α, IL-1B, IFN-γ, and GM-CSF.[39] IL-18 is secreted by a variety of cells, including monocytes/macrophages, dendritic cells, articular chondrocytes, and synovial fibroblasts, in a proform which requires enzymatic cleavage by ICE (caspase-1).[40] There is considerable interest in the role of IL-18 in RA given its ability to provoke an immune response and perpetuate inflammatory reactions. IL-18 is often detectable in the synovial tissues of patients with RA. Expression of IL-18 increases the production of TNF-α and IL-1 in RA synovial membrane and correlates with local inflammation and increased ESR.[41] There are several approaches to regulate IL-18 activity. One is to use a recombinant form of IL-18 binding protein (IL-18BP), which binds to IL-18 with high affinity as a decoy receptor and neutralizes its activity. Local intra-articular IL-18BP treatment significantly reduced the incidence of CIA. IL-18 neutralizing antibodies in animal studies, like IL-18BP, slowed disease progression and resulted in significant inhibition of TNF-α, IL-6, and IFN-γ secretion by macrophages.[42] Furthermore, IL-18 antibody treatment, but not IL-18BP, had a significant effect on established synovitis. Another approach is to target ICE, which is required to process IL-18 and IL-1ß from their precursors into active forms. Oral ICE inhibitors are still in clinical development.

T-Cell Directed Therapy

Evidence indicates that T-cell activation is central in the pathogenesis of RA. For example, the rheumatoid synovium contains a preponderance of CD4+ T-cells.[43] The association of certain major-histocompatibility-complex (MHC) class II alleles (HLA-DR1 and DR4) with RA also implicates a role for CD4+ T cells.[44] It is thought that these cells are stimulated by as yet unknown arthritogenic antigen(s) to initiate synovial inflammation. In order to trigger the inflammatory cascade, resting T-cells require at least two signals for full activation. The antigen-specific first signal occurs through the interaction between the T-cell receptor with antigen-loaded MHC molecules on antigen-presenting cell (APC). The second is a costimulatory signal provided by the engagement of CD28 on T cells with CD80 (B7-1) and CD86 (B7-2) on the surface of the APC.[45]

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) is expressed on the surface of T cells within days after they have become activated. CTLA4 is a high-affinity receptor for CD80 and CD86, binding approximately 500 to 2500 times as avidly as CD28.[46] CTLA4 prevents CD80 and CD86 from binding to CD28, thereby effectively blocking the costimulatory signal required for activation of T cells. Blocking CD28 costimulation has been shown to induce T cell anergy.[47] The fact that CTLA4 and B7 have been isolated from rheumatoid, but not from normal synovium,[48] suggests a possible role of CTLA4 in RA and makes it a potential target for therapy.

4.1 CTLA4 Immunoglobulin (abatacept)

CTLA4 immunoglobulin (CTLA4-Ig) consists of the extracellular component of CTLA4 fused to the Fc region of human IgG, which increases its half-life. CTLA4-Ig inhibits CD28/CD80-CD86 mediated costimulation. Preclinical studies demonstrated, inhibition of both primary and secondary immune responses by CTLA4-Ig administration [49] and resulted in the prevention of CIA in murine models.[50] Early evidence of clinical applicability came from an open-label study of patients with psoriasis where CTLA4-Ig significantly reduced cutaneous inflammation.[51, 52]

CTLA-4-Ig has been shown to have significant efficacy in a number of studies in patients with RA.[53, 54] In most studies, CTLA4-Ig was added to concomitant methotrexate. Importantly, this approach has also been shown to be capable if inducing clinical responses in patients who have previously failed therapy with TNF inhibitors.

B-Cell Directed Therapy

The precise function of B cells in the pathogenesis of RA is not clear although there is emerging evidence that they may play a role. B cells migrate into and accumulate within the rheumatoid synovium, with formation of B cell

aggregates and tertiary follicular structures.[55] They also produce autoantibodies such as rheumatoid factor (RF), which is found in the sera of approximately 80% of RA patients.[56] Seropositive RA patients tend to have higher disease severity, increased frequency of extra-articular involvement and mortality.[57] Other antibodies such as ones against cyclic citrullinated peptide are more specific than RF to RA[58] and may be predictive of disease development among patients with undifferentiated arthritis.[59] The disruption of humoral immune response seems to be associated with a more aggressive form of RA.

