The Journal Of The International Federation Of Clinical Chemistry And Laboratory Medicine
eJIFCC

THEORY, TARGETS AND THERAPY IN RHEUMATIC DISEASES

Dubravka Bosnić, M.D.
Division of clinical immunology and rheumatology, Department of Medicine, University Hospital Zagreb, Croatia

Autoimmunity appears to contribute significantly to many different rheumatologic diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), dermatomyositis, Sjögren syndrome, systemic sclerosis and the antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides. The best evidence for autoimmunity is the presence of autoantibodies in a large proportion of patients. These autoantibodies are class - switched, somatically mutated, often high affinity and directed against diverse epitopes on the autoantigens - all features associated with T cell dependent immune response. There are also data, particularly in SLE, demonstrating the presence of autoantibodies, and hence autoimmunity, years before the onset of clinical disease. The present paradigm for the development of these autoimmune diseases is that autoimmunity develops in four stages: genetic predisposition, initiation, perpetuation and progression and clinical disease. B cells take up and present autoantigens via specific cell surface immunoglobulins to T cells and they can help regulate and organize inflammatory responses. The importance of these latter functions has been demonstrated in murine SLE, where B cells have been found to be critical to the development of disease even when they are unable to secrete autoantibodies.

The understanding of immunopathogenic mechanisms in autoimmune disease including SLE has increased exponentially and this has led to the discovery of novel targets for which biologic or targeted therapies have been developed against. The mainstay of therapy for severe manifestations of SLE include the use of high-dose corticosteroids and cytotoxic agents such as cyclophosphamide (CYC) which have been associated with an increased risk of serious and opportunistic infections. Since the 1980s, we have argued for more judicious use of steroids and more recently, controlled studies have demonstrated that low-dose i.v. CYC and mycophenolate mofetil are equally effective and less toxic than high dose CYC in the treatment of lupus nephritis.

The potential advantage of biologic therapy is possibly, a better safety profile with less general immunosuppression. These targeted therapies may range from small molecules that specifically inhibit inflammatory processes at an intracellular, cell-cell or cell-matrix level to monoclonal antibodies (mAb), soluble receptors or natural antagonists that interfere with cytokine function, cellular activation and inflammatory gene transcription.

The immunopathogenic hallmark of SLE is the polyclonal B cell activation which leads to hyperglobulinemia, autoantibody production and immune complex formation (Figure 1). The fundamental abnormality appears to be the failure of T cells to suppress the forbidden

B cell clones due to generalized T cell dysregulation with resultants excess in CD4+ T cell activity and deficient CD8+ cytotoxic/suppressor function. In addition, B and T-cell interaction is facilitated by several cytokines such as IL-10 as well as co-stimulatory molecules such as CD40/CD40L, B7/CD28/CTLA-4 which initiate the second signal. These interactions together with impaired phagocytic clearance of immune complexes and apoptotic material perpetuate the immune response with resultant tissue injury.

