Immunology 1987 **62** 523–527

Identification of the site on IgG Fc for interaction with streptococci of groups A, C and G

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Accepted for publication 17 July 1987

SUMMARY

The interaction between living groups A, C and G streptococci and IgG Fc was studied using human IgG, IgG Fc and IgG Fc-intermediate (Fc_i) fragments, chemically modified human IgG and fragment D of staphylococcal protein A (SPA). Diethylpyrocarbonate modification of His or *N*-acetylimidazole modification of Tyr of human IgG resulted in the loss of its capacity to inhibit the binding of radiolabelled human IgG Fc to the group A streptococci types M1 and M55, and to the group C strain SC-1, indicating that the amino acids His and Tyr are involved in the binding. Lys seems not to participate in the binding of IgG to these bacteria, however, since reductive methylation of Lys did not reduce its inhibitory capacity. Fragment D of SPA also inhibited the binding of radiolabelled human IgG Fc to strains M1, M55 and SC-1. We have previously shown that these bacteria do not bind to IgG fragments consisting of only the Cγ2 or Cγ3 domains. On the basis of these results, and the known relative positions in space of the His and Tyr residues on IgG Fc, it is speculated whether streptococci with IgG Fc receptors, like SPA and rheumatoid factors, interact with IgG in the interface between the Cγ2 and Cγ3 domains and involve His 435 and one or more of Tyr 436, His 433 and His 310. The similarities in binding sites on IgG for RFs and these bacterial Fc binding proteins suggest structural similarities between them that may be relevant to the production of rheumatoid factors in rheumatoid arthritis.

INTRODUCTION

The capacity of microorganisms to bind IgG via the Fc region has emerged in the last two decades to be a widespread biological phenomenon. Such activity is found among bacteria, i.e. streptococci of groups A, C, and G (Kronvall, 1973) and *Staphylococcus aureus* (Forsgren & Sjöquist, 1966). Furthermore, IgG Fc receptors have been demonstrated on cells infected with *Herpes simplex* virus (Watkins, 1964) of both serotypes 1 and 2 (Para, Goldstein & Spear, 1982), *Varicella-zoster* (Ogata & Shigeta, 1979), Epstein–Barr virus (Yee *et al.*, 1982) and *Cytomegalovirus* (Furatawa *et al.*, 1975), as well as on cells infected with schistosomes (Torpier, Capron & Ouaissi, 1979). Using a system with solid-phase aggregated IgG, we have shown previously that purified IgG Fc binding protein (FcBP) from the M15 strain of group A streptococci binds to the same site in the interface between the Cγ2 and Cγ3 domains as IgG rheumatoid factors and staphylococcal protein A (SPA) (Nardella *et al.*, 1985, 1987). His 435 and Tyr 436 on the IgG heavy chain, and possibly one or both of His 433 and 310, were demonstrated to be involved in the binding. Such molecular mimicry may be important for the production of rheumatoid factors (Nardella *et al.*, 1985, 1987). However, so far it is unknown whether this specific binding site is involved for FcBP present on the surface of living bacteria, or may be found outside type 15 group A streptococci. In a previous report we showed that living strains of groups A, C, and G streptococci, like SPA and IgG RFs, do not bind to IgG fragments consisting of only the Cγ2 or Cγ3 domains (Schröder *et al.*, 1986). The present report extends our previous studies by using human IgG, chemically modified human IgG, Fc_i and fragment D of SPA to localize further the site on IgG molecules for interaction with living streptococci of different types and groups.

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MATERIALS AND METHODS

Bacterial strains

The following streptococcal strains were used for these studies: group A, types M1 (9198), M4 (734), M6 (8302), M8 (8324), M15 (EF1949), M22 (59/50), M30 (Quinn), M55 (100189), T27 (SF 40), T44 (Henson Glossy); group C, strains SC-1, T7 and 81 C; group G, strain 113 G. Among the strains, M1, M8, M15, M22, M55 and groups C and G strains expressed receptors for the Fc region of IgG, whereas the remaining strains were

negative for FC binding. The strains were grown overnight at 37° in Todd-Hewitt broth, washed in phosphate-buffered saline (PBS, 0·05 M phosphate, 0·12 M NaCl, pH 7·4) and suspended in the same buffer to a concentration of 2×10^{10} bacteria per ml (Christensen & Oxelius, 1974).

Immunoglobulin preparations

The human IgG and Fc fragments were isolated and prepared as described previously (Schröder *et al.*, 1986). Fc_i fragments were prepared and isolated from human Cohn Fraction II (Sigma Chemical Company, St Louis, MO) (Nardella & Teller, 1985).

