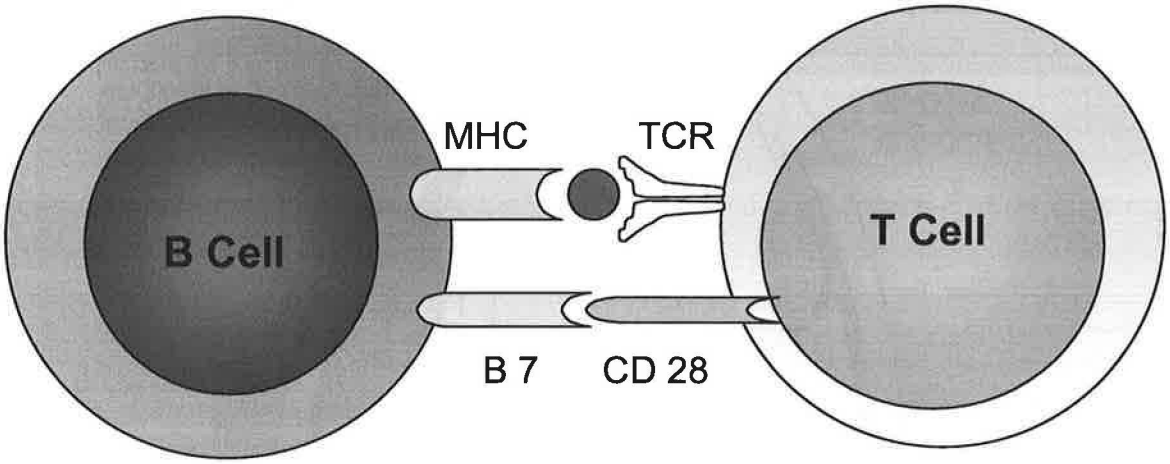
Blocking T Cell Costimulation and Depleting B Cells:

New Strategies for the Management of Rheumatoid Arthritis



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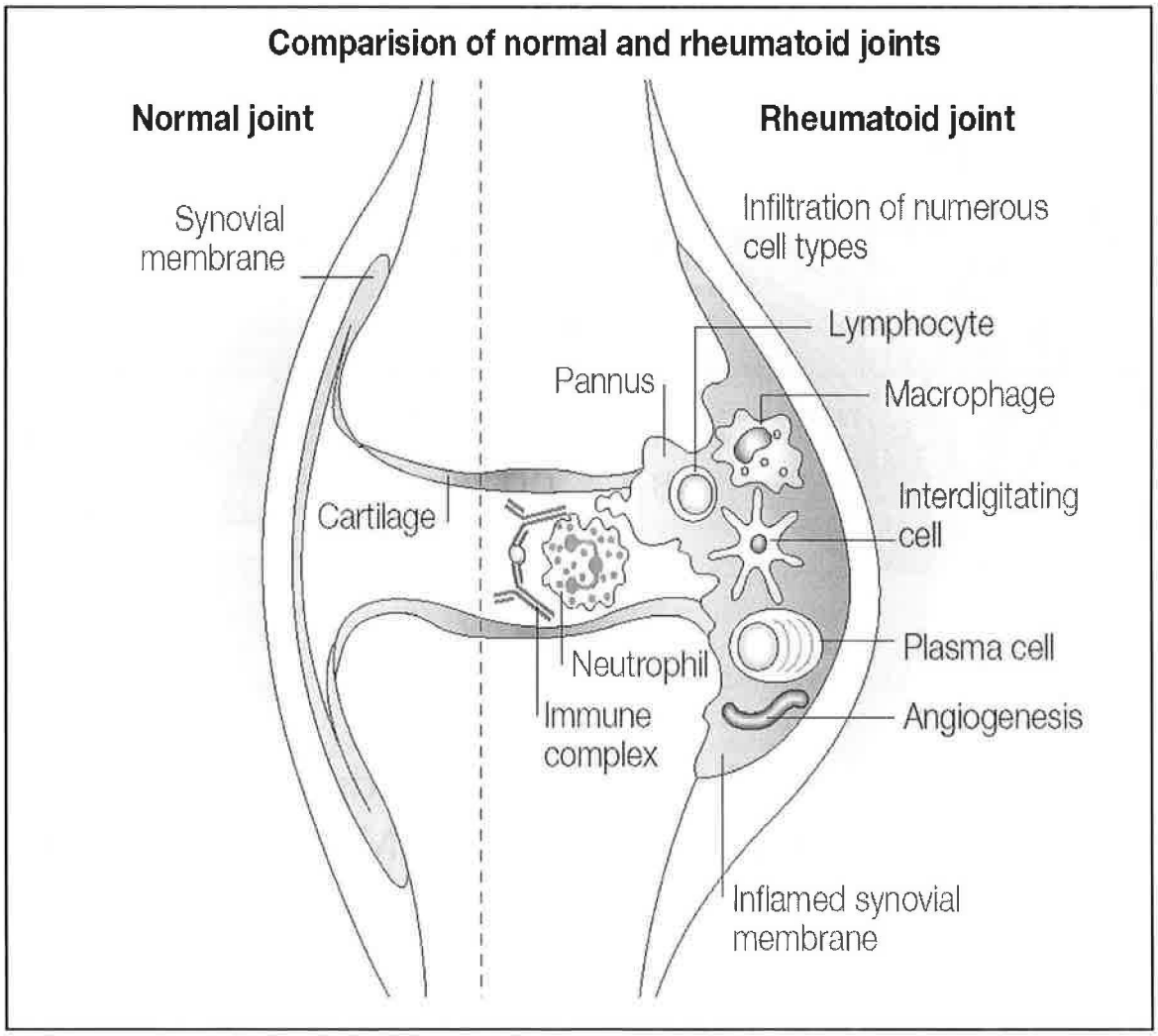
Salahuddin Kazi

