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CD20: A target antigen for immunotherapy of autoimmune diseases

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Abstract

This article reviews the role of CD20 antigen in B cell function and the effectiveness and limits of passive immunotherapy with anti-CD20 monoclonal antibody (Rituximab) in the treatment of autoimmune (or immune-mediated) diseases. Active immunotherapy is a more feasible way to control these chronic diseases. A peptide that mimics the CD20 epitope recognized by Rituximab is employed to stimulate the host immune response against CD20.

Keywords: Autoimmunity; CD20; Immunotherapy; Mimotope; Peptide; Rituximab; Vaccine

Contents

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1. Introduction

Most systemic autoimmune diseases (AD) are still associated with high morbidity and mortality despite the use of a wide range of drugs that control their symptoms, delay progression and/or improve the quality of life, but never bring about a complete cure. This failure has aroused an interest in new forms of experimental immunotherapy (IT). Treatments employing monoclonal antibodies (mAb) have made the most significant progress because: 1) they are based on consolidated experimental evidence, since the hybridoma technology has been in use for more than 25 years [1]; 2) advances in molecular biology have led to the identification of new IT target molecules; 3) biotechnology has produced modified (chimeric or humanized) mAbs with a higher therapeutic index than their mouse counterparts. The FDA's recent approval of the chimeric anti-TNF-α mAb Infliximab for the treatment of rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis and Crohn's disease is a further illustration of the validity of this approach.

Rituximab, a mAb to the anti-B-cell-associated-antigen CD20, is another valuable chimeric mAb. Approved by the FDA for the treatment of lymphomas, it has also attracted the interest of clinical immunologists because of the promising results it has achieved in the treatment of several AD in open non-controlled trials and one double-blind controlled clinical trials.

This paper reviews the extent to which B cell function is influenced by CD20-targeting by Rituximab and the latter's usefulness in the passive immunotherapy of AD. The rationale for developing peptides that mimic CD20 (mimotopes) for use in vaccine therapy is also discussed, together with the results of preliminary studies designed to identify them and determine their function.

2. CD20 antigen as target of experimental immunotherapy

2.1. Functional role of CD20 and passive immunotherapy with Rituximab

CD20 antigen is a 33–35 kDa phosphoprotein expressed on B lymphocytes from the early pre-B to the late B stage, though its expression ceases when they differentiate into plasma cells. CD20 sequence analysis predicts a four-transmembrane domain with intracellular termini and only one extracellular 44-amino-acid loop (from 142 to 182), which is the contact site of all the current anti-CD20 mAbs, including Rituximab.

CD20's natural ligand has not been defined. Studies using specific mAbs, however, have shown that this Ag is involved in B cell activation and proliferation by triggering tyrosine kinase intracellular signals [2,3] and regulating intracellular calcium [4,5]. CD20 engagement by the corresponding mAb, in fact, blocks these functions and induces apoptosis [6]. Furthermore, due to the lack of CD20 down-regulation upon mAb binding, antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity [7] are seen as additional mechanisms that partly explain Rituximab-induced B cells ablation in vivo [8].

These data, as well as the demonstration of CD20 on autoreactive B cells and recent indications that B cells have a broader pathogenetic role in the maintenance of autoreactive T cell activation [9–13], have led to the use of Rituximab for the treatment of AD.

Rituximab has indeed proved effective in the treatment of rheumatoid arthritis [14], systemic lupus erythematosus [15–18], dermatomyositis [19], refractory pemphigus vulgaris [20,21], severe autoimmune hemolytic anemia [22,23], refractory immune thrombocytopenic purpura [24–26], acquired hemophilia [27], Wegener's granulomatosis [28], and mixed cryoglobulinemia [29] in non-controlled clinical trials (Table 1), as well as recent double-blind controlled clinical trial [30]. In this trial, 161 rheumatoid arthritis were enrolled in four arms: 40 continued to receive methotrexate (>10 mg/week) (M group); 40 patients were given Rituximab (1000 mg on day 1 and 15) (R group); 41 patients received Rituximab plus 750 mg cyclophosphamide (on days 3 and 17) (RC group); 40 received Rituximab plus methotrexate (>10 mg/week) (RM group). At week 24, the number of patients achieving 50% symptom improvement was significantly higher in the RM group than in the RC and M groups, and all the ACR (American College of Rheumatology response criteria) responses were maintained at week 48 in this group. The most reassuring aspect of this trial was the low incidence of

