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Anti-IgG antibodies in rheumatic diseases cross-react with *Streptococcus mutans* SR antigen

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SUMMARY

We have previously shown that SR protein, a *S. mutans* major cell wall protein, as well as the recombinant protein SR (rSR) share common epitopes with human IgG. Since this antigenic mimicry could play a role in the induction of anti IgG, we have examined, in k-ELISA, the presence of antibodies reacting with *S. mutans* SR proteins and *S. mutans* whole cells in sera from 36 patients with rheumatic diseases. The majority of the 36 sera showed a high reactivity with rSR when compared with control sera. Eight highly positive sera were further purified on rSR and human IgG sorbents and tested against both rSR and IgG in ELISA and Western blotting. The affinity-purified antibodies reacted strongly with rSR, IgG and IgG Fab fragments but failed to react with IgG Fc fragment. In Western blotting the addition of unlabelled IgG abolished the reactivity of affinity-purified biotinylated antibodies with all antigens, confirming the existence of a common epitope shared by rSR and human IgG heavy chain. We show the existence in rheumatic diseases of high titres of anti-human IgG antibodies cross-reactive with *S. mutans* SR proteins. Those antibodies are principally IgG and react with the Fd part of the Fab fragment. We can hypothesize from the above data that this antigenic mimicry existing between *S. mutans* SR-related antigens and human IgG could play a role in the synthesis of at least a part of the anti-IgG antibodies present in rheumatic diseases sera.

Keywords SR protein rheumatic diseases anti-human IgG antibodies antigen mimicry

INTRODUCTION

Recently it has become clear that a high molecular weight cell surface protein from the 'mutans streptococci', the principal agent of dental caries, termed antigen I/II (Russell & Lehner, 1978) (equivalent to B, IF, P1, SpaA, PAc and SR) isolated from various serotypes except *S. rattus*, plays an important role in streptococcal adherence to teeth surfaces. The cell wall associated glycoprotein, which is also released in the culture medium, functions as a bacterial adhesin (McBride *et al.*, 1984) and can be considered as a major virulence factor responsible for the pathogenicity of the bacteria. Furthermore, protein I/II has been previously believed to possess antigenic mimicry with human heart components (Hughes *et al.*, 1980), a property which has hampered the development of a vaccine against dental caries. However, recent studies using affinity-purified antibodies against *S. mutans* protein I/II or heart myosin failed to reveal any cross-reactivity between both components (Bergmeier & Lehner, 1983; Russell, 1987; Swartzwelder *et al.*, 1988). Recently we have shown that a fraction of the heart-reactive antibodies induced in rabbit during immunization with SR reacts predominantly with human IgG (Wachsmann *et al.*, 1989).

Furthermore, *S. mutans* has been implicated in autoimmune disorders (Stinson, Albini & Nisengard, 1986), and molecular mimicry could be one of the possible mechanisms implicating bacteria in this pathology. Therefore it could be suggested that at least a part of the anti-IgG antibodies which are generally found in patients with rheumatic diseases could result from a constant stimulation with SR related antigens present on the cell surface of the 'mutans streptococci'.

In order to determine the possible importance of SR in induction of anti-IgG antibodies in pathological mechanisms, we investigated the existence of anti SR protein antibodies cross-reactive with IgG in sera from patients with rheumatic diseases.

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MATERIALS AND METHODS

Sera

Stored sera were from the Blood Transfusion Centre, Strasbourg, France. Thirty-six sera with IgM rheumatoid factor (RF) titres ranging from 1/32 to 1/1024 (1/32 was defined as cut-off of positivity) detected by the Waaler–Rose test, were chosen from

patients (mean age 46·4 years) with classic or definite rheumatoid arthritis according to the American Rheumatism Association (ARA) criteria (Ropes *et al.*, 1958). Ten sera with titres of IgM RF < 1/32 were used as controls.