Evidence suggests that B cells may function as Antigen Presenting Cells and may provide costimulatory signals essential in CD4+ T cell activation, clonal expansion, and effector functions.[60] Animal models have shown the development of autoimmune disease in mice that lacked secreted Ig, revealing an antibody-independent role for B cells.[61] Another study found increased production of inflammatory mediators when isolated CD4+ T cells in human RA synovial germinal centers were transferred into RA synovium-severe combined immune-deficient mouse chimeras.[62] This finding underlies the potential importance of B cells in T cell activation, which is thought to be a central aspect of RA pathogenesis. Moreover, B-cell depleting treatment of the chimeric mice with anti-CD20 monoclonal antibodies resulted in the dissociation of synovial follicular structures and suppressed IFN-γ and IL-1ß. This implies a potentially beneficial role of depleting B cells in RA.

Rituximab

Rituximab is a chimeric mAb to the B cell-specific antigen CD20.[63] CD20 is expressed on pre-B and mature B cells, but not on stem cells or on plasma cells. When rituximab binds to CD20, B cells may be depleted via mechanisms including complement-mediated lysis, antibody-dependent cytotoxicity, and apotosis. Rituximab was approved for the treatment of CD20+ B-cell non-Hodgkin's lymphoma in 1997 in the United States.

Interest in the use of rituximab for RA came after an open-label study involving five patients with RA refractory to multiple DMARDs.[64] In that study patients received prednisolone, three doses of rituximab, and two does of cyclophosphamide. In this small study and in another larger open study, some prolonged clinical responses were noted.[65, 66] Subsequently, in a double blind placebo controlled trial, rituximab was shown to be effective in conjunction with methotrexate, obviating the need for cyclophosphamide.[67] Prolonged responses were seen in some patients, even out through 2 years of followup after a single treatment course. Rituximab treatment induced depletion of peripheral-blood B cells for 6 months or much longer, but the levels of immunoglobulins did not change substantially. Interestingly, RF levels decreased substantially. Despite the prolonged depletion of peripheral-blood B cells, the overall incidence of infection was similar in the control group and the rituximab groups. The infusion-related events were higher in the rituximab group compared to the control (36% to 30%), but lower than seen in patients with non-Hodgkin's lymphoma.

Conclusions

Progress in biotechnology, a greater understanding of the immunopathogenesis of RA, and a growing appreciation of the need for more effective therapies has driven the development of novel therapies for RA. Recently, notable success has been achieved, particularly with biologic agents targeting the key pro-inflammatory cytokine TNF-α. This in turn has spurred interest in further research into other immunomodulatory approaches for this and other autoimmune systemic inflammatory disorders. With a greater understanding of the pathophysiology of these diseases, novel therapies may offer patients markedly improved outcomes and perhaps even remission.

REFERENCES

1. Hochberg MC, Spector TD. Epidemiology of rheumatoid arthritis: update. Epidemiol Rev 1990; 12:247-252.
2. Dougados D, Suurmeiher T, Krol B, et al. Work disability in early rheumatoid arthritis. Ann Rheum Dis 1995; 54:445-460.
3. Mitchell DM, Spitz PW, Young DY, Bloch DA, McShane DJ, Fries JF. Survival, prognosis and causes of death in rheumatoid arthritis. Arthritis Rheum 1986; 29:706-14.
4. Fox, D.A. The role of T cells in the immunopathogenesis of rheumatoid arthritis: new perspectives. Arthritis Rheum 1997; 40:598-609.
5. Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. Cell 1996; 85: 307-310.
6. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature 1997; 385:729-733.
7. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. N Engl J Med 2001; 344:907-916.
8. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. Nature 1986; 319:516-518.
9. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. Lancet 1999; 354:1932-1939.

10. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN. Infliximab and methotrexate in the treatment of rheumatoid arthritis. N Engl J Med 2000; 343:1594-1602.
11. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 1999; 340:253-259.
12. Moreland LW, Schiff MH, Baumgartner SW, Tindall EA, Fleischmann RM, Bulpitt KJ, Weaver AL, Keystone EC, Furst DE, Mease PJ, Ruderman EM, Horwitz DA, Arkfeld DG, Garrison L, Burge DJ, Blosch CM, Lange ML, McDonnell ND, Weinblatt ME. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. Ann Intern Med 1999; 130:478-486.
13. Genovese MC, Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, Wasko MC, Moreland LW, Weaver AL, Markenson J, Cannon GW, Spencer-Green G, Finck BK. Etanercept versus methotrexate in patients with early rheumatoid arthritis: two year radiographic and clinical outcomes. Arthritis Rheum 2002; 46:1443-1450.
14. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, Teoh LA, Fischkoff SA, Chartash EK. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 2003; 48:35-45.
15. Keystone E, Kavanaugh A, Sharp J, Tennenbaum H, Hua Y, Teoh L, Fischkoff S, Chartash E. Radiographic, clinical, and functional outcomes with adalimumab (a human anti-TNF alpha monoclonal antibody) in the treatment of patients with active rheumatoid arthritis on concomitant methorexate theapy: a randomized, controlled, 52 week-trial. Arthritis Rheum 2004; 50:1400-1411.
16. Klareskog L, van der Heijde D, de Jager JP, Gough A, Kalden J, Malaise M, Martin Mola E, Pavelka K, Sany J, Settas L, Wajdula J, Pedersen R, Fatenejad S, Sanda M. 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.
17. Eastgate JA, Symons JA, Wood NC, Grinlinton FM, di Giovine FS, Duff GW. Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. Lancet 1988; 2:706-709.
18. Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996; 87: 2095-2147.
19. Sims JE, Gayle MA, Slack JL, Alderson MR, Bird TA, Giri JG, Colotta F, Re F, Mantovani A, Shanebeck K, Grabstein KH, Dower SK. Interleukin 1 signaling occurs exclusively via the type I receptor. Proc Natl Acad Sci USA 1993; 90:6155-6159.
20. Bresnihan B, Alvaro-Gracia JM, Cobby M, Doherty M, Domljan Z, Emery P, Nuki G, Pavelka K, Rau R, Rozman B, Watt I, Williams B, Aitchison R, McCabe D, Musikic P. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. Arthritis Rheum 1998; 41:2196-2204.
21. Cohen S, Hurd E, Cush J, Schiff M, Weinblatt ME, Moreland LW, Kremer J, Bear MB, Rich WJ, McCabe D. Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2002; 46:614-624.
22. Jiang Y, Genant HK, Watt I, Cobby M, Bresnihan B, Aitchison R, McCabe D. A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis: radiologic progression and correlation of Genant and Larsen scores. Arthritis Rheum 2000; 43:1001-1009.
23. Nuki G, Bresnihan B, Bear MB. Long-term safety and maintenance of clinical improvement following treatment with anakinra (recombinant human interleukin-1 receptor antagonist) in patients with rheumatoid arthritis: extension phase of a randomized double-blind, placebo-controlled trial. Arthritis Rheum 2002; 46:2838-2846.
24. Bendele AM, Chlipala ES, Scherrer J, Frazier J, Sennello G, Rich WJ, Edwards CK 3rd. Combination benefit of treatment with the cytokine inhibitors interleukin-1 receptor antagonist and PEGylated soluble tumor necrosis factor receptor type I in animal models with rheumatoid arthritis. Arthritis Rheum 2000; 43:2648-59.
