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Role of idiotype–anti-idiotype interactions in the induction of collagen-induced arthritis in rats

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ABSTRACT

The mechanism of autoantibodies (rheumatoid factor (RF) and anti-collagen autoantibodies) induction in collagen-induced arthritis (CIA) is unknown. The study assessed the hypothesis that activation of autoantibody-producing clones is mediated by idiotype–anti-idiotype (IAI) interactions with lymphocytes on heterologous collagen. It was demonstrated that RF-containing serum of rats immunized with bovine collagen (BCII) in ELISA competes with BCII for binding to anti-BCII antibodies. Immunization of rats with Fc fragments of IgG caused not only an increase in RF levels, but also induction of antibodies to BCII and anti-collagen autoantibodies. Taken together, these facts suggest that activation of RF-producing lymphocytes in CIA model occurs through IAI interactions with anti-BCII lymphocytes.

Three populations of antibodies were detected in the blood of arthritic rats: a population of antibodies reacting only with BCII, a population of antibodies reacting only with rat collagen (RCII) and a population of antibodies that can bind to both bovine and rat collagen. It was shown that RF in relation to anti-collagen autoantibodies act as anti-idiotype antibodies, and a comparative analysis of antibody production in arthritic and resistant rats demonstrated that the level of anti-collagen autoantibody production depends on the level of RF production. This suggests that RF and RF-producing lymphocytes are involved in regulation of anti-collagen autoreactive lymphocyte activity through an IAI interaction mechanism. A direct activation of autoreactive anti-RCII lymphocytes by BCII cannot be excluded, but it can be supposed that induction of anti-collagen autoreactive lymphocytes needs a signal generated in IAI interactions by RF-producing lymphocytes. This hypothesis is based on the data obtained, and not only explains the mechanism of autoreactive lymphocytes activation in the rat CIA model, but also indicates that the key regulatory element in the development of arthritis in animals is RF-producing lymphocytes. The results allow a new insight on the role of RF in the pathogenesis of rheumatoid arthritis and on seeking more effective therapeutic means.

Introduction

An experimental model of collagen-induced arthritis (CIA) in rats has been known for a relatively long time and was accepted as one of the most adequate experimental models of human rheumatoid arthritis. Arthritis in rats is evoked by injections of bovine collagen, inducing autoimmune reaction to collagen and production of rheumatoid factor (RF) (Holmdahl et al., 1989). However, mechanisms of induction of autoreactive lymphocytes, including those producing RF and leading to arthritis, are still unknown. At present mechanisms of induction of autoreactive clones in the CIA model are explained by different hypotheses. The most common is the hypothesis of immune crossover, postulating the similarity of bovine collagen to rat autocollagen as the reason for cross-reaction of autoclones to collagen in response to administering of bovine collagen to rats. However, the immune crossover hypothesis does not explain how RF is induced and how mechanisms of controlling autoreactive clones are surmounted.

At the same time, some facts support the hypothesis that induction of autoreactive clones in the CIA model can occur indirectly through idiotype–anti-idiotype (IAI) interactions. The existence of idiotype–anti-idiotype interactions between antibodies to foreign antigens causing arthritis, and to autoantibodies, is shown in different models. For instance, P.M. Johnson et al. has shown that RF was produced in animals immunized with streptococcal peptidoglycan–polysaccharide complex, and conversely, immunization of mice with purified RF extracted from serum of patients with rheumatoid arthritis led to the production of antibodies to peptidoglycan–polysaccharide polymer of pyogenic streptococci (Johnson and Smalley, 1988). Based on this data, the author suggests that there is idiotypic complementarity

Abbreviations: BCII, bovine collagen type II; RCII, rat collagen type II; CIA, collagen-induced arthritis; CIC, circulating immune complex; IAI, idiotype-anti–idiotype; RF, rheumatoid factor

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between rheumatoid factor and anti-peptidoglycan antibodies. F. Nardella et al. has shown that the major antigenic determinants for rheumatoid factors (RFs) are in the C gamma 2–C gamma 3 interface region of IgG in the same area that binds staphylococcal protein A. Furthermore, the Fc-binding proteins of groups A, C and G streptococci, as well as the Fc-binding proteins induced on cell surfaces by herpes simplex virus type I, also bind to the same area of IgG. These findings indicate that RFs bear the conformational internal image of these microbial proteins and suggest that RFs could appear as antibodies to the idiotypic determinants on antibodies to microbial Fc-binding proteins (Nardella et al., 1988). Moreover, C. Nordling et al. has shown that monoclonal anti-idiotype antibodies C1C3 to monoclonal antibodies, recognizing epitopes of native mouse type II collagen molecule, are rheumatoid factor (Nordling et al., 1991; Holmdahl et al., 1986).

Unlike the molecular mimicry hypothesis, the hypothesis of idiotype–anti-idiotype induction of antibodies provides a number of advantages. For instance, it can explain activation of autoreactive lymphocytes, including RF-producing clones, in response to bovine collagen. Furthermore, taking into account the regulatory nature of IAI interactions, the activation of autoclones mediated by such interactions might be the reason for disruption of mechanisms that control autoreactivity.

