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Rituximab in induction therapy for anti-neutrophil cytoplasmic antibody (ANCA) vasculitis

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Summary

Anti-neutrophil cytoplasmic antibodies (ANCA) have been associated with a spectrum of vasculitis that includes granulomatous polyangiitis (formerly known as Wegener's granulomatosis), microscopic polyangiitis, the Churg–Strauss syndrome, primary pauciimmune necrotizing and crescentic glomerulonephritis and related forms of vasculitis. *In vitro*, *in vivo* and clinical evidence support the conclusion that ANCA participate in the pathophysiology of this disease spectrum. Rituximab is a potent tool that can interrupt B cell-mediated immunity without major compromise of T cell-mediated immunity. Thus, it has great appeal as a tool to interrupt antibody-mediated autoimmune disease. The results of two prospective randomized trials confirm that rituximab can be effective as part of induction therapy for active ANCA-associated vasculitis. The safety profile for rituximab appears favourable relative to cyclophosphamide and steroids. However, there remain many patients who require individualized adjustments of ancillary therapy, as breakthrough disease, relapses and infectious complications do occur. Based on our current knowledge, rituximab should now be incorporated as part of induction therapy in many patients with ANCA-associated vasculitis. However, more work is needed to determine how rituximab may best be integrated into the overall immunosuppression of these patients.

Keywords: ANCA, ANCA-associated vasculitis, rituximab

Introduction

In the 1970s, Fauci and Wolff [1] hypothesized that Wegener's granulomatosis was mediated by a hypersensitivity reaction and responded to the immunosuppressive effects of cytotoxic agents, including cyclophosphamide. Experience has shown that immunosuppression using broad-based cytotoxic therapy can dramatically help patients with Wegener's granulomatosis. However, recurrence of disease and toxicity of therapy continue to produce substantial morbidity and mortality. In the 1980s, two specific types of anti-neutrophil cytoplasmic antibodies (ANCA) were discovered to be associated closely with a restricted spectrum of vasculitis, including Wegener's granulomatosis. This spectrum is now often referred to as ANCA-associated vasculitis (AAV). After discovery of ANCA, studies to dissect the immunological mechanisms that lead to AAV were initiated. Starting in the 1990s, biological tools have been developed to interrupt or influence the immune system at specific foci. Now, in the 21st century, the question is: given our new understanding of the mechanisms of AAV, can we use these emerging immunosuppressive tools to provide a longer and higher-quality life for our patients with AAV than that provided by 'standard' cytotoxic therapy? Furthermore, is there sufficient evidence to conclude that rituximab is the first such immunosuppressive tool that should now be used routinely in the treatment of AAV?

Pathophysiology of ANCA-AAV

As noted above, there is an extremely close correlation between the presence of ANCA and a diagnosis of a restricted spectrum of vasculitis [2]. This spectrum of vasculitis includes: granulomatous polyangiitis (formerly known as Wegener's granulomatosis), microscopic polyangiitis, the Churg–Strauss syndrome and renal limited pauci-immune necrotizing and crescentic glomerulonephritis. Two specific types of ANCA, anti-proteinase 3 and anti-myeloperoxidase antibodies, have been implicated in this association and the term 'ANCA' is often used in reference to these two antibody specificities.

There is now accumulating evidence to conclude that these antibodies have a pathogenic role.

Clinical evidence suggests a role of ANCA in the pathogenesis of vasculitic injury. Not only is there a correlation between the presence of ANCA and the diagnosis of vasculitis, but some studies have shown a correlation between changes in ANCA and the activity of disease [3,4]. Furthermore, the direct removal of the ANCA by plasma exchange appears to mitigate the damage to glomeruli in severe cases of ANCA glomerulonephritis [5].

In vitro studies have shown that ANCA can activate primed neutrophils to produce reactive oxygen species and to degranulate with the release of proteolytic enzymes [6,7]. Treatment of rolling neutrophils with ANCA can cause integrin-mediated adhesion [8]. ANCA-activated neutrophils activate complement by the alternative pathway which, in turn, can prime additional neutrophils for ANCA activation [9]. Thus, *in vitro*, ANCA can interact with neutrophils leading to a series of steps that promote inflammation [10].

