Clin. exp. Immunol. (1971) **8**, 685–700.

LOCALIZATION OF Gm MARKERS TO DIFFERENT MOLECULAR REGIONS OF THE Fc FRAGMENT

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(*Received* 7 *January* 1971)

SUMMARY

pFc′ fragments (corresponding to homology region Cγ3) and smaller subfragments produced by trypsin and papain have been studied by haemagglutination inhibition techniques for the presence of a range of Fcγ allotypic markers. The markers which were identified in the pFc′ fragment included Gm(a), (x), (b^0), (b^3), (b^4), (b^5), (c^3), (c^5) and 'non a'. In addition, an IgG 4 marker which possibly occupies the same molecular region as the Gm(a) and 'non a' antigens was detected in pFc′ fragments from IgG 4 proteins.

Limited tryptic cleavage of pFc′ fragment resulting in the loss of approximately seven N-terminal residues had no effect on the antigenic expression of the genetic markers. More extensive degradation of pFc′ fragments with papain yielded Fc′-like fragments which are presumed to lack a C-terminal tridecapeptide as well as eight or nine N-terminal residues. In these fragments antigenic expression of Gm(a), (x) and 'non a' was lost, but Gm(b^0), (b^3), (b^4) and (b^5) antigens were still detectable.

The other allotypic markers of the Fc region (Gm(n), (b^1), (g) and 'non g') were not detected in any of the fragments investigated and it is probable that they are in homology region Cγ2.

INTRODUCTION

Important information about the structure and synthesis of human IgG has been gained through studies with the Gm markers. Recent work suggests that Gm(z) and Gm(f) are present in homology region Cγ1, i.e. the constant part of the Fd region (Asofski *et al.*, 1969) whereas the other Gm antigens, (a), (b), (g), (n) and (x), together with 'non a' and 'non g', are present in the Fc region (homology regions Cγ2 and Cγ3) (Steinberg, 1969; Natvig & Kunkel, 1968; Natvig, Kunkel & Joslin, 1969). We have recently reported that Gm(a) and 'non a' are present in the pFc′ fragment prepared from pooled human IgG (Turner *et al.*, 1969). The pFc′ fragment (Turner & Bennich, 1968) (synonyms: Pep III′

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(Utsumi & Karush, 1965), stFc (Utsumi, 1969) and Component II (Heimer & Schnoll, 1968) is a dimeric subunit situated at the C-terminal end of the molecule and corresponds closely to homology region Cγ3. The location of the other genetic antigens within homology regions Cγ2 and Cγ3 is not known.

We have purified pFc′ fragments from allelic variants of the four IgG subclasses and investigated each preparation for the presence of both Gm and the 'non a' and 'non g' antigens. In addition, a new IgG 4 antigen revealed by a human anti-IgG 4 antibody was studied. In some cases smaller fragments were prepared by tryptic and papain digestion of pFc′ fragments and these were also investigated for the presence of genetic antigens. A preliminary report of some of these findings has recently been presented (Turner, Natvig & Bennich, 1970).

MATERIALS AND METHODS

Isolation of IgG. Human G-myeloma proteins and the total IgG of certain selected normal individuals were isolated by appropriate combinations of the following procedures: zone-electrophoresis on Pevikon block (Fahey & McLaughlin, 1963), DEAE-cellulose chromatography (Stanworth, 1960), DEAE-cellulose gradient chromatography (Fahey & McLaughlin, 1963) and Sephadex G-200 gel filtration. Full details have recently been published (Turner, Bennich & Natvig, 1970). Pooled normal IgG was a generous gift from Kabi, Stockholm.

Pepsin digestion and fractionation of peptic digests. The optimum conditions for the preparation of pFc′ fragments from the various subclasses were defined in a separate investigation (Turner, Bennich & Natvig, 1970). These are as follows:

IgG 1, peptic digestion for 10–24 hr (Enzyme:Substrate = 1:100);
IgG 2, peptic digestion for 6 hr (Enzyme:Substrate = 1:100);
IgG 3, peptic digestion for 1–1½ hr (Enzyme:Substrate = 1:100);
IgG 4, peptic digestion for 2 hr (Enzyme:Substrate = 1:200).

All digestions were performed at 37°C in 0·1 M acetate buffer, pH 4·5. Digestion was inhibited by addition of solid Tris salt to give pH 8·0. Pepsin digestion of normal pooled IgG from individuals with rare IgG 3 genetic markers was carried out for 1 hr as with IgG 3 myeloma proteins.

Pepsin digests of IgG were fractionated by ascending gel-filtration on a column of Sephadex G-150 (3·2 × 92·4 cm; bead size 40–120 μ) using a buffer of 0·1 M Tris–HCl–0·2 M NaCl–2 mM EDTA Na_2, pH 7·7, and containing 0·02% sodium azide (Fig. 1). A constant elution rate of 15·9 ml/hr was employed throughout. The peaks of protein identified as $F(ab')_2$ fragment (see Fig. 1) were concentrated by dialysis against distilled water and lyophilization; peaks corresponding to pFc′ fragment were concentrated by ultrafiltration using collodion sacs (Sartorius Membranfilter, Gottingen, Germany) and peaks containing small peptides were lyophilized directly without previous dialysis.

Trypsin digestion and fractionation of tryptic digests. Isolated pFc′ fragments were digested with trypsin (Sigma Chemical Co., St Louis) for 4 hr at 37°C in 0·05 M Tris buffer, pH 8·4, using an enzyme:substrate ratio of 1:50. The reaction was terminated by the addition of trypsin inhibitor (Sigma Chemical Co.). Samples were dialysed against saline to remove small peptides before testing for genetic antigens.

