STUDIES OF THE Vi (γ_{2c}) SUBGROUP OF γ-GLOBULIN

A Relationship between Concentration and Genetic Type Among Normal Individuals*

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Previous studies have demonstrated that human γG-globulin consists of at least four subgroups based on antigenic differences in their heavy polypeptide chains (1, 2). Two of these, We and Vi types (γ_{2b} and γ_{2c}), have received major emphasis primarily because of a variety of genetic antigens associated with each type. It has been demonstrated that Gm(a), (x), (z), (f), and (y) are all limited to the We class and that Gm(b^1), (b^3), (b^4), (c), (s), and (t) are limited to the Vi class (3–7). In view of the extensive studies in various populations of some of the various Gm(b) factors (8) and their selective occurrence on molecules of the Vi type (9), the complete delineation of this subgroup assumed added significance.

The present studies were undertaken because a number of primate as well as rabbit antisera had become available which characterized the Vi subgroup in a variety of ways. Quantitative analyses of the amount of this protein in normal sera from several populations were carried out. In the course of these investigations a relationship was found between the genetic type of an individual and the quantity of Vi subgroup γ-globulin in the serum.

Materials and Methods

Multiple Myeloma Proteins.—Serum or plasma was obtained from patients with an established diagnosis of multiple myeloma. The globulin peak was isolated from other serum proteins by zone electrophoresis on starch medium (10), or polyvinyl copolymer (Pevikon). In certain instances further purification was carried out by gel filtration on Sephadex G-200 equilibrated with 0.1 M tris(hydroxymethyl)aminomethane (Tris) and 0.5 M sodium chloride. Isolated proteins were concentrated by vacuum or pressure dialysis.

Human Sera.—Random samples were obtained from 523 Caucasian, 21 Negro, 48 Chinese, 39 Easter Island, and 86 Indian (Bombay) donors, primarily through the aid of Dr. Alexander Bearn and Dr. Fred Allen. Sera were also obtained from patients with increased and decreased levels of γ-globulin quantitated by cellulose acetate electrophoretic scanning.

Enzymatic Cleavage of γ-Globulin.—Fc and Fab fragments of myeloma proteins were pro-

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duced by papin digestion (11) using a ratio of protein to enzyme of 100:1. Digestion for 1½ hr at 37°C was carried out in the presence of 0.01 M cysteine and 0.002 M EDTA, pH 7.5. It was stopped by removal of cysteine by dialysis against cold saline.

Immunological Techniques.—Ouchterlony agar diffusion (12), microimmunoelectrophoresis (13), and quantitative precipitin analysis (14) were carried out as described previously (1).

Antisera.—Antisera to several isolated Vi myeloma proteins were produced by immunizing animals with antigen emulsified with an equal volume of complete Freund's adjuvant. Multiple injections of 2–5 mg were given subcutaneously over 3–6 months or more. Nine different antisera to Vi subgroup proteins were used in these studies.

1–4—Rabbit anti-Vi (R Vi_1, R Vi_2, R Vi_3, R Vi_4): Animals were immunized with isolated myeloma protein from patient Vi, which was Gm(b−), R Vi_1 was used for the majority of quantitative radial diffusion analyses.

5–7—Rabbit anti-Fe and anti-Jo(R Fe, R Jo_1, R Jo_2): These antisera were prepared against Gm(b+) myeloma proteins Fe and Jo of the Vi subgroup.

8—Monkey anti-Zu (M Zu): A cynomologous monkey was immunized with isolated Gm(b+) γ-globulin peak protein from a patient with "heavy chain" disease (15) provided by Dr. Elliot Osserman.

9—Baboon anti-Vi (B Vi): Antiserum against the Gm(b−) myeloma protein, Vi, was prepared in a baboon. This antiserum, prepared by Dr. Moor-Jankowski, was used in radial diffusion plates with a selected number of sera.

Gm Typing.—Gm typing was performed using slide hemagglutination inhibition techniques. Fresh, washed O Rh+ red blood cells were coated with incomplete anti-D antiserum [Ri for Gm(a), HAD for Gm(b), at a dilution of 1:5]. Agglutinator Smej., obtained from a normal serum, was used for Gm(a) typing. Rabbit antiserum R Fe was used for Gm(b) typing as previously described (16). The Gm(b) system recognized antigens Gm(b^1), (b^3), and (b^4).