Table 1
Case reports, open non-controlled clinical trials (ONC-CT) and double-blind controlled clinical trials (DBC-CT) with monoclonal antibody anti-CD20 (Rituximab) in autoimmune diseases

Disease	References	I.V. dose[a]/infusion no.	Type of trials/ (no. of patients)	Therapeutic response/ side-effects
Rheumatoid arthritis	[30]	1000 mg/2, day 1 and 15	DBC-CT/ (161)	Excellent/ slight
Systemic lupus erythematosus	[15]	500 mg/2, weekly	ONC-CT/ (6)	Good/ absent
	[16]	375 mg m^2/4, weekly	Case reports/(2)	ND
	[17]	100–375 mg m^2/1 or 4, weekly	ONC-CT/(18)	Good/ absent
Dermatomyositis	[19]	375 mg m^2/4, weekly	ONC-CT/(7)	Good/slight
Pemphigus vulgaris	[21]	375 mg m^2/4, weekly	Case reports/(3)	ND
Severe autoimmune hemolytic anemia	[22]	375 mg m^2/3, weekly	ONC-CT/(15)	Good/ absent
	[23]	375mg m^2/6, weekly	Case reports/(1)	ND
Refractory ITP[b]	[24–26]	375mg m^2/4, weekly	ONC-CT/ (25), (12), (57)	Good/ absent
Acquired hemophilia	[27]	375 mg m^2/4, weekly	ONC-CT/ (10)	Good/ slight
ANCA-associated vasculitis[c]	[28,31]	375 mg m^2/4, weekly	ONC-CT/ (1), (11)	Good/ absent
Mixed cryoglobulinemia	[29]	375 mg m^2/4, weekly	ONC-CT/ (20)	Good/ absent
Cold agglutinin disease	[32]	375 mg m^2/4, weekly	ONC-CT/ (27)	Slight/ absent

[a] I.V.: intravenous.
[b] ITP: Idiopathic thrombocytopenic purpura.
[c] ANCA: anti-neutrophil cytoplasmic antibodies.

clinical signs of immune depression despite marked B cell depletion over the course of 24 weeks. These signs included pneumonia, septic arthritis and septicemia owing to *Staphylococcus aureus* infection. Their incidence, however, was only slightly higher in the RC, RM and R groups than in the M group.

Assessment of the overall success of passive immunotherapy (PIT) is hampered by the fact that patients have been follow for short periods compared to the duration of rheumatoid arthritis, especially since it can only be controlled by constant treatment with mAb with an increasing risk of toxicity or anaphylactic reactions. In addition, the production of antibodies to the variable region of a mAb can abrogate its effectiveness (tachyphylaxis), even when a chimeric or humanized form of an xenogenic mAb is used [33].

2.2. Active immunotherapy

An attractive alternative or complement to PIT is active immunotherapy (AIT), namely stimulation of the patient's immune system to develop a response against PIT's target Ag. AIT is indeed a major challenge, since it is presumed to be a more feasible way to control or even cure AD.

AIT, however, was initially restricted both by the practical difficulty of obtaining purified antigens and the tolerance developed by the immune system towards most molecules regarded as targets in AD. Furthermore, the risk of immune depression that might prove more difficult to control than that occasionally associated with PIT has been an additional qualm in the promotion of suitable trials. Surrogates of the original antigens (mimotopes), namely anti-idiotypic mAbs or peptides, have therefore been produced. Vaccination protocols, in fact, have employed mimotopes that mimic Ags, such as T cell receptor [34], HLA-DR1/4 [35] and CD4 [36].