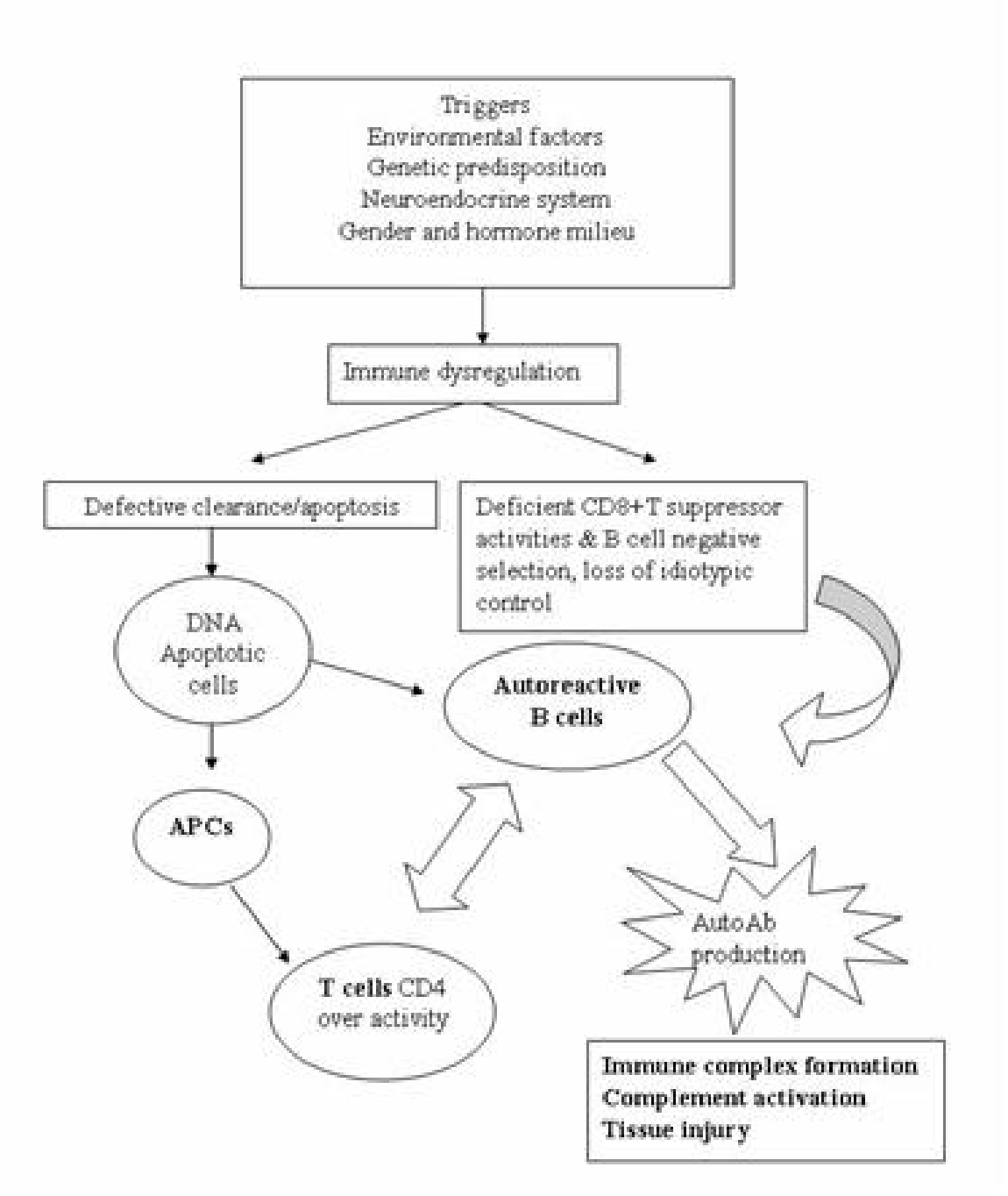

Figure 1. Immunopathogenesis of SLE (adapted from Moc CC et al.)

The “prototypic” biologic agents first approved for use in rheumatic disease were the anti-tumour necrosis factor (TNF-ά) inhibitors: etanercept and infliximab, for the treatment of rheumatoid arthritis. Since the initial success, its use has been extended to the treatment of spondyloarthropathy (ankylosing spondylitis, psoriatic arthritis) and some preliminary data has emerged suggesting benefit in other rheumatic diseases such as several forms of systemic vasculitis (Behcet's disease. Churg - Strauss syndrome, and polyarteritis nodosa) and even a certain subgroup of patients with SLE. Following this lead, a new generation of biologic agents for the treatment of SLE is currently being developed, some of which have reached clinical phase trials. The following discussion on these novel therapies have been classified according to the potential targets of the immune cascade in SLE.

