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Research Article

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One family was encountered with probable structural gene abnormalities in the autosomal Gm loci. Both parents had different abnormal gene complexes detectable by absence of specific Gm markers and the propositus received both types from the parents. Similar gene complexes have been seen previously in rare instances through population screening but only in the heterozygous state and were not associated with clinically evident hypogammaglobulinemia. Of several other families of patients with subgroup imbalance, two were informative in that structural gene defects could be excluded. Studies on 22 first degree relatives of patients […]



# Imbalances of Gamma Globulin Subgroups and Gene Defects in Patients with Primary Hypogammaglobulinemia

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ABSTRACT Analysis of immunoglobulin classes, γG subgroups, and Gm genetic markers from 59 patients with various types of immune deficiencies was undertaken to assess the function of the several cistrons concerned with synthesis of gamma globulins. 13 patients including two sibling pairs were found to have γG subgroup imbalances. All of these patients had non sex-linked disease. 11 of the 13 had preponderance of the γG3 subgroup. In most instances of γG3 preponderance it was the Gm(b) type of γG3 that was selectively retained; the Gm(g) type, controlled by the allelic gene was markedly depressed but not absent in the cases where it could be studied. Other imbalances, either seen concomitantly with γG3 preponderance or independently, included predominance of the γG2 subgroup and selective absence of single γG subgroups.

One family was encountered with probable structural gene abnormalities in the autosomal Gm loci. Both parents had different abnormal gene complexes detectable by absence of specific Gm markers and the propositus received both types from the parents. Similar gene complexes have been seen previously in rare instances through population screening but only in the heterozygous state and were not associated with clinically evident hypogammaglobulinemia. Of several other families of patients with subgroup imbalance, two were informative in that structural gene defects could be excluded. Studies on 22 first degree relatives of patients with subgroup imbalances indicated that the most common abnormality detected was in γA which was absent in 3 and markedly decreased in 2 others; other abnormalities included decreased levels of specific genetic types of γG globulin. It is concluded that γG subgroup imbalances are frequently found in non sex-linked immunoglobulin deficiency disorders and in some instances may be associated with family abnormalities suggesting either regulator or structural gene defects.

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## INTRODUCTION

Human γG immunoglobulin has been shown to contain four subgroups based on antigenic differences in the polypeptide heavy chains (1–3). The approximate percentages in normal γG are: γG1, 66%; γG2, 23%; γG3, 7% (4); and γG4, 4%. Individual Gm genetic markers are found only in molecules of a single subgroup (5, 6). Varying relationships between genetic markers, especially the new markers Gm(n) at the γG2 locus (7) and Gm(g) at the γG3 locus (8), have provided evidence for close linkage between the three cistrons for which markers are available and have led to the conclusion that some of the differences in gene complexes among population groups arose through crossover events (9–13). Gene complexes which are characteristic for the Caucasoid population group are shown in Table I. Current evidence suggests that the order of γG heavy-chain cistrons may be γG2, γG3, γG1 (12–14). A number of rare gene complexes have been observed in large surveys of families from different populations; included among these were families with either an apparent deletion of the γG3 locus (γG2, —, γG1) or of the γG1 locus (γG2, γG3, —) (15).

The availability of reagents to identify virtually all γG molecules by heavy-chain subgroup and approximately 85% as to Gm genetic markers has permitted a more detailed analysis of γG synthesis in patients with hypogammaglobulinemia. Disproportionate synthesis of γG heavy-chain subgroups resulting from genetic defects was sought for, especially in those patients showing restricted electrophoretic heterogeneity and disproportionate ratios of kappa: lambda light-chain types (16, 17). The

TABLE I

*Horizontal Rows of Gm Gene Complexes Illustrating Normal Caucasoid Gene Complexes and Associated γG Subgroups. The Rare Gene Complexes Associated with Hypogammaglobulinemia from the Present Study are Shown*

| | γG Subgroup | | |
|---|---|---|---|
| | γG2 | γG3 | γG1 |
| Usual Caucasoid *Gm* gene complexes | | g | za |
| | | g | zax |
| | n | b | f |
| | | b | f |
| Rare Caucasoid | | — | za |
| | n | b | — |

possibility that structural or regulator gene defects, recognized in associated family members, might be responsible for asynchronous gamma globulin synthesis was examined in particular detail. A preliminary report of this work has been presented (18).