25. Genovese MC, Cohen S, Moreland L, Lium D, Robbins S, Newmark R, Bekker P. Combination therapy with etanercept and anakinra in the treatment of patients with rheumatoid arthritis who have been treated unsuccessfully with methotrexate. Arthritis Rheum 2004; 50:1412-1419.
26. Flannery CR, Little CB, Hughes CE, Curtis CL, Caterson B, Jones SA. IL-6 and its soluble receptor augment aggrecanase-mediated proteoglycan catabolism in articular cartilage. Matrix Biol 2000; 19:549-53.
27. Isshiki H, Akira S, Sugita T, Nishio Y, Hashimoto S, Pawlowski T, Suematsu S, Kishimoto T. Reciprocal expression of NF-IL6 and C/EBP in hepatocytes: possible involvement of NF-IL6 in acute phase protein gene expression. New Biol 1991; 3:63-70.
28. Bravo J, Heath JK. Receptor recognition by gp130 cytokines. EMBO J 2000; 19:2399-411.
29. Brozik M, Rosztoczy I, Meretey K, Balint G, Gaal M, Balogh Z, Bart M, Mituszova M, Velics V, Falus A. Interleukin 6 levels in synovial fluids of patients with different arthritides: correlation with local IgM rheumatoid factor and systemic acute phase protein production. J Rheumatol 1992; 19:63-8.
30. Dasgupta B, Corkill M, Kirkham B, Gibson T, Panayi G. Serial estimation of interleukin 6 as a measure of systemic disease in rheumatoid arthritis. J Rheumatol 1992; 19:22-5.
31. Kotake S, Sato K, Kim KJ, Takahashi N, Udagawa N, Nakamura I, Yamaguchi A, Kishimoto T, Suda T, Kashiwazaki S. Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. J Bone Miner Res 1996; 11:88-95.
32. McInnes IB, Gracie JA. Interleukin-15: a new cytokine target for the treatment of inflammatory diseases. Curr Opin Pharmacol 2004; 4:392-7.
33. Gonzalez-Alvaro I, Ortiz AM, Garcia-Vicuna R, Balsa A, Pascual-Salcedo D, Laffon A. Increased serum levels of interleukin-15 in rheumatoid arthritis with long- term disease. Clin Exp Rheumatol 2003; 21:639–642.
34. Kotake S, Schumacher HR Jr, Yarboro CH, Arayssi TK, Pando JA, Kanik KS, Gourley MF, Klippel JH, Wilder RL. In vivo gene expression of type 1 and type 2 cytokines in synovial tissues from patients in early stages of rheumatoid, reactive, and undifferentiated arthritis. Proc Assoc Am Physicians 1997; 109:286-301.
35. Liew FY and McInnes IB, Role of interleukin 15 and interleukin 18 in inflammatory response. Ann Rheum Dis 2002; 61(Suppl 2):ii100–ii102.
36. 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; 160:5654-60.
37. Ferrari-Lacraz S, Zanelli E, Neuberg M, Donskoy E, Kim YS, Zheng XX, Hancock WW, Maslinski W, Li XC, Strom TB, Moll T. Targeting IL-15 receptor-bearing cells with an antagonist mutant IL-15/Fc protein prevents disease development and progression in murine collagen-induced arthritis. J Immunol 2004; 173:5818-26.
38. McInnes IB, Martin R, Zimmerman-Gorska I, et al. Safety and Efficacy of a Human Monoclonal Antibody to IL-15 (AMG 714) in Patients with Rheumatoid Arthritis: Results from a Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial. American College of Rheumatology 68th Annual Scientific Meeting. San Antonio, Texas. 2004: abstract 527.
39. Puren AJ, Fantuzzi G, Gu Y, Su MS-S, Dinarello CA. Interleukin-18 (IFNÁ-inducing factor) induces IL-8 and IL-1, via TNF· production from non-CD14+ human blood mononuclear cells. J Clin Invest 1998; 101:711-24.
40. Ghayur T, Banerjee S, Hugunin M, Butler D, Herzog L, Carter A, Quintal L, Sekut L, Talanian R, Paskind M, Wong W, Kamen R, Tracey D, Allen H. Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. Nature 1997; 386:619-23.