13.1 B cell targeted therapies

It is now clear that apart from autoantibody production, B cells play a critical role in amplifying the immune response through its function as antigen-presenting cells. Autoantigens are presented via specific cell surface immunoglobulins to T cells together with a second signal via co stimulatory molecules which leads to T cell activation. B cell blockade (Figure 1) can thus be directed at: 1) B cell surface receptors (CD-20, CD-22). 2) inhibition of co-stimulatory signals CTLA4 Ig, 3) inhibition of B cell survival (antiBLyS). and 4) induction of B cell anergy (B cell toleragens).

13.1.1 Blockade of B-cell surface receptors

Rituximab, a monoclonal antibody against CD-20+ B cells was first approved for use in the treatment of non-Hodgkin's cell lymphoma. It selectively depletes immature, mature, naive and memory B cells. Plasma cells do not express CD-20 and are hence unaffected. There is encouraging data from open label trials and case reports demonstrating its efficacy and safety in SLE. Notably, it appears to be beneficial in those with active refractory disease and none of the studies thus far have reported significant adverse effects, particularly that of serious infection. This observation has also been supported by other recent case reports citing successful outcomes in patients with life-threatening SLE (renal, haematological and central nervous system involvement). It appears, from the studies performed, that successful depletors (patients with <1% B cells in peripheral blood) have a more sustained clinical response compared to "poor depletors" and this variable response may be related to polymorphisms of FC γ receptors as well as the dose of rituximab.

13.1.2 Inhibition of costimulatory signals

Cell surface molecules that mediate cell-cell interaction and generate intracellular biochemical signals in the interacting cells are termed “costimulatory molecules”. These provide the much needed second signal for T cell activation by antigen-presenting cells (Figure 2). The costimulatory targets that have been or are undergoing evaluation in patients with SLE include the CD40-CD30L and CD28-CTLA4-B7 molecules. CTLA4 (cytotoxic T-lymphocyte antigen4), expressed on activated T cell surfaces, provides inhibitory signals with down regulate T cell function whereas CD28-B7 interaction promotes T-cell activation. As the former has higher affinity to B7, investigators have developed CTLA4Ig, a soluble receptor (fusion protein of the extra cellular domain of CTLA4 and Fc portion of IgGI) to

block CD28-B7 interaction and subsequent T cell dependent B cell function. Following promising animal date demonstrating improvement in lupus nephritis, survival and reduction in autoantibody and cytokine (IL-2, c and 10) production with CTLA41g monotherapy as well as in combination with CYC and encouraging results in patients with rheumatoid arthritis, a Phase I/II study in SLE patients conducted by NIAID is underway.