Chemical modifications of His in IgG with diethylpyrocarbonate, Tyr with *N*-acetylimidazole and Lys by reductive methylation and reversal of the His and Tyr modifications with hydroxylamine were performed as described in previous work (Nardella *et al.*, 1985). The degree of His modification in IgG was 52·3%, which was reduced to 12% after hydroxylamine reversal. Of the Tyr, 13·3% was modified in IgG, with 5·2% remaining after hydroxylamine reversal, and 30·3% of the Lys in IgG was modified.

Fragment D

Fragment D of staphylococcal protein A (SPA) was a gift from Dr John Sjöquist, University of Uppsala, Sweden, and was prepared from the secretory product of the V-1 mutant strain of *Staphylococcus aureus*, Cowan I (Movitz, Masuda & Sjöquist, 1979).

Binding tests

IgG and IgG fragments were radiolabelled with ^{125}I using the lactoperoxidase method (Marchalonis, 1969). In brief, 0·1 mCi of carrier-free $Na^{125}I$ (The Radiochemical Centre, Amersham, Bucks, U.K.) was added to a mixture of 2 μl lactoperoxidase (2·5 mg/ml) (lot no. L8250, Sigma) and 25 μl (25 μg) of the preparation to be labelled. The reaction was started by adding 2 μl 30% (v/v) H_2O_2 diluted 1:20,000 in PBS (E. Merck, Darmstadt, FRG) and stopped by adding 500 μl PBS containing 0·02% (w/v) sodium azide. The free ^{125}I was removed by dialysis against PBS.

The binding test was performed as described previously (Christensen & Oxelius, 1974). In brief, 50 μl (2 μg) radiolabelled IgG, IgG Fc or IgG Fc_i fragments were added to a 200 μl suspension of 2×10^9 bacteria unless otherwise stated. After incubation for 30 min at 37°, 2 ml PBS were added and the tube centrifuged at 3000 *g* for 15 min. The supernatant was aspirated and the radioactivity bound in the pellet counted and expressed as a percentage of the total radioactivity added. Binding below 10% was considered negative, i.e. to be due to entrainment of the radiolabelled test material in the pelleted bacteria. Under the test conditions given, a substantial excess of possible bacterial binding sites is present in the test system (Christensen & Oxelius, 1974).

Inhibition experiments

Unlabelled immunoglobulin preparations, i.e. chemically modified IgG, chemically modified and hydroxylamine-reversed IgG, human IgG, IgG Fc and IgG Fc_i or fragment D of staphylococcal protein A, were added in amounts from 10 to 50 μg to the bacterial suspensions and incubated for 1 hr at ambient temperature followed by the addition of 2 μg ^{125}I-labelled human IgG Fc or ^{125}I-labelled human IgG Fc. The capacity of the bacteria to bind the radioactive fragments was then determined as described above. In order to obtain optimal sensitivity, however, the number of bacteria was lowered to 2×10^8. The capacity of the fragments to inhibit the binding was expressed as percentage inhibition, and was calculated from the formula:

$$\% \text{ inhibition} = \frac{Ba - Bp}{Ba} \times 100$$

where Ba is the binding in the absence and Bp in the presence of the fragment to be tested for inhibitory capacity.

Table 1. The capacity of streptococci groups A, C and G to bind radiolabelled human IgG and IgG Fc and IgG Fc_i fragments*

Bacterial strains		Binding (±SEM) of: IgG	IgG Fc	IgG Fc_i
Group A streptococci				
With IgG Fc receptors				
Types	M1	86±1·4	79±3·0	13±0·4
	M8	49±3·0	66±0·7	13±1·8
	M15	55±5·8	34±8·0	<10
	M22	74±14·1	70±2·1	17±0·4
	M55	67±5·3	66±4·7	19±1·5
Without IgG Fc receptors				
Types	M4	<10	<10	<10
	M6	<10	<10	<10
	T27	<10	<10	<10
	M30	<10	<10	<10
	T44	<10	<10	<10
Group C streptococci				
Strains	SC-1	88±7·0	87±6·8	64±6·3
	T7	82±8·6	73±8·3	26±1·6
	81C	100±2·2	74±5·2	16±1·2
Group G streptococci				
Strain	113 G	93±1·3	76±5·5	20±2·2

*Two micrograms of immunoglobulin added to 2×10^9 bacteria.

