Review

Developments in lupus 2006

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Abstract

Published reports in 2006 on systemic lupus erythematosus are reviewed with regard to preclinical and clinical studies on disturbances of the immune system including co-stimulation, cytokines and recent insights into new therapeutic approaches. Increasing knowledge of components of the innate immune system, such as certain receptors (Toll-like receptors, Fc receptors and complement receptors) and cytokines as well as immune cells (dendritic cells, plasmacytoid cells and neutrophils) supports their immunopathogenic relevance and enhance our understanding of the pathogenic complexity of lupus. Although it remains to be shown which of those could be targets for therapy or biomarkers, lymphocyte-directed therapy is currently under promising clinical investigation.

Introduction

It is always tempting to look at what has been achieved during a year and evaluate the speed, quality and extent of research data in systemic lupus erythematosus (SLE). Although it will be impossible to determine the impact of these new data, we will try to critically review relevant published peer-reviewed research of the year 2006. Because there were several data directly or indirectly linked to mechanisms of innate immunity, we will highlight these aspects because it will help our understanding of activated cells in systemic inflammation beyond interactions between T and B lymphocytes.

There is an increasing number of potential new immunotherapeutic agents under investigation, such as monoclonal antibodies directed toward lymphocyte surface antigens and co-stimulatory signals, cytokines and modulatory agents of immune receptors. It is apparent that the recognition of unmet medical needs of severely ill patients with SLE and of research in the field of immunology has begun to translate into innovative drugs for improved treatments for patients.

This review is separated into specific categories: preclinical studies in lupus mice, clinical studies on immunopathogenesis, genetics, environmental factors and biomarkers, and finally therapy.

Preclinical studies in murine lupus

SLE is a typical autoimmune disorder and has been considered to result from disturbed tolerance to self-antigens. Although many cell types apparently contribute to autoimmune disorders, lymphocytes are considered to be key effector cells in the initiation, propagation and maintenance of specific autoimmunity. During normal lymphopoiesis, few self-reactive B lymphocytes emerge [1]. B-cell-activating factor (BAFF) is an important B-cell survival factor [2] produced by myeloid cells, T cells and different stromal cells [3]. This member of the TNF superfamily acts via three distinct BAFF receptors: B-cell maturation protein (BCMA), transmembrane activator and calcium modulator ligand interactor (TACI) and BAFF receptor (BAFF-R). Mice overexpressing BAFF develop SLE/Sjögren's-like autoimmunity [4]. Because BAFF is triggering two distinct NF-κB-signalling pathways (the classical and alternative NF-κB pathways), a recent study [5] was able to dissect which NF-κB pathway and which B-cell subsets are involved in developing autoimmunity, by using BAFF-Tg mice and other genetically engineered mice. Interestingly, they found that CD40-dependent germinal center (GC) formation was not required for the development of SLE-like disease. In contrast, another splenic B-cell compartment, the marginal zone (MZ), was found to be enlarged in BAFF-Tg mice. In these MZ cells, survival

BAFF = B-cell-activating factor; BAFF-R = BAFF receptor; BILAG = British Isles Lupus Assessment Group; BLyS = B lymphocyte stimulator; DC = dendritic cell; dsDNA = double-stranded DNA; GC = germinal center; hnRNP = heterogeneous nuclear ribonucleoprotein; ICOS = inducible co-stimulator; IFN = interferon; IL = interleukin; IRF = interferon regulatory factor; MZ = marginal zone; NF = nuclear factor; pDC = plasmacytoid dendritic cell; PML = progressive multifocal leukencephalopathy; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; TACI = transmembrane activator and calcium modulator ligand interactor; TGF = transforming growth factor; TLR = Toll-like receptor; TNF = tumor necrosis factor.

depended mainly on the alternative NF-κB pathway and contained the majority of autoreactive B cells. The study showed in particular that the alternative NF-κB pathway is indispensable for enhanced survival of peripheral B cells and for the manifestation of SLE in BAFF-Tg mice. SLE development in BAFF-Tg mice was clearly dependent on both NF-κB pathways. The interesting finding that neither CD40-mediated interactions between B cells and T cells nor GC formation had an important role in lupus pathogenesis of BAFF-Tg mice indicates a substantial role of innate immunity as well as T-cell-independent activation of B cells [6]. It will be interesting to see to what extent other pathways resulting in classical or alternative NF-κB activation can also influence B-cell survival as well as B-cell functions, and whether blocking certain pathways will provide an efficacy outranging toxicity. Nevertheless, the identification of the source of enhanced BAFF production in SLE and its regulation remains open. This may provide additional clues about the role of 'innate compartments' in lupus.

