STRUCTURAL STUDIES OF HUMAN γG-MYELOMA PROTEINS OF DIFFERENT ANTIGENIC SUBGROUPS AND GENETIC SPECIFICITIES*

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In 1956 Grubb (1) discovered individual differences in human γG-globulins (7Sγ) detectable by a complex serologic reaction based on the ability of individual γ-globulins to inhibit the interaction of selected anti-γ-globulins with γG-incomplete anti-Rh antibodies, coating Rh_0+ erythrocytes. This finding led to the subsequent delineation of several genetically controlled variants of human γG-globulins, collectively known as the Gm factors (2).[1] In spite of the complex assay system required for their detection, and as yet incomplete knowledge of their precise mode of inheritance (3, 4), a great deal is known about their molecular localization. The Gm factors are present only on certain types of heavy polypeptide chains of IgG (γ-chains), and not found in the IgA and IgM fractions (5–8). The Gm(a) and Gm(b) factors are found in the Fc fragment of the γ-chain (5, 6) while Gm(f) activity appears to be located in the Fd fragment (9).

The precise mode of inheritance of the Gm factors is controversial. Population studies suggest that the Gm factors are determined by multiple codominant alleles at the Gm locus. They are phenotypically recognizable as a series of serologically detectable factors; of these, those studied in greatest detail to date are Gm(a) [Gm(1)], Gm(b) [Gm(5)], and Gm(f) [Gm(4)] (2). A large number of additional Gm factors have been described during the past 10 yr, and new ones are being found with increasing frequency. Two of these Gm(y) and Gm(z), are of greatest immediate significance since they appear to be under the control of the alleles of Gm^a and Gm^f respectively (10).

The discovery of several antigenically distinguishable subgroups of γ-chains, each associated with different Gm factors, has led to another theory of their inheritance (4),

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[1] A complete summary of the currently recognized Gm factors, as well as a recommended new nomenclature for these factors, is listed in the report of the World Health Organization Scientific Committee on Genes, Genotypes and Allotypes of Immunoglobulins. As this report has not yet been generally accepted, both terminologies will be used in this report.

namely that a series of closely linked loci exist, each associated with one of these subgroups. These γ-chain subgroups have been termed We, Vi, Ne, and Ge by Grey and Kunkel (11), and γ_{2b}, γ_{2c}, γ_{2a}, and γ_{2d} in an alternate terminology by Terry and Fahey (12). According to this hypothesis, the We locus controls the synthesis of the Gm(a), (f),(y), and (z) factors; the Vi locus that of Gm(b) and the recently described Gm(g) [21] (38) factor; and the Ne and Ge loci determine the elaboration of genetic factors that have yet to be identified (4). Neither concept (a single locus or four loci) satisfactorily explains all of the serologic and biochemical data presently available. Accordingly, the present studies were designed to provide additional data correlating some structural features with the serologic (Gm) differences and the antigenic heterogeneity of the γ-chains of the human IgG fraction.

Previous studies of peptide maps of γ-globulins from normal Caucasian individuals who were Gm(a+b−), Gm(a+b+), or Gm(a−b+) have clearly demonstrated certain characteristic differences related to the Gm type (13–16). The subsequent identification of a new Gm factor, Gm(f) [4] or (bw) [3], (17, 18), which is universally present in those Caucasians who are Gm(b+) but does not occur in Negroes all of whom are Gm(b+), has clearly pointed out the limitations of studies of normal γ-globulins from a single ethnic group. Furthermore, isolated γG-preparations, even from a homozygous subject, consist of mixtures of at least four subclasses of IgG and may carry, in addition to the currently recognized Gm factors, other genetic markers which have not yet been delineated. Since the more homogeneous proteins produced by patients with neoplastic dyscrasias of plasma cells (myeloma proteins) consist of only a single type of γ-chain (11, 12) and usually carry no more than one of the genetic markers, Gm(a) [1], (b) [5], or (f) [4] (4, 7), it seemed probable that study of such proteins would provide more definitive information on the structural variations of γ-globulins differing in Gm type.

The present study presents the results of peptide map analyses of 36 myeloma proteins and two "heavy chain disease" proteins of the four major γ-chain subtypes and including proteins that were phenotypically Gm(a+b−f−), Gm(a−b−f+), Gm(a−b−f−), and Gm(a−b+f−). In addition, the results of amino acid analyses of some of the peptides characteristic of Gm(a), (b), and (f), and (−) molecules are presented and possible mechanisms for their variations are suggested. The results of these studies of myeloma proteins confirm and, in several areas, extend those previously obtained with normal γG-globulins, and raise a number of questions about the currently postulated mode of inheritance of the Gm factors.

Materials and Methods

Isolation of Myeloma Proteins and Normal γ-Globulin.—γG-myeloma proteins were isolated from sera of 36 patients with multiple myeloma by starch zone electrophoresis (19). Two additional proteins (Zuc; Cra) obtained from patients with "heavy chain disease", were isolated from the urine. Purity was determined by immunoelectrophoresis, using antisera specific for each class of immunoglobulin. In most preparations, only a single type of light or γ-chain was seen on immunoelectrophoresis, and contaminating normal γ-globulin never exceeded 10% of the total protein.

Antigenic Typing.—The four major subtypes of γ-chains were determined by agar double diffusion with antisera prepared to a myeloma protein or heavy chain disease protein belonging to each class and rendered class-specific by absorption with a pool of myeloma proteins of the other three classes and Bence-Jones proteins of Type k and λ.