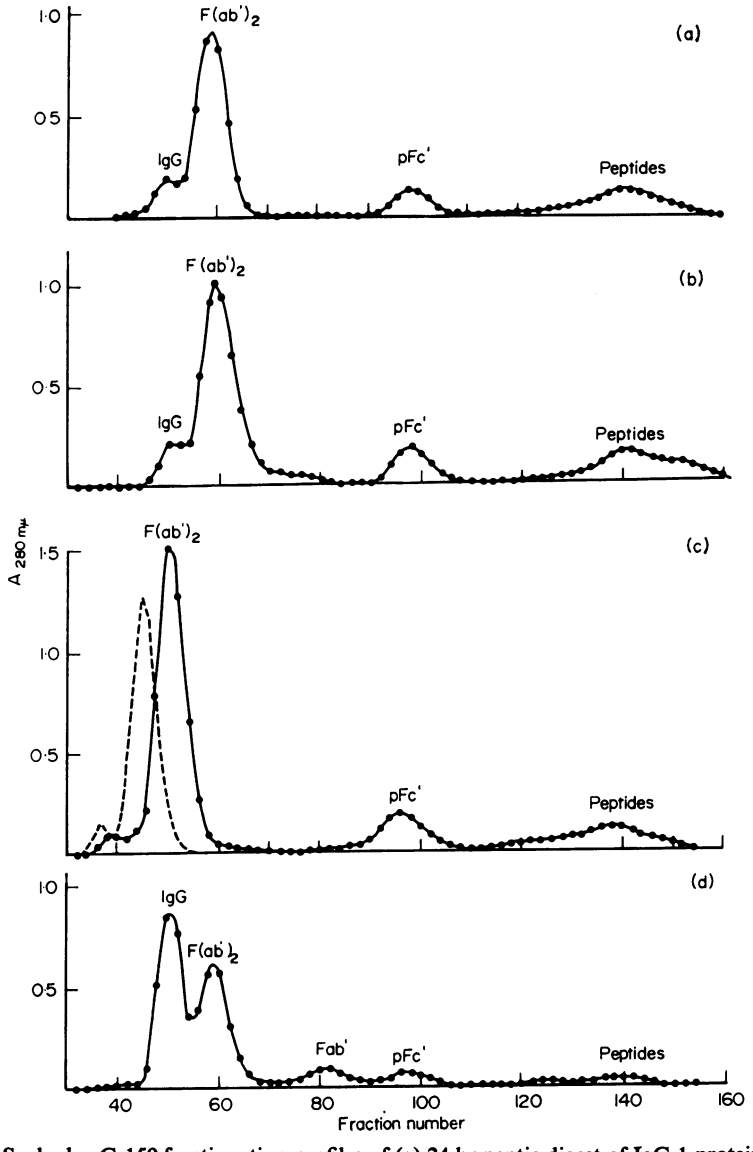

FIG. 1. Sephadex G-150 fractionation profiles of (a) 24 hr peptic digest of IgG 1 protein Hoo; (b) 6 hr peptic digest of IgG 2 protein Pet; (c) 1 hr peptic digest of IgG 3 protein Hus; (d) 2 hr peptic digest of IgG 4 protein Joh. (For conditions of digestion see text.) The elution position of intact IgG 3 protein is shown by the dashed line. Digestion products were identified in an independent study (Turner, Bennich & Natvig, 1970).

Tryptic digests of pooled normal pFc' fragment were fractionated on a column of Sephadex® G-50 (3·2 × 89·5 cm; bead size 20–80 μ) using Tris buffer, pH 7·7, as above. The major protein peak was lyophilized.

Papain digestion. Isolated pFc' fragments from various myeloma proteins were digested with mercuripapain (Sigma Chemical Co.) at 37°C and pH 7·7, using an enzyme:substrate

ratio of 1:200. Digestion was performed in the presence of 0·01 M L-cysteine. The reaction was terminated by the addition of iodoacetamide and samples were dialysed against saline to remove small peptides. In the case of Gm(b) myeloma protein (Lev) samples were taken after 2, 4 and 6 hr of digestion.

Genetic typing. The genetic antigens present in intact proteins and fragments were determined by haemagglutination-inhibition methods with tube or slide techniques employing red cells coated with incomplete anti-Rh antibodies of known genetic specificity. The

TABLE 1. Main test reagents used for Gm typing

Subclass	Genetic antigen	Anti-Gm code No.	
IgG 1	Gm (a)	3070(H)*	and 6(B)
	Gm(z)	953(R)	
	Gm(x)	RO(H)	and Wi(H)
	Gm(f)	876(R)	and A.J.(H)
IgG 2	Gm(n)	2(M)	
IgG 3	Gm(g)	3978(H)	and 755(B)
	Gm(b^0)	2357(H)	and 881(R)
	Gm(b^1)	2247(H)	and Ros(H)
	Gm(b^3)	2277(H)	and Th
	Gm(b^4)	Bu(H)	
	Gm(b^5)	Bu(H)	
	Gm(s)	2624(H)	
	Gm(t)	2939(H)	
	Gm(c^3)	S(H)	
	Gm(c^5)	W(H)	
	other antigens		
IgG 4	'γ non a'	2872(H)	and 4243(H)
IgG 1-2-3	'non a'	4(B)	and 746(B)
IgG 2-3	'non g'	746(B)	

* H, of human origin; R, of rabbit origin; B, of baboon origin, and M, of cynomologous monkey origin.