Quantitative Radial Diffusion Technique.—Concentrations of Vi protein in whole serum were determined chiefly by radial immunodiffusion (17, 18). Antiserum R Vi_1, absorbed with a We subgroup myeloma protein of the λ type, was mixed with molten 2% agarose agar and 0.1 M Veronal buffer, pH 8.6, in a 56°C constant temperature bath in volume ratios of 1:5:4. Agar plates of uniform depth (either 1 or 2 mm) were made by pipetting the mixture into molds consisting of two glass 8 × 10 cm slides separated by a three-sided metal frame machined to a uniform thickness and coated with silicone lubricant to ensure a tight seal. Thirty wells 2.8 mm in diameter were filled with standard solutions of isolated Vi subgroup meloma proteins or serum specimens using 5 μl (plates of 1 mm thickness) or 10 μl (plates of 2 mm thickness) pipettes. The filled plates were incubated in a humid chamber for 16 hr at 37°C and photographed in a dark field. The diameter of the precipitin rings was measured to the nearest 0.1 mm. The minimum concentration of Vi protein detectable without staining the plates was 10 μg/ml. A standard curve was prepared for each plate by plotting precipitin ring diameter vs. log concentration of six standards ranging from 0.2 to 4.0 mg/ml. Standards were prepared from isolated Vi subgroup meyloma proteins giving a discrete precipitin arc with subgroup-specific antisera and showing no contaminant arcs at high concentration with antisera for other γG-, γM-, or γA-immunoglobulins. Protein concentrations were determined using Folin phenol reagent (19) and by nitrogen determination.

Within 95% confidence limits, ring diameter for standard solution varied from 3.2 to 9.8% of the total diameter on multiple determinations, with an average variation for all standards of 4.8%. Repeated determinations of individual serum specimens on single or multiple plates gave a standard error of 5.5% when four or more determinations were done, and 8% with duplicate determinations of 130 specimens.

Proteins of the Vi subgroup were found to be more labile than those of the Ne (γ_{2a}), We (γ_{2b}), and Ge ($\gamma 2_d$) subgroups. Both isolated Vi myeloma proteins and Vi proteins in normal

sera were found to split when stored at 4°C, especially with repeated exposures to room temperatures. Special precautions to prevent this were taken, and most studies were conducted on fresh sera or sera stored at −4°C for short periods with sodium azide 0.1% used as a preservative.

RESULTS

Reactions with Different Antisera.—All of the nine antisera gave similar results after absorption with myeloma proteins of the major We subgroup. They reacted specifically with a panel of eight Vi myeloma proteins and failed to react with proteins of the We and Ge subgroups. The antigens specific for Vi myeloma proteins detected by these antisera were of two general types which depended in part on the character of the antigen used for immunization. In most instances where immunization was carried out with whole Vi myeloma proteins, the specific antigen was found in the Fab fragment produced by papain splitting. This antigen, as described previously (1), was very sensitive to sulfhydryl reduction. Two antisera to whole myeloma proteins, R Fe and B Vi, not only recognized the Fab antigen but also showed specificity for the Fc fragment. Antiserum M Zu showed only Fc specificity.

The antisera reacting with the Vi antigen on the Fab portion of the molecule gave identical results with all of the Vi proteins studied and with normal sera. However, two of the three Fc-specific antisera showed small differences among the myeloma proteins and normal sera. These Fc-specific antisera were produced by absorption of the antiserum with We-type myeloma proteins and with the pepsin-treated myeloma protein used for immunization. Slight reactions with proteins of the Ne class were obtained, indicating that the Vi and Ne types possessed antigens in common, not found in the major We group. This finding was brought out more clearly with several antisera to Ne proteins, which showed strong cross-reactions with Vi proteins after absorption with We proteins. These observations will be published in further detail later.[1] Absorption with both We and Ne proteins was required for complete specificity with certain of the antisera reacting with the Fc antigens.