## METHODS

*Patients.* Sera were collected from 59 patients with hypogammaglobulinemia of various types. All of the patients studied were Caucasoid. From clinical criteria and family studies patients were classified as to type of hypogammaglubulinemia (Table II) (19). Only specimens before treatment or specimens taken several weeks or months after an injection of gamma globulin were studied. Normal concentrations and percentages of γG subgroups were determined from sera selected from a group of 600 normal blood donors provided by Dr. Fred Allen of The New York Blood Center. For each of the 13 patients with γG subgroup imbalance, 2 normal persons matched for age, sex, and race were studied. In addition, 10 normal children between the ages of 3 and 9 months were studied to assess levels at the nadir of γG levels after birth.

*Myeloma proteins.* Several myeloma proteins from each of the four γG subgroups and the two γA subgroups, γD proteins and Waldenström macroglobulins were isolated by zone electrophoresis (20) on polyvinyl copolymer (Pevikon) (21). In some instances further purification was effected either by gel filtration on Sephadex G-200 equilibrated with 0.1 M [tris(hydroxymethyl)aminomethane hydrochloride] (Tris-HCl) and 0.15 M sodium chloride, or by DEAE cellulose chromatography. Fragments from papain (22) or pepsin digestion of myeloma proteins were isolated by zone electrophoresis on Pevikon followed by gel filtration. Isolated proteins were reduced in 0.55 M Tris-HCl buffer made 0.1 M with β-mercaptoethanol for 1 hr at room temperature, and then alkylated by the addition of recrystallized iodoacetamide to a final concentration of 0.11 M. Heavy and light chains were separated by filtering the reduced and alkylated proteins through Sephadex G-100 equilibrated with 0.5 M propionic acid. Isolated heavy chains were concentrated by ultrafiltration and then exhaustively dialyzed against phosphate buffered saline, pH 7.4, before use in animal immunizations.

*Antisera.* Antisera specific for γG, γA, γM, γD, kappa, lambda, γG heavy-chain subgroups, and genetic factors were prepared by immunization of rabbits, cynomolgus or rhesus monkeys or baboons with isolated whole immunoglobulins, polypeptide chains, or enzymatic digestion fragments. The antisera were absorbed with isolated Bence Jones proteins, myeloma proteins, or digestion fragments, fraction II, isolated normal γG or selected whole sera in order to obtain the light-chain type or heavy-chain class, subgroup, or Gm specificity sought as has been described previously (1, 4, 7, 9, 14, 15). The baboon antisera were prepared through the cooperation of Dr. J. Moor-Jankowski and the Laboratory for Experimental Medicine and Surgery in Primates. Quantitation of γG, γA, γM, γD, kappa, lambda and γG2, γG3, and γG4 subgroups was carried out by radial immunodiffusion (23, 24) modified as previously described for the γG3 subgroup (4). The isolated immunoglobulins used as standards were tested in high concentration on radial diffusion plates for concentrations of contaminant proteins and were found to be less than 1% contaminated by proteins of other classes, subgroups or of the opposite light-chain type. Protein concentrations of standard solutions were determined by the Folin-Lowry technique and Kjeldahl nitrogen determination (25). Of many antisera showing γG subgroup specificity, only a few were suitable for quantitative radial immunodiffusion (26). Because most γG1 antisera were not ideal for quantitative radial immunodiffusion, γG1 levels were determined by two alternative procedures, direct hemagglutination inhibition with an anti γG1 Fc antiserum and by subtracting levels of γG2, γG3, and γG4 from the total γG.

*Gm typing.* Gm typing was carried out using hemagglutination-inhibition systems (9, 14). Gm(n) typing was performed by coupling Gm(n+) myeloma proteins to group O Rh positive red blood cells using bis-diazotized benzidine (BDB) (15). In similar fashion, antigens specific for γG1-Fc, γG1-kappa, γG3-Fc, γG3-Fab, γG2-Fc, γG2-kappa, γG4, and γD molecules were measured with more sensitive hemagglutination-inhibition techniques (14).