The following reagents were kindly provided from other laboratories: Anti-Gm(f) A.J. and anti-D Vai from Dr L. Mårtensson; anti-Gm(b^0), (b^1), (b^3), anti-Gm(s) and (t) from Dr E. van Loghem and partly from Dr C. Ropartz, anti-Gm(b^3) and (b^4) and anti-D Wa from Dr A. G. Steinberg, and anti-Gm(c^3) and (c^5) from Dr H. Borel.)

Gm reagents used are listed in Table 1. Some of the anti-Gm reagents of human origin were sera from patients with rheumatoid arthritis but most of the reagents were either from selected healthy individuals or were of animal origin (heteroantisera).

Heteroantisera were made specific for a particular Gm antigen by absorption with selected human sera or isolated myeloma proteins negative for that genetic antigen. The precipitate obtained was spun down and the supernatant used as the anti-Gm serum. Several anti-Gm reagents, subtyping antisera and reagents used for the genetic antigens 'non a' and 'non g' were the same as those described previously (Natvig, Kunkel & Joslin, 1969; Natvig *et al.*, 1968).

In the present investigation intact proteins, $F(ab')_2$ fragments, pFc' fragments and small peptides from each allotypic variant of the four subclasses of IgG were tested for the presence of the following genetic antigens: Gm(a), (x), (z), (f), (b^1), (b^0), (g), (n), 'non a' and 'non g'. Two or three examples of each allotype were tested for all the antigens.

The protein concentrations of the samples to be tested were determined by UV absorption, assuming $E^{1\%}_{280nm} = 14{\cdot}5$. This extinction coefficient represents an approximation since exact E values have still to be determined for the proteins and fragments of each subclass. In some instances there was insufficient sample for UV absorption and the protein concentration was determined by a micro-Folin procedure. A range of doubling dilutions was then prepared (drop-wise) to give the following scale: 500, 250, 120, 60, 30, 15, 8, 4, 2 μg/ml. The lowest concentration of protein or fragment giving inhibition was taken as the end point of each titration.

No anti-Rh antibody belonging to the IgG 2 subclass was available and Gm(n) typing was therefore performed using Gm(n+) myeloma proteins coupled to red blood cells by bisdiazotized benzidine.

The $CrCl_3$ method (Vyas *et al.*, 1968) was used for the detection of the IgG 4 antigen described in this paper. Red cells coated with IgG 4 proteins were shown to be specifically agglutinated by certain rheumatoid sera and the reaction could only be inhibited by other IgG 4 proteins. Similar results were obtained when red cells were coated with an IgG 4 anti-Rh (van Loghem & de Lange, 1970) kindly provided by Dr Erna van Loghem.

EXPERIMENTS AND RESULTS

Genetic markers of pFc' fragments from the four IgG subclasses

In Table 2 are given the results of the haemagglutination-inhibition titrations with intact IgG myeloma proteins, $F(ab')_2$ fragments and pFc' fragments representing each subclass and allotype of human IgG. Two or three examples of each class and allotype were studied and no significant difference was observed in the inhibition obtained between either proteins or fragments of the same allotypic class.

The Fd genetic markers (Gm(z) and (f)) were expressed by appropriate $F(ab')_2$ fragments but were absent from all pFc' fragments. Conversely all $F(ab')_2$ fragments were negative for the Fc genetic markers.

pFc' fragments from Gm(z+ a+) proteins retained Gm(a) activity and, in addition, Gm(x) typing of fragments from pooled IgG and Gm(a+ x+) myeloma proteins showed that the Gm(x) antigen was also present in the pFc' fragment (Table 3). pFc' fragments from IgG 1 Gm(f+) 'non a'+, all IgG 2 and all IgG 3 proteins retained 'non a' activity. In the case of the IgG 2 and IgG 3 proteins studied there was no evidence of contamination with other subclass markers which might bring into question the source of the 'non a' activity (see Table 2).

Although present in all the unsplit IgG 2 and IgG 3 Gm(b) proteins the 'non g' antigen was not detected in any of the pFc' fragments. Similarly, Gm(n) activity was not found in the pFc' fragments of two IgG 2 Gm(n) proteins and no inhibition was obtained using IgG 3 Gm(g) pFc' in the Gm(g) system.

The Gm(b) system is a complex antigenic mosaic which includes Gm(b^0), (b^1), (b^3), (b^4) and (b^5). Of these only Gm(b^0) is found in all Gm(b) proteins. As shown in Table 2 the Gm(b^0) marker was present in the pFc' fragment of three Gm(b) myeloma proteins. Further

TABLE 2. Results of haemagglutination-inhibition titrations with IgG, F(ab')$_2$ and pFc' fragments from the four IgG subclasses