Bacterial strains and antigen preparation

S. mutans serotype f cells were grown in D1-20 synthetic medium of Carlsson (1972). The recombinant SR (rSR) protein was prepared from cell extract of *Escherichia coli* HB101 harbouring the pHBsr-1 recombinant plasmid. Purification was performed by gel filtration on Sephacryl S300 and immunoaffinity chromatography as described by Ogier *et al.* (1989).

Human IgG and chromopure Fab and Fc fragments were from Jackson Immunoresearch (Immunotech, Marseille, France).

Isolation of cross-reactive anti-IgG antibodies

rSR, human IgG and bovine serum albumine (BSA) were bound to cyanogen-bromide-activated Sepharose CL-4B as described by the manufacturer (Pharmacia, St-Quentin en Yvelines, France) to a ratio of approximatively 5 mg of protein per 1 ml of beads. Batches of 0·5 ml of serum samples from eight patients with high anti-SR antibodies were first purified by affinity chromatography on either rSR or BSA Sepharose column. After extensive washing, the column-bound antibodies were eluted with 0·1 M sodium acetate, 0·5 M NaCl (pH 4) and immediately neutralized with 1 M K_2HPO_4. After dialysis against phosphate-buffered saline (PBS), part of the antibodies eluted from the rSR column were fractionated on a human IgG Sepharose column. After removal of non-bound antibodies, the fixed antibodies were eluted with acetate buffer and treated as above. The purified anti-IgG antibodies were then reconstituted to their original volume.

Biotinylation of the anti IgG antibodies

Biotinyl-*N*-hydroxysuccinimide (Calbiochem, Meudon, France) was used to link covalently biotin to human affinity-purified anti-IgG antibodies according to the procedure of Kendall, Ionesco-Matiu & Dreesman (1983).

Electrophoresis and Western blotting

Electrophoresis was carried out on homogeneous 12·5% phast gels in the presence of SDS using the Phastsystem (Pharmacia, Uppsala, Sweden). Electrophoretic transfer and immunochemical analysis were carrried out essentially as described by Wachsmann *et al.* (1989). Blocking of unspecific binding was carried out by incubation of the nitrocellulose sheets with PBS-Tween 20 (PBS-T) and 3% BSA. The blots were then sequentially incubated with affinity-purified biotinylated anti-IgG, alkaline phosphatase (AP) streptavidin (GIBCO, Cergy-Pontoise, France) and enzyme substrate as previously described.

ELISA

The reactivity of the various sera, against whole *S. mutans* or purified rSR was determined by k-ELISA as described by Fischetti & Windels (1988). Briefly, microtitre plates were coated with *S. mutans* (10^9 cells/ml) or rSR (1 μg/ml) as previously described (Ogier *et al.*, 1986; Wachsmann *et al.*, 1989). After the remaining binding sites were blocked by PBS-T–BSA, the plates were sequentially incubated with test or control sera diluted to 1/200 (1 h, 37°C), rabbit anti-human Ig, IgG, IgA and IgM sera diluted 1/10000 (1 h, 37°C) and AP-labelled anti-rabbit immunoglobulin (1 h, 37°C). After addition of the substrate (*p*-nitrophenyl phosphate), the individual wells were automatically monitored every 2 min with a Dynatech MR 5000 reader and a kinetics program cartridge. The results are expressed as mean absorbance 405 nm/h of triplicate determinations. The reactivity of the affinity-purified antibodies against rSR, IgG, Fab or Fc fragments was determined by direct ELISA as previously described (Wachsmann *et al.*, 1989). Plates coated with the various antigens (1 μg/ml) were incubated with serial dilutions of either unlabelled or labelled affinity purified antibodies (1 h, 37°C). Antibody binding was detected with AP–anti-human IgG diluted 1/10000 or AP–streptavidin diluted 1/10000 (1 h, 37°C) followed by incubation with substrate. The absorbance at 405 nm was read after incubation of the plates for 1 h at 37°C.