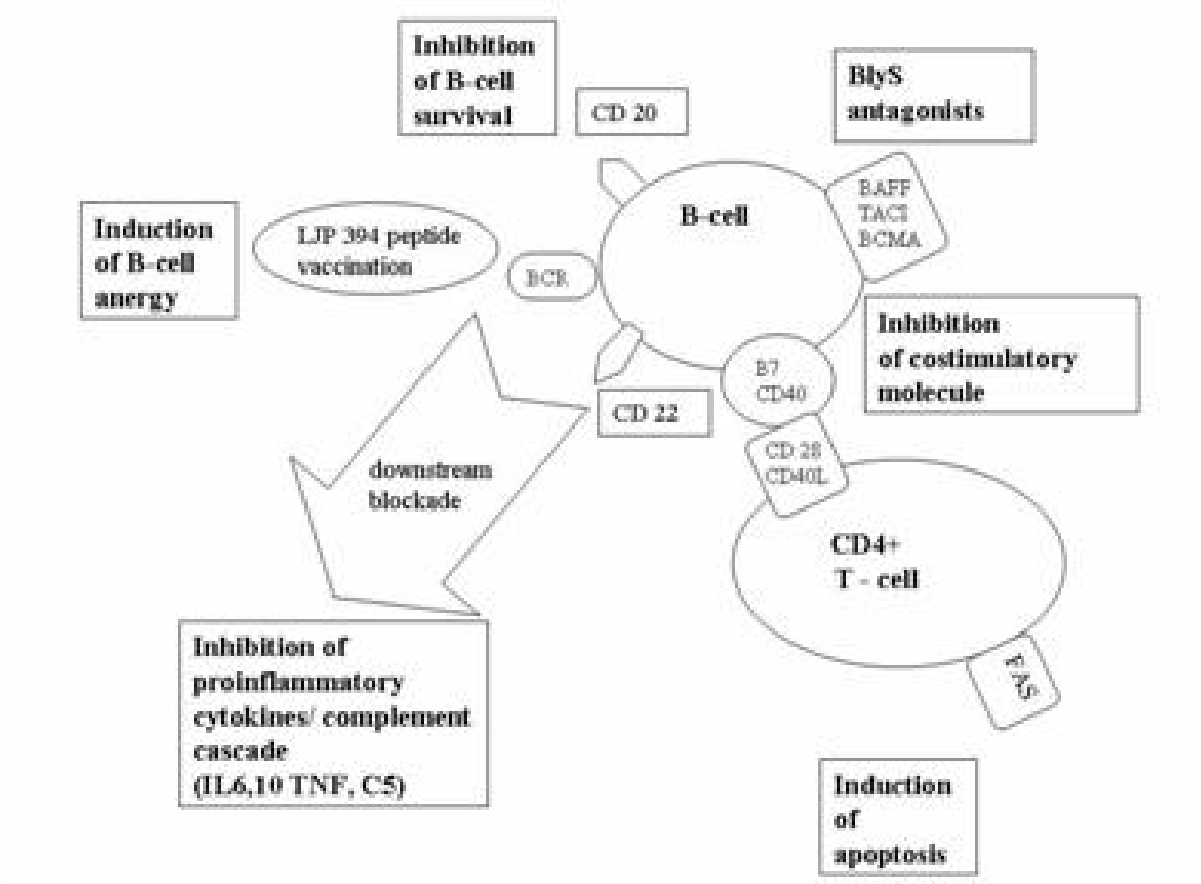

Figure 2. Targets for SLE therapy (adapted from Vasoo and GRV Hughes)

Unfortunately, the anti-CD40L mAb treatment approach in human lupus was not fruitful, as short-term administration of the anti-CD40L mAb, ruplizumab in lupus nephritis was associated with life-threatening prothrombotic activity despite initial encouraging data in the serology and renal function of the patients. Ironically, treatment with another anti-CD40L mAb (designated as IDEC-131) did not prove to be clinically effective in human, SLE, despite being well tolerated. A glimpse of other potential targets include monoclonal antibodies against CD137 costimulatory molecules on T cells in NZB/WF1 SLE-prone mice which have reversed the progression of established SLE-like disease and prolonged survival.

13.1.3 Inhibition of B cell survival

B-lymphocyte stimulator (BLyS) also known as BAFF, is a member of the TNF family of cytokines. Expression of BLyS receptors (BCMA, TACI, BAFFR) is largely restricted to B cells. It has been shown in animal models that over expression of BLyS results in a lupus-like state

and knock-out mice models for BLyS ameliorates the disease. In human SLE, over expression of BLyS is common (persistent elevation in up to 50% of patients over a one year follow-up period). The results of a recent Phase I trial of fully humanized monoclonal IgG antibody against BLyS were encouraging. There was a significant decrease in peripheral B-cells and treatment was a significant decrease in peripheral B-cells and treatment was well tolerated with no serious adverse reactions. However, no clinical or serological improvement was detected in this cohort study. Currently, a Phase II clinical trial is ongoing and other BLyS antagonists are being explored for use in humans. They include BAFFR-Ig (preclinical trials) and TACI-Ig (Phase I in normal subjects).