Class	Allotype	No. of proteins studied	Protein or fragment	Lowest concentration (μg/ml) of protein or fragment giving inhibition								
				Fd markers		Fc markers						
				f	z	a	n	b^0	g	New IgG 4 marker	'non a'	'non g'
IgG 1	Gm(f)	3 (Hoo, Cus, Mar)	IgG	2	—	—	—	—	—	—	2–8	—
			F(ab')$_2$	2–8	—	—	—	—	—	—	—	—
			pFc'	—	—	—	—	—	—	—	8	—
IgG 1	Gm(za)	3 (Cha, Hog, Mel)	IgG	—	8	15	—	—	—	—	—	—
			F(ab')$_2$	—	2–8	—	—	—	—	—	—	—
			pFc'	—	—	15	—	—	—	—	—	—
IgG 2	Gm(n−)	2 (Tsh, Mon)	IgG	—	—	—	—	—	—	—	8–15	8–15
			F(ab')$_2$	—	—	—	—	—	—	—	—	—
			pFc'	—	—	—	—	—	—	—	8	—
IgG 2	Gm(n+)	2 (Bri, Pet)	IgG	—	—	—	8	—	—	—	15	8
			F(ab')$_2$	—	—	—	—	—	—	—	—	—
			pFc'	—	—	—	—	—	—	—	8	—
IgG 3	Gm(g)	2 (Hus, Jon)	IgG	—	—	—	—	—	2–8	—	15–30	—
			F(ab')$_2$	—	—	—	—	—	—	—	—	—
			pFc'	—	—	—	—	—	—	—	8–15	—
IgG 3	Gm(b)	3 (Lev, Evl, Man)	IgG	—	—	—	—	8	—	—	30	30
			F(ab')$_2$	—	—	—	—	—	—	—	—	—
			pFc'	—	—	—	—	15	—	—	8–15	—
IgG 4	Gm(−)	3 (Joh, Heb, Wil)	IgG	—	—	—	—	—	—	30–60	—	—
			F(ab')$_2$	—	—	—	—	—	—	—	—	—
			pFc'	—	—	—	—	—	—	30–60	—	—

— means no inhibition at 500 μg/ml.
In each case small peptides (see Fig. 1) were tested and shown to be negative for all the genetic markers (no inhibition at 2000 μg/ml).

studies of two of these proteins (Lev and Man) revealed that the antigens (b^3), (b^4) and (b^5) were also present in the pFc' fragment whereas the (b^1) antigen was absent (Table 4).

Additional studies on rare Gm factors related to Gm(b)

Further studies were performed on myeloma proteins or isolated IgG from normal individuals carrying rare Gm markers related to the Gm(b) system. One myeloma protein (Per) having Gm(b^0), (s), (b^3), (b^5) markers and normal IgG from an individual (Gil) having Gm(b^0), (s), (t), (b^3), (b^5) markers were used. pFc' fragments obtained from both samples were shown to lack Gm(s) and (t) activities, while Gm(b^0), (b^3) and (b^5) were retained (Table 4). Thus, besides Gm(b^1), the antithetic markers Gm(s) and (t) (which substitute for Gm(b^1) in some populations) were absent from the pFc' fragments of IgG 3 Gm(b) molecules.

TABLE 3. Results of haemagglutination-inhibition titrations with intact IgG and various fragments from IgG 1 Gm(a+ x+) myeloma protein Mel*

	Lowest concentration (μg/ml) of protein or fragment giving inhibition		
	anti-Gm(x)	anti-Gm(x)	anti-Gm(a)
	Ro	Wi	B6
IgG	15	30	15
$F(ab')_2$	—	—	—
pFc'	30	30	15
Tryptic fragment	30	30	30
Fc'-like fragment	—	—	—

— means no inhibition at 500 μg/ml.

* Similar results were obtained with fragments of pooled IgG and Gm(a+ x+) myeloma protein De.

No myeloma proteins with the IgG 3 Gm(c^3) and (c^5) markers were available for study and therefore four Nigerian sera with these activities were pooled and the IgG isolated for pepsin digestion. pFc' fragments isolated from this pooled IgG retained the Gm(c^3) and (c^5) activities (Table 4). The immunoglobulins carrying the Gm(s) and (t) or the Gm(c^3) and (c^5) antigens showed similar patterns to other Gm(b) myeloma proteins with respect to other Gm(b) markers and 'non a' and 'non g'.

New antigen of IgG 4 pFc' fragments. During the screening of 315 strongly seropositive rheumatoid sera, forty-eight sera were found to react with IgG 4 coated red cells. The haemagglutination activity present in ten of these forty-eight sera was inhibited only by IgG 4 myeloma proteins and not by IgG 1, IgG 2, IgG 3, IgA or IgM proteins. One agglutinating serum (2872) was particularly studied (Table 5). The anti-IgG 4 activity in this serum was not inhibited by Fab or $F(ab')_2$ fragments from IgG 4 proteins. In contrast, inhibition was seen with IgG 4 Fc fragment. Since the Gm(a) and 'non a' pFc' antigens are often involved in the specificity of rheumatoid factors and are known to be absent from IgG 4, the question arose whether the IgG 4 marker revealed by the above rheumatoid sera might be related to the pFc' region of IgG 4.

TABLE 4. Haemagglutination-inhibition titrations with various intact IgG 3 Gm(b^0) proteins and their pFc′ fragments

Designation of protein	Allotype	Protein or fragment	Lowest concentration (μg/ml) of protein or fragment giving inhibition										
			b^0	b^1	s	t	b^3	c^3	b^4	b^5	c^5	'non a'	'non g'
Lev	Gm	IgG	30	30	—	—	30	—	30	8	—	15	15
	(b^0 b^1 b^3 b^4 b^5)	pFc′	30	—	—	—	8	—	15	8	—	8	—
Man	Gm	IgG	30	60	—	—	30	—	30	8	—	15	15
	(b^0 b^1 b^3 b^4 b^5)	pFc′	30	—	—	—	15	—	30	8	—	8	—
Per	Gm	IgG	2	—	2	—	8	—	—	n.t.	—	30	30
	(b^0 s b^3 b^5)	pFc′	15	—	—	—	15	n.t.	n.t.	n.t.	n.t.	15	—
Gil	Gm	IgG	30	—	8	15	30	—	—	15	—	15	15
	(b^0 s t b^3 b^5)	pFc′	30	—	—	—	30	n.t.	n.t.	n.t.	n.t.	30	—
C.C.	Gm	IgG	60	120	—	—	n.t.	60	n.t.	n.t.	120	30	15
	(b^0 b^1 c^3 c^5)	pFc′	60	—	n.t.	n.t.	n.t.	120	n.t.	n.t.	60	30	—

—means no inhibition at 500 μg/ml.

n.t. indicates fractions not tested because of scarcity of material.

