Review

Targeting B cells for the treatment of rheumatoid arthritis

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Received: 30 Jul 2003 Revisions requested: 4 Aug 2003 Revisions received: 15 Aug 2003 Accepted: 19 Aug 2003 Published: 2 Oct 2003

Arthritis Res Ther 2003, **5(Suppl 4)**:S7-S11 (DOI 10.1186/ar1006)

Abstract

The role of B cells in rheumatoid arthritis (RA) has been debated for decades. However, recent clinical trial data indicating that depletion of B cells in RA patients is of therapeutic benefit has validated the importance of this cell type in the pathogenesis of the disease. Elucidation of the molecular basis of B cell development and activation has allowed the identification of a number of possible therapeutic targets that are appealing for drug development. This review discusses briefly a number of these molecules and the rationale for targeting them for the treatment of RA.

Keywords: autoimmunity, arthritis, B cells

Introduction

Rheumatoid arthritis (RA) is a complex disease of unknown etiology. Decades of research into the pathogenesis of the disease have elucidated a number of the key pathways involved in the generation of the disease state. From this research it is clear that many cell populations are involved in disease pathogenesis. These include B cells, T cells, dendritic cells, macrophages, monocytes, and fibroblasts. The exact contribution of each of these cell types in RA is unclear but it is likely that the resultant disease is due to significant interplay among these cell populations [1]. The observation in clinical trials that depletion of B cells from RA patients results in a significant therapeutic effect suggests that B cells play an important role in disease pathogenesis [2,3].

The observation that B cell depletion in RA patients has been efficacious in initial clinical trials suggests that other B cell targeted therapies may also be of benefit in RA. Molecular dissection of the pathways that regulate B cell development and function has identified many possible avenues, apart from B cell depletion, for modulating B cell function in RA patients. These include strategies that are aimed at inhibiting B cell signaling and/or B cell trafficking. Although we briefly touch on the state of B cell depletion techniques, the main thrust of this article is to discuss some of the more prominent targets that allow modulation of the B cell response.

B cell depletion

The technology to deplete B cells in RA patients is already clinically validated. The ability to deplete B cells selectively in RA patients was made possible through the development of rituximab. Known commercially as MabThera®/Rituxan® (Roche Pharmaceuticals, Basel, Switzerland; Genentech, South San Francisco, USA; IDEC Pharmaceuticals, San Diego, USA) and marketed globally for the treatment of malignant B cell lymphoma, rituximab is a chimeric human/mouse monoclonal antibody that targets the CD20 molecule found on the surface of B cells [4]. The CD20 molecule is a 32 kDa nonglycosylated phosphoprotein that is present on B cells at all stages of development before plasma cell differentiation. CD20 is not found on other cell types, including stem cells [5]. Rituximab binds to the CD20 molecule on the surface of B cells and facilitates the depletion of B cells from patients largely by invoking host effector mechanisms [6,7]. Initial clinical trials in RA patients indicated that circulating B cells are undetectable after a brief dosing regimen with rituximab [2]. The treatment is well tolerated, and develop-

BAFF = B cell activating factor belonging to the TNF superfamily; BCR = B cell antigen receptor; Btk = Bruton's tyrosine kinase; BLC = B lymphocyte chemokine; CXCR = CXC receptor; RA = rheumatoid arthritis; *xid* = X-linked immunodeficiency.

ment of antibody responses against the rituximab molecule is low [2]. Rituximab treatment in RA is discussed at length elsewhere in this supplement.

Although rituximab is highly effective at depleting B cells, other reagents for the depletion of B cells are currently under development. Some of these agents could be applicable to indications outside oncology. Among these drugs in development are other antibodies that target the CD20 molecule. One example of these is Humax-CD20 (currently under development; Genmab, Copenhagen, Denmark). This molecule differs from rituximab in that it is a fully human monoclonal antibody produced in transgenic mice, in which the mouse genes for creating antibodies have been inactivated and replaced by human antibody genes [8].

The CD19 molecule represents another attractive target for future B cell depletion reagents. Its expression is restricted to B cells and follicular dendritic cells. CD19, like CD20, is present at all stages of B cell development up until differentiation to plasma cells [9]. A molecule is currently being developed that targets both CD19 and CD3, found on B cells and T cells, respectively. This recombinant bispecific antibody (bscCD19 × CD3) is composed of two single chain antibodies each against the individual target and is aimed at inducing T cell mediated depletion of B cells [10]. Initial cell culture experiments with the antibody indicate that the molecule is capable of inducing T cell mediated killing of normal peripheral B cells [11].