Another study [7] analyzed the potential of targeting BAFF and its homologue APRIL ('A proliferation-inducing ligand') as therapeutic targets. The authors studied the effects of BAFF receptor–immunoglobulin, which blocks only BAFF, with those of TACI–immunoglobulin, inhibiting both BAFF and APRIL, in an NZB/NZW mouse model. Both reagents led to prolonged survival of NZB/NZW F_1 mice when administered either before or after disease onset. These treatments showed comparable B-cell subset depletion and prevention of T-cell activation as well as dendritic cell accumulation without substantial effects on the emergence of the IgG anti-double-stranded DNA response. Blockage of both BAFF and APRIL, but not that of BAFF alone, reduced the serum levels of IgM antibodies and the frequency of plasma cells, and inhibited the IgM response to a T-cell-dependent antigen. Although the antagonism of BAFF and APRIL is a promising therapeutic approach for B-cell-mediated autoimmunity, it still is not quite clear whether blockage of survival factors is sufficient to inhibit immune reactions significantly. However, an important result of that study was the confirmation of the dominant role of BAFF/BAFF-R interactions for the survival of MZ and follicular B cells in these mice. From our perspective, this study also suggests that selective targeting of MZ cells seems to be very challenging by a blockage of BAFF and/or APRIL.

The development of B cells and their control mechanism and pathways remain subjects of great interest. Despite apparent intrinsic abnormalities, the influence of 17β-estradiol has been analyzed in detail in BALB/c mice [8]. It is noteworthy that this factor, previously known to induce lupus, was found to enhance the maturation of pathogenic naive B cells, whereas the development of a protective B-cell repertoire was disturbed. In particular, this study has shown that an important check point of selection during B-cell transition was impaired at high dosages of this estrogen. This report is consistent with other studies indicating that at earlier stages of B-cell development (pre-GC stages), autoreactive B cells can already emerge and circumvent negative selection. This expands previous hypotheses that most autoreactive cells are generated in the GC with a subsequent lack of appropriate negative selection.

Several reports have identified soluble factors with the potential to influence disease outcome; one of these is hepatocyte growth factor, which is able to prevent lupus nephritis, autoimmune sialadenitis and autoimmune cholangitis [9]. The mechanism of action of hepatocyte growth factor is considered to inhibit Th2 cell functions. Another promising approach is blockage of IL-6 [10-12] initially identified as B-cell growth factor, as well as modulating Toll-like receptor (TLR) signalling by oligomers [13]. IL-6 targeting seems to be unique because data indicate its clinical value in rheumatoid arthritis and SLE, which has not been seen for other biological agents.

Considerable progress has been reported in the field of TLRs. The potential of activating innate immunity by TLR-9 agonists that recognize bacterial DNA has been demonstrated in a genetically predisposed mouse (MRL lpr/lpr) [14], triggering the onset of lupus nephritis. In these mice, CpG DNA, a specific agonist for TLR-9 but – surprisingly – no other ligands of TLR-3 or TLR-7, was able to induce lupus in this strain. Both TLR-7 and TLR-9 are expressed by B lymphocytes, a critical feature for the induction of nucleic-acid-specific autoantibodies. Pawar and colleagues [14] found that only CpG DNA induced sufficient B lymphocyte proliferation and anti-double-stranded DNA (dsDNA) IgG2a production. In contrast with the findings of this study [14], TLR-9 has been found on a C57BL/6 background to protect against the development of lupus nephritis [15]. However, a spontaneous model [16] using the same strain for TLR-7 or TLR-9 knockout mice provided evidence that TLR-7 is protective. The titer of anti-dsDNA autoantibodies was strikingly higher in TLR-$9^{-/-}$ mice. It is important to note that Pawar and colleagues [14] used an exogenous agonist to activate TLR-9, whereas previous studies used endogenous agonists of TLR-9. Interestingly, the current data support the notion that TLRs have different functions in autoimmunity and are not themselves promoting autoimmunity. Thus, TLR-7 recognizing single-stranded RNA promotes autoimmune disease, whereas TLR-9 recognizing DNA was found to protect against lupus-like disease in mice [16]. Although TLR-9 can induce the secretion of anti-chromatin autoantibodies, there is clear evidence that the TLRs have regulatory functions involving B cells, T cells, dendritic cells (DCs) and soluble mediators that are by far more complex than initially thought and need further exploration. However, TLR-9 function represents an example that autoantibody production and the development of a systemic autoimmunity do not essentially overlap. This is supported by clinical observations in some patients.