RESULTS

Binding of radiolabelled human IgG, IgG Fc and IgG Fc_i fragments to different streptococci

In repeated experiments, group A streptococci with IgG Fc receptors bound 49–86% of the ^{125}I-labelled human IgG, and 34–79% of the ^{125}I-labelled human IgG Fc fragments compared with 0–19% of the ^{125}I-labelled human IgG Fc_i fragments (Table 1). The group A streptococci without IgG Fc receptors did not take up any of the preparations. The groups C and G streptococcal strains, all possessing IgG Fc receptors, revealed a somewhat higher uptake of the IgG preparations, 82–100% of the intact IgG, 73–87% of ^{125}I-labelled human IgG Fc fragments, and 16–64% of the ^{125}I-labelled human IgG Fc_i fragments (Table 1).

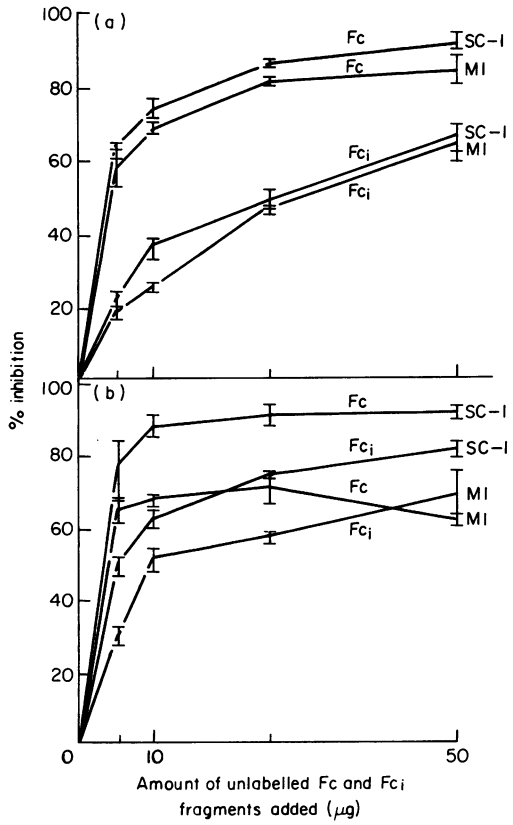

Figure 1. The capacity of unlabelled human IgG Fc and IgG Fc_i to inhibit the binding of ^{125} I-labelled human IgG Fc (a) and ^{125}I-labelled human IgG Fc_i (b) to streptococci group A type M1 and group C strain SC-1.

Inhibition of the binding of radiolabelled human IgG Fc and IgG Fc_i fragments to streptococci by the corresponding unlabelled fragments

The capacity of unlabelled human IgG Fc and IgG Fc_i fragments to inhibit the binding of ^{125}I-labelled IgG Fc and ^{125}I-labelled IgG Fc_i to streptococci group A type M1 and group C strain SC-1 was tested using 5, 10, 25 and 50 μg of each of the unlabelled preparations (Fig. 1). Five micrograms of unlabelled IgG Fc inhibited the binding of ^{125}I-labelled IgG Fc to the strains M1 and SC-1 better than unlabelled Fc_i. When the amounts of unlabelled Fc and Fc_i were increased to 50 μg, similar relations between the capacity of the two preparations to inhibit were found (Fig. 1a). In the test system with ^{125}I-labelled IgG Fc_i, the inhibitory capacity for the binding to the streptococcal strain M1 was better for Fc than for Fc_i. For the SC-1 strain, Fc and Fc_i produced similar degrees of inhibition but exhibited a more pronounced inhibitory effect on the binding of ^{125}I-IgG Fc_i to the SC-1 strain than to the M1 strain (Fig. 1b).

Inhibition of the binding of radiolabelled human Fc fragments to different streptococci by unlabelled chemically modified human IgG and fragment D of SPA

Virtually no inhibition of the binding of radiolabelled human IgG Fc to the streptococci group A strains M1 and M55 was obtained with 10–50 μg human IgG on which 52·3% of the His