The Fc-specific antisera showed small spurs between Vi myeloma proteins and between normal sera which were related to the genetic type of the proteins under study. Antiserum R Fe, which was produced by immunization with a Gm(b+) myeloma protein, showed that Gm(b+) myeloma proteins reacted more intensely than Gm(b−) myeloma proteins of the Vi class, with suggestions of spur formation. This distinction was made more evident by partial absorption of the antiserum with the isolated Gm(b−) myeloma protein, Vi. Normal sera also showed this difference, and complete agreement between the Gm(b) type and the precipitation pattern was obtained. Similar differences in just the reverse direction were obtained with antiserum B Vi, which was made against the Gm(b−) myeloma protein, Vi. Here again the antiserum was partially

[1] W. J. Yount, S. D. Litwin, and H. G. Kunkel. Unpublished observations.

absorbed with a homozygous Gm(b+) serum after previous absorption rendered it specific for the Fc fragment of Vi proteins. Under the conditions of the experiments a distinct line was given by the Gm(b−) sera and little or no precipitation was observed with the Gm(b+) sera. The line observed with the Gm(b−) sera showed a reaction of complete identity with myeloma protein Vi, which

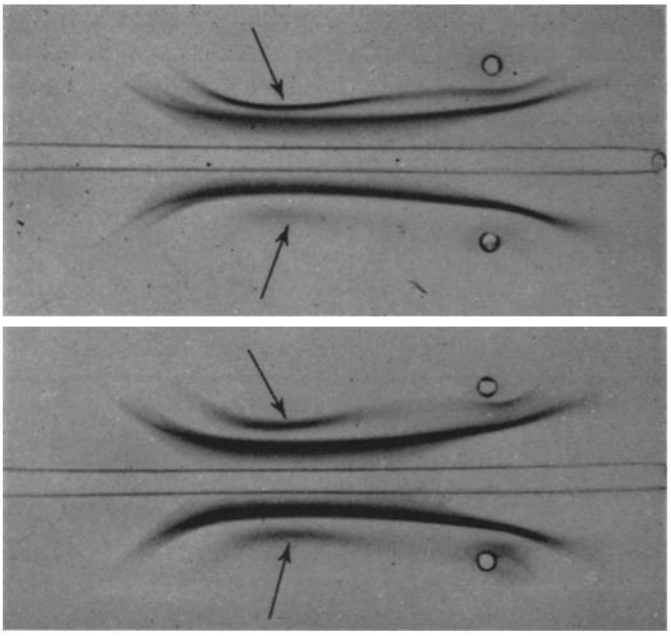

FIG. 1. Immunoelectrophoresis patterns of Vi subgroup proteins in four normal sera (1–4 in descending order). Each trough contains unabsorbed antiserum R Vi, made against an isolated Vi subgroup myeloma protein. The precipitin arcs specific for the Vi subgroup are indicated by arrows. Wells 1 and 3 contained Gm(b−) sera; wells 2 and 4, Gm(b+) sera. The lower two wells contain normal sera representative of the extremes in concentration of Vi subgroup proteins.

was also Gm(b−). In a study of 80 normal sera, there was complete correlation with the Gm(b−) type. This antigen, detected by precipitation methods, appeared to be identical with the Gm(g) factor described recently by Natvig (20).

Differences in Concentration of Vi Protein in Normal Sera.—Since all myeloma proteins of the Vi class, as well as normal sera, showed reactions of complete identity when antisera specific for the Fab portion of Vi proteins were used, an antiserum specific for this area of the molecule was utilized for quantitation of Vi-type proteins in normal sera. This antiserum was considerably stronger than those detecting the Fc antigens. Some differences in concentration were apparent from immunoelectrophoresis experiments with unabsorbed antisera. Fig. 1

illustrates the reaction of four normal sera with R Vi_1, and the Vi component is visible in each serum. The upper slide shows the component in a Gm(b−) serum and a Gm(b+) serum. The lower slide illustrates extreme levels selected from a group of normal sera. All sera studied showed the Vi component but it appeared that Gm(b−) sera tended to have lower concentrations. Another characteristic of the Vi proteins in both normal sera and in myeloma sera was partial absorption near the well of origin. This is seen particularly well in the bottom pattern of Fig. 1. Vi myeloma proteins isolated by electrophoresis in a polyvinyl medium from a sharp, slowly migrating peak also showed this biphasic pattern in the agar system. Five different antisera (R Vi_1, R Vi_2, R Vi_3, R Jo_1, R Jo_2) made against both Gm(b+) and Gm(b−) myeloma proteins of the Vi