The $F(ab')_2$ fragments isolated from each of the IgG preparations were negative for all the markers shown.

pFc′ fragments isolated from IgG 4 myeloma proteins were negative for all known genetic markers. However, using the test system with IgG 4 coated red cells and the rheumatoid factor with anti-IgG 4 activity, inhibition was obtained with pFc′ fragments from IgG 4 proteins (Tables 2 and 5).

Effect of tryptic digestion of pFc′ fragment. pFc′ fragments from pooled IgG, IgG 1, IgG 2, IgG 3 and IgG 4 proteins were subjected to trypsin digestion for 4 hr and the products of digestion examined by immunoelectrophoresis. The digested fragment was more anodic than the parent preparation in all the samples studied, including the electrophoretically fast IgG 4 pFc′ fragment (Figs 2 and 4). Partial characterization of the tryptic fragment from pooled pFc′ fragment has included determination of amino acid composition, C-terminal amino acid analysis and N-terminal amino acid analysis. This suggests that during tryptic digestion a basic peptide, consisting of approximately seven amino acid residues, is

TABLE 5. Results of haemagglutination-inhibition titrations of new IgG 4 marker* (see text)

Proteins and fragments tested	No. of proteins tested	Lowest concentration (μg/ml) of protein or fragment giving inhibition
IgG 1	10	> 500
IgG 2	10	> 500
IgG 3	10	> 500
IgG 4	10	30–120
IgA	3	> 500
IgM	3	> 500
IgG 4 Fab		> 500
Fc		30
pFc′		60

* Using antiserum 2872 (Table 1).

lost from the N-terminal end of pFc′ fragment (unpublished observations). A similar cleavage is presumed to occur in all the IgG subclasses and the fragments obtained will subsequently be called 'tryptic fragments'.

As expected, the genetic markers 'non g' Gm(g), Gm(n) and Gm(b^1), which were absent from the pFc′ fragments, were also lacking following tryptic digestion. All the markers which were present in pFc′ fragments Gm(a), (x), (b^0), (b^3), (b^4), (b^5) and 'non a' were retained following tryptic digestion (Table 6). The IgG 4 antigen was also retained in the tryptic fragment of IgG 4 pFc′ fragment.

Effect of papain digestion of pFc′ fragments. Papain digestion of pooled pFc′ fragment removes both a C-terminal and an N-terminal peptide to produce Fc′ fragment (Turner & Bennich, 1968). Detailed investigation of the papain susceptibilities of pFc′ fragments from each subclass was not possible in the present study. However, immunoelectrophoretic analysis of samples removed after 2, 4 and 6 hr of papain digestion of IgG 3 Gm(b) pFc′ fragment showed that a fast migrating fragment was readily detectable at 2 hr but not there-

TABLE 6. Results of haemagglutination-inhibition titrations with pFc′ subfragments from the four IgG subclasses

Class and designation of protein	Allotype	Fragment	Lowest concentration (μg/ml) of fragment giving inhibition							
			a	x	b^0	b^3	b^4	b^5	'non a'	IgG 4
IgG 1 (Mel)	Gm(ax)	pFc′	30	30	—	—	—	n.t.	—	—
		Tryptic fragment	60	30	—	—	—	n.t.	—	—
		Fc′-like fragment	—	—	—	—	—	n.t.	—	—
IgG 2 (Pet)	Gm(n)	pFc′	—	—	—	—	—	n.t.	30	—
		Tryptic fragment	—	—	—	—	—	n.t.	30	—
		Fc′-like fragment	—	—	—	—	—	n.t.	—	—
IgG 3 (Lev)	Gm(b)	pFc′	—	n.t.	15	15	8	8	8	—
		Tryptic fragment	—	n.t.	30	15	15	<50	15	—
		Fc′-like fragment	—	n.t.	30	30	60	<50	—	—
IgG 4 (Wil)	Gm(−)	pFc′	—	—	—	—	—	n.t.	—	60
		Tryptic fragment	—	—	—	—	—	n.t.	—	60
		Fc′-like fragment	—	—	—	—	—	n.t.	—	—

— means no inhibition at 500 μg/ml.
n.t. = not tested.