This is to acknowledge that Salahuddin Kazi has disclosed financial relationships with commercial concerns related directly or indirectly to this program. Salahuddin Kazi will be discussing off-label uses in this presentation.

Salahuddin Kazi, MBBS
Associate Professor of Internal Medicine
Division of Rheumatic Diseases
UT Southwestern Medical Center at Dallas
Dallas VA Medical Center



Rheumatoid arthritis is a chronic autoimmune inflammatory disease that affects 2.1 million people in the United States of America [1]. While it is a systemic disease, the main pathology is found in the synovium of affected joints [2]. Untreated, synovitis flourishes and results in irreversible tissue damage and loss of function. Eventually rheumatoid arthritis causes disability, deformity and shortens life [3, 4].

The last decade has witnessed remarkable advances in the management of rheumatoid arthritis. While there is no cure for the disease, the ability to suppress inflammation has been greatly facilitated by the development and use of disease modifying anti-rheumatic drugs (DMARDs) and by the adoption of better diagnostic tools and aggressive therapeutic strategies. The most notable of these have been the emphasis on early diagnosis and the recognition that early treatment may prevent permanent joint damage [5-8]. Earlier diagnosis has been facilitated by the discovery of antibodies to cyclic citrullinated peptides [9] and the development of new imaging techniques [10]. Much of this progress has been driven by the availability of a new class of therapeutic agents referred to as biologic response modifiers (BRMs) [11]. The strategy of assessing patients frequently and adjusting therapy more frequently has resulting in earlier and tighter control of disease with demonstrated improvements in radiographic and functional outcomes [12, 13].

Case Presentation

Sandra is a 58-year-old woman with rheumatoid arthritis first diagnosed in 1994. She was treated with methotrexate and subsequently sulfasalazine and hydroxychloroquine were added, but she continued to have active disease with radiographic progression. In 2000 etanercept was added and her disease was controlled for one year. She then developed a rash, thought to secondary to etanercept which was then stopped. In 2001, infliximab was administered and in 2002 she transferred her care to the Dallas VA Medical Center in June 2002.