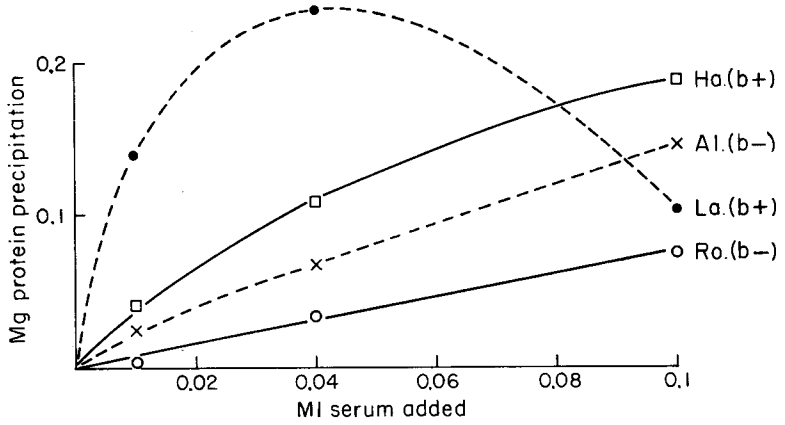

FIG. 2. Quantitative precipitin analysis of the four normal sera shown in Fig. 1 with absorbed rabbit anti-Vi antiserum (R Vi_1). Sera La. and Ro. again reflect extremes in concentration of Vi protein. The Gm(b) type for each serum is shown.

type all showed immunoelectrophoresis patterns similar to those illustrated for antiserum R Vi_1. The relative concentration of the Vi line for the four sera was similar, with all antisera showing the same sera to be high and the same to be low. Similar results were also obtained with M Zu antiserum even though this detected antigens only in the Fc area of the molecule, in contrast to the other antisera described above.

Quantitative precipitin curves with absorbed antiserum R Vi_1 gave further evidence for the quantitative differences noted in the immunoelectrophoresis experiments (Fig. 2). The sera with the highest and lowest Vi protein concentration are the same as those shown in the bottom of Fig. 1, which represent the extremes in concentration as seen on immunoelectrophoresis. Similar quantitative analyses were carried out on 40 Gm(b+) and Gm(b−) sera and confirmed the trend of higher concentrations in Gm(b+) sera.

Quantitation of Vi Protein by Radial Immunodiffusion.—Because both immunoelectrophoresis and quantitative precipitin curves suggested that levels of Vi protein were lower in Gm(b−) individuals, and also to establish normal levels of Vi proteins, quantitative radial immunodiffusion studies on a large scale were done on normal sera. Similar results were obtained using two different isolated Vi subgroup myeloma proteins as standards. Two different antisera against a Gm(b−) Vi subgroup myeloma protein (R Vi_1 and B Vi) were used with relatively good agreement of duplicate determinations (Table I). Serial determinations in a limited number of normal and diseased individuals revealed only small fluctuations in measured levels. A portion of one of the radial diffusion plates is shown in Fig. 3.

TABLE I

Duplicate Determinations of Vi Subgroup γ-Globulin Concentration Using Two Different Antisera on Radial Diffusion Plates with 25 Normal Caucasian Sera

Antiserum R Vi_1	Antiserum B Vi
mg/ml	*mg/ml*
0.25	0.37
0.28	0.40
0.38	0.53
0.44	0.39
0.46	0.54
0.51	0.52
0.51	0.56
0.60	0.64
0.63	0.61
0.66	0.61
0.69	0.70
0.75	0.63
0.82	0.83
0.84	0.80
0.92	0.85
0.92	0.77
0.94	1.17
0.96	0.85
1.00	1.03
1.15	0.94
1.25	1.25
1.30	1.15
1.35	1.35
1.55	1.40
1.80	1.75

Correlation of level of Vi protein and Gm(b) type: Differences in concentration of Vi subgroup protein within population groups were found to be associated

with genetic differences involving the Gm(b) system. In Caucasians, after 144 random sera were studied a second group of 523 sera was screened to increase the number of Gm(b−) sera since these appeared of particular interest. A total of 37 Gm(b−) sera were found and an additional 8 sera from known Gm(b−) normal donors were added. Significant differences in level of Vi protein were noted between three groups of normal Caucasians (Fig. 4). Mean levels were highest in individuals homozygous for genes synthesizing Gm(b+) proteins and lowest in those homozygous for genes synthesizing Gm(b−) Vi proteins ($P < 0.001$). The mean level in heterozygous Gm(a+b+) individuals was intermediate but significantly different from both the Gm(b+) ($P < 0.01$) and Gm(b−) ($P < 0.001$) individuals. Similar results, also showing