Gm typing was performed by standard inhibition of agglutination techniques, exactly as described previously (20). Myeloma proteins were tested at concentrations of 2.5, 0.6, and

TABLE I

Summary of 38 Myeloma Proteins Studied

Name	γ-chain subclass	Race*	Gm type‡	Name	γ-chain subclass	Race*	Gm type‡
Lew	We(γ_{2b})	W	a+	Gla	Vi(γ_{2c})	W	b+
Tru	" "	W	a+	Cha	" "	W	b+
Mac	" "	P.R.	a+	Mai	" "	W	b+
Hic	" "	N	a+	Bur	" "	W	b+
Bur	" "	N	a+	Nie	" "	W	(−)
Dee	" "	N	a+	Vi	" "	W	(−) (g+)
Chu	" "	N	a+	Fel	" "	W	b+
Cab	" "	N	a+	Zuc‖	" "	W	b+
Pre	" "	W	f+	Sha	" "	N	b+
Nag	" "	W	f+	Mar	" "	N	b+
Fri	" "	W	f+				
Coo	" "	W	f+	Hof	Ne(γ_{2a})	W	(−)
Schw	" "	W	f+	Wat	" "	W	(−)
Bre	" "	W	f+	Lew	" "	N	(−)
Sch	" "	W	f+	Erw	" "	N	(−)
Vog	" "	W	f+				
Bor	" "	W	f+	Dun	Ge(γ_{2d})	W	(−)
Bac	" "	N	f+	Wur	" "	W	(−)
Burr	" "	N	f+	Moo	" "	W	(−)
Fra	" "	N	f+				
Cra §, ‖	" "	N	(−)				

* N, Negro; W, White; and P.R., Puerto Rican.

‡ Gm a, b, and f tested. Positive results listed. Only Vi was tested for Gm(g).

§ The precise antigenic type is difficult to determine. It most closely resembles the We subtype though it has been previously classified as Ge (7).

‖ "Heavy Chain" Disease.

0.15 mg/ml. "Inhibitors" gave clear inhibition at the lowest concentration while "noninhibitors" failed to inhibit at the highest one.

Preparation of Fc Fragments.—Fc fragments were prepared from myeloma proteins with papain, EDTA, and cysteine exactly as described (21), and separated from the Fab fragments by adsorption to DEAE-cellulose at pH 8, μ 0.01 and elution with 0.3 M NaCl pH 8. Digestion was allowed to proceed for 18 hr in the case of the Ge and We proteins, 24 hr in the case of Ne molecules, and 1 to 4 hr in the case of Vi molecules because of the known lability of their Fc fragment to papain digestion (22, 23). Fc fragments were prepared from all the We(γ_{2b}) and Ge(γ_{2d}) myeloma proteins. Because of their rapid electrophoretic mobility, two of the latter were isolated by starhch zone electroporesis. Only six Vi(γ_{2c}) proteins yielded sufficient Fc

fragments to permit their isolation. Insufficient amounts of protein were available to prepare Fc fragments from two of the Ne(γ_{2a}) proteins. In two others, papain digestion was incomplete, and the Fc fragment could not be separated from the Fab fragment and residual partially digested myeloma protein. Purity of Fc fragments was checked by immunologic techniques The naturally occurring Fc fragments from two patients with heavy chain disease were used as such. Patient Zuc belonged to the Vi class and was Gm(b+) (4). Cra, though previously reported as belonging to the Ge(γ_{2d}) subclass (7) was difficult to classify but resembles We(γ_{2b}) molecules most closely antigenically and by peptide mapping.

Preparation of Heavy Chains.—Heavy chains were prepared from each of the myeloma proteins by reduction in Tris pH 8.2, 0.55 M buffer with 0.75 M mercaptoethanol, followed by the addition of an equal amount of iodoacetamide. Heavy and light chains were separated on Sephadex G-100 in 1 N propionic acid (24). Purity was checked by Ouchterlony analysis with antisera to γ-chains and light chains.

Fingerprinting.—Two dimensional chromatography and electrophoresis of trypsin digests was done (25), using performic acid oxidized preparations. The details of the procedure have been described (13).

Amino Acid Analyses.—In order to obtain sufficient amounts of peptides for amino acid analyses, eight to twelve peptide maps were prepared under identical conditions. One was stained with ninhydrin and used as a reference pattern. The appropriate spot in the same position in the unstained maps was cut out from each of the others and eluted with distilled water and dried on an Evapo mix (Buchler Instruments, Inc., Fort Lee, New Jersey). The rest of the paper was then stained with ninhydrin as a check of the purity of the spot. Each amino acid analysis was performed on the pooled eluates obtained from six to twelve maps. Each peptide was subjected to acid hydrolysis in 6 N HCl at 105°C for 24 hr in sealed glass vials, and amino acid analyses were done on a Beckman Spinco auto analyser, Beckman Instruments, Inc., Palo Alto, California, according to the method of Spackman, Stein, and Moore (26).

RESULTS

Table I lists the antigenic γ-chain subtype and the Gm type of the proteins studied. None of the myeloma proteins was positive for more than one of the factors Gm(a), (b), or (f), and a number were negative for all three. Reagents to test for Gm(y) and (z) were not available (10). However, it seems likely that all Gm(a+) molecules were also Gm(z+) and that Gm(f+) proteins were Gm(y+).

