LOCALIZATION OF THE XG ALLOTYPIC DETERMINANTS, IN WHICH CARBOHYDRATE STRUCTURES TAKE PART, TO THE SEPARABLE PARTS OF THE RABBIT IgG MOLECULE.

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SUMMARY

Enzyme-immunologic and radio-immunologic techniques made it possible to localize the determinants responsible for the xg allotypic specificity (in which carbohydrate structures are involved) to the heavy polypeptide chains and the Fc fragment of rabbit IgG, but not to the light chains nor the Fab fragment, and in part to the F(ab')2 fragment obtained by pepsin digestion. The relationship between the xg and the e14 allotypic patterns, to date always found together in the same individuals, is discussed in light of these results.

Since the first observation of allotypy of rabbit immunoglobulins (1) and since the description (2.3) of the two series of allotypic specificities later designated as "a" and "b", many allotypic specificities have been described, some of which are peculiar to rabbit IgG, such as d11 and d12 (4.5), and e14 and e15 (6.7). Each time a correlation has been established between allotypic specificities and differences in immunoglobulin structure, these differences have concerned the primary structure of either one or the other polypeptide chain. The first example of the participation of carbohydrate structures in an allotypic specificity (provisionnally designated as "xg") has been observed in rabbit immunoglobulins (8). The localization of the determinants responsible for this allotypic specificity of the IgG molecule is studied in the present paper.

MATERIAL AND METHODS

Preparation of the reagents. The anti-xg anti-sera were prepared by injecting xg^- rabbits with IgG purified from sera xg^+ rabbits by chromatography on diethyl-amino-ethyl (DEAE) cellulose. The injected IgG had been before injection either polymarized by glutaraldehyde (9.10), or mixed with a

small amount of somatic antigen of Salmonella typhi (8).

The Fab and Fc fragments of xg^+ IgG were prepared by papain hydrolysis of 100mg of IgG, and fractionation on carboxymethyl (CM) cellulose in acetate buffer, pH 5.3 (11). The Fc fragment was purified by gel filtration on Sephadex G100 in acetate buffer, pH 5.4, then crystallized 3 times in a neutral solution at 4°C. The F(ab')2 fragments were prepared by pepsin digestion of 50mg of IgG, and gel filtration on Sephadex G200 in phosphate buffer, pH 7.3 (12).

The polypeptide chains were separated by reduction of the IgG with dithiotreitol (final concentration: 0.01M) and gel filtration on Sephadex G200 in the presence of 5M guanidine (13).

Antigen-antibody reactions and their inhibition. xg^+ IgG was purified by adsorption and elution from an immunoadsorbent of glutaraldehyde-insolubilised anti-xg serum, (9) and labelled as follows:

a/ with E.Coli alkaline phosphatase (14); the IgG-enzyme conjugate (IgG-Pase), isolated by filtration on G-200 Sephadex, had a specific activity of 0.8 to 1.5 unit/mg of conjugate (15).

b/ with ^{125}I by the chloramine T method (16) so that the specific activity was 3000 cpm/ng of IgG.

Insolubilisation of the antibodies was accomplished by binding to: a/ xg^+ IgG adsorbed to the walls of polystyrene tubes (17), or b/ xg^+ IgG covalently linked to cyanogen-bromide activated Sepharose 4B.

RESULTS

The binding of the IgG-Pase conjugate to antibodies bound to xg^+ IgG adsorbed on polystyrene tubes was inhibited (96% or 98%) by the heavy chain preparation, which demonstrates the localization of the xg determinants to the heavy chains. This fixation was not inhibited by the

preparation of light chains except at a concentration of 2800 x 10^{-5} µM, and only in the proportion of 30% (table I).

µM. of heavy chain ($\times 10^5$)	16.8	168	1680
% of inhibition	70	96	98
µM. of light chain ($\times 10^5$)	28	280	2800
% of inhibition	2	6	30

Table I. Inhibition, by increasing amounts of heavy and light polypeptide chains, of the binding of IgG-Pase to anti-xg antibodies bound to xg^+IgG adsorbed on polystyrene tubes.

