Novel treatment strategies for antibody-mediated diseases: targeting long-lived plasma cells

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'Whenever pathogenic antibodies are predominantly secreted by long-lived plasma cells, good treatment responses become unlikely.'

Autoantibodies are believed to contribute to the pathogenesis of multiple immune-mediated diseases. However, there are only a few diseases in which a critical role of antibodies for the pathogenesis has been convincingly demonstrated. A good correlation of autoantibody titers with the disease activity, and the detection of such antibodies in most patients with a certain disease or disease manifestation, as well as the detection of autoantibodies bound to damaged organ structures, may provide an initial indication of a contribution of antibodies to the pathogenesis. Further evidence might come from *in vitro* experiments with exposure of isolated antibodies to primary cells or cell lines. Efficacy of immune adsorption, that is, elimination of immunoglobulins by extracorporal adsorption to an affinity matrix, such as immobilized recombinant protein A, further substantiates the role of autoantibodies in a disease entity [1,2]. The induction of disease by the transfer of autoantibodies proves their importance for the pathogenesis [2,3].

Autoantibodies may cause disease by a variety of mechanisms, the most important being:

- Direct damage of cells by the antibodies binding to surface structures, and consecutive lysis of cells by antibody-dependent cellular cytotoxicity or – presumably less important – complement-mediated lysis. The different forms of autoimmune hemolytic anemia and immune thrombocytopenia are predominantly mediated by these mechanisms. Similarly, pathogenic antibodies to double-stranded (ds) DNA may crossreact with α-actinin on the surface of mesangial cells and podocytes, and, thereby, cause kidney damage [4–6];
- Modulation of cellular function by antibodies against cell surface receptors. On the one hand, antagonistic antibodies can block binding of ligands, as is the case with antibodies directed against the acetylcholine receptors of the skeletal muscles, thereby causing symptoms of myasthenia gravis [7]. On the other hand, agonistic antibodies may induce inappropriate signaling, as has been demonstrated in Grave's disease with antibodies against the receptor of thyroid-stimulating hormone;
- Organ and tissue damage can also be caused by immune complex formation, either in the blood or *in situ* within the target organ. Complement activation with formation of C3a and C5a attract inflammatory cells. In addition, phagocyte activation via stimulating Fc receptors causes proinflammatory cytokine release. Immune complex formation appears to be critically involved in the pathogenesis of lupus nephritis and other forms of immune-mediated nephritides, including Goodpasture's syndrome [8,9];
- Autoantibodies neutralizing functionally important soluble biomolecules can induce life-threatening conditions. For instance, antibodies to coagulation factor VIII may cause severe bleedings [10];
- So-called penetrating antibodies directed against intracellular antigens appear to cross the cytoplasmic membrane of intact cells and mediate intracellular effects [11,12].

Despite important advances during the past few decades, antibody-mediated diseases are still difficult to treat. Current therapeutic approaches including high-dose glucosteroids, cyclophosphamide, azathioprin, mycophenolate mofetil, methotrexate, high-dose intravenous immunoglobulins, plasmapheresis and immunoadsorption, even in combination, often cannot induce remission of the disease. The reasons why autoantibody production can resist even high-dose chemotherapy and allogeneic stem cell transplantation was elucidated by several highly important findings from Radbruch, Manz, Slifka, Ahmed and Hiepe: long-lived plasma cells were identified as a source of antibody memory, their role in autoimmune diseases was investigated and their surprisingly high resistance to current treatments was characterized

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[13–18]. Whenever pathogenic antibodies are predominantly secreted by long-lived plasma cells, good treatment responses become unlikely. Hence, the remaining autoantibodies produced by long-lived plasma cells can perpetuate the pathogenic process.

Recent data provide evidence for the beneficial effects of B-cell-targeted therapies. The chimeric anti-CD20 antibody rituximab, which induces long-lasting depletion of CD20-positive B cells, but does not attack most plasma cell populations, has been approved for the treatment of rheumatoid arthritis (RA) [19]. Despite complete depletion of peripheral blood B cells, total immunoglobulin concentrations and many autoantibody specificities are only slightly reduced. Whereas rheumatoid factor in patients with rheumatoid arthritis and antibodies against thrombocytes in patients with refractory immune thrombocytopenia are usually markedly decreased upon rituximab treatment, in patients with systemic lupus erythematosus (SLE), antibodies to dsDNA, nucleosomes, Ro/SSA and RNP/Sm are only slightly or moderately decreased [20,21]. This discrepancy could be explained by a predominant production of autoantibodies by short-lived versus long-lived plasma cells. Whereas short-lived plasma cells need to be continuously renewed from the B-cell pool, long-lived plasma cells can survive for years [15,18]. Furthermore, amelioration of disease activity by rituximab in SLE may rather depend on inhibition of B-cell functions, such as antigen presentation and cytokine production, than on the modest reduction of autoantibody levels. Importantly, at present, placebo-controlled trials proving the efficacy of rituximab in classical antibody-mediated diseases such as SLE are still warranted.

CD22 is another B-cell antigen that can be therapeutically targeted. The humanized monoclonal antibody epratuzumab is less efficient in depleting B cells compared with rituximab; its effects may rather be due to modulation of B-cell function via ligation of the inhibitory CD22 molecule. In a small open-label trial, epratuzumab has shown durable benefits on most body systems in SLE [22]. However, there were no significant changes in the concentrations of autoantibodies or serum immunoglobulins observed.