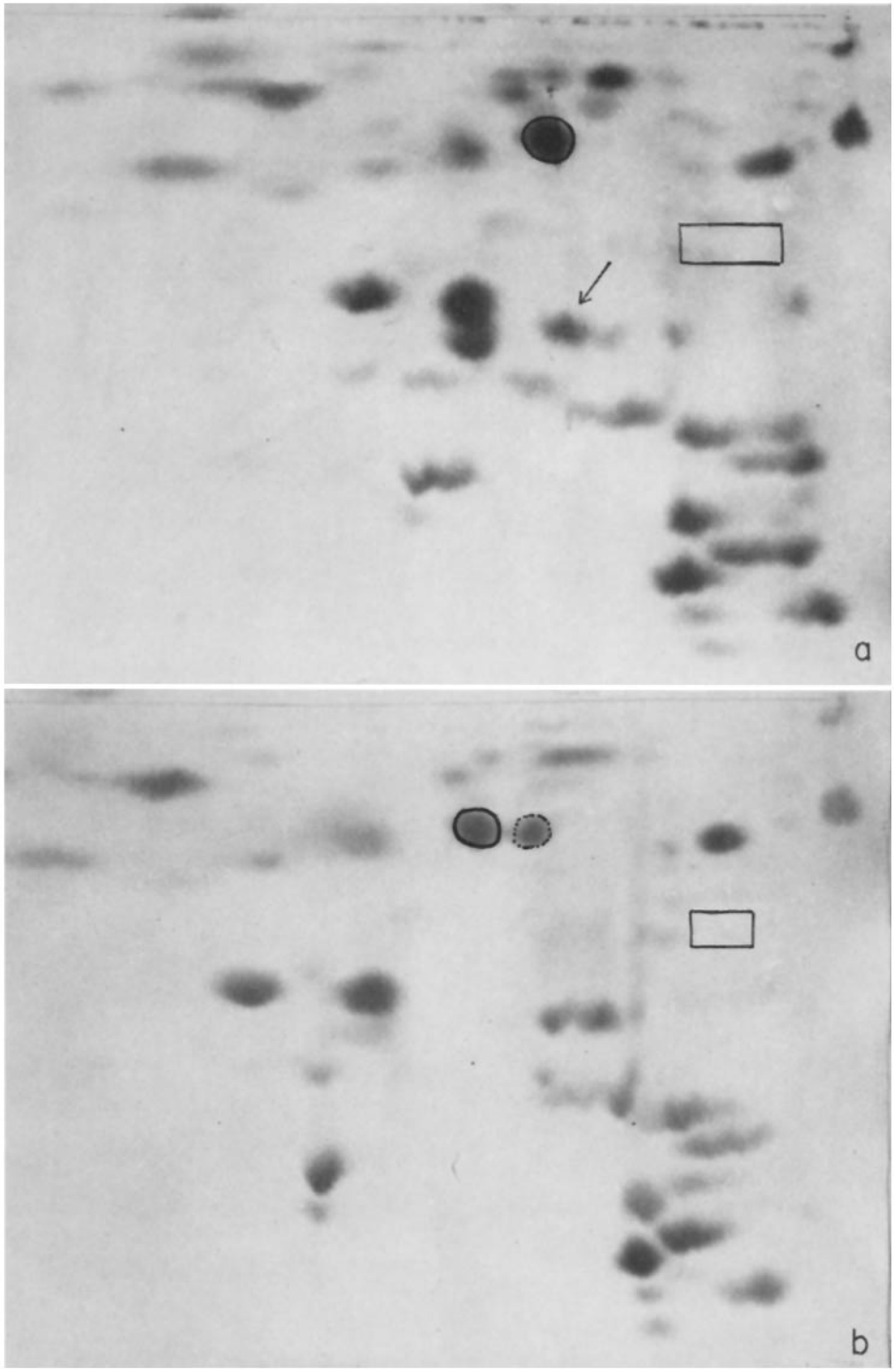
Peptide Maps of Gm(a+) We Myeloma Proteins.—The overall appearance of each of the peptide maps of Fc fragments of eight Gm(a+) myeloma proteins was strikingly similar. Each of the fingerprints had about 25 dark and several lighter peptides. One peptide, encircled in Fig. 1 *a*, and previously correlated with Gm(a) in normal γ-globulins, was present in all of the maps; this peptide was absent in all the maps of Fc fragments prepared from Gm(a−) myeloma proteins or from γG-globulin from normal Gm(a−) subjects. In addition to this major variable, which correlated with Gm(a) positivity or negativity, other minor differences were noted. For example, peptide maps of three proteins (Bur, Dee, and Hic), all obtained from Negro donors, had an extra small spot close to the Gm(a) peptide which could not be related to the other minor Gm factors (Fig. 1 *b*). One of these (Hic) was Gm(c+) while the other two were Gm(c−).

In addition, the peptide map of Dee lacked three peptides usually seen in the area above the Gm(a) spot. Similar results were obtained in three of twelve peptide maps from Fc fragments from normal Gm(a+) donors (13). The significance of this finding is not known, but appears to be related to the differences in the susceptibility of heavy chains of different We proteins to the action of papain (27). Some variability in peptide maps of We Fc fragments was also seen in a group of five to six peptides with variable locations and marked with arrows in Figs. 1 *a* and 1 *c*. In this region, the peptide marked by the arrow was prominent in each of the twelve Gm(a−f+) We maps, but seen in only three of eight of the peptide maps prepared from Gm(a+) We proteins (Fig. 1 *c*). In contrast, peptide maps of heavy chains from the same myeloma proteins showed no variation in this area. This discrepancy suggests that this, too, may be related to the enzymatic digestion with papain.

Peptide maps of heavy chains from the eight Gm(a+) We type myeloma proteins, like those of the Fc fragments, all contained the characteristic "Gm(a)" spot. The map of the H chains of Dee, like that of the Fc fragment of Dee, lacked the three peptides above the Gm(a) spot. In Dee, the other area of variability in the middle of the map was not present. However, this region in maps prepared from heavy chains, as well as the region near the "a" spot (which showed an extra peptide in two of the Negro Fc fragments), are difficult to evaluate due to the variability in the "Fd piece" of different myeloma proteins demonstrated previously (28). None of the peptide maps prepared from Gm(a+) myeloma proteins contained the peptide associated with Gm(b) in previous studies with normal γ-globulins. Thus, the studies with Gm(a+) myeloma proteins are in keeping with the results obtained previously with normal Gm(a+) γ-globulins (13).