Treatment with infliximab and methotrexate were continued. She responded well but had gradual increase in disease activity necessitating two dose increases in infliximab. Again, after an initial response her disease continued to progress and methotrexate was stopped and leflunomide was added. There was an improvement in disease activity, but by June 2004, she still had evidence of moderate disease activity. Infliximab was stopped and adalimumab was added. She responded well to adalimumab but in December 2004 had a major disease flare. Adalimumab was stopped and Rituxan was administered in January 2005. Within three months her disease became quiescent. Another course of Rituxan was administered in June 2005 and repeated in March 2006.

This case illustrates the central conundrum of rheumatoid arthritis.

Despite periods of responsiveness to therapy, many patients experience disease progression, often requiring new agents or new combinations of therapy.

Therapeutic Success and Failure

Methotrexate is the anchor drug of any successful treatment regimen for rheumatoid arthritis. It is often the first (and only) drug used when initiating therapy. Over time, an increasing proportion of patients will stop taking this drug either because of inefficacy or because of intolerability [12, 14].

The anti-TNF (tumor necrosis factor) agents (etanercept, infliximab and adalimumab) when first used in patients with methotrexate failure showed remarkably good therapeutic efficacy and relatively high retention rates [15-18]. Experience from European registries has suggested that all three biological agents show excellent drug survival, and that discontinuations due to treatment failure are approximately the same across the three anti-TNF agents [19-21]. However all three agents have shown progressive loss of efficacy with time [22-25].

Several trials of switching between anti-TNF agents when one or more have failed have shown restoration of efficacy in many such patients [26, 27]. The reasons for such differential dug resistance have been reviewed by Sidiropoulos [28]. It is not known whether this is due to progression of the disease (with change in the relative importance of pathogenic mechanisms of inflammation, allowing circumvention of anti-TNF agents) or due to drug resistance or immunogenicity.

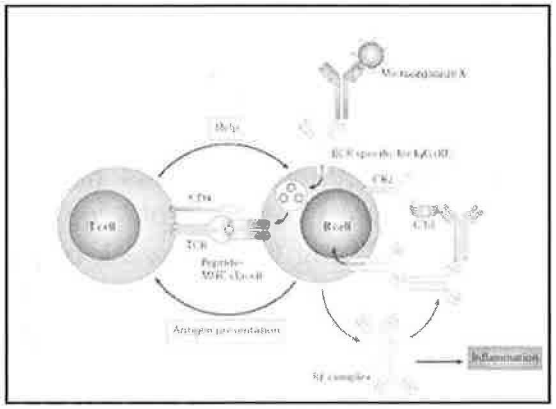
Pathogenesis of Rheumatoid Arthritis: Historical Perspective

Rheumatoid arthritis is a multisystem disorder characterized by inflammation in synovial joints. The most consistently observed immunological abnormalities in rheumatoid arthritis are the presence of rheumatoid factor and antibodies to citrullinated peptides [9, 29, 30]. The synovitis shows infiltration of macrophages, neutrophils and lymphocytes. The positive clinical response to biological therapy with TNF-neutralizing agents illustrates that the inflammation is largely TNF dependent, at least in the synovium [31].

During the 1980s and 1990s, there was a partial consensus that both the underlying immune response and the inflammatory effector mechanisms in rheumatoid arthritis were T-cell driven [32]. This view was to some extent supported by the fact that certain types of experimentally induced arthritis could be transferred by T cells, and in part by the unpredictable relationship between autoantibodies and clinical disease. However, evidence for a T-cell effector mechanism remained inconsistent [33] and T-cell-targeted therapies failed to provide benefit [34].

The Re-Emergence of B Cells

In 1998, it was proposed that the underlying response was driven by self-perpetuating B cells [35] and that the initiation of inflammation was primarily due to ligation of the low-affinity IgG receptor FcγRIIIa by immune complexes.