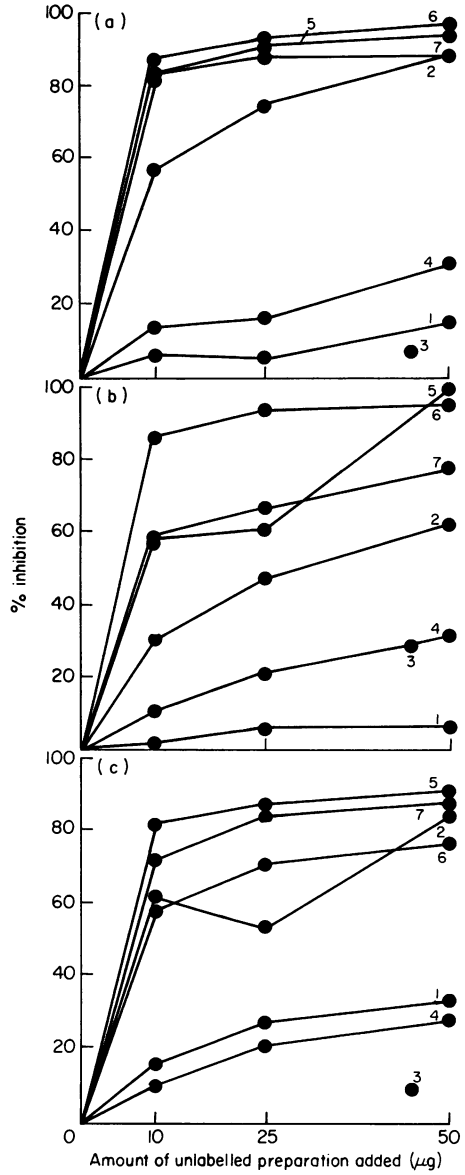

Figure 2. The capacity of unlabelled diethylpyrocarbonate modified His on human IgG (1), diethylpyrocarbonate-modified and hydroxylamine-reversed IgG (2), *N*-acetylimidazole-modificated Tyr on human IgG (3), *N*-acetylimidazole-modified and hydroxylamine-reversed IgG (4), reductively methylated Lys on human IgG (5), fragment D of SPA (6), and unmodified human IgG Fc (7) to inhibit the binding of ^{125}I-labelled human IgG Fc to streptococci group A types M1 (a) and M55 (b), and group C strain SC-1 (c).

had been modified with diethylpyrocarbonate, whereas 15–32% inhibition was found for strain SC-1 (Fig. 2a, b and c). When 77% of the His modifications were reversed with hydroxylamine, the capacity to inhibit the binding of ^{125}I-labelled human IgG Fc to the bacteria was partially regained for all three strains. *N*-acetylimidazole modification of 13·3% of the Tyr on human IgG resulted in the loss of capacity to inhibit the binding of ^{125}I-labelled human IgG Fc fragments to strains M1 and SC-1 (this part of the experiments was only performed with 40 μg of modified IgG because of shortage of material). With hydroxylamine reversal (61% of modified Tyr reversed), the capacity to

inhibit increased to 13–28% for the M1 strain and to 10–27% for the SC-1 strain (Fig. 2a and c). Reductive methylation of 30·3% of the Lys on human IgG had no effect on the inhibitory capacity for the binding of ^{125}I-labelled human IgG Fc to strains M1, M55 and SC-1. Unmodified human Fc was strongly inhibitory in these systems (Fig. 2).

The monovalent subunit fragment D of staphylococcal protein A added in amounts from 10 to 50 μg inhibited the binding of radiolabelled human IgG Fc to strains M1, M55 and SC-1 (Fig. 2a, b and c).

DISCUSSION

We have shown previously that group A, C and G streptococci with IgG FcBP do not bind human or rabbit IgG fragments that contain only the Cγ2 or Cγ3 domains (Schröder *et al.*, 1986). Subsequently, we showed that the isolated Fc binding protein from the M15 strain of group A streptococci binds to the same site in IgG as SPA and IgG rheumatoid factors and involves His 435, Tyr 436 and one or both His 310 and 433 on the gamma chains of IgG (Nardella *et al.*, 1987). The present results extend previous observations to other group A strains and the group C streptococci. These results also apply to group G streptococci, since group C and G streptococcal Fc receptors have the same terminal 15 amino acid residues and thus may be identical (Reis, Hansen & Björck, 1986).

In this report we show that the monovalent fragment D of SPA inhibited the binding of radiolabelled human IgG Fc to strains M1, M55 and SC1-1, providing further evidence to suggest that streptococcal FcBP bind to the same site on IgG as is involved in the binding of SPA. Furthermore, diethylpyrocarbonate and *N*-acetylimidazole modification of human IgG resulted in the loss of its ability to inhibit the binding of radiolabelled human IgG Fc to the group A streptococci types M1 and M55 and to group C strain SC-1, indicating that the amino acids His and Tyr were involved in the binding. It has to be taken into account that only 52% of the His and 13% of the Tyr residues were modified and the reactive residues not identified. Hydroxylamine reversal of the Tyr-modified IgG did not allow even partial return of inhibitory capacity of the binding of Fc to M55, although it did for binding strains M1 and SC-1. The reasons for this are not known, but point to the slight heterogeneity of the specific binding determinants for the different strains within the same general Cγ2-Cγ3 locale. Another explanation might be that modification outside the binding site results in conformational change at the binding site, and this may not be reversed on deblocking. In contrast, Lys seemed not to participate in the binding of IgG to group A streptococci types M1 and M55 or the group C strain SC-1 as reductive methylation of Lys did not reduce inhibiting capacity; only 30% of Lys residues were modified, and hence there could be a Lys in the binding site that was not affected. Coupled with previous data, and the known relative positions in space of His and Tyr residues on IgG Fc, these results indicate that the FcBP on group A, C and G streptococci involve His 435 and one or more of Tyr 436, His 433 and His 310. This is the same site that binds SPA (Deisenhofer, 1981) and rheumatoid factors (Nardella *et al.*, 1985). Probably the best evidence for the involvement of His 435 in the binding site for SPA is from 'natures' experiments with IgG3 allotypes, i.e. the Arg/His interchange between Caucasian and Mongoloid populations—this is also reflected in RF binding (Matsumoto *et al.*, 1983).