Peptide Maps of Gm(f+) We Myeloma Proteins.—The peptide maps of the Fc fragments of the twelve Gm(f+) myeloma proteins were remarkably similar to those of the Gm(a+) proteins, since virtually all the peptides were similar in location. The major difference was the absence of the "a" spot in each of the Gm(f+) maps and the presence of another peptide in the maps from Gm(f+) proteins which was lacking in the Gm(a+) maps (Fig. 1 *c*). For reasons cited below, this peptide will be called "non-a" rather than "f". Fingerprints of heavy chains of the Gm(a−f+) myeloma proteins also showed the presence of the "non-a" spot in all of them. A second, less striking and somewhat inconstant area of variability occurred in the middle of the map and is indicated by arrows in Figs. 1 *a* and 1 *c*. In this region, a peptide, lacking in five of eight Gm(a+) Fc fragments, is clearly visible in each of the twelve Gm(f+) Fc fragments.

Peptide Maps of Gm(a−b−f−) Ge Myeloma Proteins.—Peptide maps of the Fc fragment and heavy chains from three Gm(a−b−f−) myeloma proteins of the Ge type clearly differed from all the others in a number of ways. Firstly,


a
b

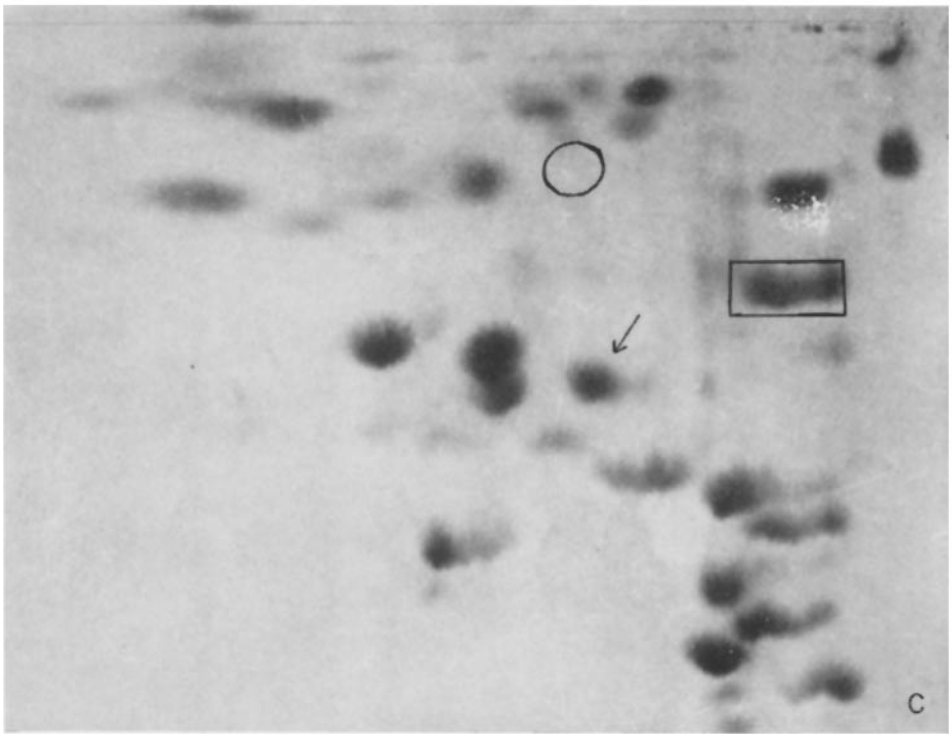


FIG. 1. Peptide maps of Fc fragments prepared from the following: Fig. 1 *a*, Gm(a+) We myeloma (Cab) showing the characteristic single "a" spot (○) and absence of the "non-a" spot (□); Fig. 1 *b*, Gm(a+) We myeloma (Bur) showing the extra peptide (◌) next to the "a" spot (○); and Fig. 1 *c*, Gm (f+) We myeloma (Pre) showing the "non-a" spot (□) and absence of the "a" peptide (○). The arrows (Figs. 1 *a* and 1 *c*) point to the peptide present in all Gm (f+) and only a few of the Gm (a+) maps.

they lacked both the "a" spot and the "non-a" spot, but each had a peptide which moved somewhat further than the "non-a" spot on chromatography but appeared to have the same electric charge as the "non-a" spot (Fig. 2). In addition, they lacked two peptides in the middle of the map and one in the basic region which were prominent in all the Gm(a+), (b+), or (f+) maps. The