RESULTS

In this study we examined the presence, in rheumatic diseases sera, of antibodies that cross-react with *S. mutans* SR protein, by testing the reactivity of these sera with purified rSR and whole *S. mutans* cells in k-ELISA. As seen in Fig. 1a, most of the 36 rheumatic diseases sera showed a high reactivity with rSR at a serum dilution of 1/200, when compared with the reactivity of control sera. Isotype testing of the reactive antibodies with class-specific anti-immunoglobulins identified principally IgG and to a lower extent IgA; however, no anti-rSR IgM antibodies could be detected. The sera were also tested for reactivity against *S. mutans* whole cells. The results showed that the binding pattern of the rheumatic diseases sera to *S. mutans* whole cells was strikingly similar to that obtained with purified rSR but the reactivity was much lower (Fig. 1b). In control experiments, immobilized antigens failed to react with a rabbit anti-human IgG (1/10000) and AP–goat anti-rabbit IgG (1/10000).

In order to characterize further the anti-rSR antibodies present in rheumatic diseases sera, we selected eight sera found to be highly reactive with rSR. The sera were loaded separately onto a rSR or a BSA Sepharose column. Affinity-purified antibodies from all individual rheumatic diseases sera showed a strong reactivity in ELISA against rSR (Fig. 2), whereas BSA Sepharose purified fractions from rheumatic diseases sera as well as affinity-purified antibodies from normal sera reacted only weakly with rSR (Fig. 2).

In order to discriminate between the presence of anti-rSR antibodies, and antibodies cross-reacting with both rSR and IgG, we further purified separately the different anti-rSR antibodies on a human IgG Sepharose column. Adsorption with IgG of the anti-rSR antibodies originated from the eight sera resulted in a decrease of their reactivity with rSR (data not shown). The antibodies eluted from the column reacted strongly with rSR and IgG and the results of a typical serum are shown in Fig. 3. Since we previously showed the absence of cross-reactivity between rSR and light chains (Wachsmann *et al.*, 1989), we tested the reactivity of the affinity purified antibodies with IgG fragments. All the antibodies reacted strongly with the IgG Fab fragments, but failed to react with Fc fragment in ELISA (Fig. 3).

In order to confirm this cross-reactivity, we tested the ability of biotinylated affinity purified antibodies to react with rSR, IgG, Fab and Fc fragments in Western blotting. As expected,