TABLE II

*Patients with Primary Hypogammaglobulinemia Studied for Imbalances of γG Subgroups*

| Diagnosis | Imbalance found | Number studied |
|---|---|---|
| Infantile sex-linked agammaglobulinemia (Bruton)* | 0 | 8 |
| Non sex-linked primary immunoglobulin deficiency with variable onset and expression | | |
| (*a*) low γG, γA, and γM | 10 | 39 |
| (*b*) low γG and γA, normal γM | 2‡ | 6 |
| Congenital rubella syndrome | 1 | 1 |
| Immune deficiency with thrombopenia and eczema | 0 | 1 |
| Alymphocytic agammaglobulinemia (Swiss) | 0 | 2 |
| Unclassified | 0 | 2 |
| Total | 13 | 59 |

* In some of these cases the sex-linked pattern could not be clearly established and the diagnosis is presumptive.

‡ An associated thymic abnormality was present in one of these patients.

## RESULTS

*γG subgroup imbalance.* A total of 59 patients were studied which were grouped according to a recently recommended classification of the primary immunoglobulin deficiency syndromes (19) (Table II). Except for one patient with the congenital rubella syndrome, all the patients with subgroup imbalance were from patients with non sex-linked primary immunoglobulin deficiency of variable onset and expression. This group includes "acquired" deficits and "congenital" non sex-linked forms of hypogammaglobulinemia (19). 13 patients showed imbalances of γG subgroups and these are shown in Table III. Predominance of the γG3 subgroup was the most common imbalance and was seen in 11 of the 13 patients. Subgroup concentrations and percentages for normal caucasoid adults and for sera from two normal controls for each patient with subgroup imbalance matched for age, sex, and race are shown. The γG3 subgroup comprised 7.3 ±3.8% of total γG in 145 normal sera with no specimen exceeding 18% of total γG. In the remaining 46 sera from patients with hypogammaglobulinemia and in the matched control sera, percentages equivalent to those in caucasoid adults were found. In five patients with γG3 preponderance (Bon., Rod., And., Mes. M., and Mes. B.) (Table III), the remaining γG subgroups were all reduced proportionately. The most striking members of this group were two siblings, patients M. Mes. and B. Mes., with epispadias, bony abnormalities, and the adult onset of propensity for infections. Levels of γG3 were in the high normal range, and despite spontaneous fluctuations in total γG, percentages of γG3 subgroup in the range of 50–65% were consistently seen in both siblings.

In six patients with predominance of γG3, a variety of associated subgroup imbalances were seen (Table III). In patient Hx., for example, nearly half the γG molecules were γG3 subgroup, and no γG2 or γG4 molecules could be detected, even when sensitive hemagglutination inhibition techniques were employed to confirm the radial immunodiffusion results. γG1 molecules were present but reduced approximately 20-fold as determined by hemagglutination inhibition. The high level of γG3 subgroup was confirmed by inhibition studies employing both antigens of the Fc portion of the heavy chains and antigens of the Fd portion. These findings were consistently present in serum specimens collected over a period of 2 yr. Selective suppression of γG1, the major subgroup, was an associated abnormality in patients Tia., Gau., J. Tu., and G. Tu. This was most striking in patient Tia. where γG1 molecules were undetectable instead of the normal 66%; the genetic marker, Gm(f), which with the system employed is detectable in normal sera at dilutions of 1:10,000, was reduced approximately 1000-fold indicating trace amounts of Gm(f$^+$) γG1 molecules. In patient Gau. 57% of the total serum gamma globulin was γG3 subgroup, and γG1 was markedly reduced in absolute concentration and relative amounts (12% instead of the expected 66%). The γG4 subgroup was not detected. Patients J. Tu. and G. Tu. also showed γG1 suppression with the total γG composed primarily of γG2 and γG3 molecules. These sera were from a pedigree shown to have low levels of γA in the same two siblings. Patient Lind. showed a preponderance of γG3 and γG4 which together comprised 70% of his low level of total γG. This was the only patient in whom a relative preponderance of γG4 was observed.

Two of six sera from patients with non sex-linked primary immunoglobulin deficiency having normal γM and low γG and γA levels (dysgammaglobulinemia type I) showed unusual types of γG subgroup imbalances. One serum (Geo., Table III) was almost exclusively γG2 subgroup and totally lacked γG1 and γG3 subgroup markers both by radial diffusion and by hemagglutination inhibition. The genetic markers associated with γG1 and γG3 subgroups including Gm(za, f, b, and g) were undetectable, but the Gm(n) marker known to occur on the Fc portion of γG2 molecules was present. In subsequent specimens the γM also was diminished, but the γG remained predominantly γG2 subgroup. Other features concerning this patient have been described in a previous communication (27). The second patient (Feld.) showed selective paucity of γG2 subgroup and γA molecules with moderately diminished total γG but normal levels of γG3 and γG4 subgroups. The patient, a young boy, also manifested persistent neutropenia and died at age 3 of varicella pneumonia. No detectable thymus could be found at postmortem examination.