The Fab fragments obtained by papain digestion (designated as FabI or FabII according to their order of elution from CM cellulose) did not inhibit the binding of xg^+ IgG labelled with ^{125}I to anti-xg antibodies coupled to Sepharose beads (Fig.1). On the other hand, this binding was completely inhibited by the preparation of Fc fragment. This preparation, at a concentration of 2mg/ml, was not precipited by an anti-b4 serum which precipitates the IgG at a concentration as low as 6 µg/ml, thus indicating that undegraded IgG is not present in the Fc preparation at a concentration larger than 0.3%.

The binding of the labelled IgG to the same antibody preparation was partly inhibited by F(ab')2 fragments obtained by pepsin digestion (Fig.2), thus indicating that a part, and only a part, of the determinants of the xg allotypic pattern is present on these fragments. The following characters of the F(ab')2 fragments contained in the preparation were checked: a/ the reaction of the F(ab')2 preparation in gelled medium [double diffusion in cells with parallel walls (18)] with a goat anti-

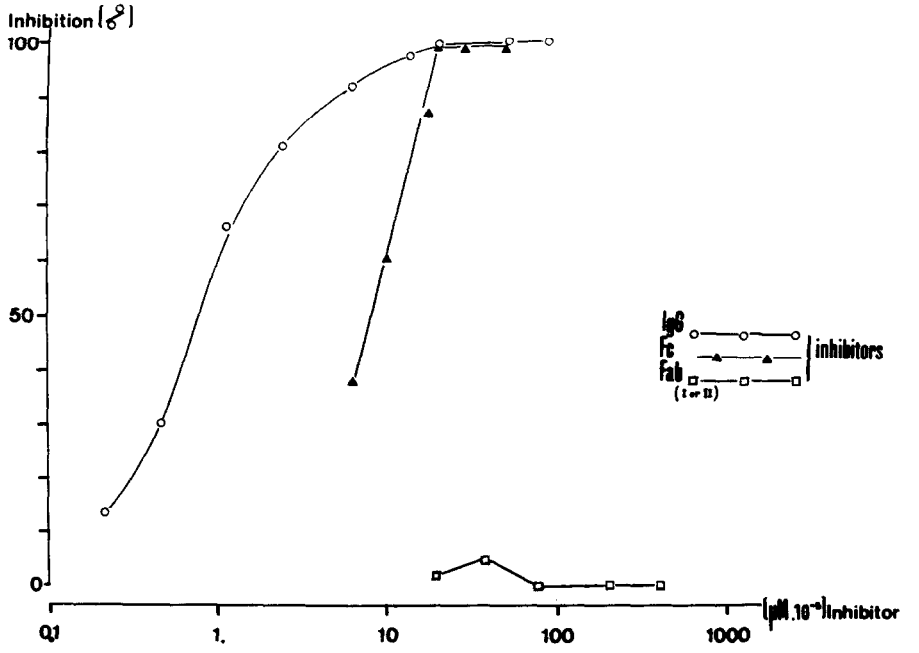

Fig.1. Inhibition, by FabI, FabII, and Fc fragments, obtained by papain digestion of xg^+ IgG, of the binding of ^{125}I-labelled xg^+ IgG to anti-xg antibodies bound to xg^+ IgG coupled to Sepharose beads.