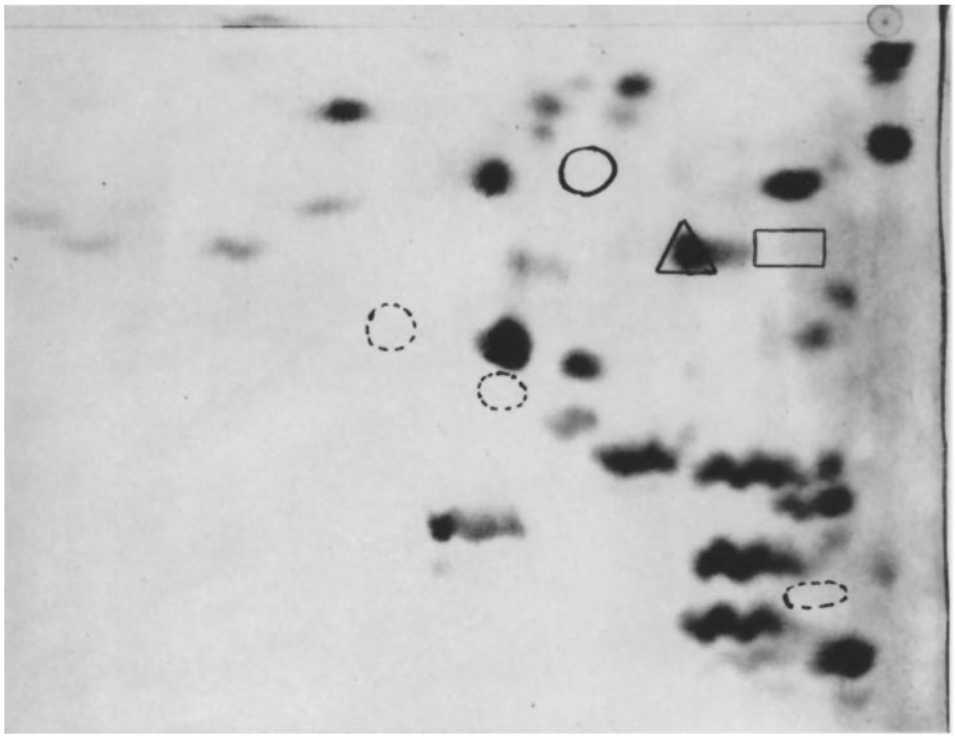


FIG. 2. Peptide map of Fc fragments of Ge protein (Dun) showing the absence of the "a" spot (○) and "non-a" spot (□) as well as three other peptides (◌) characteristic of the Fc fragments from We and Vi proteins shown in Figs. 1 and 3. The peptide marked (△) was present in all three proteins.

heavy chains had eight additional peptides characteristic of the Fd fragment, none of which could be related to the missing ones.

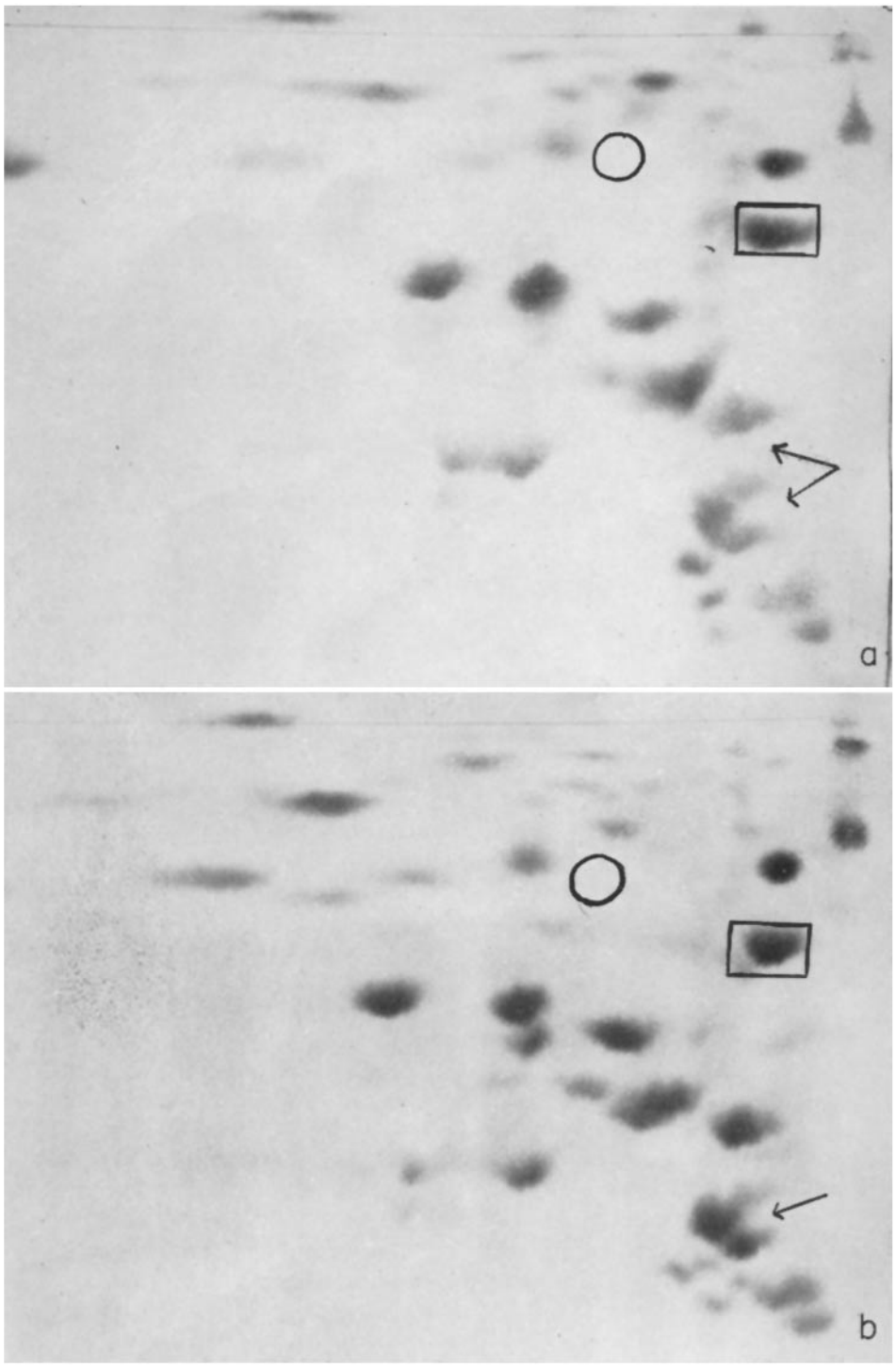
Peptide Maps of Vi Myeloma Proteins.—Each of the fingerprints of Fc fragments of four Gm(b+f−) myeloma proteins lacked the "a" peptide and contained a peptide spot located in the same position as the "non-a" spot, already described for Gm(f+) We proteins (Figs. 3 *a* and 3 *b*). This "non-a" spot appeared identical to the spot previously termed the Gm(b) spot in normal γ-globulin (13). However, since these studies of myeloma proteins show that the