Other recently published studies support a role for TLR-7 as a receptor for RNA in the generation of autoantibodies and lupus nephritis [17]. In this context, the Y-chromosome-linked autoimmune accelerating (*yaa*) locus has been mapped to a translocation and duplication of the TLR-7 locus, indicating that gene dosage and expression of TLR-7 also can contribute to SLE [18,19]. The identification of this gene duplication with its functional consequences is one of the most important recent achievements, but its clinical impact on human disease needs more investigation.

Ehlers and colleagues [20] further studied downstream effects of the TLR-9–MyD88 pathway in detail and found that its signalling is required for the generation of pathogenic anti-DNA/polyreactive IgG2a and IgG2b autoantibodies. These subclasses efficiently trigger inflammatory responses by their ability to preferentially engage the activation receptor FcγRIV on macrophages [21]. An important observation of these experiments was that TLR-9–MyD88 signalling is able to promote class switching. Moreover, IgG2a and IgG2b seemed to be important pathogenic agents to activate macrophages because a lack of TLR-9, IgG2a and IgG2b autoantibodies led to a similar reduction of autoimmunity to that found in MyD88-deficient mice. Although the data are very compelling, the role of TLR-9 in lupus autoimmunity is considered rather protective against lupus (see above), and involvement of other TLRs and/or complement receptors cannot be ruled out.

In another report [22] the implications of TLRs were analyzed using purified nucleosomes, which are major autoantigens in SLE. This particular study showed that physiological concentrations of nucleosomes were endocytosed and induced the activation of human neutrophils. As a result, these cells upregulated CD11b/CD66b, induced IL-8 and increased their phagocytic activity. Here nucleosomes could induce activation of neutrophils independently of unmethylated CpG DNA motifs and also independently of the formation of immune complexes. Interestingly, neutrophil activation was independent of TLR-2 and TLR-4. Although the exact pathway has yet to be identified, activated neutrophils are suspects for the link between innate and adaptive immunity that results in antinucleosomal antibodies, a characteristic feature of disturbed tolerance in SLE patients. After years of studying T-cell and B-cell aspects in lupus, the interesting data of this study finally support the concept that neutrophils could have an important inflammatory function, in particular in final tissue destruction.

In summary of this section, there is increasing literature on the role of TLRs, especially TLR-7 and TLR-9, with some conflicting data. Thus, the role of TLRs in lupus autoimmunity remains to be elucidated. Given that most data were obtained on selected inbred strains of mice that have unique phenotypes and requirements for disease development, understanding human lupus is even more challenging. Another important aspect is that the nature of TLR ligands determines whether an oligomer is activating or inhibitory. This quality can be changed by very subtle modifications and needs to be considered in the interpretation of studies.

Despite the studies on macrophages [20] and neutrophils [22] as compartments of innate immunity, DCs are the subject of intensive research, and a large body of evidence supports their central role in lupus pathogenesis. In that context, Colonna and colleagues [23] analyzed the phenotype of dendritic cells in different backgrounds of lupus mice. Importantly, this study identified an altered co-stimulatory profile with significantly enhanced expression of CD40 and decreased expression of CD80 and CD54, whereas the expression of another member of this family, CD86, was normal. Similar data about defective CD80 expression on DCs have been obtained by previous studies on patients with lupus. Interestingly, and in contrast with available data in patients with SLE, the study identified an overexpression of CD40 before disease onset. This indicates that DCs in these mice are prone to escape from tolerance and have a key role in very early immune activation.

The role in lupus of type I IFN, in particular IFN-α, which is a candidate as a key cytokine in lupus, has been further explored. The emergence of lupus-like disease in patients treated with IFN-α has been reported [24,25] and resolved on discontinuation of treatment with IFN-α. Rönnblom and colleagues [26] first identified the involvement of this cytokine in SLE. However, a study in MRL mice by Hron and Peng [27] showed that IFN-RII protected against the development of lupus, but IFN-RI-deficient mice worsened lymphoproliferation and organ damage. Similarly, studies by Li and colleagues [28] analyzed the effects of IFN-I and blockage of IFN-I in B6.Sle2 mice. Interestingly, treatment with IFN-α led to an improvement in B6.Sle2 congenics, which contradicts the notion that this cytokine has such a central pathogenic role in lupus.