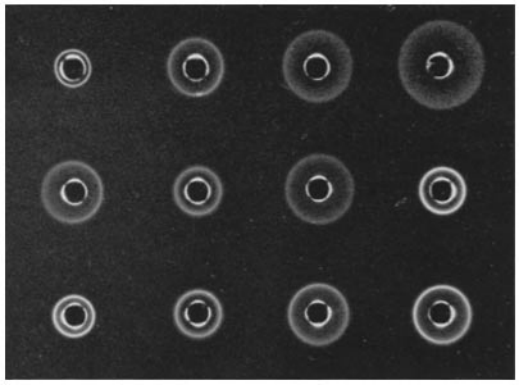

FIG. 3. Representative plate for the quantitation of Vi subgroup γ-globulin in normal sera by radial immunodiffusion. Absorbed antiserum specific for Vi subgroup is incorporated in the agar. The four wells in the upper row contain standard solutions of an isolated Vi subgroup myeloma protein. The remaining wells in the lower two rows contain normal sera.

significant differences between genetic groups, were obtained with a group of 170 sera collected from a random group of hospital patients.

In Caucasians, although inherited antigenic determinants Gm(a) and Gm(b) are found on separate subgroups of γG heavy chains (3) they segregate as if controlled by allelic genes. Therefore, individuals in whose sera Gm(a) antigens were present and Gm(b) absent [Gm(a+b−)] or vice versa [Gm(a−b+)] were considered homozygous, and those with Gm(a+b+) sera as heterozygous. Further confirmation of homozygous and heterozygous individuals in 80 of the sera was made by the positive identification of a genetic antigen present on Gm(b−) Vi molecules by measurements of the Gm(g) antigen as mentioned above. In Negroes, the gene for Gm(b) is distributed differently than in Caucasians. All pure-blooded Negroes are Gm(a+b+), and Gm(a) and Gm(b) are linked rather than in repulsion. Concomitantly, a Gm(g+) synthetic site for

Vi subgroup proteins is absent in Negro sera studied thus far. Differentiation of Gm(b^1), Gm(b^3), and Gm(b^4) was not made in this study, but an evaluation of levels of Vi protein in Negroes lacking one or more of these antigens would be of interest.

Significant differences between genetic groups were also noted in other populations. In the Indian sera, Gm(a+b+) individuals had levels of Vi protein

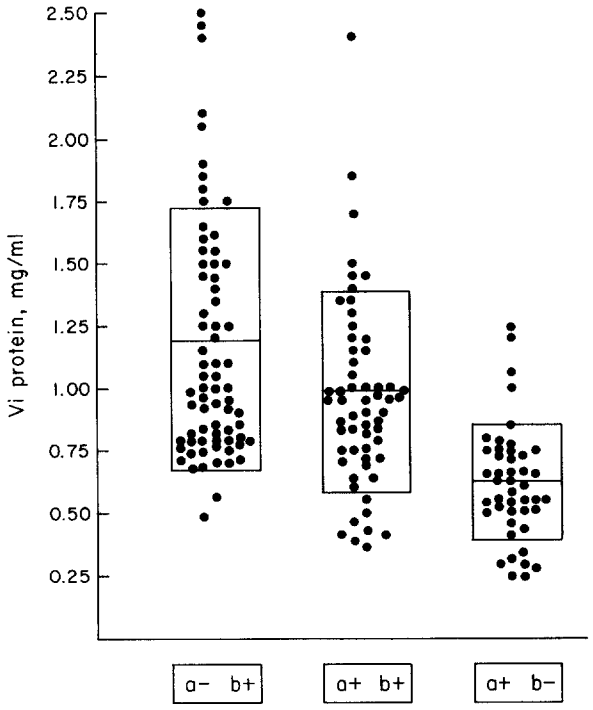

FIG. 4. Scatter diagram of concentrations of Vi subgroup γ-globulin in 175 normal Caucasian sera charted according to Gm phenotype. Mean values $\pm$ 1 SD are indicated. Significant differences were seen between homozygous Gm(b+), heterozygous, and homozygous Gm(b−) sera.

significantly higher than in Gm(a+b−) individuals ($P < 0.01$). In the Chinese and Easter Island populations, the total numbers of Gm(b−) sera were small, but levels of Vi protein were uniformly below the mean for each population.