Blocking B cell activation

The molecular signaling pathways involved in the activation of B cells are rapidly becoming elucidated [12]. From this work a number of potential targets for modulating B cell function have been identified. Some of these molecules and the rationale for targeting them in RA or other autoimmune disorders are discussed below.

Bruton's tyrosine kinase

Bruton's tyrosine kinase (Btk) plays a key role in B cell development and activation [13]. The maturation and response of B cells to antigen is regulated by the cell surface complex called the B cell antigen receptor (BCR) [14]. The interaction of antigens with the BCR activates signaling pathways through nonreceptor protein tyrosine kinases, including the Tec family member Btk [12]. The activation of these signaling pathways is required for proper development and function of B cells, and Btk plays a central role in this [15].

The importance of Btk in the activation process is demonstrated most clearly by mutations that inactivate the kinase function of the molecule. Loss of function mutations in Btk affect B cell development and B cell activation in response to antigen [16]. In humans this type of mutation results in the disease X-linked agammaglobulinemia [16,17]. This disorder is characterized by the absence of mature B cells in the periphery and a serious deficiency of serum antibodies [18]. In mice, Btk inactivation results in a disorder called X-linked immunodeficiency (*xid*) [19,20]. The murine disorder results in an approximately 50% reduction in the number of mature B cells. In addition, the *xid* B cells present are functionally abnormal in that BCR signaling is severely compromised. Further genetic experiments have shown that partial replacement of Btk activity (25%) in *xid* animals is sufficient to normalize B cell development but not BCR signaling [21]. This result strongly suggests that the degree of Btk function is critically important in allowing effective B cell activation in response to antigen. That modulation of Btk function can allow normal development of B cells but not be sufficient for B cell activation leads to the compelling hypothesis that a complete block in Btk protein kinase function is not required to impair B cell signaling, and a window may exist between halting B cell development and reducing B cell signaling. These data suggest that targeting the kinase function of Btk could result in desensitization of B cell signaling and possibly provide a therapeutic effect in autoimmune disorders, including RA.

CD19

CD19 is a 95 kDa member of the immunoglobulin superfamily expressed on B cells during most stages of development until plasma cell differentiation. CD19 is part of a multimolecular complex called the B cell coreceptor [9]. CD19 regulates the intrinsic B lymphocyte signaling thresholds [22]. CD19 functions to mediate the amplification of Src family protein kinase activity involved in BCR signaling and plays a crucial role in the pathways that regulate intrinsic and antigen receptor induced signals [23]. CD19 mediates this signal modulation through a complex series of phosphorylation events, referred to as progressive amplification [24]. Experiments addressing the function of CD19 *in vivo* suggest that therapies that inhibit CD19 function might be therapeutic in RA and other autoimmune disorders. Specifically, an elegant series of genetic and molecular experiments have shown that the level of functional CD19 is critical for B cell activation. Using genetically modified mice, it was demonstrated that mice that are deficient for CD19 are hyporesponsive to BCR signaling [25]. In contrast, animals that over-express CD19 develop spontaneous autoimmunity [26]. This finding is even more interesting in light of the fact that patients with certain autoimmune disorders exhibit an increase in CD19 levels comparable with that required to induce autoimmunity in mice [27]. These findings suggest that therapies that inhibit CD19 function may be useful in the treatment of autoimmune disorders.

B cell activating factor

The B cell activating factor belonging to the TNF superfamily (BAFF; BlyS, TALL-1, THANK) molecule is a homotrimer of the tumor necrosis factor superfamily that is

found either on the cell surface as a type II transmembrane protein or as a soluble molecule [28,29]. BAFF is produced by macrophages, monocytes and dendritic cells, and plays a key role in B cell survival, maturation and activation through its interaction with its receptors TACI, BAFF-R and BCMA [30,31]. Several lines of evidence suggest that BAFF plays a role in the induction of autoimmunity. Transgenic mice engineered to over-express BAFF break tolerance and develop autoantibodies, including anti-DNA antibodies and rheumatoid factor [32,33]. This autoimmune response results in the development of a systemic lupus-like condition. Furthermore, increased levels of BAFF have been detected in the sera of patients with various autoimmune diseases including systemic lupus erythematosus and RA, further implicating this molecule in the pathology of the disease [34,35].