Recently, Feng and colleagues [29] analyzed five type I IFN-inducible genes (*LY6E*, *OAS1*, *OASL*, *MX1* and *ISG15*) in 48 patients with SLE, 48 normal controls and 22 patients with other rheumatic diseases (14 patients with rheumatoid arthritis and 8 patients with Wegener's granulomatosis) for their mRNA expression levels. All genes were highly and uniquely expressed in patients with SLE, compared with all controls. Moreover, SELENA/SLEDAI scores and the physician's global assessment score were correlated with the expression levels and confirmed previous experiences that glucocorticoids can downregulate the expression of IFN-inducible genes. Notably, patients with lupus nephritis and flaring had higher IFN-inducible gene expression. One particular gene, LY6E, showed a correlation of its levels with lupus nephritis and was suggested as biomarker for lupus nephritis.

However, IFN-α was also found at enhanced levels in patients with rheumatoid arthritis, Sjögren's syndrome [30] and

dermatomyositis [31], as well as in unaffected relatives of lupus patients. Therefore, the 'IFN signature' is apparently not uniquely linked to SLE. The role of IFN-α and its receptor will remain of central interest in lupus but requires additional exploration.

IFN-α is produced mainly by plasmacytoid dendritic cells (pDCs), which have been found at reduced frequencies in the blood of patients with SLE but are likely to reside in the tissues [26,32,33]. It is noteworthy that TLR-7 and TLR-9 seem to be involved in pDC activation and can induce the production of IFN-α [34]. Currently, it is suggested that activation of TLRs remains an early event and results in the activation of innate and adaptive immunity, with IFN-α being the key cytokine. Why lupus patients respond so differently, namely with enhanced IFN-α production compared with controls, remains unclear. Although the IFN signature in lupus seems to be related to pDC activation by TLR-7 or TLR-9 agonists, it remains to be determined whether pDCs initiate or amplify the pathogenic circle.

'From bench to bedside' has gained new data in 2006, especially giving insight into innate autoimmunity. It will be interesting to see how, and to what extent, immunomodulation of blocking specific receptors or ligand–ligand interactions, targeting of cellular compartments, and soluble or insoluble factors of immune activation will translate into future clinical practice.

Discoveries on genetics, environmental factors and biomarkers

Genetics: relatively stable differences

Because the induction of human SLE is clearly dependent on an interplay between hereditary factors and exposure to environmental agents, it is crucial to identify underlying genes of lupus susceptibility. Studies in mice [35] explored the possibility that the presence of *Sle1z*/*Sle1z* within the susceptibility locus is important for B-cell regulation, including the gene Ly108.1. Ly108.1 was highly expressed on immature B cells of B6.Sle1z mice candidates as a critical censor of self-reactive B cells.

Graham and colleagues [36] identified a common haplotype of the interferon regulatory factor 5 (IRF5) gene that regulates mRNA splicing and the expression of IFN-α and was strikingly associated with SLE. This replicate study showed the association of the IRF5 rs2004640 T allele and SLE in four case-control cohorts and family-based transmission disequilibrium test analysis. If more functional data can be obtained after this association, the pathogenic impact of type I IFN may become more robust and may help to overcome some contradictions as noted above.

A recent review [37] summarized certain genes identified within different pathways and immune compartments that all contribute to the induction of specific autoantibodies and pathogenic autoimmunity, finally leading to end organ damage. A meta-analysis of genome-wide linkage studies in patients with SLE [38] summarized putative susceptibility loci for SLE by independent studies. Suggestive regions on 6p21.1–q15, 20p11–q13.13 and 16p13–q12.2 represent the highest relation to the disease, with the region on chromosome 6 containing HLA. It remains an open question whether this class II susceptibility is more related to autoantibody production or to the disease itself.

Another genetic variable for the course of the disease is the relation to gene polymorphisms and the response to specific drugs, as is known for cytotoxic drugs in oncology. A study by Lopez and colleagues [39] provided evidence that the clinical efficacy of antimalarial drugs depends on polymorphisms of the cytokines TNF-α and IL-10. The combined genotype of high producers of TNF-α and low producers of IL-10 responded better to antimalarial treatment and had milder disease courses among the 192 patients studied. This study may be just the beginning of a complex analysis in which certain polymorphisms of several variables need to be considered for the dissection of unique characteristics of the disease from individual profiles. These profiles have the potential to result in patient-tailored therapies, which becomes more important when the therapeutic possibilities enlarge.