Thus, in addition to producing soluble antibodies for immune defense, these antibodies appear to be crucial for the regulation of B cell survival and for B-T cell interactions. While B cells are undoubtedly involved in the pathogenesis of rheumatoid arthritis by virtue of autoantibody production, some of which appear to very clearly related to disease amplification [36], B cells may be crucial to disease amplification and persistence in their role as antigen presenting cells. The latter (newly discovered) role may be much more important than the traditional role in autoantibody production. In the rheumatoid synovium lymphoid aggregates can develop germinal centers with usual concentrations of B cells [37]. Experiments performed in severe combined immunodeficiency (SCID) mice in whom rheumatoid synovium is transplanted, the B cells persist. If they are removed with rituximab (a monoclonal antibody directed against CD 20, a B cell specific surface marker), the inflammation is diminished [38]. It is thus very likely that B cells in rheumatoid arthritis have more complex functions than simply being the precursors of antibody producing plasma cells [39]. B cells can regulate T cells, dendritic cells and other B cells. They can produce an array of cytokines, including interleukin 4 (IL-4) and interleukin 10 (IL-10) [40]. Furthermore they are superb antigen presenting cells [41].

B cells as Therapeutic Targets for Autoimmune Diseases

The growing body of knowledge concerning the role of B cells in the generation and perpetuation of autoimmunity afforded a strong rationale to target these cells in the treatment of diseases such as systemic lupus erythematosus and rheumatoid arthritis. Such therapy first became available with the development of rituximab, a monoclonal antibody directed against CD20, a B cell specific surface marker [42]. CD 20 is present in all but the earliest stages of B cell development. Its function is largely unknown. In fact CD20 knockout mice have no obvious B cell deficits [43]. Yet, CD 20 expression is high; it is not shed and does not endocytose when bound by antibody. Additionally it does not exist in soluble form [44]. This made it an excellent target for the treatment of B cell malignancies, such as non-Hodgkin's lymphoma [42]. Rituximab was ap-

proved in 1997 for the treatment of low-grade non-Hodgkin B cell lymphoma and is now part of first-line treatment regimens for this disease [45].

Encouraging reports of the success of rituximab in a variety of autoimmune conditions led to its use in rheumatoid arthritis [46, 47]. Subsequently, randomized control trials in rheumatoid arthritis confirmed the efficacy of rituximab in this disease, even in patients who had failed anti-TNF therapy [48-50].

Clinical Trials of Rituximab in Rheumatoid Arthritis

Edwards reported a study of 161 patients with active rheumatoid arthritis despite treatment with methotrexate who were randomized to one of four treatments: oral methotrexate; rituximab (1000 mg on days 1 and 15); rituximab plus cyclophosphamide (750 mg on days 3 and 17); or rituximab plus methotrexate. Results showed that a single course of two infusions of rituximab, alone or in combination with either cyclophosphamide or continued methotrexate, provided significant improvement in disease symptoms at 24 and 48 weeks [49]. In this trial patients received large doses of steroids.

A second trial examined the role of steroids and different doses of steroids and did not find that steroids contributed to the clinical response, but that steroids did reduce the incidence of infusion reactions [50]. The study that led to the FDA approval of rituximab in rheumatoid arthritis was a trial of rituximab in patients who had failed combination therapy with methotrexate and an anti-TNF agent. In this trial, 517 patients with rheumatoid arthritis were randomized to receive either rituximab (1000 mg on day 1 and 15) or placebo. In this study, inadequate response to treatment with an anti-TNF could have been due to inefficacy or toxicity. To qualify as having had an inadequate response, patients had to have received etanercept for ≥3 months at 25 mg twice a week or infliximab for at least 4 infusions at ≥3 mg/kg, or adalimumab for ≥3 months at 40 mg every other week. Patients also received 100 mg of methyl prednisone intravenously with each infusion along with oral steroids between infusions. Fifty one percent of patients achieved the primary endpoint of ACR 20 (compared with 18% on methotrexate alone) and 27% and 12% achieved the secondary endpoints of ACR 50 and 70 respectively (compared with 5% and 1% on methotrexate alone) respectively [48].