In the "CD20 system", a conjugate mimotope of the CD20 extracellular domain recognized by Rituximab would induce an immune response and be expected to generate biological effects similar to those that follow the passive administration of Rituximab, with the additional advantage that the polyclonal response would be more effective at recruiting effector cells [37].

Roberts et al. [38] showed that tolerance to CD20 can be broken by a CD20-derived KLH-conjugated 40-mer peptide corresponding to the extracellular domain of mouse and human CD20 (amino acids 142–182). However, the abnormal length of this peptide meant that it was likely to assume a three-dimensional conformation different from that of the naïve protein and result in the expression of novel epitopes. Sera from immunized mice, in fact, weakly bound naïve CD20, despite their high reactivity against the

immunizing peptides. Similar considerations apply to the results obtained in mice by Huang et al. [39], who used a protein containing the all extracellular domain of mCD20 fused to a foreign IgG Fc fragment.

3. Identification and characterization of CD20 mimotopes

The phage-display random peptide library (PDPL) has quite recently emerged as a powerful technique for isolation of mimics of antigens successfully utilized as PIT targets. We have used this approach to define mimotope(s) of the CD20 epitope recognized by Rituximab. Biopanning of either a phage-displayed random cystein (c)-constrained-heptapeptide library (c7c PDPL), or a linear dodecapeptide library (12-mer PDPL) with Rituximab resulted in the isolation of specific phage clones. Affinity selection and immunoscreening were performed according to previously described procedures [40]. The deduced amino acid sequences of their insert have been used to synthesize cyclic and linear peptides that specifically recognize Rituximab and inhibit its binding to $CD20^+$ cells.

We have used the linear peptide Rp10-L to immunize mice and determine whether they develop antibodies reacting with $CD20^+$ cells. Sera from two BALB/c mice immunized with Rp10-L specifically reacted with $CD20^+$ Raji cells (representative results are shown in the Fig. 1), whereas they failed to react with $CD20^-$ CEM cells. Furthermore, anti-Rp10-L