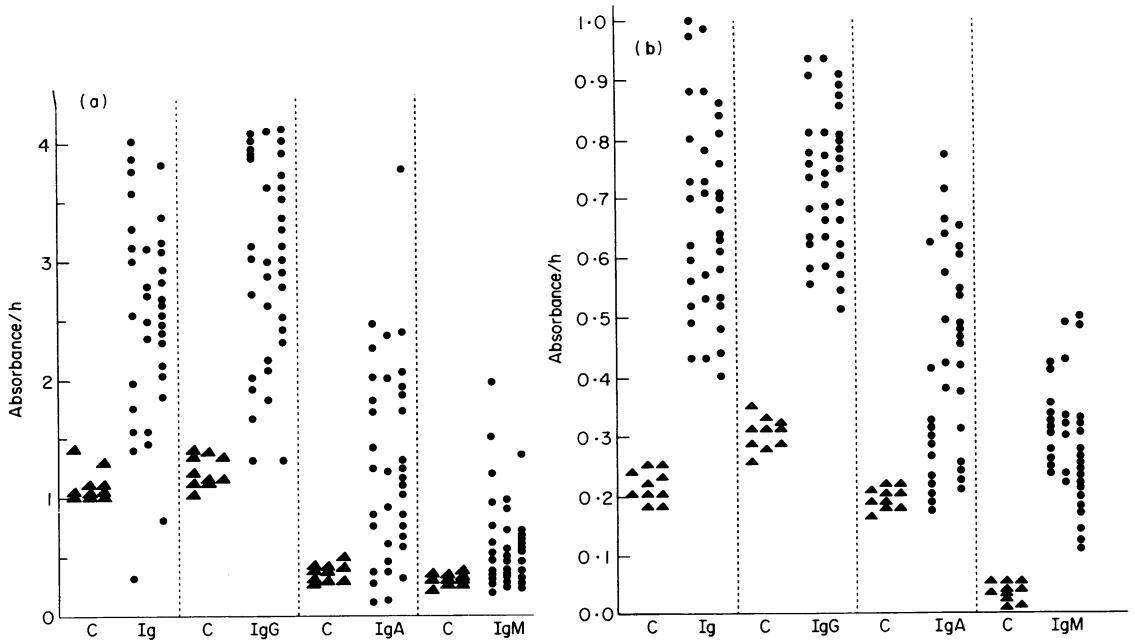

Fig. 1. k-ELISA analysis of rheumatic diseases (●) and control sera (▲) with purified rSR (a) and whole *S. mutans* cells (b). Sera were titrated individually (diluted 1/200) with anti-human immunoglobulins, IgG, IgA and IgM (1/10000) followed by AP–anti-rabbit immunoglobulin (1/10000) on both antigens. Results are from triplicate experiments.

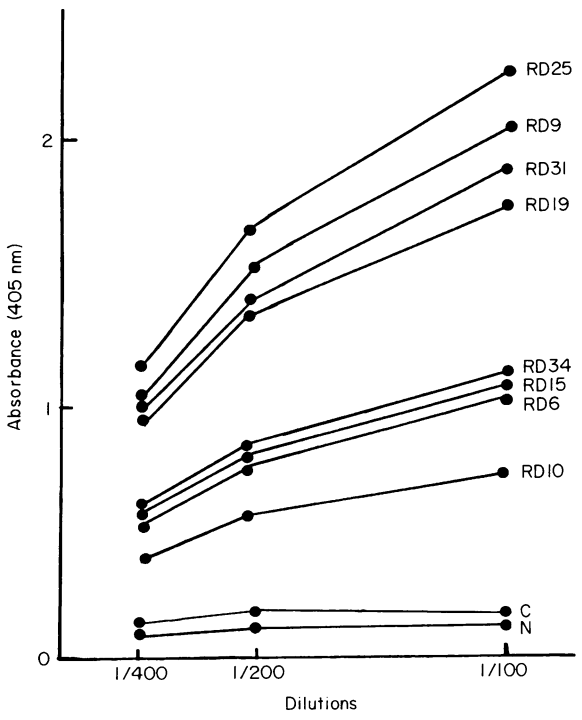

Fig. 2. Dose-dependent binding of anti-rSR antibodies isolated from 8 different rheumatic diseases sera (RD) or normal sera (N) to rSR; C, control binding activity to rSR of the rheumatic sera adsorbed to BSA Sepharose. The antigen binding activity of each type of antibody is expressed as absorbance at 405 nm. Results are expressed as arithmetic means of triplicate experiments.