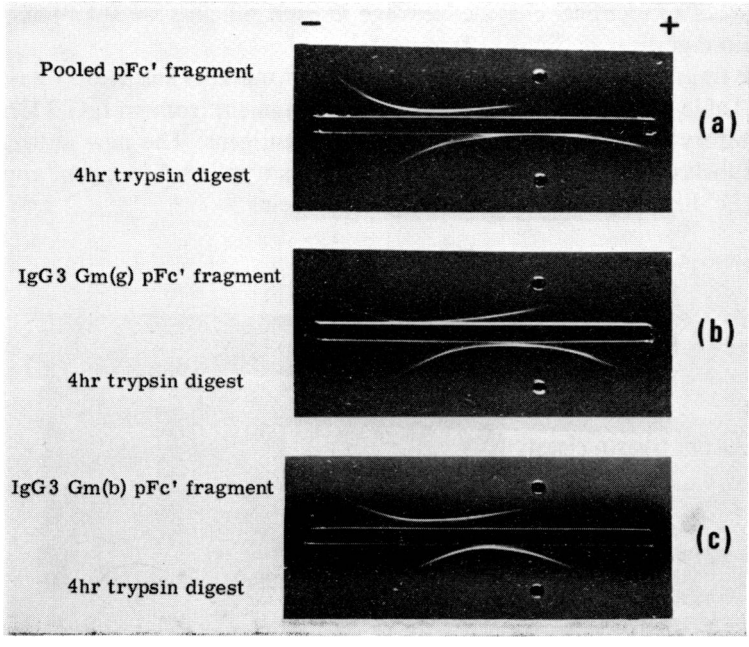

FIG. 2. Immunoelectrophoretic analyses showing effect of trypsin digestion on pFc′ fragments from pooled normal IgG, IgG 3 Gm(g) myeloma protein (Hus) and IgG 3 Gm(b) myeloma protein (Lev). All the troughs contained specific antiserum to pooled pFc′ fragment.

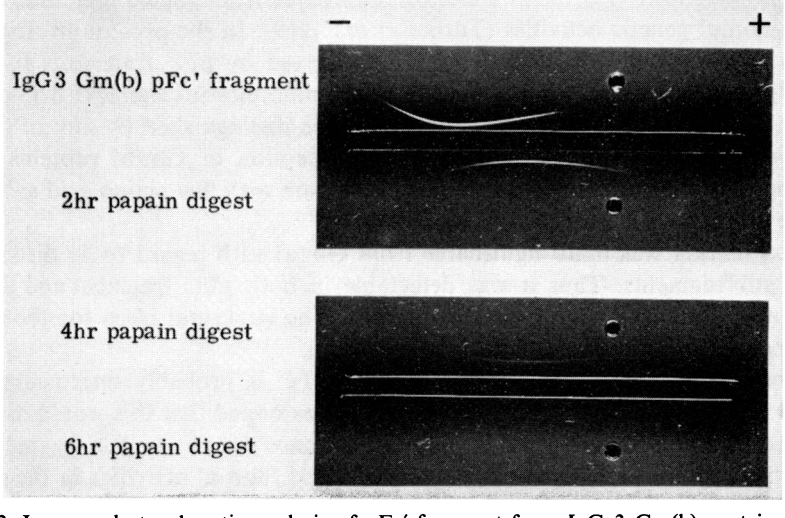

FIG. 3. Immunoelectrophoretic analysis of pFc′ fragment from IgG 3 Gm(b) protein (Lev) undergoing papain digestion. Troughs contained specific antiserum to pooled pFc′ fragment.

after (Fig. 3). On the basis of this result, pFc′ fragments from all subclasses were digested with papain for 2 hr and the products checked by immunoelectrophoresis. A fast migrating fragment was obtained in every case, including IgG 4 pFc′ (see Fig. 4). Since we do not have

biochemical proof of identical enzyme cleavage in each subclass we shall refer to the products of papain digestion as 'Fc′-like fragments'.

The Fc′-like fragments lacked the Gm(a) and 'non a' markers and Gm(x) was also shown to be absent (Table 6). In contrast, isolated Fc′-like fragment from an IgG 3 Gm(b) protein was still inhibitory for Gm(b^0), (b^3), (b^4) and (b^5) antigens. The new antigen described in the IgG 4 subclass which was present in pFc′ and its tryptic subfragment appeared to be absent in the Fc′-like fragment of isolated IgG 4 proteins.

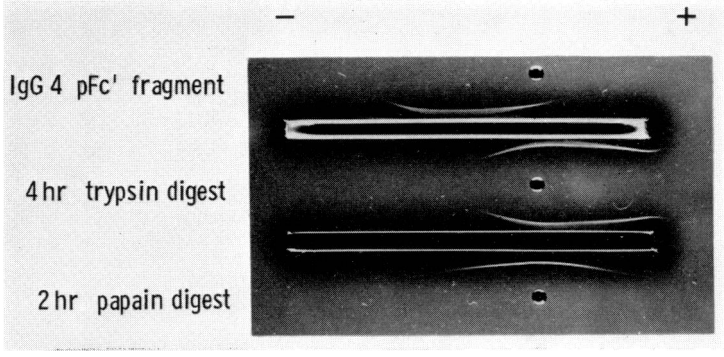

FIG. 4. Immunoelectrophoretic analysis of pFc′ fragment from IgG 4 protein (Wil) undergoing trypsin and papain digestion. Troughs contained specific antiserum to pooled pFc′ fragment.

DISCUSSION

Previous work has shown that the pFc′ fragment prepared from pooled IgG retains both the Gm(a) and 'non a' genetic activities (Turner *et al.*, 1969). In the present investigation the 'non a' marker was found to be antigenically expressed by pFc′ fragments from IgG 1 Gm(f), all IgG 2 and all IgG 3 proteins. All such molecules (except IgG 3 Gm(b) pFc′) carry only the 'non a' genetic marker and cannot be distinguished by any of the known genetic typing reagents. Thus with the possible exception of Gm(b) proteins the pFc′ fragment appears to have retained its antigenic structure with few amino acid substitutions during the evolution of the subclasses.

The Gm(x) marker was indistinguishable from Gm(a) with regard to its distribution in the various subfragments. Thus it was detectable in both pFc′ fragment and the tryptic fragment but was absent in the Fc′-like fragment. The structural basis for the difference between Gm(a+ x+) and Gm(a+ x−) is not known.