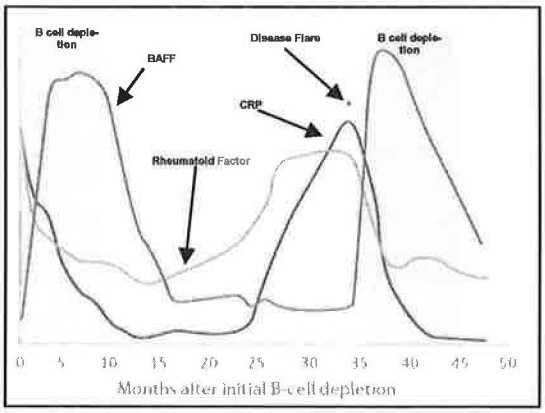
Rituximab: Mechanism of Action

Rituximab targets B cells that express the CD 20 antigen. Thus is spares the hematopoietic stem cell and the plasma cell, both populations of which do not express CD 20. B cell killing is largely mediated by antibody-dependent cell-mediated cytotoxicity (ADCC) [51]. In systemic lupus erythematosus, B cell depletion

with rituximab seems to depend on the FcγRIII allotype [52]. Other mechanisms of B cell killing may include complement mediated lysis or the induction of apoptosis [53]. Interestingly germinal center B cells and marginal-zone B cells appear to be resistant to killing [53].

Immunodynamics of Rituximab

When patients with rheumatoid arthritis are treated with rituximab, the clinical benefit generally persists for the period of B cell depletion, typically 7-8 months. In as many of 50% of cases, clinical benefit persists for several months beyond the period of B cell depletion [54]. Rheumatoid factor levels decrease following administration of rituximab with IgG rheumatoid factor decreasing by 60% [55]. Similarly antibodies to citrullinated peptides also decrease and often the C-reactive protein closely parallels the decrease in antibody levels. As mentioned above, B cells in the marginal zone may be resistant to rituximab, which best explains the persistence of antibacterial antibodies.



Following B cell depletion, levels of B cell activating factor (BAFF; also known as BLyS), typically rise markedly and remain elevated for 1-2 months. BAFF levels decrease after the return of B cells. Interestingly in patients with prolonged clinical benefit BAFF rises more gradually. The patterns of B cell depletion, serum BAFF and antibody levels, and clinical relapse for each BCDT cycle were remarkably similar in re-treated patients [56].

Implications of Repeat Courses of Therapy

With repeat courses of therapy, there appears to be a cumulative reduction in total serum immunoglobulins, with IgG levels noted to be as low as 3.5 g/L and IgM becoming undetectable in some cases [54]. Up to 5 cycles of re-treatment have been administered in patients with rheumatoid arthritis over a period of 7 years [57]. Additionally, 279 patients from the REFLEX trial [48] received a second course of therapy without an increase in infections while demonstrating higher proportions of clinical response [58]. It remains to be seen if such repeat cycles of treatment will be safe and whether the disease might be cured with successive cycles of B cell depletion resulting in elimination of self-perpetuating B cell clones with replacement by more tolerant ones.

Other B cell targeted agents, none of which have been approved for use in rheumatoid arthritis have been reviewed by Edwards [54] and are shown in the table below.