Vi-type γ-globulin was found in all of more than 600 sera tested. Quantitative data in normal populations are shown in the first column of Table II. In Caucasians the mean values $\pm$ 1 SD were 0.97 $\pm$ 0.47 mg/ml. If the additional Gm(b−) sera are excluded, the true mean for 144 random Caucasian sera is 1.08 $\pm$ 0.46 mg/ml. This represents 8.5% of reported values for total γG using radial diffusion methods (16).

Determinations of Vi protein in several preparations of Fr II globulin of 10–11.6 mg/ml showed concentrations of Vi proteins of 6.9 to 8.7% of the total γ-globulin. The percentages of Vi protein agree with the incidence of Vi subgroup myeloma proteins reported previously of 10% (1), and the more recent figure of 8.4% for 130 γG-myeloma proteins studied to date. Small but statistically significant differences were noted between population groups: Caucasians vs. Negroes ($P < 0.001$), Caucasians vs. Chinese ($P < 0.001$), Caucasians vs. Easter Islanders ($P < 0.05$), Easter Islanders vs. Negroes ($P < 0.05$) or Chinese ($P < 0.05$). These differences between population groups of concentration of Vi subgroup γ-globulins are probably based not only on variation of gene frequency for Gm(b+) Vi subgroup proteins, but also on variations in total γ-globulin of population groups.

TABLE II

The Concentration of Vi Subgroup Proteins in Several Population Groups

Population group	Total	a − b +	a + b +	a + b −
	mg/ml	*mg/ml*	*mg/ml*	*mg/ml*
Caucasian	0.97 ± 0.47 (177)	1.19 ± 0.52 (72)	0.98 ± 0.40 (60)	0.62 ± 0.23 (45)
Negro	1.48 ± 0.58 (21)	—	1.48 ± 0.58 (21)	—
Chinese	1.45 ± 0.48 (48)	—	1.49 ± 0.44 (46)	0.51 (2)
Easter Island	1.25 ± 0.40 (39)	—	1.28 ± 0.41 (35)	1.01 (4)
Indian (Bombay)	1.01 ± 0.35 (86)	1.07 (2)	1.18 ± 0.33 (39)	0.86 ± 0.30 (45)

Mean ± 1 SD. The total number of sera studied in each group is given in parentheses.

Family studies: A number of Caucasian and Easter Island families were studied, and selected families are shown in Table III. Two were of particular interest. Families 11 and 14 had several members with elevation ($P < 0.05$) of Vi subgroup proteins. Four of nine members studied in family 14 had significantly high levels. Levels for five of the nine family members are shown in Table III; the others were paternal siblings. Lower levels of Vi protein ($P < 0.05$) were also noted among the Gm(b−) sera studied. In families with one parent with an elevated level of Vi protein (families 1, 8, 11, 13, and 14), 5 of 19 children had elevated levels. In families with both parents in the normal range, none of 28 children showed elevated levels. This difference in incidence of elevated levels in children is statistically significant ($P < 0.05$).

Pathological sera: In a group of patients with diffuse hypergammaglobulinemia, correlation of concentration of Vi protein and total γ-globulin was generally seen, with occasional striking exceptions. 59 sera from patients with

systemic lupus erythematosus, sarcoidosis, a variety of tumors or liver disease with elevated γ-globulin were studied. In some sera, levels of Vi proteins were disproportionately low or high compared to the total γ-globulin. One Gm(b+) patient with systemic lupus erythematosus and total γ-globulin of 50.1 mg/ml had a level of Vi protein greater than 10 mg/ml, and a Gm(b−) patient with a total γ-globulin of 26.5 mg/ml had only 0.31 mg/ml of Vi protein. These patients, with Vi protein representing more than 20% and only 1.1% of the total γ-globulin, respectively, represent extreme examples. No Gm(b−) individuals

TABLE III

Levels of Vi Protein in 15 Families Correlated with the Gm Type for Each Individual