same spot exists in Gm(f+) proteins and certain proteins that are Gm(a−b−f−) (see below), the peptide has been renamed the "non-a" spot. Furthermore, it is evident that this peptide alone cannot distinguish Gm(b+) from Gm(f+) proteins belonging to the We and Vi subclasses as well as certain Gm(−) proteins belonging to the Vi and Ne subclasses. Thus, it is important to note that Gm(b+) myeloma proteins differed in the number and location of several positively charged peptides at the bottom of the map. When compared with We Fc fragments from either Gm(a+) or Gm(f+) proteins, the Gm(b+) proteins lack one peptide, differ in the distribution of the spots beneath this missing peptide, and contain another small extra peptide at the bottom of the map (Figs. 3 *a* and 3 *b*). Therefore, the appearance of the group of peptides at at the bottom of the maps in Figs. 1 *a* to 1 *c* and 3 *a* and 3 *b* and the single peptide just above them generally serve to distinguish We from Vi heavy (γ) chains and Fc fragments. However, there was striking variability also in the appearance of this group of basic peptides in different Vi proteins.

The relation of this area to the "non-a" spots and to the serologic specificity of the parent proteins is not known. A fingerprint of the naturally occurring Fc fragment obtained from a patient (Zuc) with "heavy chain disease" (29) contained the "non-a" peptide and had the same appearance as some of the other Gm(b+) proteins at the bottom of the map (Fig. 3 *b*). Peptide maps of heavy chains of ten different Vi myeloma proteins (eight of which were Gm(b+) and two Gm(g+)) always contained the "non-a" spot. However, this peptide is not as clearly defined in peptide maps of H chains as in maps of the Fc fragments, since in the former the "non-a" area is surrounded by several other peptides derived from the variable Fd fragment. The appearance of the "non-a" spot in Vi proteins was variable, ranging from a single homogeneous, round dark spot (Mai) to a definite doubling in one (Vi) who was Gm(a−g+f−). A number of "non-a" spots from other Vi proteins appeared to be doubled, but not as distinctly as in Vi. This may be due to incomplete digestion of this peptide (30). In general, the greater variability, both in the appearance of the "non-a" and the basic peptides at the bottom in the Vi proteins, is consistent also with the serologic heterogeneity of Gm(b+) proteins (2, 3, 31).

Peptide Maps of Gm(a−b−f−) Ne Myeloma Proteins.—Peptide maps of heavy chains and Fc fragments of four Gm(a−b−f−) myelomas, belonging to the Ne type, contained a peptide in the "non-a" region and lacked the "a" spot (Fig. 4). Although the fingerprints generally resembled those of the We type, they were difficult to interpret since in all the proteins belonging to this group only heavy chains or heavily contaminated Fc fragments were available for study. This appears to be in part due to the incomplete digestion of these proteins even after 24 hr of papain digestion and in part to the rapid mobility of the Fab fragment in several of these proteins.

Amino Acid Composition of "a" and "non-a" Peptides.—The amino acid


a
b

recoveries obtained after 20 hr of acid hydrolysis of "a" and "non-a" peptides are shown in Table II. The data have been expressed as moles of amino acid per mole of lysine, the C-terminal residue in both the tryptic peptides. Values are given only for those amino acids whose yields were at least 10% of that obtained for lysine.

The "a" peptide was isolated from the Fc fragment of a Gm(a+) myeloma (Chu). Its hydrolysate contained one residue of lysine, aspartic acid, threonine, and leucine in addition to one or possibly two residues of glutamic acid. Significant amounts of contamination were apparent in the fractional recoveries of arginine, serine, proline, and glycine; their presence is consistent with the location of several peptides in close proximity to the "a" spot, some of which may have been eluted with the "a" spot. When the "a" peptide from the Fc fragment of pooled normal γ-globulin was analyzed, a similar composition was observed for the neutral and acidic amino acids. However, the amounts of the normal "a" peptide available were too small to determine the content of its basic amino acids.

Analyses were carried out on a representative series of "non-a" spots. The peptides were isolated from: (*a*) the Fc fragment of one Gm(f+) myeloma (Pre); (*b*) the Fc fragment of a Gm(b+) myeloma (Gla) and the heavy chain disease protein (Zuc); and (*c*) from the heavy chain of a Gm(a−b−f−) Ne myeloma (Lew). The results show that the major components in all the "non-a" peptides were identical, each hydrolysate containing one residue of lysine, methionine sulfone, and threonine, and two residues of glutamic acid. Again, significant impurities were present; as expected, the amounts of contaminants in the peptides prepared from the entire heavy chain were larger than those in the peptides prepared only from the Fc portion of the chain.

On the basis of these amino acid analyses, both the "a" and "non-a" spots appear to be pentapeptides which contain three amino acids in common, lysine, threonine, and glutamic acid, and differ in the remaining two. The "a" peptide is characterized by a residue of aspartic acid and leucine while the "non-a" peptide is characterized by a methionine and an additional glutamic acid. It should be emphasized, however, that any asparagine or glutamine present in the peptides would be converted to the corresponding carboxy amino acid after acid hydrolysis. Thus, final definition of the amino acid compositions of these peptides cannot be made until their amide content has been determined.

FIG. 3. Peptide maps of Fc fragments prepared from the following: Fig. 3 *a*, Fc fragment of Gm(b+) Vi myeloma (Gla); and Fig. 3 *b*, naturally occurring Fc fragment (Zuc) from patient with heavy chain disease. Both of these show the presence of the "non-a" peptide (□) and absence of the "a" peptide (○). The arrows point to the region of variability in the basic peptides which generally allows them to be distinguished from maps prepared from We proteins.