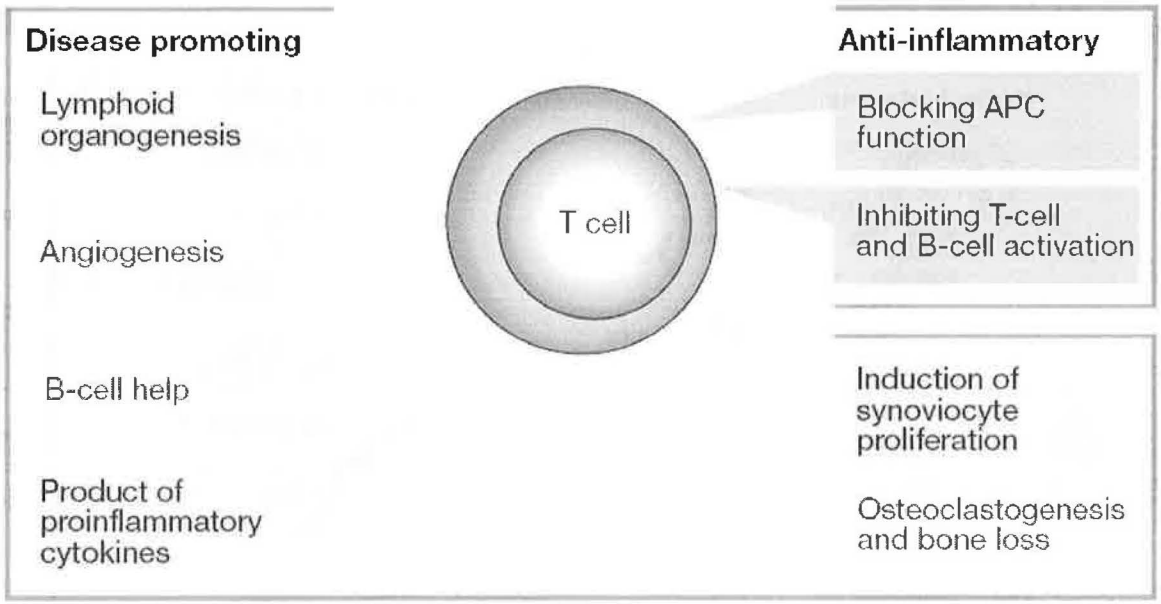
B Cell Targeted Agents Relevant to Autoimmune Diseases

Agent	Target	Target Characteristics	Mode of Action
Epratuzumab	CD22	Membrane protein	Cytolysis
DT2219	CD 19 & CD 22	Membrane protein	Cytolysis
Belimumab	BAFF	B-cell survival factor	Sequestration Neutralization
TACI - Ig	BAFF & APRIL	B-cell survival factor	Sequestration Neutralization
LJP394	BCR	Cell-surface ligand	Antigen decoy

Adapted from [54]

Targeting T Cells in Rheumatoid Arthritis

As noted above, initial experiments targeting T cells in rheumatoid arthritis displayed inconsistent results. Antibody-based protocols had successfully depleted T cells but were complicated by long-term lymphopenia and selective survival of memory T cells [59].Despite this, there is no doubt that CD4+ T cells dominate the rheumatoid synovium and are crucial elements in the inflammatory process in the rheumatoid synovium. This role has been confirmed in experiments in the SCID mouse model [60] and by the well described relationship between numbers of T cells in the synovium and the severity of joint damage in rheumatoid arthritis [61]. T cells have many functions in the rheumatoid synovium; these include roles in the development of the tissue infrastructure necessary for inflammation, promotion of angiogenesis, providing cognate help to B cells, production of inflammatory cytokines, synoviocyte proliferation and osteoclast activation. T cells also serve as regulators of inflammation by providing negative signals. The biochemical basis of this last role is not fully understood.



