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The crystal structure of the pathogenic collagen type II-specific mouse monoclonal antibody CIIC1 Fab: Structure to function analysis

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Abstract

Monoclonal anti-collagen type II antibody CIIC1 is an arthritogenic autoantibody, which induces arthritis in mice. We crystallized and solved the structure of CIIC1 Fab molecule. Analysis of structure revealed an interaction between the CDR regions of one Fab to the CH1 domain of another Fab, which resembles an antibody–antigen interaction. ELISA experiments confirmed the cross-reactivity of both the full CIIC1 antibody and a single chain Fv fragment to other anti-collagen antibodies which are of different isotypes and epitope specificity. The rheumatoid factor like reactivity of CIIC1 antibody together with its collagen type II specificity may explain the pathogenicity of this antibody.

Keywords: Arthritis; Rheumatoid factor; Collagen type II; Autoantibodies

1. Introduction

The relation between the autoantibodies and rheumatoid arthritis (RA) pathogenicity is yet to be clearly ascertained but certain therapies that target B cells, especially B cell depletion therapy shows not only promises for treatment but also highlights the role of B cells and antibodies in the disease development (Edwards and Cambridge, 2001). Besides targets of specific treatments, autoantibodies have turned out to be valuable diagnostic disease markers. Rheumatoid factor (RF) antibody with a reactivity towards the Fc region of IgG antibodies has been used in RA diagnosis since the existence of RF was stated in ACR classification criteria in 1987 for rheumatoid arthritis (Arnett et al., 1988). Although the presence of RF has a remarkable sensitivity (75–85%) it has a low specificity since RF is also detected in other inflammatory diseases (SLE, Sjögren's syndrome, osteomyelitis) and even in some healthy people. It is believed that RFs, by binding to the Fc domain of other IgGs, can form immune complexes in joints and activate complement and FcγR systems, which may finally lead to inflammation. RF appears before the clinical onset of RA and high serum levels are associated with severe arthritis suggesting a role in the pathology of the disease (Vaughan, 1993). In addition to RF, several other autoantibodies have been described. Some of the identified self-antigens are collagen type II (CII), fibrin(ogen), vimentin, fibronectin and (pro)filaggrin. Among these antigens only filaggrin is not expressed in the synovium. In most of the cases, the antibodies directed against these antigens also specifically recognize a posttranslational modification, which is mainly citrullination (Schellekens et al., 1998). It has also been shown that antibodies against glucose-6-phosphate isomerase (G6PI), which is a ubiquitous self-antigen precipitating in the joints, induce arthritis in the K/BxN mouse model (Bockermann et al., 2005; Kouskoff et al., 1996).

Apart from other self-antigens, CII deserves more attention because of its joint specificity. Moreover, CII is the dominant protein constituting 50% of the articular cartilage. CII-specific

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B cells and autoantibodies have been detected in the sera, synovial fluid and synovium of RA patients (Rudolphi et al., 1997). An arthritic disease resembling in many aspects human RA can be provoked in mouse by immunization with type II collagen (CII), this is the so-called collagen-induced arthritis (CIA) (Holmdahl et al., 1990). The histopathology of the affected joints observed in CIA reflects an erosive form of arthritis and CII-specific autoantibodies are detected in mouse serum. In addition, the epitopes are highly conserved across the species (Burkhardt et al., 2002). Monoclonal antibodies specific for these epitopes also trigger arthritis in naïve mice (collagen antibody-induced arthritis, CAIA), which suggests a role for CII-specific antibodies in RA (Nandakumar et al., 2003b) and this antibody-mediated arthritis was enhanced by CII-reactive T cells *in vivo* (Nandakumar et al., 2004). Furthermore, production of antibodies to CII is dependent on the activation of MHC restricted T cells, that are critical to elicit joint-specific immune responses that finally lead to the development of CIA (Holmdahl et al., 1995). In the complex immunopathogenesis of CIA B cells may be important not only for producing pathogenic autoantibodies but also for presenting antigenic peptides to the T cells. Thus, B cell deficient mice are resistant to CIA (Svensson et al., 1998). Conclusively, the antibody-mediated pathway is probably one of the major pathogenic mechanisms in both CIA and RA (Nandakumar and Holmdahl, 2006).

