ISOLATION AND ANTIGENIC CHARACTERIZATION OF pFc' FRAGMENTS FROM SIX GENETICALLY DIFFERENT HUMAN G MYELOMA PROTEINS

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1. Introduction

The pFc' fragment* is a dimeric subunit of immunoglobulin G (IgG) released from the carboxyterminal end of the molecule following pepsin digestion. It contains about 230 amino acids, has a molecular weight of 26,000 and thus represents approximately one-half of the Fc region of IgG [4]. It has been actively investigated for several biological [2, 5, 6] and genetic [2, 7] activities known to reside in the Fc region, but so far only limited experiments have been performed with IgG of different subclasses and genetic types.

The genetic (Gm) markers of the γ-chains of the four subclasses of human IgG are as follows: γ_1 chains from the IgG 1 subclass are either Gm (a+ z+) or Gm (f+) "non a" + [8] : γ_2 chains from the IgG 2 subclass are either Gm (n+) or (n-) and both types are, in addition, "non a" + and "non g" + [8] : γ_3 chains from the IgG 3 subclass are either Gm(g+) "non a" + or Gm (b+) "non a" +, "non g" +: γ_4 chains from the IgG 4 subclass do not possess any of the known Gm markers. With the exception of Gm (z) and Gm (f) these genetic antigens are all present in the Fc region [8, 9]. Reagens are available for testing all of the markers except (n-) for which no test system is known.

Recently, we reported that pFc' fragment prepared from pooled human IgG retained both the Gm (a) and "non a" genetic activities [7]. There was also a weakly positive reaction with the Gm (b^o) reagent but we were unable to reach any definite conclusion regarding the antigenic markers of the γ_2 and γ_3 chains. Furthermore, it was not established whether the "non a" activity was derived from each of the IgG 1, 2 and 3 subclasses or whether it originated entirely from the IgG 1 subclass. This uncertainty arises because pooled IgG contains approximately 70% IgG 1 and only 18% IgG 2 and 8% IgG 3.

We now report studies on isolated G-myeloma proteins of the IgG 1, IgG 2 and IgG 3 subclasses and the identification of the "non a" antigen in the pFc' fragments of all these subclasses. In addition, the Gm (b^o) antigen was localized in the pFc' fragment of Gm (b) proteins.

2. Materials and methods

Human G-myeloma proteins were isolated by two-step procedures as follows: zone-electrophoresis on Pevikon block [10] followed by DEAE cellulose batch chromatography [11] for IgG 1 and IgG 3 proteins; zone electrophoresis on Pevikon block followed by DEAE-cellulose gradient chromatography for IgG 2 proteins. The purity of isolated proteins was assessed by (1) immunoelectrophoresis with a polyvalent antiserum to human proteins and (2) Gm tests for contaminating IgG of other subclasses. When detectable,

* pFc' fragment: syn. Pep III' [1] : Component II [2] stFc [3]. No internationally approved nomenclature exists at the time of writing.

Table 1

Class and designation of protein	Protein or fragment tested	Genetic antigens								
		z	a	f	n	g	b^0	b^1	"non a"	"non g"
IgG 1 Gm (f)	IgG	–	–	+	–	–	–	–	+	–
	pFc′ fragment	–	–	–	–	–	–	–	+	–
IgG 1 Gm (a)	IgG	+	+	–	–	–	–	–	–	–
	pFc′ fragment	–	+	–	–	–	–	–	–	–
IgG 2 Gm (n-)	IgG	–	–	–	–	–	–	–	+	+
	pFc′ fragment	–	–	–	–	–	–	–	+	–
IgG 2 Gm (n+)	IgG	–	–	–	+	–	–	–	+	+
	pFc′ fragment	–	–	–	–	–	–	–	+	–
IgG 3 Gm (g)	IgG	–	–	–	–	+	–	–	+	–
	pFc′ fragment	–	–	–	–	–	–	–	+	–
IgG 3 Gm (b)	IgG	–	–	–	–	–	+	+	+	+
	pFc′ fragment	–	–	–	–	–	+	–	+	–

Preparations were tested for inhibitory capacity over the concentration range 0.002–0.5 mg/ml. Positive end-points were all within the range 0.002–0.015 mg/ml and negative end points were all $\geqslant$ 0.5 mg/ml.

such contamination was below a level of approximately 1%.

The optimum conditions for the preparation of pFc′ fragment were determined for each subclass. Pepsin digestion was carried out at pH 4.5 and a temperature of 37°C using an enzyme: substrate ratio of 1:100 in all cases. The maximum yield of pFc′ fragment was obtained at widely different times depending upon the subclass [12]. Thus for IgG 1 proteins, 10–24 hr of digestion was optimum; for IgG 2 proteins 6 hr was used and for IgG 3 proteins 1 hr digestion was sufficient. In every case enzyme action was terminated by adjusting the pH to 8.0 by addition of solid tris salt.