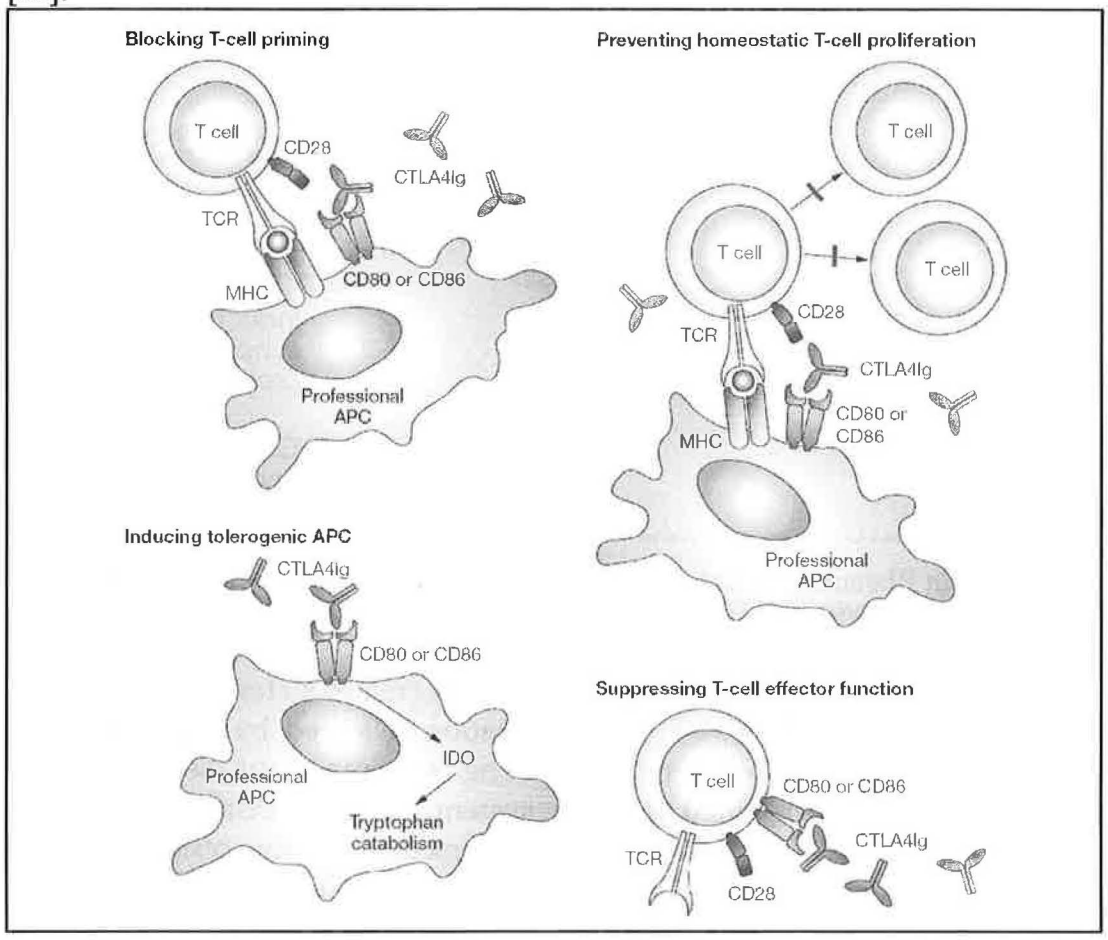
Roles of T cells in Rheumatoid Arthritis (Weyand and Goronzy; Nature Clinical Practice Rheumatology April 2006)

Blocking T cell Costimulation

T cell activation is a two-step process. The first signal is provided by the engagement of the human lymphocyte molecule bearing the immunogenic antigen with the T cell receptor. This signal imparts specificity but in itself is not sufficient to induce complete T cell activation. Complete T cell activation requires a second signal. This second signal is delivered when B7-1/B7-2 (also known as CD80/86) engages CD28. Once this occurs, T cell activation ensues. Inhibition of the system ensues when the activated T cell displays cytotoxic-T-lymphocyte-associated protein 4 (CTLA4). This inhibitory molecule most likely acts by competing with CD28 for B7 and is successful because it has a higher affinity for B7. Thus the sequential T cell display of CD 28 followed by the display of CTLA4 creates a closed system of activation followed by inhibition, creating an important self-regulatory system. The importance of this is underscores by the observations in animal models; CTLA4 knockout mice develop a fatal syndrome of multiorgan lymphocytic infiltrates and severe enlargement of lymphoid organs [62]. CTLA4 can also bind to B7 on the antigen presenting cell (APC), where is appears to induce the production of indoleamine 2,3-dioxygenase (IDO), an intracellular enzyme that breaks down tryptophan. Breakdown products of tryptophan can induce T cell apoptosis.

This ability of CTLA4 to transmit an anti-inflammatory signal was harnessed by fusing the extracellular domain of human CTAL4 to the Fc portion of human immunoglobulin G1 (IgG1), to create a fusion protein,

CTLA4 Ig now known as abatacept [63].



Immunosuppressive Mechanisms Mediated By CTLA4 Ig (Weyand and Goronzy; Nature Clinical Practice Rheumatology April 2006)

Abatacept Mechanism of Action

By inhibiting costimulation of T cells, abatacept affords the novel approach of suppressing T cell responsiveness without completely eliminating it. This potentially allows the fine-tuning of T cell stimulation without causing severe immunosuppression.