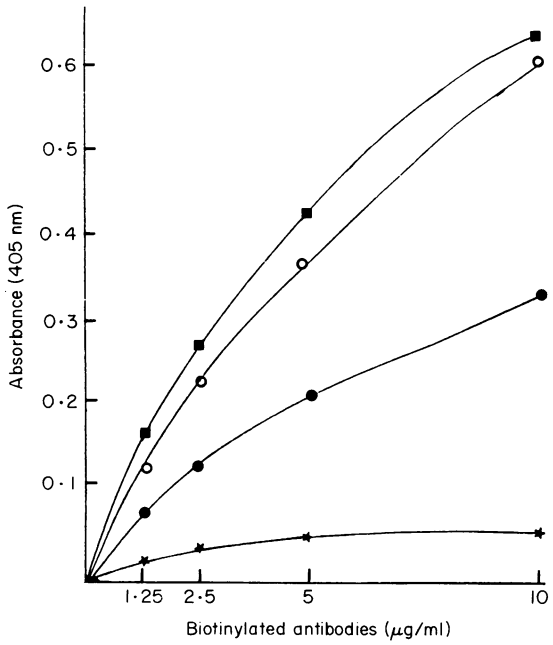

Fig. 3. Dose-dependent binding of biotinylated affinity purified antibodies to plates coated with rSR (●), human IgG (○), Fab fragments (■) and Fc fragment (★). The antibody binding activity is expressed as absorbance at 405 nm. Results are expressed as arithmetic means of triplicate determinations.

the biotinylated antibodies reacted with rSR (Fig. 4a), human IgG heavy chain (Fig. 4b), Fab fragments (Fig. 4c) but not with the Fc fragment of IgG (Fig. 4d).

To ensure that the observed reactivity of the biotinylated antibodies towards the antigens was due to a true cross-

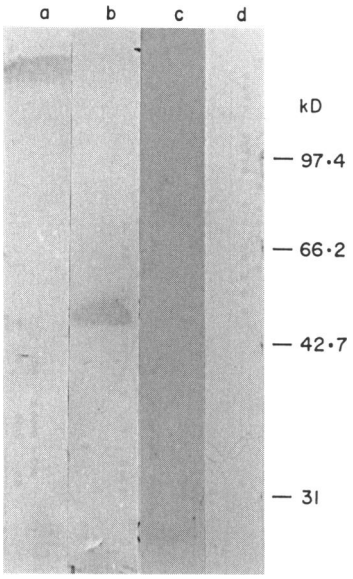

Fig. 4. Western blot analysis of biotinylated affinity purified antibodies from one of the rheumatic diseases sera with rSR (a), human IgG (b), Fab fragments (c) and Fc fragment (d). Molecular mass standards are indicated.

reactivity, we incubated the Western blots in the presence of large excess of soluble IgG. The addition of IgG abolished the reactivity of the antibodies with all antigens, confirming the existence of a common epitope shared by rSR and human IgG heavy chain.

DISCUSSION

Our results confirm the presence of high titre of anti-rSR antibodies in rheumatic diseases sera as compared with control sera, and are consistent with previous studies showing high level of antibodies to streptococcal components in patients with inflammatory diseases (Avila *et al.*, 1987; Moore, El-Najdawi & Dorner, 1989).

Furthermore, at least a portion of the natural anti-rSR antibodies from rheumatic diseases patients cross-reacted with human IgG.

These results are in agreement with our earlier studies (Wachsmann *et al.*, 1989) which showed the existence of anti-rSR antibodies cross-reactive with human heavy chains in rabbit sera after immunization with rSR.

Those antibodies are principally IgG and react with the Fd part of the Fab fragment and studies are underway in our laboratory to identify the localization of the cross-reactive epitope on the rSR antigen. As the rheumatic diseases sera reacted with *S. mutans* whole cells we can postulate that the cross-reactive epitope of the SR protein is located and accessible on the bacterial cell surface.

From the above data we can hypothesize that the antigenic mimicry existing between *S. mutans* SR related antigens and human IgG could play a role in the synthesis of at least a part of the anti-IgG antibodies present in rheumatic diseases sera. This mechanism differs from that previously proposed, where anti-IgG antibodies specific for the Fc portion of human and animal IgG could be anti-idiotypic antibodies specific for primary antibodies originally induced against the Fc receptor present on the cell surface of some streptococcal strains (Nardella *et al.*, 1987), which has been implicated in the pathogenesis of various autoimmune disorders. Although the synthesis of the anti-IgG antibodies could result from a constant stimulation with SR antigens present on the surface of 'mutans streptococci', the exact mechanism of anti-IgG synthesis as well as the serological relevance to rheumatic diseases remain unknown, and studies are now undertaken to determine the presence of such cross-reactive antibodies in sera from patients with various autoimmune diseases.

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