Environmental factors

Despite the known role of ultraviolet exposure and estrogens, from our perspective two aspects need particular emphasis in 2006. First of all, smoking [40-42] is clearly documented as a risk factor for SLE and the production of anti-dsDNA antibodies. The interaction of smoking as environmental factor with underlying genetic predispositions for immune activation is known for SLE, rheumatoid arthritis (RA) and ulcerative colitis, whereas the presence of HLA-DRB1SE is a cofactor in RA, and HLA-DR3 and IRF5 [36] represent risk factors for anti-DNA production in SLE.

Discoveries of immunopathogenesis

SLE as a classic autoimmune disease has multiple facets of disturbances of the immune system, and the search to identify additional abnormalities continues.

Regulation of the immune system follows an interplay between molecules and their receptors by balancing activation and inhibition in a timely manner. Among important regulating receptors, Fc receptors largely expressed on very different cells have attracted great interest, particularly the unique inhibitory FcγRIIB. After demonstrating its importance in mouse models, Mackay and colleagues [43] analyzed the expression of FcγRIIB in human SLE and found that upregulation of FcγRIIB was significantly decreased in memory B cells of patients with SLE. Notably, some African-American patients failed to upregulate FcγRIIB, which is consistent with the known higher susceptibility of severe SLE in those patients. It is not quite clear which (genetic) factor or

distinct regulatory mechanism leads to these functional differences in FcγRIIB regulation. The data from this study, however, support the notion that FcγRIIB is a prime candidate for the regulation of B-cell check points involved in susceptibility to lupus.

A recent Dutch study [44] studied the prevalence and course of anti-chromatin antibodies (anti-nucleosome, anti-dsDNA and anti-histone) and anti-C1q autoantibodies in 52 patients with proliferative lupus nephritis who were enrolled in a randomized controlled trial with either cyclophosphamide or azathioprine plus methylprednisolone. Patients with higher SLEDAI had higher levels of anti-nucleosome autoantibodies, anti-C1q autoantibodies and serum creatinine. A comparable rapid decline of anti-nucleosome, anti-dsDNA and anti-C1q autoantibodies was seen in both treatment arms. Renal flares were not preceded by rises in autoantibody titres. The authors found that measurement of anti-chromatin and anti-C1q autoantibodies is useful for diagnosing lupus nephritis. However, these antibodies did not reliably allow monitoring the disease course and did not seem to be a useful biomarker; this remains a matter of debate [45].

Studies on autoimmune T and B cells

Analysis of T-cell abnormalities in SLE has been expanded by the identification of the spliceosomal autoantigen heterogeneous nuclear ribonucleoprotein (hnRNP)-A2 as a major T-cell autoantigen [46] with the use of T-cell clones. A large number of $CD8^+$ T-cell clones not expressing CD28 showed anti-hnRNP-A2 reactivity despite the large number of autoreactive $CD4^+$ clones. The value of $CD8^+$ T cells in SLE autoimmunity and the capability of $CD8^+$ cells for antigen presentation remain less understood.

Co-stimulation by a member of the CD28 family, the inducible co-stimulator (ICOS), has been reported in patients with lupus [47]. By using a different antibody for detection, the study confirmed the enhanced expression of ICOS on $CD4^+$ and $CD8^+$ T cells in patients with SLE compared with normal controls, as reported previously [48]. Moreover, the humanized antibody used (JTA009) led to enhanced production of IFN-γ, IL-4 and IL-10 both in T cells from normal controls and in those from lupus patients; it was also able to induce immunoglobulin and anti-DNA antibody production in co-cultures with B cells. Thus, blocking interaction between ICOS and ICOS ligand is a potential candidate for therapeutic intervention that has already been shown to be effective in mice [49].

Of recent interest, checkpoints of B-cell development have been studied by studying re-expressed immunoglobulin receptors obtained from normal controls and from lupus patients. In this regard, Wardemann and colleagues [1] found that most early immature B cells were self-reactive, suggesting inefficient checkpoint regulation in lupus. The same group reported that patients with SLE in clinical remission continue to produce elevated numbers of self-reactive and polyreactive antibodies in the mature naive B-cell compartment. Although the frequency of B cells expressing autoreactive immunoglobulin was lower than during active disease, the data suggest that early checkpoint abnormalities are an integral feature of SLE [50].