In CIA the antibody response to CII, which precedes the onset of arthritis, mainly targets some immunodominant triple helical CII determinants. These major epitopes share a common motif consisting of R-G-hydrophobic amino acids that is possibly located in a repetitive way on the cartilage or in the CII aggregates, known to occur in an inflamed synovia (Schulte et al., 1998). In this respect the C1 epitope is one of the most frequently recognized CII epitopes in arthritogenic autoantibody response to CII. A B cell hybridoma producing the IgG2a autoantibody CIIC1 that binds the triple helical C1 epitope was originally generated from a CII immunized DBA/1 mouse in the phase of arthritis development (Holmdahl et al., 1986). The CIIC1 antibody induces synovitis and even clinical arthritis if injected in higher doses or together with other anti-CII antibodies with different epitope specificities (Nandakumar et al., 2003a,b; Nandakumar and Holmdahl, 2005). It also impairs cartilage formation by chondrocytes (Amirahmadi et al., 2004), inhibits CII fibril formation in vitro (Gray et al., 2004), caused disorganization of CII fibrils in the ECM (Amirahmadi et al., 2005), had adverse effects on preformed cartilage (Crombie et al., 2005) and induced relapses in chronic arthritic mice (Bajtner et al., 2005). To further investigate the interaction between pathogenic anti-CII autoantibodies and CII, we crystallized and solved the structure of the mouse autoantibody CIIC1 Fab molecule. The contacts between symmetry related Fab fragments were unusual in the structure because all the CDR loops of variable domains have contacts with a region on the constant domain of the symmetry related Fab molecule. These interactions are very similar with observed interactions in antigen and antibody complexes (Davies and Cohen, 1996). Further investigation by using both the full length antibody and a single chain Fv fragment of CIIC1 (C1scFv) in ELISA experiments revealed that the antibody may bind not only to CII but also to other IgG1, IgG2a and IgG2b antibodies, thus behaving as a RF (Sutton et al., 2000).

2. Materials and methods

2.1. Peptide synthesis

Peptide 0207, which contains C1[1] epitope and terminal glycine proline hydroxyproline triplets (GP-HyP-GP-HyP-**GARGLT**-GP-HyP-GP-HyP-GP-HyP) was purchased from peptide synthesis company SHAFER-N Denmark.

2.2. Preparation of CIIC1 Fab

CIIC1 Fab fragments were prepared by using an ImmunoPure Fab preparation kit (PIERCE, Rockford, USA) according to manufacturer's instructions. Collected Fab fragments were concentrated and further purified by gel filtration on a superdex 200 (10/30) column (Amersham Biosciences, Uppsala, SWE). The gel filtration column was previously equilibrated with 30 mM Tris buffer pH 8.0.

2.3. DNA sequencing

RNA was extracted from 10^7 cells of the murine B cell hybridoma C1 by using the RNeasy total RNA isolation kit (Qiagen AB, SWE). Total RNA was reverse transcribed into cDNA by using a first-strand cDNA synthesis kit (Amersham Biosciences, Uppsala, Sweden). DNA fragments that encode heavy and light chain were PCR amplified using primers for heavy chain: 5′-CAGGTCCAACTGCAGCAGCCTGGG-3′, 5′-GCTCAATTTTCTTGTCCACCTT-3′; for light chain: 5′-GACATTGTGATGACACAGTCTCCA-3′, 5′-AAGAGCTTCAACAGGAATGAGTGT-3′.

PCR fragments were cloned into the PCR-4 TOPO (Invitrogen AB, SWE). The DNA sequences of the heavy and light chain including the variable and constant domains were determined by automated sequencing using M13 sequencing primers (Invitrogen AB, SWE) with BigDye sequencing reagent (Applied Biosystems, CA, USA) and ABI 3100 Automated Capillary DNA Sequencer (Applied Biosystems, CA, USA).

2.4. Crystallization

10 mg/ml CIIC1 Fab in 30 mM Tris buffer (pH 8.0) was used for crystallization alone or in the presence of the collagen peptide 0207 using the hanging drop method with a Peg/Ion screen (Hampton Research, CA, USA). For the peptide Fab complex crystallization, collagen peptide 0207 and CIIC1 Fab were mixed in a 5:1 molar ratio and left at 4 °C overnight. The hanging drop experiments contained 2 μl of precipitant solution and 2 μl of protein solution and have been incubated at 20 and 4 °C. Crystals appeared after 3–4 weeks only at 20 °C.

Diffracting quality crystals of CIIC1 Fab alone grew in conditions #1 (0.2 M sodium fluoride, 20% PEG 3350, pH 7.1) and #29 (0.2 M potassium acetate, 20% PEG 3350, pH 7.8).