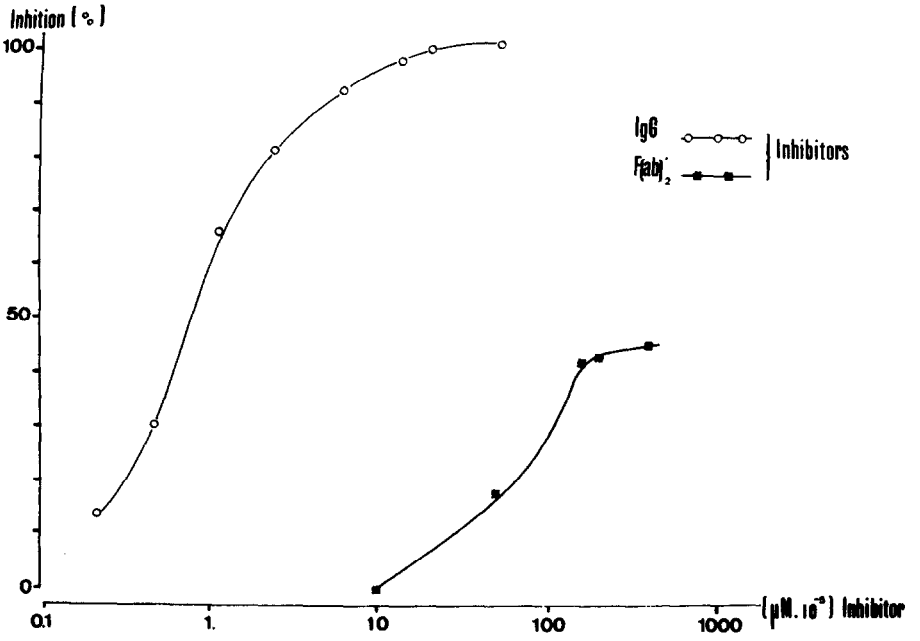

Fig.2. Inhibition of the same reaction as in Fig.1 by F(ab')2 fragment obtained by pepsin digestion of xg^+ IgG.

rabbit IgG anti-serum indicated that only a part of the isotypic determinants of the IgG is present on these fragments; further b/ the elution volume of these fragments on Sephadex G200 indicated a molecular weight in the range of 100 000.

DISCUSSION

The complete inhibition of the antigen-antibody reaction by the preparation of heavy polypeptide chains demonstrates the presence of the xg determinants on these chains. The partial inhibition by large concentrations of the light chain preparation is almost certainly due to slight contamination of this preparation by heavy chains. The absence of inhibition by the Fab fragments agrees with the absence of xg determinants on the light chains. The fact that the amount of Fc fragments needed to obtain complete inhibition is in the range of 3 times the number of IgG molecules which gives the same effect (Fig.1) might be tentatively explained by a slight modification of part of the determinants by hydrolysis; alternatively, some of the determinants located close to the site of enzyme action might be randomly destroyed during digestion.

The definite differences in the behavior of 1/ the Fab fragments, which do not inhibit the reaction at all, 2/ the F(ab')2 fragments, which inhibit partially, and 3/ the Fc fragments, which inhibit completely, lead to the conclusion that some of the xg determinants are located on the region of the heavy chains which is destroyed by pepsin digestion, and which, after papain digestion, is on the Fc fragments; the rest of of these determinants appear to be located in the region of the heavy chains which is not destroyed by pepsin digestion.

The only allotypic patterns known so far to be located on the Fc fragments of rabbit IgG are those of the e series, e14 and e15 (7). The location, in position 309 on the γ chains, of the amino acid residue which differs in e14 and e15 makes it unlikely that the e14 and e15

determinants, which are not found on the Fab fragments, would be present on the F(ab')2 fragments. Now the xg allotypic pattern is found in all $e14^+$ rabbits, and only in them (19.20.21). The difference in behavior of the fragments obtained by digestion agrees with the hypothesis that certain xg determinants are part of the e14 pattern, while others are not. It thus seems that the xg pattern includes a number of determinants larger than the e14 pattern; this agrees with the observation that anti-e14 sera are rarely precipitating, while anti-xg sera are nearly always precipitating. The determinants which are part of the xg pattern and not of the e14 pattern might be of carbohydrate nature; this would agree with the initial observation of the participation of carbohydrate structures in the xg pattern (8), which has been confirmed and extended by recent observations (22).

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