DISCUSSION

The conclusions drawn from previous studies (13–15) of chemical differences associated with different Gm factors (Gm(a) and Gm(b)) in normal Caucasian γG-globulins have been limited by the marked heterogeneity of these molecules and the subsequent discovery of new genetic factors some of which, like Gm(f),

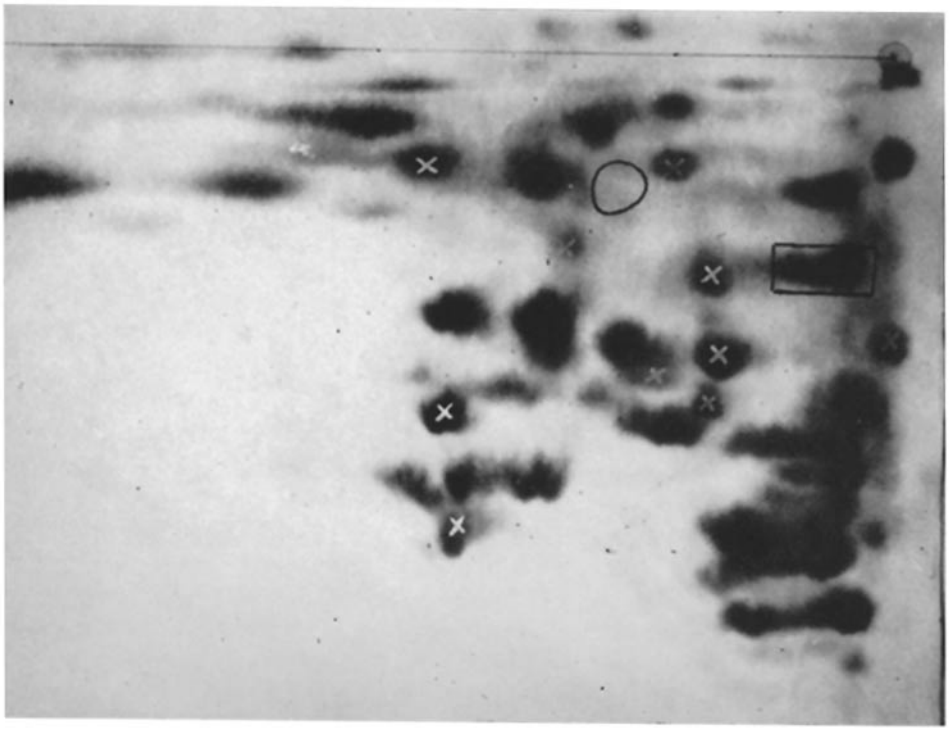


FIG. 4. Peptide map of the heavy chain of a Gm(−) protein of the Ne group showing the presence of the "non-a" spot (□) and absence of the "a" spot (○). The basic peptides are poorly resolved and difficult to evaluate. The peptides marked X were derived from the Fd fragment.

are closely linked to Gm(b) in Caucasians (17, 18). Studies with myeloma proteins circumvent some of the ambiguities inherent in this heterogeneity since any one myeloma protein contains only one of the four recognized subtypes of γ chains (11, 12) and carries only one of the major Gm factors (4, 7, 32). The current studies of peptide maps of 38 such proteins (including representatives of all four antigenic groups), none containing more than one of the factors Gm(a), Gm(b), or Gm(f), confirm the existence of a peptide characteristic of Gm(a+) γG-immunoglobulin molecules. However, the peptide thought to be

characteristic of Gm(b+) γG-globulin was found in all Gm(a−) myeloma proteins of the We, Vi, and Ne subtypes, but not in three myeloma proteins of the Ge(γ_{2d}) type. Since all Gm(b+) Caucasian subjects are also Gm(f+) and since the We Gm(f+) molecules probably make up more than 80% of the total "non-a" molecules of IgG of normal subjects, it seems likely that the major contributors to this "non-a" spot in normal subjects were Gm(f+) molecules, while Gm(b+) molecules, belonging to the Vi subgroup, contributed to a lesser extent to this spot in peptide maps of normal γ-globulins (13–15). Further, the

TABLE II

Comparison of the Amino Acid Recoveries from "A" and "Non-A" Peptides

Peptide	"A"	"Non-A"			
γ-Chain	We	We	Vi		Ne
Gm type	a+	f+	b+		−
Source material	Chu-Fc	Pre-Fc	Zuc-Fc	Gla-Fc	Lew-H
Lys	*1.0*	*1.0*	*1.0*	*1.0*	*1.0*
His		0.33			0.35
Arg	0.18				
Met. sulfone		*1.1*	*1.1*	*0.99*	*0.93*
Asp	*1.2*	0.28	0.15	0.41	0.54
Thr	*1.1*	*0.96*	*1.0*	*0.99*	*1.0*
Ser	0.43	0.28	0.17	0.24	0.38
Glu	*1.4*	*2.1*	*2.0*	*2.2*	*1.9*
Pro	0.25				0.12
Gly	0.44	0.20	0.23	0.36	0.49
Ala		0.21			0.30
Val		0.25			0.37
Leu	*1.1*	0.30			0.24

amino acid composition of the "non-a" spot was identical in all Gm(a−) myeloma proteins belonging to the We(γ_{2b}), Vi(γ_{2c}), or Ne(γ_{2a}) subclass, whether positive for Gm(b), positive for Gm(f), or negative for both factors. Two of the five amino acid residues of the "non-a" spot (glutamic acid and methionine) differed from the residues in the "a" peptide (aspartic acid and leucine). Thus, these studies show that the single peptide difference previously observed can distinguish only between Gm(a+) and Gm(a−) globulins belonging to three of the subclasses. Independently of the work presented here, Thorpe and Deutsch (30) have isolated the "a" and "non-a" peptides from two We myeloma proteins and determined their sequence. Their findings, and the amino acid compositions reported above, are in excellent agreement.