In vivo studies have added overwhelming support for the pathogenic role of ANCA. In 2002, Xiao *et al*. described an animal model of ANCA-AAV where myeloperoxidase (MPO) knock-out mice were immunized with recombinant mouse MPO. Immunoglobulin was then harvested and infused into T and B cell-deficient mice and wild-type mice. Necrotizing and crescentic glomerular lesions developed by day 6 [11]. Furthermore, the process is dependent on bone marrow-derived target cells – specifically, neutrophils [12,13].

Thus, there is overwhelming evidence that ANCA play a pathogenic role. Meanwhile, the triggers of ANCA are largely unknown. A minority of patients have AAV triggered by certain drugs that have been implicated in the induction of autoantibodies. These include hydralazine, propylthiouracil, minocycline and the combination of cocaine and levamisole. Nevertheless, somehow, something stimulates B cells with specificity for proteinase 3 or MPO to replicate and differentiate into plasma cells, which in turn produce ANCA, which can then lead to vasculitis.

Rituximab

B cells reside in lymphoid tissue and in the circulation. In response to specific antigens, B cells activate, replicate and differentiate into plasma cells. Activated B cells and plasma cells produce antibodies. Rituximab targets the CD20 antigen on the surface of B cells and clears circulating B cells from the circulation. While rituximab clears the B cells, it does not affect plasma cells, nor does it clear antibodies. After a single course of rituximab there is no change in the serum levels of immunoglobulin [14,15]. Nevertheless, with time, immunoglobulin (Ig)M levels fall [16], and with repeated dosing of rituximab IgG levels also fall [17].

While rituximab's effect on existing IgG levels is slow, it does suppress humoral responses [14]. In patients on treatment for rheumatoid arthritis, humoral responses to influenza vaccination and multivalent pneumococcal vaccination were impaired after rituximab [15,18]. Response to influenza vaccine was restored modestly after 6–10 months [18].

Even though B cells in the circulation are cleared rapidly, B cells in lymphoid tissue are not eliminated, and memory B cells may again respond to exposure to antigens as the rituxan effect wears off [15]. Similarly, recurrence of rheumatoid arthritis after rituximab occurs in conjunction with the return of circulating memory B cells [19].

Thus, while rituximab has little immediate effect on established antibody levels it blocks new or recurrent antibody responses temporarily, but its effect wears off in approximately 6–9 months with the return of responsive B cells [19].

The immunosuppressive effect of rituximab may also change the risk of infection. Astoundingly, studies of rituximab have shown minimal effect on infection rates from the addition of rituximab to other immunosuppression or in comparison to alternative immunosuppression in rheumatoid arthritis [20]. One of the known complications of rituximab is late-onset neutropenia. While the neutropenia is responsive to granulocyte monocyte colony-stimulating factor, it poses a high risk of sepsis when unrecognized. Reactivation of hepatitis B can occur, hence screening is essential prior to rituximab. Lastly, reports of JC virus infection with progressive multi-focal leucoencephalopathy have appeared with rituximab. The rates are very low, and the disease has not appeared in any of the randomized trials of rituximab for autoimmune disease. A report in solid organ transplant immunosuppression suggests that B cell suppression alone does not foster JC virus replication, but it takes combined T and B cell suppression to create an environment for JC virus to propagate [21].

Rituximab for induction therapy for AAV

Our understanding of the mechanisms of AAV suggest a treatment strategy that blocks the effect of ANCA on neutrophils, removes ANCA from the circulation, clears ANCA-specific B cells and plasma cells from the body and prevents repopulation of circulating ANCA-specific B cells. Our understanding of rituximab suggests that, by itself, rituximab can accomplish part, but not all, of this goal. Rituximab does not interrupt the effect of ANCA on neutrophils. It does not directly affect the circulating ANCA. It also does not directly affect the plasma cells, which are the main cells making ANCA. Lastly, it does not eliminate tissue-bound memory B cells which can ultimately replace the circulating B cell. Thus, how well can it work? What are the synergies of rituximab with other immunosuppressive treatments? Should it be used along with plasma exchange, pulse steroids, pulse cyclophosphamide or other biological agents? How much should be used, and how often should it be given? Furthermore, the question is not simply whether rituximab can work in the short term, but can we integrate rituximab

into our therapy for some or all of our patients with AAV in a way that provides better disease control and fewer side effects over a longer period of time?