Cardiovascular risk and SLE

Patients with SLE have a disease-related enhanced risk for the development of cardiovascular complications. As a result, accelerated atherosclerosis is a major cause of mortality and morbidity in SLE, with 6 to 10% of patients developing premature clinical coronary heart disease. In 2006, increased intima-media thickness, an easy test in clinical practice was confirmed to be associated with age, systolic blood pressure, disease duration and a systematic coronary risk evaluation [51]. Importantly, interaction between endothelial cell activation, vascular remodeling, lipid profiles and enhanced blood pressure and interaction with thrombocyte activation are all critical factors and have yet to be elucidated for early intervention to prevent cardiovascular complications.

Another cardiovascular study in SLE analyzed 200 patients [52] and demonstrated that high titers of IgG anticardiolipin antibodies (more than 80 IU/ml) were associated with the development of mitral valve nodules and significant mitral regurgitation but were not related to systolic dysfunction or signs of atherosclerosis or myocardial hypertrophy. Because of enhanced levels of vascular cell adhesion molecule (VCAM)-1 and TNF receptors, a mechanistic relationship between local endothelial cell activation and TNF receptors was concluded. This is of interest because it follows a proposed separation of distinct endothelial cell subsets and may explain why vascular lesions have preferred sites; that is, where characteristic arthritis manifests itself. This was supported by recent animal studies [53] demonstrating that local endothelium defines where arthritic lesions develop. These data have methodological implications because results on epithelial cells are widely derived from umbilical vein endothelial cells that may not allow the acquisition of reliable data.

Transforming growth factor (TGF)-β_1

TGF-β_1 is a potential factor involved in the balance of the immune system and atherosclerosis. It is also considered to be a potent naturally occurring immunosuppressant produced by all immune cells and has a fundamental role in controlling proliferation and the fate of cells through apoptosis. TGF-β_1 in the vascular wall functions to maintain the normal vascular wall structure; it controls the balance between inflammation and extracellular matrix deposition in atherosclerosis and inhibits smooth muscle and endothelial cell proliferation. Because impairment in the TGF-β_1 pathway has been associated with both an SLE-like illness and enhanced atherogenesis, a recent study [54] measured the efficiency of TGF-β_1 activation in SLE: patients with SLE had low to normal TGF-β_1 activation and were linked with increased

lymphocyte apoptosis, irreversible organ damage, disease duration, low-density lipoprotein levels and increased carotid intima-media thickness. Inappropriate TGF-β_1 activation in SLE may therefore lead to disturbances of immune tolerance and enhanced atherosclerosis, which connect vascular biology and the immune system.

New advances in treating lupus

After the demonstration that mycophenolate mofetil is more effective than intravenous cyclophosphamide in inducing remission in a 24-week non-inferiority trial [55], its use for the induction and maintenance of lupus nephritis has been shown in larger databases [56] and provides advantages in safety.

The search for other innovative treatment options comprises several promising compounds that are under investigation and have been nicely summarized recently [57,58]; these include B-cell tolerogens (LJP394), anti-B-cell-directed antibodies (CD20 and CD22), cytokine blockage in SLE (anti-IL-10, anti-TNF-α, anti-BAFF and anti-IL-6), anti-C5, cytotoxic T lymphocyte-associated antigen 4 immunoglobulin for co-stimulator blockage, and TV4710 as a DNA antibody neutralizing agent as well as autologous stem cell transplantation. Considerably more drugs are in early developmental stages.

Additional progress has been achieved in B-cell-targeted therapies. SLE is widely accepted as a disease of B cells, with a plethora of autoantibodies as a hallmark of this entity. On the surface of B cells, the B-cell receptor, as part of the adaptive immune system, is expressed and linked with several other extracellular and intracellular receptors of innate immunity, such as TLRs, Fc receptors and complement receptors. B cells are therefore a common denominator of pathways of both the innate and adaptive immune systems, which is impressively unique [6,59].

Data on Lymphostat B, a human monoclonal antibody that blocks the bioactivity of BAFF or B lymphocyte stimulator (BLys), have been reported, including its effect on B-cell depletion of blood and tissue B cells in cynomolgus monkeys [60,61]. The safety profile, including a lack of treatment-related infections in animals repeatedly treated and followed for 34 weeks after treatment, is considered favorable. Publications of clinical trials on the use of this drug in RA and lupus patients are awaited.