Digested material was fractionated by gel-filtration on Sephadex G-150 as previously described [7] but using experimental conditions giving a high order of resolution. Thus it was not necessary to recycle the pFc′ fragment on Sephadex G-50 as was the case previously [7] because, under the present conditions, the fragment was well separated from the potential contaminant-Fab′ fragment.

The purity of the pFc′ fragment was established by (1) a constant elution position on Sephadex G-150 gel filtration; (2) starch gel analysis in acid urea pH 3.0 [12]; (3) immunological tests using specific antisera and (4) the results of Gm typing.

3. Results

Fig. 1 shows gel filtration profiles of pepsin digested G-myeloma proteins of different subclasses. Preparations of pFc′ fragment were obtained following concentration by ultrafiltration of appropriately pooled fractions.

Haemagglutination-inhibition procedures [13] were used to investigate preparations for the following activities: Gm (z), (a), (f), (n), (g), b^0) and (b^1). In addition, haemagglutination-inhibition assays of the recently described "non a" and "non g" markers [8] were also included. All preparations were tested for inhibitory capacity over a concentrations range of 0.002–0.5 mg/ml and the results of the tests are shown in table 1.

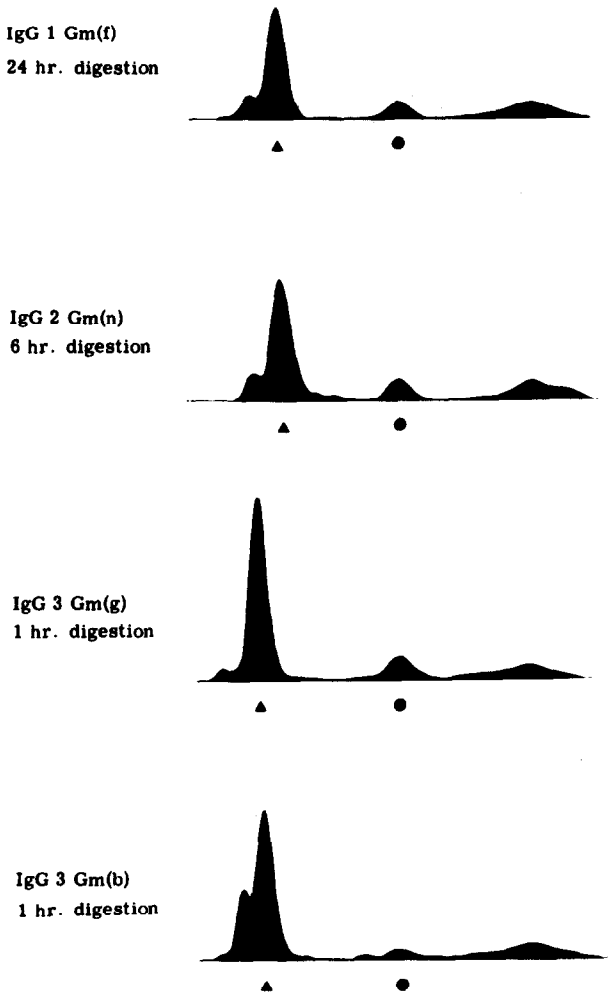

Fig. 1. Sephadex G-150 gel filtration profiles of pepsin digested human G-myeloma proteins. In each case the digestion time employed to give maximum yield of pFc′ fragment (●) is indicated. The elution position of the $F(ab')_2$ fragment (▲) is also shown. Both IgG 3 proteins produced $F(ab')_2$ fragments having elution positions different from those of IgG 1 and IgG 2 $F(ab')_2$ fragments. Possible reasons for these differences are currently under investigation.

Two examples of each genetic variant were tested and results within groups were always consistent. In the IgG 1 subclass the Gm (f+) proteins gave a pFc′ fragment with "non a" activity whilst (a+ z+) proteins gave fragments with Gm(a) activity. These findings confirm those already published for pooled IgG [7]. In the IgG 2 proteins the pFc′ fragments possessed "non a" activity but lacked both the Gm(n) and "non g" antigens. Fragments prepared from IgG 3 Gm (g+) proteins were also "non a" positive but were Gm (g) negative. Finally, fragments from IgG 3 Gm (b+) proteins were "non a" positive and "non g" negative which was in agreement with the findings in the other subclasses. However, these fragments were also Gm (b°) positive whilst lacking Gm (b^1) activity. These results are summarized schematically in fig. 2.

4. Discussion

Several important conclusions may be drawn from these findings. Firstly, pFc′ fragments can be prepared from IgG 1, IgG 2 and IgG 3 proteins. Secondly, the pFc′ fragments from these three subclasses are of similar molecular size since each is eluted in the same position following Sephadex G-150 gel filtration. In addition, starch gel analysis [12] shows that the fragments have similar charge characteristics. Thus it seems that this part of the IgG molecule is very similar in all the major subclasses of IgG and a common feature of all such proteins is a peptide bond susceptible to pepsin digestion near residue 332.