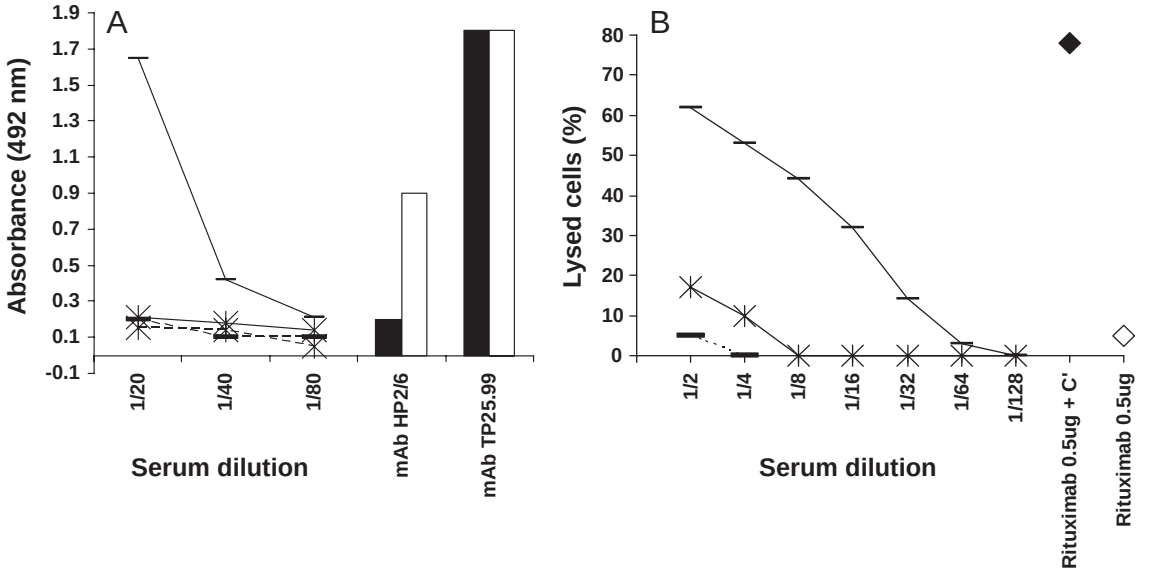

Fig. 1. Binding (A) and cytotoxic effect (B) displayed by Rp10-L immunized sera on CD20+Raji cells. (A) Fifty microliters of a two-fold dilution of sera drawn on day 28 from BALB/c mice immunized with Rp10-L (-) and BSA (*) were incubated with Raji human B lymphoid cells (continuous line) and CEM human T cells (dashed line) (5×10^5 cells/well) for 2 h at 4 °C. Cells were then washed twice with PBS and incubated with 50 μl of an appropriate dilution of HRP-conjugated xeno-antisera to mouse IgG (Fc portion). Following 90-min incubation at 4 °C and three washings with PBS, serum reactivity with cells was detected by addition of OPD-solution. Background binding was determined by absorbance generated in wells incubated with plain PBS. Bindings of the anti-HLA class I mAb TP25.99 and of anti-CD4 mAb HP2/6 to Raji (closed bar) and CEM (open bar) were included as specificity controls. (B) Ten microliters of a two-fold dilution of complement-inactivated sera drawn on day 28 from Balb/c mice immunized with Rp10-L (-) and BSA (*)(negative control) were added to wells of a round-bottom 96-well plate (Corning Costar) containing Raji cells (1×10^4/well in 10 μl of complete medium). After 30-min incubation at 4 °C, 50 μl of complete medium containing an appropriate dilution of rabbit complement (BAG, Germany) were added to the mixture and incubation was prolonged for 1 h at 25 °C. Cells were assessed for viability by trypan blue exclusion and counted with a hematocytometer. Lysis was calculated according to the following formula: 100 × (% viable cells with Ab in the absence of complement − % viable cells with Ab in the presence of complement)/(% viable cells with Ab in the absence of complement). Lysis obtained by incubation of immune sera in the absence of complement (dashed line), of Rituximab (500 ng) in the presence (♦) and in the absence (◇) of complement were included as specificity controls.

sera were cytotoxic if incubated with Raji cells in the presence of complement, while no cytotoxicity was detected in the absence of complement or when Raji cells were incubated with sera from mice immunized with BSA. The results indicate that Rp10-L elicited B-cell-specific cytotoxic antibodies. Further experiments are obviously required to demonstrate that the target Ag of these cytotoxic antibodies is CD20 and that they induce biological effects similar to those of Rituximab. If this is shown, they could be used to target CD20 in an AIT setting.

4. Conclusions

This review emphasizes the encouraging results already obtained and those potentially obtainable with CD20-targeting-based experimental IT in the treatment of AD. Subject to the provision that a better understanding of the pathogenetic mechanisms of AD, or those that determine their course, is the indispensable premise to the attainment of satisfactory therapeutic goals, four final objectives must be pursued: i) reduction of the allergizing and/or toxic effects to tolerability levels; ii) definition of the disease stage during which one form of management may be more appropriate than another; iii) optimization of the treatment protocols; iv) assessment of combined treatments (AIT and/or PIT and/or conventional drugs).

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Take-home messages

- Systemic autoimmune diseases are still associated with high morbidity and mortality despite the administration of a wide range of drugs.
- Passive immunotherapy with monoclonal antibodies (mAb) to selected antigen(s) is another way of controlling disease progression.
- Targeting of CD20 antigen with Rituximab is a promising approach in the treatment of rheumatoid arthritis.
- Active immunotherapy is a more feasible way to control disease by dispensing with chronic mAb administration.
- A peptide that mimics the Rituximab-specific epitope can be used to induce a CD20-specific immune response.

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