B-lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL) are closely related members of the TNF superfamily and mediate maturation, proliferation, survival and differentiation of B lymphocytes, and support plasma cell survival [23]. Overexpression of BLyS induces hypergammaglobulinemia and autoantibody production, leading to a lupus-like disease [24,25]. Moreover, BLyS serum concentrations are elevated in many patients with SLE. Hence, the humanized BLyS neutralizing antibody belimumab was tested in a clinical Phase III trial in patients with SLE. There was a slightly reduced disease activity and less flares in belimumab-treated patients. Within 1 year of treatment, the concentrations of most autoantibodies decreased to approximately 50% of their previous concentrations. However, there was no further decrease after 3 years of belimumab treatment [26]. The TACI–Ig fusion protein atacicept employs a similar mechanism of action. TACI is one of the receptor molecules for BLyS and APRIL [23]. Therefore, the TACI-Ig fusion protein neutralizes both BLyS and APRIL. In a Phase Ib placebo-controlled trial in RA patients, atacicept has been shown to be well tolerated, and there was a trend toward clinical improvement within the 3-month treatment period. Consistent with the supposed mechanism of action, in patients who had received the highest dose of atacicept, total serum IgM decreased by approximately 50%, IgM, IgG and IgA rheumatoid factors by approximately 40%, and antibodies against cyclic citrullinated peptides by approximately 25% [27]. The modest decrease in autoantibodies achieved by neutralization of BLyS and/or APRIL will not be sufficient for an efficient treatment of antibody-mediated diseases. Nevertheless, this principle may be used as add-on therapy or for the maintenance of remission.

'The proteasome inhibitor bortezomib eliminates both short-lived and the otherwise treatment-resistant long-lived plasma cells.'

All treatments mentioned above cannot efficiently deplete long-lived plasma cells, a fact that might be responsible for their limited efficacy in autoantibody-mediated diseases. In very severely affected patients not responding to conventional therapies, high-dose chemotherapy with subsequent autologous stem cell transplantation may be an option. Only if this regimen is combined with anti-thymocyte globulin treatment can long-lived plasma cells also be eliminated. This procedure might allow a 'reset' of the immune system, and can achieve long-term remissions, including disappearance of autoantibodies. However, it is connected with a substantial risk of morbidity and mortality [28].

Apart from biologics there has been an increasing interest in research on small molecules for the

treatment of autoimmune diseases. In this context, we recently described that the proteasome inhibitor bortezomib eliminates both short-lived and the otherwise treatment-resistant long-lived plasma cells in mice [29]. Bortezomib has been approved as a second-line medication for multiple myeloma, and is currently investigated in various clinical trials for the treatment of non-Hodgkin's lymphomas and other malignancies. We and others have recently demonstrated that proteasome inhibitors induce cell death of multiple myeloma cells by activation of the terminal unfolded protein response. Our laboratory demonstrated a clear correlation between the extent of antibody production, which inherently results in defective ribosomal products and unfolded proteins, and the susceptibility of myeloma cells toward proteasome inhibition [30]. Also, normal plasma cells synthesize enormous numbers of antibodies (2000 to 10,000 molecules per second), and therefore need to degrade a large number of unfolded proteins in their proteasomes. Furthermore, Roberto Sitia and colleagues demonstrated a strong decrease of the proteasome activity during differentiation of B cells into plasma cells *in vitro* and *in vivo*, a fact that makes plasma cells even more sensitive toward proteasome inhibitors, and that may be crucial to limit the lifespan of short-lived plasma cells [31,32].

To investigate if proteasome inhibitors could be used to treat autoantibody-mediated diseases, we used bortezomib in murine models of lupus. In contrast to cyclophosphamide and dexamethasone, bortezomib was able to deplete long-lived plasma cells very efficiently from spleens and bone marrows of NZB/W F1 lupus mice. Autoantibodies to dsDNA disappeared, and lupus nephritis with proteinuria was ameliorated upon bortezomib treatment. Most importantly, bortezomib-treated lupus mice survived much longer than controls. To our knowledge, this is the first therapeutic approach that can deplete virtually all plasma cells without causing overt toxic side effects in mice. Antibodies to dsDNA and cells secreting dsDNA antibodies almost completely disappeared upon bortezomib treatment, whereas total IgG concentrations were reduced by only 50%. Based on these promising results, we are currently planning a Phase I clinical trial with bortezomib in SLE patients that are refractory to conventional therapy.

The elimination of plasma cells producing pathogenic autoantibodies represents a key therapeutic goal for efficient treatment of antibody-mediated diseases. In contrast to other therapeutic regimens, bortezomib can efficiently and quite selectively eliminate plasma cells in mice. However, long-lived plasma cells secreting protective antibodies against viral or bacterial pathogens are also affected. Hence, an ideal treatment strategy would just eliminate such plasma cells secreting pathogenic antibodies. A first step in this direction was carried out in the laboratory of Tchavdar Vassilev: B cells producing autoantibodies against dsDNA were eliminated using an engineered antibody-like molecule that crosslinks the DNA-specific surface immunoglobulins on autoreactive B cells with inhibitory FcγRIIb receptors. In the MRL/lpr lupus model, the appearance of anti-DNA antibodies and the disease onset were markedly delayed by intravenous injection of this bispecific antibody-like molecule [33]. However, plasma cells themselves do not express surface immunoglobulins and, hence, cannot be directly targeted by this fascinating new approach.

'A key to a successful treatment with a long-lasting response is the depletion of pathogenic antibodies producing long-lived plasma cells.'

Taken together, new treatment strategies for antibody-mediated diseases are currently being developed, which hopefully can induce remissions with acceptable side effects in most of these difficult-to-treat patients. A key to a successful treatment with a long-lasting response is the depletion of pathogenic antibodies producing long-lived plasma cells. In particular, combinations of immune adsorption to immediately reduce autoantibody load with plasma cell depletion by proteasome inhibitors, and then maintenance therapy with conventional immunosuppressants such as glucosteroids, azathioprine and methotrexate to prevent relapses, may represent a promising approach for future clinical studies.

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