Circumstances of the individual patient may affect our choices of therapy. Patients who present with immediately life-threatening disease of the lung and kidney need a very rapid induction of remission to preserve renal function and avoid respiratory failure. Patients with diabetes or severe bone disease may warrant an approach that minimizes steroid use. Young women who wish, some day, to become pregnant may warrant different immunosuppression than men, or women who do not desire to become pregnant. Elderly patients may have different considerations for the long-term toxicities of therapy than young patients.

Two recent prospective randomized controlled trials have shown an ability of rituxan to induce remission of ANCA-AAV when used in conjunction with steroids (Stone *et al.*; RAVE trial [22]) or steroids with a short course of cyclophosphamide (Jones *et al.*; RITUXVAS trial [23]). Both studies used a cyclophosphamide and steroid control arm. In the RAVE trial, one patient died in each arm. Sixty-four per cent of the patients achieved complete remission, off all treatment at 6 months, compared to 53% in the cyclophosphamide control arm. Another 22% of the rituximab patients required additional low-dose steroids only. The remaining 15% had one or more events that led to withdrawal from the treatment arm. Most of these were due to the need for additional immunosuppression. In RITXVAS, 18% of the patients in the rituximab arm died *versus* 9% in the control arm (difference not statistically significant). Among survivors, 93% of the rituximab patients achieved a sustained remission *versus* 90% in the cyclophosphamide control arm. In both studies, disease control was achieved as rapidly as with the standard cyclophosphamide therapy arm. Furthermore, in both studies, ANCA titres frequently became negative.

Treatment-related and disease-related adverse effects were similar in both arms in both studies. In RAVE, one patient had an infusion reaction leading to discontinuation of rituximab. Leukopenia was less frequent than in the control arm. An equal number of serious infections occurred in the two arms. In RITUXVAS, severe adverse events (1·0 per patient-year *versus* 1·1 per patient-year) and infection rates (0·66 per patient-year *versus* 0·60 per patient-year) were similar in the rituximab *versus* control arms, respectively. Other studies also suggest that the safety profile of rituximab for autoimmune disease rates favourably [24].

In the RAVE trial, we do not yet know how many of those who entered remission have maintained their remission after 6 months. In RITUXVAS, 15% of patients who attained sustained remission subsequently relapsed prior to 1 year. Other reports suggest that relapse rates are high, and that additional treatment will be needed in many, if not most, of these patients [25,26]. Thus, an appropriate transition from induction therapy to maintenance therapy appears necessary. Maintenance therapy with rituximab has appeared promising. Options include continuous B cell depletion with scheduled rituximab dosing [27], or awaiting the return of B cells or a rise in ANCA prior to repeat dosing [26].

Conclusion

Rituximab is a potent tool that can interrupt B cell-mediated immunity without major compromise of T cell-mediated immunity. Thus, it has great appeal as a tool to interrupt antibody-mediated autoimmune disease. The results of two prospective randomized trials confirm that rituximab can be effective as part of induction therapy for active AAV. The safety profile for rituximab appears favourable relative to cyclophosphamide and steroids. However, there remain many patients who require individualized adjustments of ancillary therapy, as relapses and infectious complications do occur. Furthermore, a strategy for conversion to maintenance therapy is also needed.

Based on our current knowledge, I believe rituximab can and should be used as part of induction therapy in many patients with AAV. However, more work is needed to determine how to best incorporate rituximab into the overall care of these patients.

Disclosure

None.

References

1 Fauci AS, Wolff SM. Wegener's granulomatosis: studies in eighteen patients and a review of the literature. Medicine (Balt) 1973; **52**:535–61.

2 Choi HK, Liu S, Merkel PA, Colditz GA, Niles JL. Diagnostic performance of antineutrophil cytoplasmic antibody tests for idiopathic vasculitides: metaanalysis with a focus on antimyeloperoxidase antibodies. J Rheumatol 2001; **28**:1584–90.

3 Han WK, Choi HK, Roth RM, McCluskey RT, Niles JL. Serial ANCA titers: useful tool for prevention of relapses in ANCA-associated vasculitis. Kidney Int 2003; **63**:1079–85.

4 Tervaert JW, van der Woude FJ, Fauci AS *et al.* Association between active Wegener's granulomatosis and anticytoplasmic antibodies. Arch Intern Med 1989; **149**:2461–5.