The mechanism through which BAFF appears to mediate B cell activation is qualitatively different from that observed with CD19 and Btk. In contrast to Btk and CD19, BAFF does not function to potentiate the activation of B cells directly through enhancement of BCR signaling. Instead, the proliferation signal that is mediated by BAFF results in more cells surviving to enter the cell cycle, rather than the actual costimulation of B cells [36]. The prevailing hypothesis is that pathologic concentrations of BAFF can overcome the negative selection of autoreactive B cells in the periphery. These autoreactive B cells then survive and subsequently produce self-reactive antibodies. Based on this hypothesis, reagents that reduce the effective concentration of BAFF either through interaction with BAFF itself or one or more of its receptors could be expected to be of clinical benefit in the treatment of RA and other autoimmune disorders.

Blocking B cell trafficking

The trafficking of B cells is critical for proper B cell development and activation. The molecules responsible for B cell trafficking are called chemokines [37]. This large family of molecules promotes the trafficking of different cell types via concentration gradient attraction [38]. The chemokine system has been shown to play a key role in mediating inflammatory cell infiltration. Several B cell associated chemokines have been described. These molecules function to promote B cell maturation by directing B cell localization to the germinal centers as well as the localization of B cells to sites of inflammation [39]. An ability to inhibit the function of chemokines that are involved in B cell localization could allow downregulation of both the local and systemic immune responses. The most well characterized B cell chemokine system is B lymphocyte chemokine (BLC) and its receptor CXC receptor (CXCR)5.

B lymphocyte chemokine/CXC receptor 5

The chemokine BLC, also known as BCA-1 and CXCL13, is constitutively produced by stromal cells in lymphoid tissue and serves as a homing signal that attracts B cells and memory T cells [39]. BLC is an 88 amino acid member of the CXC chemokine family that interacts with its receptor CXCR5. The CXCR5 receptor is found on all mature B lymphocytes as well as on a subpopulation of memory T cells [40]. Transgenic mice that have been rendered deficient for either BLC or CXCR5 exhibit several characteristics that demonstrate the important role of the BLC/CXCR5 system in the trafficking of B cells *in vivo* [41,42]. These mice lack most inguinal lymph nodes and Peyer's patches. Furthermore, the normal organizational architecture observed in the spleen is disrupted in these animals. The fact that mice deficient for the BLC signaling system fail to organize lymphoid tissue compartments correctly has implications for the importance of the target in RA pathogenesis. Analysis of tissues harvested from active RA joints indicated that tissue infiltrating lymphocytes can be found to be arranged in sophisticated microorganizations identified as germinal centers, which are normally restricted to secondary lymphoid organs [43–49]. Examination of the expression of BLC in the synovium of RA patients suggest that the chemokine is upregulated in these tissues and the levels of expression correlate with the level of ectopic germinal center formation and B cell aggregation [43]. These data strongly implicate aberrant production of BLC in the formation of these structures.

The expression of BLC and CXCR5 in germinal centers within RA synovium suggest that therapies that effectively target the functioning of this B cell homing system could significantly impact on the progression of the disease by inhibiting the formation of autoantibody producing structures within the joint. The effect of eliminating B cells from the lymphoid structures was addressed through the use of human synovium/severe combined immunodeficient mouse chimeras [48]. In these experiments, treatment with rituximab demonstrated that the presence of B cells is required for germinal center formation and T cell activation. Taken together, these results suggest that therapies that prevent B cell trafficking and the subsequent organization of B cells into lymphoid structures may act to inhibit the pathology observed in RA joints.

Conclusion

Data from clinical trials indicating that B cell depletion with rituximab is highly therapeutic, and well tolerated, in RA patients have validated the contribution of B cells in the pathogenesis of RA [2]. These observations will probably lead to more therapies aimed at the modulation of B cell responses in patients. Elucidation of the pathways that regulate B cell development and activation has identified a number of molecules that are attractive from a drug discovery perspective. These include molecules involved in B cell activation, survival and trafficking, as well as targets other than CD20 for B cell depletion. In this review we

discuss some of the most obvious of these potential targets. The question of whether modulation of these targets will be of therapeutic benefit in RA remains to be determined clinically.

Competing interests

TJO is currently an employee of Roche Pharmaceuticals. SAD is a former employee of Roche Pharmaceuticals.

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