13.1.4 Induction of B cell anergy

The introduction of synthetic molecules which have the ability to crosslink with antidsDNA antibodies on the surface of B cells leading to anergy or apoptosis is another novel therapeutic approach that is undergoing further evaluation in human SLE. LJP 394 is one such agent, composed of four deoxynucleotide sequences bound to a triethylene glycol backbone that has shown promising results in the Phase II/III clinical trials. The two large randomized, DBPC studies in patients with lupus nephritis showed that there was a significant delay in the time and incidence of renal flares, as well as reduction in anti-dsDNA Ab levels in the subgroup that demonstrated high affinity binding of anti ds-DNA to LJP 394 could be useful adjunct to current therapies for lupus nephritis but the requirement for it to be administered weekly may limit its utility. The recruitment for the Phase IV DBRCT evaluating high dose LJP 394 (100mg and 300mg) in patients with active lupus nephritis is ongoing. A similar approach is also being investigated for the treatment of antiphospholipid syndrome using LJP1082.

13.2 Complement inactivation

In SLE, complement activation follows immune complex formation and the focal point on the final common pathway is C5 with resultant formation of the membrane attack complex (MAC) C5b-9 that causes tissue damage. The blockade of C5 in SLE was first studied in murine lupus which showed significant amelioration of renal disease and marked increase in survival. A Phase I clinical trial of anti C5mAb revealed that it was safe and well tolerated with a trend to improvement in disease assessment scores in the high dose group (8mg/kg).

13.3 Cytokine modulation

Cytokines are low molecular weight mediators of cell-cell communication and include interleukins (ILs), interferons (IFNs), growth factors and others. They are heterogeneous and function in an overlapping manner. The key principle is that the net biologic response in any tissue is the result of balance between local levels of proinflammatory or anti-inflammatory cytokines. SLE is considered by some to be a Th-2 driven disease with documented elevations in serum IL-4, 6,10.

13.3.1 Anti -IL 10 mAb

Interleukin-10 (IL-10) levels are elevated in patients with active SLE and correlate with

disease activity. These alterations in IL 10 and TGF beta regulation appear to result in T-cell dysfunction (accelerated T -cell apoptosis and dysregulation of T- cell dependent B- cell function). The results of a pilot study of six patients with active SLE treated with anti IL-10 mAb for three weeks and followed up for a period of six months were promising. There was marked reduction in circulating IL10 levels with corresponding clinical improvement (decline in MEX-SLEDAI and mean daily prednisolone dose) which were maximal at two months and sustained at the end of the six month review. Apart from one case of mild transfusion reaction, no serious adverse events were reported.

13.3.2 Anti IL6mAb

Interleukin-6 (IL-6) levels are elevated in both human and murine systemic lupus erythematosus (SLE). IL-6 is a potent proinflammatory cytokine that has a wide range of biological activities including terminal differentiation of B - lymphocytes into antibody - forming cells and T cells to effector cells. IL-6 blockade ameliorates disease activity in murine models of SLE. MRA is a humanized monoclonal antibody against the human IL-6 receptor. Data from clinical trials in patients with rheumatoid arthritis suggest that MRA may be an effective and relatively safe agent to block the effect of IL-6.Mild and transient transaminitis, leucopoenia and diarrhoea were observed in the treatment group. Hence, its role could possibly be extended to the treatment of SLE. An open label, dose-escalating, Phase I study of MRA in patients with moderately active SLE is currently underway to address its safety and efficacy.

13.3.3 Interferon -alfa (IFN alfa) antagonism

Recent evidence suggests that IFN-alfa may play a role in murine lupus models and human SLE. The clinical observation that some patients with malignancy or hepatitis C treated with IFN alfa developed autoimmune conditions such as SLE led to a new area of research of IFN alfa antagonism as a potential target in SLE therapy. IFN alfa has numerous biological activities. It enhanced T-cell activation, differentiation and cytokine production (IL10) which in turn activates B cells and autoAb production through a variety of mechanisms. Data from murine lupus models lends further support to this observation. IFN/ betaR knock out NZB/W F1 mice demonstrated significant improvement in serological and clinical manifestations of SLE. Theoretically, there are numerous potential levels at which IFN-alfa antagonism can be targeted but several questions need to be answered such as the possible differential regulation of INF alfa, the role of IFN-alfa subtypes in SLE, the concern of compromising anti-viral immune responses.