IgG Fc binding proteins on a number of different streptococcal strains have different molecular weights (Schröder *et al.*, 1986). The possible existence of differences in the amino acid sequences of the Fc receptors and in the number of receptors may explain differences in avidity for the binding of immunoglobulin preparations to the streptococci.

The capacity of the IgG Fc receptor-positive streptococci to bind the intermediate Fc fragment (Fc_i) was generally only 20% of the capacity for IgG Fc and intact IgG, with the exception of the group C strain SC-1, which bound a three to four times higher proportion of Fc_i (Table 1). In inhibition experiments, the M1 and the SC-1 strain also showed higher affinity for Fc than for Fc_i. The Fc_i is composed of two polypeptide chains of unequal molecular weight, where the larger polypeptide chain has both Cγ2 and Cγ3 domains, the smaller is composed on only a Cγ3 domain (Nardella & Teller, 1985). Since the interaction between the streptococci and human IgG takes place in the interface between Cγ2 and Cγ3 domains, Fc_i is monovalent, whereas the intact Fc fragments are divalent. One explanation for the difference in binding capacity might be that the group A strains and at least strain 81 C and 113 G interacted with both Cγ2–Cγ3 interfaces, because these strains showed considerably lower affinity for Fc_i as compared to Fc. On the other hand, the capacity of strain SC-1 to bind Fc_i was practically the same as for Fc. This strain might therefore only bind to one of the two Cγ2–Cγ3 interfaces on an intact IgG, or, in other words, IgG Fc reacted monovalently with strain SC-1. Another interpretation of these data is that both Fc and Fc_i fragments interacted monovalently with all the strains of streptococci, but that the interaction energy was less with Fc_i. Nardella, Teller & Mannik (1981) found that the interaction of Fab fragments of IgG rheumatoid factor with Fc_i was of lower interaction energy than the binding of these fragments to intact IgG. Thus, it is possible that conformational changes are induced in the Cγ2–Cγ3 interface region of one gamma chain of Fc_i fragment when the Cγ2 domain is absent from the other gamma chain, which has a greater effect on the binding of all the strains except SC-1. In addition, these differences suggest that although all strains bind to the same general locale, there is microheterogeneity in the exact binding determinants.

The Cγ2–Cγ3 interface region of Fc is the site of interaction for SPA, the FcBP of the groups A, C and G streptococci, and is likely to be the site of interaction of Fc receptors induced on cell surfaces by HSV-1 (Johansson *et al.*, 1986). Why is this region of Fc the site of interaction for all of these substrates? On purely biochemical grounds, this region offers groupings of residues that form exposed hydrophobic patches (Burton *et al.*, 1980), an important component of protein–protein interactions. It also has large probe-accessible convex surface areas, which are important to antigenic sites (Novotny, Handschumacher & Bruccoleri, 1987). Furthermore, immunologically, this area was one of the three major sites on the Fc region of human IgG to which mouse monoclonal antibodies were directed (Jafaar *et al.*, 1984). An important question is what these binding site similarities mean in terms of RF production in rheumatoid arthritis. The binding site similarities suggest structural similarities between these microbial FcBP and RF, and suggest that RF antibody-combining sites carry the internal image of these bacterial Fc-binding structures. We (Nardella *et al.*, 1985, 1987)

and others (Mouritsen, 1986) have proposed that RF could arise as anti-idiotypic antibodies to antibodies to FcBP of microbial agents. Alternatively, the FcBP could present IgG to the immune system in such a manner that renders that region of self-Fc immunogenic.

ACKNOWLEDGMENTS

This work was supported by research grants AM-12849 from the National Institutes of Health and the Townsend-Henderson Fund, the Swedish Medical Research Council, the Rheumatism Association in Sweden, and the Österlunds Fund.

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