The fragment produced by tryptic digestion of pFc′ is probably intermediate in size between the pFc′ and Fc′ fragments (Fig. 5) and it was hoped that this would assist in the localization of some of the antigens. In only one instance was the tryptic fragment able to provide such information. The loss of Gm(a), (x) and 'non a' activities in the pFc′→Fc′ conversion but not in the pFc′→'tryptic fragment' conversion may be cited as further support for our earlier hypothesis (Turner *et al.*, 1969) that certain C-terminal amino acids of the γ-chain (perhaps including residue 434 in the Eu numbering (Edelman *et al.*, 1969)) are involved in the antigenic expression of these genetic markers.

The tyrosine–phenylalanine interchange seen at residue 11 (numbered from the C-terminus) of γ_3 chains (Prahl, 1967) is regarded as an expression of allotypic differences

within the IgG 3 subclass. However, from the present study it is unlikely that this is related to the Gm(g) and 'non g' antigens since these are apparently absent from the pFc' fragment. Nevertheless, the possibility that the Gm(g) and 'non g' antigens incorporate residue 11 cannot be totally excluded. If these antigens are particularly dependent upon tertiary structure they would be readily destroyed during proteolysis and subsequent isolation. This has previously been discussed by Natvig *et al.* (1968) who studied the inhibitory activity of C-terminal octa-decapeptides obtained by cyanogen bromide cleavage between residues 428 and 429 (Eu numbering) (Edelman *et al.*, 1969). The octadecapeptide from Gm(g) heavy

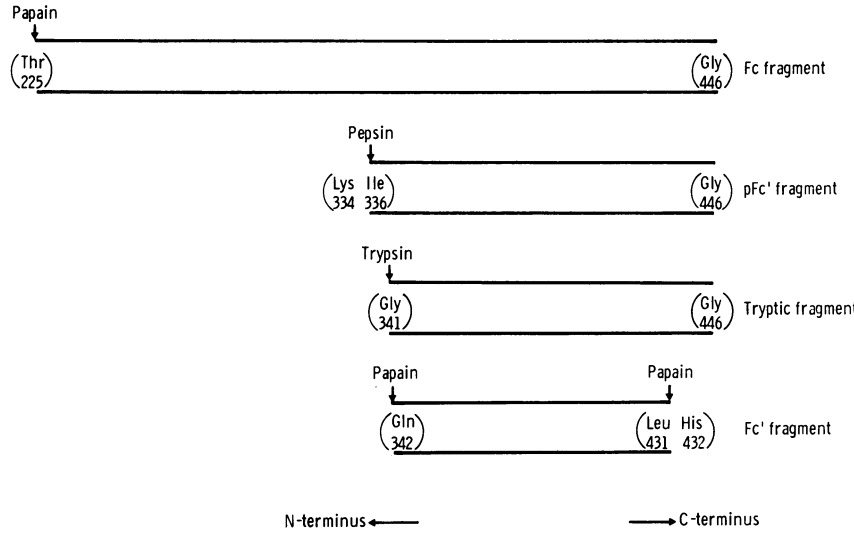

FIG. 5. Schematic illustration of different Fc subfragments showing probable molecular locations. The numbering of residues is based on the Eu sequence (Edelman *et al.*, 1969). Some C- and N-terminal residues are probably of limited variability and dependent on the exact conditions of digestion and the specificity of the enzyme used. Thus glutamic acid (333), lysine (334) and isoleucine (336) have all been detected as N-terminal residues of the pFc' fragment but lysine is generally the predominant residue. Similarly, tryptic digestion of the pFc' fragment may remove only an N-terminal peptide (our observations) or may additionally remove a C-terminal peptide (Matthews, Stewart & Stanworth, 1971). The C- and N-terminal residues of pFc' and Fc' fragments are based on comparison of the Eu sequence with the work of Turner & Bennich (1968), Grey & Abel (1967), Irimajiri, Franklin & Woods (1968); Frangione, Milstein & Franklin (1968).

chains did not inhibit anti-Gm(g) reagents and the octadecapeptide from Gm(b) heavy chains also failed to inhibit several anti-Gm(b) reagents including Gm(b^0), (b^1), (b^3) and (b^4). A recent study of similar peptides from human γ-chains of differing allotype did not include the γ_3 chain markers (Harrington, Hood & Terry, 1970).

The Gm (b^0), (b^3), (b^4), (b^5), (c^3) and (c^5) markers were all found in the pFc' fragment and appear to be related to structural differences between Gm(b) and other IgG molecules. The same markers are also retained in the smaller Fc'-like fragment (see Table 6). It would appear that the Fc' region of Gm(b) molecules has been subjected to several genetic events to give rise to this mosaic of antigens. Their exact location will, of course, require precise

amino acid sequence data. Some of these markers might be related to the interchange in position 436 of the C-terminal octadecapeptide described by Prahl (1967). Although most of the C-terminal octadecapeptide of pooled pFc′ fragment is degraded by papain under the conditions used, it is not yet clear whether this holds for papain digestion of IgG 3 pFc′ fragment

The IgG 3 genetic markers Gm(b^1), (s) and (t) were all absent from pFc′ fragments and this supports the view, based on family and population studies, that these markers are antithetic