The objective of our work was to determine the role of IAI interactions in the induction of autoreactive clones in the rat CIA model.

It is established that activation of lymphocytes through IAI interactions has its own features that are manifested by specific kinetics of antibody production. This feature is the sequential type of activation of idiotypic and anti-idiotypic lymphocyte clones with reciprocal changes in the level of antibodies they produce (Paul, 1984). Such a feature is determined by the mutual regulatory influence of lymphocytes involved in IAI interactions, and is realized through production of antibodies by these lymphocytes. Thus, antibody production allows judgments not only about the activity of antibody-producing lymphocytes, but also about the character of interactions between lymphocytes. Therefore, one of the approaches to study of the role of IAI lymphocytes in the induction of autoreactive lymphocytes in rat CIA model was to study the kinetics of the antibodies they produce.

Materials and methods

Rats

Female Wistar rats (150 ± 20 g body weight) were used. They were brought from Pushino nursery farm (Russia) and housed under standard laboratory conditions with food and water at a constant temperature of 20 ± 5 °C.

CIA induction

Bovine nasal native collagen type II (4 mg/ml; Sigma) was dissolved in acetic acid (0.1 M) and then emulsified with the same volume of ice-cold Freund's incomplete adjuvant (Sigma). On day 0 rats (n=22) were injected intradermally into the back and at the tail base with BC II/adjuvant suspension (400 μg of BC II per rat). Booster immunization was not conducted. Animal experiments were performed in accordance with the local animal care commission.

Preparation of Fc pieces of rats' IgG

The globulin fraction of rats' plasma, obtained with precipitation by ammonium sulphate, was further processed in ion-exchange chromatography (pH 7.0, $\lambda = 1.2 \times 10^3$ microsiemens/cm). IgG was further subjected to proteolysis with ratio of papain (12,000 mU): protein – 1:800, 20 h at 37° in Tris–HCl, pH 7.0, with addition of β-mercaptoethanol up to a final concentration in the sample of 0.1%, and of 0.25 M solution of Trilone B up to a final concentration in the sample of 1 mM. Hydrolysis reaction was stopped be adding 1 M iodoacethamide to the hydrolysate up to the concentration of 25 mM. Hydrolysate was further dialysed against 0.01 M Tris–HCl buffer with pH 7.0 and anion-exchange chromatography conducted (pH 7.0, $\lambda = 1.2 \times 10^3$ microsiemens/cm). A Fc-enriched fraction was obtained in desorbed peak, which was further purified with exclusion chromatography. Purity of pieces was detected in disk-electrophoresis in polyacrylamide gel by number and localization of colored fractions. Purity of Fc pieces of rats was 91%.

Immunization with Fc of rats' IgG

Fc pieces were injected (n=10) once at a dose of 500 μg in incomplete Freund's adjuvant intradermally/subcutaneously.

Samples

Blood samples were taken from each animal through cardiac puncture with anesthesia application before immunization. In CIA model blood samples were taken weekly for 10 weeks to constitute individual antibody kinetics. In experiment with Fc pieces injection blood samples were taken in a similar way for 4 weeks after immunization with Fc pieces.

Determination of antibodies to bovine collagen by ELISA

Plates (Corning-Costar) were coated overnight at 4 °C with 50 μl of native BC II (50 μg/ml in 0.01 M PBS). Plates were blocked with 150 μl 0.01 M PBS/0.05% BSA/Tween. Individual sera were analyzed. Serum samples were added in serial dilution with PBS/Tween and incubated for 1 h at room temperature (rt). The plates were then incubated for 1 h at rt with 100 μl of rabit anti-rat Ig (IgG, IgM, IgA), conjugated to horseradish peroxidase (IMTEC, Russia) diluted 1:5000 in PBS/Tween. At very step, plates were washed three times with 0.01 M PBS containing 0.05% Tween 20. Then substrate mixture was added (5 ml of citrate buffered solution (pH 5.0)/3 mg ortho-phenylenediamine/15 μl 3% H_2O_2). Absorbances were read after 15 min at 492 nm.

Determination of autoantibodies to rat collagen by ELISA

Native collagen from rat joint cartilage for rat collagen II antibodies determination was obtained by the method, described in the work of Trentham et al. Then ELISA was conducted according to the above-described ELISA method for bovine collagen antibodies determination, the only difference being that the plate was coated with RC II instead of BC II.

Measurement of RF

RF titer was determined in a rat-IgG-loaded erythrocytes agglutination test. Erythrocytes were fixed with glutaric dialdehyde in order to avoid warming the tested serums. For this purpose Group 0 human erythrocytes were cooled to 0 °C. 25% glutaric dialdehyde was diluted to 1% with the following solution: 1 part of 0.15 M Na_2HPO_4 (pH 8.2)/9 parts of 0.15 M NaCl/5 part of water. The cooling was conducted with an ice bath. The erythrocytes deposit was diluted with 1% glutaric dialdehyde to