41. Joosten LA, Radstake TR, Lubberts E, van den Bersselaar LA, van Riel PL, van Lent PL, Barrera P, van den Berg WB. Association of interleukin-18 expression with enhanced levels of both interleukin-1beta and tumor necrosis factor alpha in knee synovial tissue of patients with rheumatoid arthritis. Arthritis Rheum 2003; 48:339-347.
42. Plater-Zyberk C, Joosten LA, Helsen MM, Sattonnet-Roche P, Siegfried C, Alouani S, van De Loo FA, Graber P, Aloni S, Cirillo R, Lubberts E, Dinarello CA, van Den Berg WB, Chvatchko Y. Therapeutic effect of neutralizing endogenous IL-18 activity in the collagen-induced model of arthritis. J Clin Invest 2001; 108:1825-32.
43. Cush JJ, Lipsky PE. Phenotypic analysis of synovial tissue and peripheral blood lymphocytes isolated from patients with rheumatoid arthritis. Arthritis Rheum 1988; 31:1230-1238.
44. Stastny P. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. N Engl J Med 1978; 298:869-871.
45. Koulova L, Clark EA, Shu G, Dupont B. The CD28 ligand B7/BB1 provides costimulatory signal for alloactivation of CD4+ T cells. J Exp Med 1991; 173: 759-762.
46. Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, Peach R. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. Immunity 1994; 1:793-801.
47. Tan P, Anasetti C, Hansen JA, Melrose J, Brunvand M, Bradshaw J, Ledbetter JA, Linsley PS. Induction of alloantigen-specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB1. J Exp Med 1993; 177:165-73.
48. Verwilghen J, Lovis R, De Boer M, Linsley PS, Haines GK, Koch AE, Pope RM. Expression of functional B7 and CTLA4 on rheumatoid synovial T cells. J Immunol. 1994; 153:1378-85.
49. Warner GL. Exploratory repeat-dose intravenous efficacy study in cynomolgus monkeys, study 94648. Bristol-Myers Squibb Pharmaceutical Research Institute Report Number 51377, 1995.
50. Webb LMC, Walmsley MJ, Feldmann M. Prevention and amelioration of collagen-induced arthritis by blockade of the CD28 co-stimulatory pathway: requirement for both B7-1 and B7-2. Eur J Immunol 1996; 26: 2320-8.
51. Abrams JR, Lebwohl MG, Guzzo CA, Jegasothy BV, Goldfarb MT, Goffe BS, Menter A, Lowe NJ, Krueger G, Brown MJ, Weiner RS, Birkhofer MJ, Warner GL, Berry KK, Linsley PS, Krueger JG, Ochs HD, Kelley SL, Kang S. CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. J Clin Invest 1999; 103:1243-1252.
52. Abrams JR, Kelley SL, Hayes E, Kikuchi T, Brown MJ, Kang S, Lebwohl MG, Guzzo CA, Jegasothy BV, Linsley PS, Krueger JG. Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. J Exp Med 2000; 192:681-694.
53. Moreland LW, Alten R, Van den Bosch F, Appelboom T, Leon M, Emery P, Cohen S, Luggen M, Shergy W, Nuamah I, Becker JC. Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4 and LEA29Y eighty-five days after the first infusion. Arthritis Rheum 2002; 46: 1470-1479.
54. Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S, Russell A, Dougados M, Emery P, Nuamah IF, Williams GR, Becker JC, Hagerty DT, Moreland LW. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. N Engl J Med 2003; 349: 1907-1915.
55. Weyand CW, Kurtin PJ, Goronzy JJ. Ectopic lymphoid organogenesis: a fast track for autoimmunity. Am J Pathol 2001; 159:787-793.
56. Schrohenloher RE, Bridges SL, Jr, Koopman WJ: Rheumatoid factor. In Koopman WJ (ed): Arthritis and Allied Conditions: A Textbook of Rheumatology, ed. 13. Baltimore, Williams & Wilkins, 1997, pp 1109-1130.
57. van Zeben D, Hazes JM, Zwinderman AH, Cats A, van der Voort EA, Breedveld FC. Clinical significance of rheumatoid factors in early rheumatoid arthritis: results of a follow up study. Ann Rheum Dis 1992; 51:1029-1035.
58. Baeten D, Peene I, Union A, Meheus L, Sebbag M, Serre G, Veys EM, De Keyser F. Specific presence of intracellular citrullinated proteins in rheumatoid arthritis synovium: relevance to antifilaggrin autoantibodies. Arthritis Rheum 2001; 44:2255–2262.
59. 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.
60. Dorner T, Burmester GR. The role of B cells in rheumatoid arthritis: mechanisms and therapeutic targets. Curr Opin Rheumatol 2003; 15:246-252.
61. Chan OT, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ. A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. J Exp Med 1999; 189:1639-1648.
62. Takemura S, Klimiuk PA, Braun A, Goronzy JJ, Weyand CM. T cell activation in rheumatoid synovium is B cell dependent. J Immunol 2001; 167:4710-4718.
63. Edwards JC, Leandro MJ, Cambridge G. B lymphocyte depletion therapy with rituximab in rheumatoid arthritis. Rheum Dis Clin North Am 2004; 30:393-403.
64. Edwards JC, Cambridge G: Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes. Rheumatology 2001; 40:205-211.
65. Leandro MJ, Edwards JC, Cambridge G. Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. Ann Rheum Dis 2002; 61:883-888.
66. De Vita S, Zaja F, Sacco S, De Candia A, Fanin R, Ferraccioli G. Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis: evidence for a pathogenetic role of B cells. Arthritis Rheum 2002; 46: 2029-2033.
67. 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.