After early experiences of using B-cell depletion with anti-CD20 (rituximab) combined with intravenous cyclophosphamide in otherwise refractory patients, colleagues from University College London reported seven patients who had relapsed and subsequently received repeated cycles of this combination (up to three cycles) since 2000 [62]. In this first study of repeated B-cell depletion in lupus, there was a consistent decrease in disease activity measured by the British Isles Lupus Assessment Group (BILAG) scoring in consecutive treatment cycles with a mean duration of B-cell depletion of 6 months (range 5 to 7), which is lower than that in RA (7 months; range 6 to 8). Interestingly, the duration of clinical benefit was frequently longer than the period of B-cell depletion. With the exception of one patient with mild serum sickness, re-treatment was safe, especially taking into account the disease severity of those patients.

It is noteworthy that data are accumulating to show that rituximab treatment in lupus nephritis may provide a promising new agent, although data from randomized clinical trials are awaited. Though rituximab induces important depletion of B cells in almost all patients with SLE, it has been described that therapy with rituximab led to changes in titers of serum autoantibodies but had no significant effect on plasma immunoglobulin levels. This is consistent with the concept of different lifetimes of distinct plasma cell subsets [63].

One study found that patients with SLE receiving this biological agent showed a diminished expression of the co-stimulatory molecules CD40 and CD80 by B cells [64]. In another open clinical trial [65], 22 patients with active SLE and lupus nephritis (mainly WHO classes III and IV) were studied; a significant reduction in disease activity and reduced proteinuria at days 60 and 90 after rituximab therapy were found. In 20 of 22 patients, B-cell depletion was observed. One patient died at day 70 with invasive histoplasmosis. No other important adverse effects of this therapy were registered. Significant enhancement in the levels of different $CD4^+$ regulatory cells (T_{reg}, Th3 and Tr1), but not $CD8^+$ T lymphocytes, was seen at day 30. This increase was sustained for T_{reg} cells at day 90, and increased apoptosis of T cells was seen at day 30. These observations provide indirect evidence that B and T cells cross-talk continuously and this can apparently not be completely replaced by any other immune cell type.

Other immunological studies of lupus patients under B-cell depletion therapy [66] revealed that antinucleosome antibodies and anti-dsDNA antibodies decline significantly after B-cell depletion for 6 to 8 months, with clinical improvement in all 16 patients enrolled. Although this needs to be confirmed with more data, it was the first study in SLE demonstrating that antimicrobial immunity (anti-tetanus toxoid and anti-pneumococcal antibodies) remained unchanged, whereas $9G4^+$ antibodies encoding anti-DNA reactivity declined under B-cell depletion and increased before lupus flares. 9G4 is an interesting biomarker, but because not all patients with SLE produce this idiotype, its wider usage in clinical practice is prevented.

Follow-up of BAFF and APRIL levels in patients with SLE ($n = 10$) and RA ($n = 9$) treated with rituximab provided some very interesting distinctions between these entities under B-cell depletion [67]. Whereas BAFF increased in both patient groups, it decreased after B-cell repopulation. APRIL

levels in patients with SLE were normal at baseline and decreased significantly under B-cell depletion. Patients with RA had a 10-fold higher APRIL level at baseline, which did not change under therapy with rituximab. If these data are confirmed, our understanding of the role of BAFF, APRIL and B cells in RA versus SLE will be enhanced.

A recent report [68] analyzed BAFF/Blys isoforms and mRNA level in samples from 60 patients with SLE, 60 patients with RA and 30 controls. Although there was no robust correlation between BlyS/BAFF protein levels and disease activity, full-length and ΔBLyS/BAFF mRNA were elevated in SLE, and this was correlated with disease activity. Further studies will be required to determine whether this molecule is useful as biomarker.

Despite these encouraging data on B-cell depletion with rituximab in SLE, including lupus nephritis and neuropsychiatric SLE, two deaths of patients with SLE were reported who developed progressive multifocal leukencephalopathy (PML) [69]. It has been estimated that about 10,000 patients with SLE have been exposed to rituximab. Further careful clinical recording will be needed to evaluate the actual risk for PML, because the development of PML might also be related to the underlying disease itself.