Nevertheless, it remains difficult to evaluate whether a 24-hr pepsin digestion of a pool of normal IgG, as used previously [7], gives rise to pFc′ fragment from either IgG 2 or IgG 3 molecules. Studies on isolated myeloma proteins show that very little or no pFc′ fragment from IgG 2 and IgG 3 proteins remains intact after 24 hr of digestion but the enzyme: substrate ratios for IgG 2 and IgG 3 molecules in a normal pool are very far from 1:100. These facts justify our previous caution and emphasize the value of studying isolated proteins of each subclass.

The detection in IgG 3 pFc′ fragments of the Gm (b°) but not the Gm (b^1) antigen is of great interest because it is the first indication of the possible molecular location of the b antigens. These are a group of factors of which only the Gm (b°) is constantly present in all Gm b proteins. Others, such as the Gm (b^1) (b^3) (b^4) and (b^5), may or may not be present in association with Gm (b°).

The failure to detect Gm (n) (b^1) (g) and "non g" activities in pFc′ fragments may indicate that the

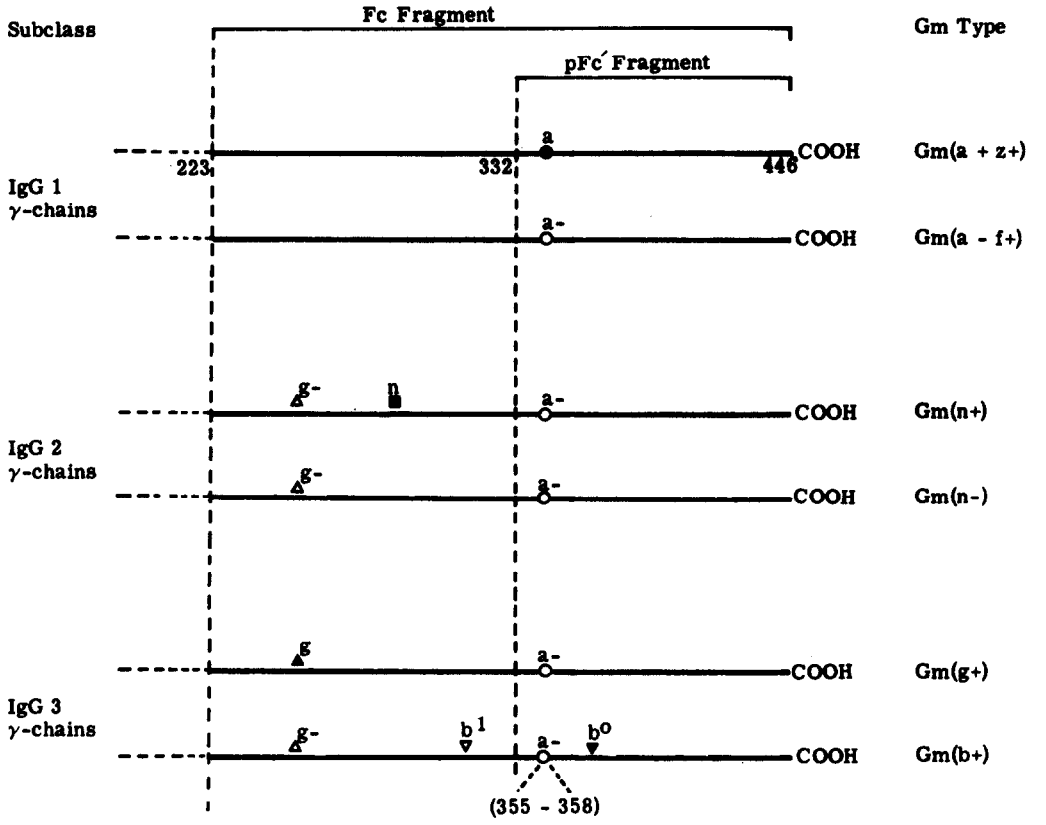

Fig. 2. Schematic diagram of the Fc region of six different γ-chains showing presumed molecular segregation of genetic markers. The approximate locations of the Gm (a) and "non a" sites only are known. The "non a" and "non g" markers are represented as a- and g- respectively. Residue numbers are approximate and based on previously published work [4] and recent sequence data of Edelman et al. [14].

sites responsible for these activities reside in the *N*-terminal half of the Fc region (see fig. 2). Alternatively these activities may require the presence of both halves of the Fc fragment or they may be in the pFc' region but depend for their expression upon some feature of tertiary structure which is destroyed during digestion. The latter explanation is less likely because (1) three other antigens (Gm (a), (b°) and "non a") all survive the digestion process and produce good inhibition in the test systems and (2) there is good evidence that rabbit Pep III' fragment (= pFc' fragment) retains considerable tertiary structure since it may be crystallized [1].

In our previous study [7] we also found b° activity in the smaller, related Fc' fragment (Mol. Wt. 21,000). We are currently investigating this and other similar fragments in the hope of further localizing some of the Gm (b) complex of antigens.

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