It is important to note that T-cell recognition events occur in two main settings; naive T cells are primed in lymph nodes and memory and effector T cells are recruited to the inflamed peripheral tissue. T cells entering the synovium are end differentiated memory T cells and in contrast to young T cells, they often undergo accelerated aging and lose CD28 expression [64]. Thus, blocking CD28 in the rheumatoid joint may be inconsequential. Abatacept may also act by providing reverse signaling to antigen presenting cells (APCs) by binding to B7. This causes induction of indoleamine 2,3-dioxygenase (IDO). IDO metabolizes tryptophan.

The breakdown products of tryptophan can induce T cell apoptosis [65]. In summary, abatacept probably works in rheumatoid arthritis in two microenvironments. In the lymph node, naïve T cell costimulation can be blocked, while in the synovium memory T cells may undergo apoptosis by reverse APC signalling.

Abatacept Clinical Trials

In a pilot study of CTLA4 Ig (abatacept), Moreland demonstrated a dose-dependent response in patients with rheumatoid arthritis [66]. In a 6 month trial of patients failing methotrexate Kremer reported that patients given 10 mg/Kg of CTLA Ig (abatacept) were more likely to have an ACR 20 than were patients who received placebo (60 percent vs. 35 percent, P<0.001) [67]. Twelve month results of this study were reported in 2005, showing that similar efficacy levels were maintained at one year [68]. This study was followed by a phase III trial to evaluate the efficacy and safety of abatacept in patients with active rheumatoid arthritis and an inadequate response to at least three months of anti-TNF-alpha therapy. Results showed that after six months, the rates of ACR 20 responses were 50.4 percent in the abatacept group and 19.5 percent in the placebo group (P<0.001). Additionally, the respective rates of ACR 50 and ACR 70 responses were also significantly higher in the abatacept group than in the placebo group (20.3 percent vs. 3.8 percent, P<0.001; and 10.2 percent vs. 1.5 percent, P=0.003) [69].

Belatacept

Belatacept (LEA29Y) is also a selective costimulation blocker. Belatacept was derived from abatacept, differing from abatacept by two specific amino acid substitutions, which conferred greater binding avidity to B7, and more potent inhibition of T-cell activation. Belatacept was tested as part of the first pilot study of CTLA4 Ig (abatacept) in rheumatoid arthritis by Moreland in 2002 [66] but its development is now being directed toward use in renal transplantation [70]

Summary

The last decade has witnessed the unprecedented success of the anti-TNF agents in the treatment of rheumatoid arthritis. But as experience with the use of these agents has accumulated, it has become evident that substantial numbers of patients either do not respond to these therapies, or acquire resistance. Two new biologic drugs, rituximab and abatacept have shown effective responses in patients who have failed anti-TNF therapy.

While these are significant advances in the management of rheumatoid arthritis, the disease is still simply suppressed and not cured. Rheumatoid arthritis appears to a complex autoimmune disease with multifaceted inflammatory pathways. Targeted drugs have continued to

drive reinterpretation of disease pathogenesis while providing important therapeutic avenues.

The availability of multiple agents (both traditional and biologic) has created a therapeutic challenge for rheumatologists. Trials examining therapeutic strategies will continue to be needed. Two trials have nicely illustrated the importance of this issue. The TICORA study [13] suggests that tight control of rheumatoid arthriticus is beneficial, analogous to the strategies employed in the management of diabetes and the BeSt study [12] suggests that early intervention with combination therapy (with or without a biologic) is superior to sequential monotherapy and to a step up strategy.

The future may answer the following important questions. Given the disease heterogeneity and differences in therapeutic response, can we reliably predict the course of rheumatoid arthritis and thus tailor treatment regimens early on? Will ongoing trials of therapeutic strategy provide helpful information for the clinical rheumatologist? Will the adaptive immune system continue to evade targeted drugs?

Current evidence based management of rheumatoid arthritis suggests that the disease should be diagnosed early because institution of treatment without delay has a better chance of suppressing disease activity. There is also compelling evidence that the disease should be controlled tightly to improve outcomes.

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