One study has chosen a different pathway for targeting B cells and has conducted an open phase IIa trial using a humanized anti-CD22 antibody targeting mature B cells [70] after it had been studied in patients with B-cell non-Hodgkin's lymphoma. The mechanism of action of this antibody is different from that of rituximab because only about 30% of peripheral B cells are depleted under this agent and its activity seems to be through the negative regulation of B-cell receptor signalling, the strongest B-cell activation signal [71]. This open clinical trial included 14 moderately active (BILAG score 6 to 12) patients using 360 mg/m^2 epratuzumab intravenously every 2 weeks for a total of four doses. The BILAG scores decreased by at least 50% in all 14 patients at some point during the study; 92% of patients showed decreases until 18 weeks, at which time 38% showed a decrease of at least 50%. Almost all patients (93%) experienced improvements in at least one BILAG B-level or C-level disease activity at 6, 10 and 18 weeks. On the basis of these data, multicenter controlled studies have been initiated to test the value of anti-CD22 strategies.

With regard to lupus flare management, two major studies were reported [45,72]. The use of hormones, in particular oral contraceptives, is of continuous and great interest in SLE. There are concerns about potential negative side effects, especially inducing lupus flare. A double-blind, randomized, non-inferiority trial [72] evaluated the effect of oral contraceptives on lupus activity in 183 premenopausal lupus patients receiving either oral contraceptives (triphasic ethynyl estradiol plus norethindrone) or placebo over a 12-month period with the primary endpoint of a severe lupus flare. These flares were reported in 7.7% of patients receiving oral contraceptives compared with 7.6% under placebo, giving similar flare rates of 0.084 and 0.087, respectively. In terms of thrombosis, the rates were not significantly different during the observation period. Although the study showed that oral contraceptives do not lead to higher flare rates among women with lupus, the relatively short observation period did not address long-term risks such as thrombembolic event rate and enhanced risks for breast cancer. Moreover, the study excluded patients with lupus anticoagulant, a patient population at even greater risk for vascular occlusions. In this group of patients, however, we are in great need for better management; data under long-term anticoagulation are not robust.

Another randomized placebo controlled trial led by Tseng and colleagues [45] could identify risk factors for lupus flare among 154 patients studied serologically by elevated C3a levels and raised anti-dsDNA antibodies before lupus flare. The use of short-term corticosteroid therapy at the time of serologic flares prevented disease activation significantly, which may fundamentally support clinical decisions. This confirms a former study [73] showing that relapses can be prevented by giving prednisone when an increase in anti-dsDNA occurs.

Curative therapy might require the elimination of long-lived plasma cells secreting pathogenic autoantibody memory without need for restimulation. These cells are refractory to immunosuppressive drugs and B-cell depletion. So far, the depletion of these cells has been achieved by complete immunoablation in combination with autologous stem cell transplantation. The development of novel approaches for specific elimination of autoreactive plasma cell memory remains a challenge for the future [74,75].

From a very different perspective, new drugs and regimens teach us continually about underlying mechanisms of autoimmunity. In this regard, we have learned that graft-versus-host immunity can induce scleroderma and Sjögren-like diseases. Most recently, Burt's group reported the development of a secondary autoimmune disorder after hematopoietic stem cell transplantation in autoimmune patients [76]. Of 155 patients transplanted, 6 developed some sort of secondary autoimmune disorder. Two patients with SLE developed factor VIII inhibitors, four patients (two with multiple sclerosis, one with lupus and one with systemic sclerosis) developed autoimmune cytopenias. This complication occurred in 15% of patients treated with alemtuzumab (Campath, anti-CD52) and in 1.9% of patients treated with antithymocyte globulin (ATG). There was no link with gender, type of ATG, CD34 selection or the development of secondary autoimmunity, in contrast with an association with the use of alemtuzumab. Thus, anti-CD52 depletion seems to disrupt important negative regulators of autoimmunity.

Because this molecule is expressed on a variety of leukocytes involved in innate and adaptive immunity, detailed studies will be needed to identify the underlying mechanisms of action involved.

Conclusion

This review seeks to highlight some reports published in 2006 about lupus and is by its nature restricted. After years of major contributions by studies of B and T lymphocytes that led to innovative therapies in lupus, such as B-cell depletion, blockade of co-stimulation or cytokines, there are new insights into the pathogenic role of innate immunity. As examples, the disputed role of TLRs, complement receptors and Fc receptors partly expressed on neutrophils, dendritic and plasmacytoid dendritic cells may open new fields of lupus research and may finally change pathogenic and therapeutic concepts.

Competing interests

TD was involved in the clinical phase IIa trial of epratuzumab in SLE, which was sponsored by Immunomedic, Inc. The work was supported by DFG grants (SFB421 and 650).

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