Family No.	Father			Mother			Children		
	a−b+	a+b+	a+b−	a−b+	a+b+	a+b−	a−b+	a+b+	a+b−
	mg/ml	*mg/ml*	*mg/ml*	*mg/ml*	*mg/ml*	*mg/ml*	*mg/ml*	*mg/ml*	*mg/ml*
1		*2.6*			1.3			1.3 1.4 1.4	
2		1.5			0.9			1.8 1.4 1.6 1.3	0.6
3		1.5			1.1			1.2	0.9
4		1.4			1.8			1.2	0.5
5		1.3			1.3			1.0 1.2	0.5 0.7
6		1.3				0.8		1.1	
7		1.0				1.3			1.0
8		0.8			*2.1*			0.7 0.7 0.7	
9			0.6		1.3			1.2 0.8 1.5 1.0	0.5 0.6
10			0.3		1.9			1.2 1.1	
11	*2.7*					0.8		*2.1* 1.7 *2.0*	
12	1.1				0.9			0.5 0.8 0.1*	
13	0.6			*2.1*			1.1 1.0 1.6 0.8 *2.7*‡ 1.4		
14		*2.1*§		?			0.7 *2.6* 1.0 *2.3*		
15		0.9			1.4			1.1 .7	
Mean.	1.5	1.4	0.5		1.4	1.0	1.5	1.2	0.7

Concentrations greater than 2 SD from the mean for the population italicized.

* Child with agammaglobulinema.

‡ Patient with chronic hepatic congestion and diffuse hypergammaglobulinemia.

§ One of three paternal siblings also had significant elevation of Vi proteins.

have as yet been encountered with diffuse hypergammaglobulinemia and marked elevations of Vi proteins.

Sera from patients with multiple myeloma of the Vi type occasionally had concentration of Vi protein in excess of 100 mg/ml. Sera from patients with γG-myeloma proteins of the We, Ne, and Ge subgroups or γA-myeloma proteins had low but detectable levels of Vi proteins, with lowest levels generally seen in sera with the highest protein peaks. Several patients with hypogammaglobulinemia or congenital agammaglobulinemia have had low but discernible levels of Vi proteins. The very low level of Vi subgroup protein (0.1 mg/ml) in one Gm(a+b+) child from family 13 (Table III) was associated with congenital agammaglobulinemia.

In 20 paired maternal and fetal sera, Vi proteins were present in all specimens and good agreement between maternal and fetal levels was observed in most pairs.

DISCUSSION

The quantitative differences in the absolute amount of Vi subgroup globulin with dependence on genetic phenotype were apparent with a number of different immunological techniques as well as with different antisera. Measurements by radial diffusion lent themselves best to large scale studies of different populations. Most of these studies were carried out with antisera against a Gm(b−) Vi-type protein which could not possibly measure Gm(b+) antigens and thus could not in this fashion contribute to the higher levels found in the Gm(b+) individuals. It was also apparent that antisera to Gm(b+) proteins gave completely parallel results, indicating further that the genetic antigen itself was not contributory. The major Vi antigen measured was localized to the Fab portion of the molecule, and both sera and myeloma proteins gave identical precipitation patterns whether they were Gm(b+) or Gm(b−).

In the course of these studies two antisera were encountered which showed specific reactivity with the Fc as well as the Fab fragments of Vi proteins. When the antisera were absorbed with such Fab fragments and thus made specific for the Fc portion of the molecule, genetic antigens could be detected by precipitation reactions. Antiserum R Fe detected Gm(b+) sera and myeloma proteins by showing a more intense precipitin line with these than with Gm(b−) samples. The difference was relatively small and no spurs were visible. Absorption with Gm(b−) proteins also removed the precipitation with the Gm(b+) types. Antiserum B Vi, absorbed in a similar fashion utilizing the Fab fragment of the immunizing protein, specifically detected Gm(b−) proteins and gave exactly the reverse reaction from the R Fe antiserum. Again the differentiation was on the basis of intensity of the precipitin lines in agar diffusion analysis and both systems were similar to those described recently for Gm(n).[2] The B Vi antiserum appeared to detect a genetically transmitted antigen very similar to or identical with the Gm(g) antigen recently described for a hemagglutination inhibition system by Natvig (20).