13.3.4 TNF alfa inhibition

The exact role of TNF alfa in the pathogenic pathway of SLE remains unclear. TNF blockade in patients with RA or Crohn s disease has led to the development of lupus-like illness, development of antinuclear and anti-ds DNA Abs (although invariably IgM, not IgG) in some patients. On the contrary, treatment of murine lupus with anti TNF therapy resulted in therapeutic benefit and the preliminary results of a recent open pilot study of six refractory SLE patients (four with nephritis, three with arthritis) were give four doses of IV Infliximab at 300mg each, showed a 60% reduction in proteinuria at the end of the follow-up period as

well as remission of arthritis and disease activity, despite the expected rise in anti-dsDNA Abs. It is noteworthy however that three out of the six patients developed urinary tract infections, complicated by E.coli bacteraemia in one patient. A plausible explanation may be that a subset of SLE patients exists in which TNF over activity is pre-eminent and hence respond to TNF blockade. At present however, there are insufficient data to recommend widespread use of such agents in SLE.

13.4 Gene therapy

Preclinical studies have provided proof of concept that gene therapy in SLE is feasible and effective. Successful efforts include gene constructs that alter the expression of cytokines via i.m. injection of naked DNA encoding cytokines or adenoviral mediated gene transfer to CTLA4-Ig into murine lupus models with resultant clinical improvement. Other effort may include gene modified gene transfer such as autologous B cells transfected with toleragenic constructs or T cells in which specific molecular aberrations have been corrected.

Literature

1. Vasoo S, Huges GRV. Perspectives in the changing face of lupus mortality. Autoimmun Rev 2004; 3:415-17.
2. Bompas DT, Furie R, Manzi S et al. For the BG 9588 Lupus Nephritis Trial Group. A short course if BG9588 (anti CD40 ligand Ab) improved serologic activity and decreased hematuria in patients with proliferative lupus GN. Arthritis Rheum 2003; 48:719-27.
3. Looney RJ, Anolik JH, Cambell D et al. B cell depletion as a novel treatment for systemic lupus erythematosus. Arthritis Rheum 2004; 50:2580-9.
4. Tackey E, Lipsky PE, Illei G. Rationale for interleukin-6 blockade in systemic lupus erythematosus. Lupus 2004; 13: 339-43.
5. Schmidt KN, Ouyang W. Targeting interferon alfa in SLE therapy. Lupus 2004; 13: 348-52.
6. Aringer M, Zimmermann C, Graninger WB, Steiner G, Smolen JS. Tumor necrosis factor alfa blockade in systemic lupus erythematosus: an open-label study. Arthritis Rheum 2004; 50:3161-9.

E-mail addresses:

Dubravka Bosnic, M.D.
miro.mayer@gmail.com

Prof. Xsavier Bossuyt, Ph.D.
xavier.bossuyt@uz.kuleuven.ac.be

Assist. Prof. Borut Bozic, Ph.D.
borut.bozic@ffa.uni-lj.si

Prof. Vesna Brinar, Ph.D.
vesna.brinar@zg.tel.hr

Olga Gabela, B.Sc.
ogabela@pharma.hr

Prof. Manfred Herold, M.D., Ph.D.
manfred.herold@uibk.ac.at

Tanja Kveder, Ph.D
tanja.kveder@kclj.si

Prof. Branko Malenica, Ph.D.
b_malenica@yahoo.com

Tea Marcelić, B.Sc.
marcelictea@yahoo.com

Prof. Mladen Petrovečki, Ph.D.
Mladen.Petrovecki@mzos.hr

Prof. Harald Renz, Ph.D.
renzh@med.uni-marburg.de

Prof. Blaž Rozman, M.D.
kc.lj.rozman@siol.net

Wilhelm H. Schmitt, M.D., Ph.D.
wilhelm.schmitt@med5.ma.uni-heidelberg.de

Prof. Sándor Sipka, M.D., Ph.D.
sipka@iiibel.dote.hu

Andrea Tesija-Kuna
andrea.tesija@zg.htnet.hr asw@dadlnet.dk