5 Jayne DR, Gaskin G, Rasmussen N *et al.* Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. J Am Soc Nephrol 2007; **18**:2180–8.

6 Falk RJ, Terrell RS, Charles LA, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals *in vitro*. Proc Natl Acad Sci USA 1990; **87**:4115–9.

7 Rarok AA, Limburg PC, Kallenberg CG. Neutrophil-activating potential of antineutrophil cytoplasm autoantibodies. J Leukoc Biol 2003; **74**:3–15.

8 Radford DJ, Savage CO, Nash GB. Treatment of rolling neutrophils with antineutrophil cytoplasmic antibodies causes conversion to firm integrin-mediated adhesion. Arthritis Rheum 2000; **43**:1337–45.
9 Schreiber A, Xiao H, Jennette JC, Schneider W, Luft FC, Kettritz R. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. J Am Soc Nephrol 2009; **20**:289–98.
10 Kallenberg CG. Pathophysiology of ANCA-associated small vessel vasculitis. Curr Rheumatol Rep 2010; **12**:399–405.
11 Xiao H, Heeringa P, Hu P *et al.* Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. J Clin Invest 2002; **110**:955–63.
12 Schreiber A, Xiao H, Falk RJ, Jennette JC. Bone marrow-derived cells are sufficient and necessary targets to mediate glomerulonephritis and vasculitis induced by anti-myeloperoxidase antibodies. J Am Soc Nephrol 2006; **17**:3355–64.
13 Xiao H, Heeringa P, Liu Z *et al.* The role of neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibodies. Am J Pathol 2005; **167**:39–45.
14 van der Kolk LE, Baars JW, Prins MH, van Oers MH. Rituximab treatment results in impaired secondary humoral immune responsiveness. Blood 2002; **100**:2257–9.
15 Bingham CO III, Looney RJ, Deodhar A *et al.* Immunization responses in rheumatoid arthritis patients treated with rituximab: results from a controlled clinical trial. Arthritis Rheum 2010; **62**:64–74.
16 Keystone E, Fleischmann R, Emery P *et al.* Safety and efficacy of additional courses of rituximab in patients with active rheumatoid arthritis: an open-label extension analysis. Arthritis Rheum 2007; **56**:3896–908.
17 Popa C, Leandro MJ, Cambridge G, Edwards JC. Repeated B lymphocyte depletion with rituximab in rheumatoid arthritis over 7 yrs. Rheumatology (Oxf) 2007; **46**:626–30.
18 van Assen S, Holvast A, Benne CA *et al.* Humoral responses after influenza vaccination are severely reduced in patients with rheumatoid arthritis treated with rituximab. Arthritis Rheum 2010; **62**:75–81.
19 Leandro MJ, Cambridge G, Ehrenstein MR, Edwards JC. Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. Arthritis Rheum 2006; **54**:613–20.
20 van Vollenhoven RF, Emery P, Bingham CO III *et al.* Longterm safety of patients receiving rituximab in rheumatoid arthritis clinical trials. J Rheumatol 2010; **37**:558–67.
21 Kamar N, Mengelle C, Rostaing L. Incidence of JC-virus replication after rituximab therapy in solid-organ transplant patients. Am J Transplant 2009; **9**:244–5.
22 Stone JH, Merkel PA, Spiera R *et al.* Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med 2010; **363**:221–32.
23 Jones RB, Tervaert JW, Hauser T *et al.* Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. N Engl J Med 2010; **363**:211–20.
24 Fleischmann RM. Safety of biologic therapy in rheumatoid arthritis and other autoimmune diseases: focus on rituximab. Semin Arthritis Rheum 2009; **38**:265–80.
25 Jones RB, Ferraro AJ, Chaudhry AN *et al.* A multicenter survey of rituximab therapy for refractory antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 2009; **60**:2156–68.
26 Keogh KA, Ytterberg SR, Fervenza FC, Carlson KA, Schroeder DR, Specks U. Rituximab for refractory Wegener's granulomatosis: report of a prospective, open-label pilot trial. Am J Respir Crit Care Med 2006; **173**:180–7.
27 Rhee EP, Laliberte KA, Niles JL. Rituximab as maintenance therapy for anti-neutrophil cytoplasmic antibody-associated vasculitis. Clin J Am Soc Nephrol 2010; **5**:1394–400.