The concentration of Vi subgroup protein in normal sera exhibited a range from 0.25 to 2.7 mg/ml, with all sera showing a measurable level. The higher levels (above 1.25 mg/ml) were only found in Gm(b+) individuals. Studies with pathological sera with hypergammaglobulinemia other than myeloma showed levels of Vi protein as high as 10.5 mg/ml. Such high levels were also limited to Gm(b+) sera although the number of pathological Gm(b−) sera was relatively small. It appeared as if the structural genes for Vi proteins in-

[2] H. G. Kunkel, W. J. Yount, and S. D. Litwin. 1966. A genetically determined antigen of the Ne subgroup of γ-globulin; detection by precipitin analysis. *Science.* **154:** 1041.

volved in the synthesis of the Gm(b+)-type heavy chains were more susceptible to antigenic or other stimuli resulting in increased production. The unusual skewed distribution of the levels of Gm(b+) individuals seen in Fig. 3 probably reflects similar effects in a small percentage of normal individuals. However, some evidence was obtained that, at least in part, the high levels tended to be clustered in particular familial groups. Further studies relating to this aspect of the problem are currently in progress.

Subgroups of γG-globulin similar to those found in humans have been described for a variety of species (21–23) and have been shown to differ strikingly in such properties as ability to elicit passive cutaneous anaphylaxis and complement binding. The Ne (γ_{2a}) subgroup in humans has been shown to lack skin-sensitizing properties in guinea pigs (24). Two types of γA-globulin have also been described in humans (25). The exact biological activity of the Vi subgroup has not been established. However, it appears highly likely that antibodies of this type have a special physiological function. It is therefore of particular interest that the quantitative level was influenced by the specific genetic type of the individual. Evidence has been obtained that similar differences are also found for other subgroups (19), indicating that the genetic constitution of an individual plays a distinct role in the relative composition of his γ-globulin and, in all probability, in the population of his antibodies. The possibility is raised that resistance to infections may in turn show a similar relationship.

The level of synthesis of a number of proteins has been found to be influenced by single mutations in their structural genes. Perhaps the best example is that involving substitutions in the β-chain of hemoglobin. Less of the mutant form of hemoglobin is usually synthesized than of the normal hemoglobin A. In the case of hemoglobin S this is very apparent in heterozygous individuals, in whom less hemoglobin S than hemoglobin A is produced. The problem in the case of the γ-globulins is complicated by the fact that the products of allelic genes are formed in separate cells in contradistinction to the situation for the hemoglobins. Studies are currently underway to determine the number of cells forming the two types of Vi proteins in heterozygous individuals. It seems possible that the relative synthetic rate may still be involved despite the dependence on cell number. Some evidence has been obtained that the concentration differences described are not the result of variation in rate of breakdown. Irrespective of the mechanism involved, the large number of classes and subgroups in the γ-globulin system which appear to be under the control of separate structural genes would present even more possibilities for variation of the relative concentrations depending on the specific genes operative in a given individual.

SUMMARY

Further delineation of the antigens characteristic of the Vi or γ_{2c} subgroup of γ-globulin was carried out utilizing a number of rabbit and primate antisera.

Two genetic antigens characteristic of this subgroup, Gm(b) and Gm(g), were also detected by precipitation techniques with certain of the antisera. These were clearly differentiated from antigens common to all proteins of this subgroup.

The concentration of Vi protein in normal and pathological sera from several population groups was measured quantitatively utilizing a variety of immunological procedures. All sera studied showed measurable levels. The mean value for Caucasian sera was 1.06 mg/ml, representing approximately 8% of γG-globulin. This agreed closely with a figure of 8.4% for the incidence of myeloma proteins of the Vi subgroup among all γG-myeloma proteins in Caucasians.

A relationship was found between the Vi subgroup concentration and the specific genetic type of a given individual. Measurements of the Gm(b) genetic determinants, which are found solely in Vi-type proteins, brought forward this relationship. Gm(b+) individuals showed higher concentrations of Vi-type γ-globulin than those who were Gm(b−), and this difference was statistically significant for both the homozygous and heterozygous states. It appeared that the structural genes for Gm(b+) polypeptide chains showed a greater synthetic capacity than those for Gm(b−) types. The possible significance of such effects in governing the relative composition of the antibody population in a given individual is discussed.

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