In addition, these studies of myeloma proteins furnish new information on

several points. Firstly, the "non-a" spots in the peptide maps of Fc fragments and heavy chains do not readily distinguish between Gm(b+) and (f+) proteins, thus suggesting strongly that the "non-a" peptide is not responsible for the serologic specificity. Since Gm(f) activity is localized to the Fd fragment of the heavy chain while Gm(b) resides in the Fc fragment (9), it seems possible that a peptide characteristic of Gm(f) activity may, indeed, occur in the Fd fragment, but the marked variability of this portion of the chain renders identification of this specific peptide extremely difficult. In the case of Gm(b), a peptide characteristic of Gm(b) activity would be expected to exist in the Fc fragment and, indeed, may be present in the variable basic spots in the bottom of the map which distinguish We proteins from Vi molecules, but do not readily lend themselves to accurate interpretation; or it may reside elsewhere in the peptide map, but obscured by other peptides.

Another possibility is that the peptide characteristic of Gm(b) activity arises from a sequence of the five amino acids, different from that present in the Gm(b−) "non-a" peptides. However, this interpretation would require at least three different sequences for the "non-a" peptides, one for the Fc fragment from Gm(f+) We(γ_{2b}) proteins, one for the Fc fragment for Gm(b+) Vi(γ_{2c}) proteins, and one or more for the Fc fragment from Gm(b−f−) proteins of the Vi(γ_{2c}) and perhaps of the Ne(γ_{2a}) subclass. Therefore, an explanation based on identical amino acids arranged in different sequence seems improbable, and it seems unlikely that this peptide is responsible for serologic specificity. Information on the sequences of all these peptides is currently not available since Thorpe and Deutsch (30) have reported the sequence of the "a" spot and the "non-a" spot only from a Gm(f+) protein, but have not yet studied this peptide from a Gm(b+) or a Gm(b−f−) protein.

The second type of information revealed by peptide mapping of the myeloma protein Fc fragments are the differences correlated with the antigenic subclasses. In general, there were striking similarities in the majority of the peptides in all four classes currently described. For example, maps of all We(γ_{2b}) Fc fragments were very similar except for the single peptide difference between Gm(a+) and Gm(f+) myeloma proteins discussed above, and some minor variability in the middle of the map. Maps from the Fc fragments of Vi(γ_{2c}) proteins differed from the Gm(f+) We molecules only in a group of basic peptides at the bottom of the map. Studies on proteins of the Ne(γ_{2a}) type were limited because of the small number of pure samples available. However, in general, the peptide maps resembled those of We Gm(f+) molecules. Of special interest is the striking difference in the peptide maps of three Ge(γ_{2d}) type Fc fragments which lack several peptides characteristic of the other three classes. Though additional examples are necessary to confirm this observation, the data suggest that the Ge subgroup differs strikingly from the others. Since Ge proteins appear to be of more rapid mobility, the Ge group is perhaps analogous to the γ_1-fraction in other species (33).

Thirdly, minor differences were observed in Fc fragments belonging to the same class, which might perhaps be related to unknown Gm classes or to intra-class variation on another basis. These were most striking in the case of the Vi(γ_{2c}) molecules which are also most heterogeneous by serologic tests. However, some variability was also noted in certain peptides within each of the other three subgroups. Whether these reflect true structural differences or possibly artifacts of the fingerprinting technique remains to be determined.

Sufficient data are not yet available to permit detailed interpretations of the observed structural differences related to the Gm factors. Comparison of the amino acid composition and sequence of the "a" spot with that of "non-a" spots shows similarities in three of the amino acids (lysine, threonine, and one residue of glutamic acid), and the replacement of an aspartic acid or asparagine and a leucine residue in the "a" spot by a methionine residue and a glutamic acid or glutamine in the "non-a" spots. The possibility exists that these changes may be the result of point mutations of a structural gene, as is the case for hemoglobins (34). However, in the absence of definitive proof for such a mechanism, alternative possibilities must be considered. Among these is the possibility that the two peptides are derived from each other by other mechanisms recently thought to account for the variability in antibody structure, e.g. non-homologous crossing over, unequal homologous crossing over, interchromatid inversion with reversed complementarity (35, 36), insertion of inactive DNA (37), and possibly other mechanisms. Another possibility which may explain the existence of at least three nucleotide substitutions is the possibility that the "a" and "non-a" peptides are not directly related but have evolved from each other through a number of intermediate stages which remain to be identified. Further speculation seems unwarranted until the amino acid sequence of the pertinent peptides is known and the precise mode of inheritance of these factors is fully elucidated.

SUMMARY

1. Peptide maps of Fc fragments or heavy chains of 36 G myeloma proteins and two "heavy chain disease" proteins belonging to the four γ-chain subgroups revealed very striking similarities between them. However differences in a few peptides were noted. This was most pronounced for the Ge(γ_2d) subgroup which lacked three peptides characteristic of the other three subgroups. While Fc fragments from different proteins belonging to the same subgroup appeared very similar, minor differences in addition to those based on currently recognized Gm factors were occasionally noted.

2. Fc fragments from Gm(a+) We(γ_2b) proteins had a peptide previously shown to be characteristic of normal Gm(a+) γG-globulins. Fc fragments from Gm(a−) molecules belonging to the We(γ_2b), Vi(γ_2c), or Ne(γ_2a) subgroups, whether Gm(b+), Gm(f+), or Gm(−), had the peptide previously identified in Gm(b+f+) normal γG-globulin. This "non-a" peptide was absent in pep-

tide maps from Gm(−) molecules of the Ge(γ_2d) subgroup which contained instead another peptide with the same electrophoretic mobility but migrating slightly further on chromatography.

3. Both the "a" and "non-a" peptides were pentapeptides having three amino acids in common, and differing in the other two. The "a" peptide contained one residue of lysine, aspartic acid, threonine, leucine, and glutamic acid. The "non-a" peptides prepared from Gm(b+), Gm(f+), and Gm(−) proteins were identical and contained one residue of lysine, threonine, and methionine sulfone, and two residues of glutamic acid.

4. Several possible mechanisms for the origin of these differences, and their possible role in serologic specificity are discussed.

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