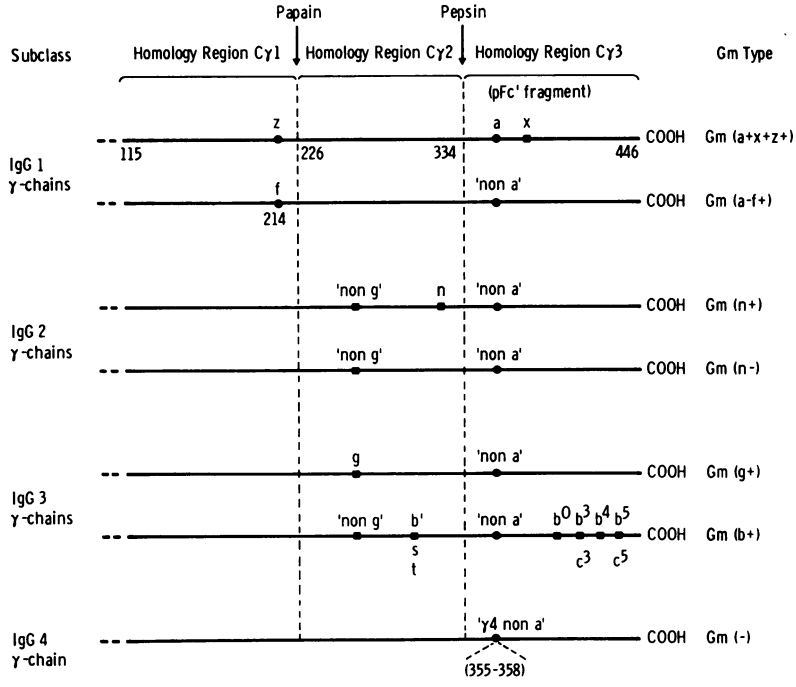

FIG. 6. Schematic representation of the three constant homology regions of the four IgG subclasses. The probable location of Gm(z), (f) and (a) markers as well as the 'non a' and 'γ4 non a' activities are shown by round symbols (●). Square symbols (■) are used to denote genetic markers for which there is no precise sequence location. These markers are segregated according to the data contained in this communication.

(Mårtensson *et al.*, 1966; van Loghem & Mårtensson, 1967; Natvig & Kunkel, 1968). Gm(g) and 'non g' are also presumed to be antithetic and both were absent from pFc′ fragments. Finally, the detection in pFc′ fragments of both (c^3) and (c^5) as well as (b^3) and (b^5) substantiates the view that (c^3) and (b^3) behave as one set of alleles and (c^5) and (b^5) as another.

The pFc′ fragments from IgG 4 molecules were found to be inhibitory in the rheumatoid factor test system specific for IgG 4. There is a strong possibility that this antigenic marker of IgG 4 molecules corresponds to the structural region responsible for Gm(a) and 'non a' antigens in IgG 1, IgG 2, and IgG 3 proteins. IgG 4 proteins have a glutamine residue at position 355 in the Eu numbering (Edelman *et al.*, 1969) whereas all the other IgG subclasses have an arginine residue (Frangione, Milstein & Pink, 1969). Since it is the region between

residues 355 and 358 which primarily determines Gm(a) and 'non a' specificity it seems likely that the IgG 4 marker studied is related to this same region, e.g.:

355	358	Residue number (Eu sequence)
– Arg – *Asp* – Glu – *Leu* –		Gm(a)
– Arg – Glu – Glu – Met –		'non a'
– *Gln* – Glu – Glu – Met –		'γ_4 non a'

Recently two new IgG 4 antigens determined by allelic genes have been described by Kunkel *et al.* (1970) These antigens are not identical to the IgG 4 antigen described in this study (unpublished observations).

Recent sequence data for an IgG 4 protein (Pink *et al.*, 1970) has revealed only three amino acid differences between the γ_1 and γ_4 chains in the pFc' region. This also strengthens the view that the IgG 4 antigenic marker is in the region of residues 355–358 which now appears to be potentially antigenic in the rheumatoid process whatever the subclass of IgG (Gaarder & Natvig, 1970; Natvig & Turner, 1970).

The location of several Gm markers within the pFc' fragment strongly suggests that these antigens are related solely to the protein structure since the fragment is know to lack carbohydrate (Turner & Bennich, 1968). This conclusion is particularly relevant to the Gm(b^0), (b^3), (b^4), (b^5), (c^3), (c^5) and (x) markers for which there is at present no structural data available. Grey & Abel (1970) recently suggested that some of the genetic antigens of human immunoglobulins might be related to carbohydrate residues. The present study has shown that the Gm(n), (b^1), (s), (t), (g) and 'non g' markers are probably associated with structures in the N-terminal half of the Fc region (homology region Cγ2) (see Fig. 6) and this might include certain oligosaccharide residues. However, the strong linkage between all the genetic markers of the Cγ homology regions of all four IgG subclasses (Kunkel *et al.*, 1970) makes it highly probable that these markers are determined by the amino acid sequence.

ACKNOWLEDGMENTS

We thank Mrs Bodil Lunden and Mr A. Medlen for skilled technical assistance. We are indebted to Dr H. Borel, Dr H. Bennich, Dr H. Fudenberg, Dr H. M. Grey, Dr J. R. Hobbs, Dr S. G. O. Johansson, Dr H. G. Kunkel, Dr E. van Loghem, Dr L. Mårtensson, Dr C. Ropartz and Dr A. G. Steinberg for providing some myeloma sera and for supplying certain Gm test reagents. This work was supported by the Norwegian Rheumatism Council and the Anders Jahres Foundation for the advancement of science. M.W.T. gratefully acknowledges receipt of an E.M.B.O. Travel Fellowship.

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