In addition to the 59 patients listed in Table II with primary deficiencies of γG, five patients with selective absence of γA, two patients with ataxia telangiectasia and absence of γA, and eight patients with secondary hypogammaglobulinemias (nephrosis, protein-losing enteropathy, lymphomas) were also studied and no subgroup imbalances were detected. Proportionate levels were also present in a group of 10 infants ranging in age from 3 to 9 months.

*Genetic studies.* Immunoglobulin concentrations and studies for the autosomal Gm genetic markers were carried out in 22 first degree relatives of 7 propositi with γG subgroup imbalance and 52 first degree relatives of 18 propositi without subgroup imbalance in a search for possible genetic explanations for the hypogammaglobulinemia. One family was of special interest (Fig. 1). The propositus (Hx., Table III) had a preponderance of γG3 subgroup and lack of γG2 and γG4 subgroup molecules. The propositus and mother both exhibited an abnormal $Gm^a$ $Gm^b$ $Gm^-$ gene complex which lacked the normally associated $Gm^x$ marker at the major or γG1

TABLE III

*Immunoglobulin Concentrations in Patients with Non Sex-Linked Immunoglobulin Deficiency with Variable Onset and Expression and Imbalance of γG Subgroups*

| | | | H-Chain subgroup* (per cent of γG) | | | | Class of immunoglobulin | | | | Light-chain type | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Age | Sex | γG1‡ | γG2 | γG3 | γG4 | γG | γA | γM | γD | kappa | lambda | kappa/lambda ratio |
| | *yr* | | | | *mg/ml* | | | | *mg/ml* | | | | |
| Bon. | 5 | M | 1.61 (42) | 0.28 (7) | 1.62 (43) | 0.32 (8) | 3.83 | 0.025 | 0.050 | 0 | 2.45 | 2.03 | 1.2 |
| Rod. | 42 | M | 1.23 (56) | 0.27 (12) | 0.66 (30) | 0.03 (1) | 2.19 | 0.20 | 0.063 | 0 | 1.45 | 0.38 | 3.8 |
| And. | 1½ | F | 4.37 (80) | 0.28 (5) | 0.85 (16) | 0 | 5.50 | 0.034 | 1.95 | 0 | 3.6 | 2.4 | 1.5 |
| Mes., M. | 24 | M | 0.75 (30) | 0.15 (6) | 1.62 (64) | 0 | 2.52 | 0.38 | 0.056 | 0.018 | 1.51 | 1.25 | 1.2 |
| Mes., B. | 21 | M | 0.77 (31) | 0.13 (5) | 1.55 (63) | 0 | 2.45 | 0.38 | 0.041 | 0.022 | 1.30 | 1.04 | 1.3 |
| Hx. | 18 | M | 0.54 (52) | 0 | 0.49 (48) | 0 | 1.03 | 0.030 | 0.030 | 0 | 0.55 | 0.60 | 0.9 |
| Tia. | 20 | M | 0 | 0.26 (46) | 0.31 (54) | 0 | 0.62 | 0.31 | 0.088 | 0 | 0.40 | 0.30 | 1.3 |
| Gau. | 30 | M | 0.08 (12) | 0.21 (31) | 0.39 (57) | 0 | 0.68 | 0.10 | 0.110 | 0 | 0.37 | 0.34 | 1.1 |
| Tu., J. | 15 | M | 0.23 (6) | 1.95 (53) | 1.49 (41) | 0 | 3.67 | 0.025 | 0.075 | 0.034 | 1.60 | 0.84 | 1.9 |
| Tu., G. | 14 | M | 1.27 (31) | 1.75 (42) | 1.12 (27) | 0 | 4.14 | 0.29 | 0.153 | 0.116 | 2.70 | 1.20 | 2.2 |
| Lind. | 37 | M | 0.08 (17) | 0.06 (13) | 0.17 (35) | 0.17 (35) | 0.48 | 0 | 0.030 | 0 | 0.25 | 0.07 | 3.6 |
| Feld. | 3 | M | 4.33 (76) | 0.10 (2) | 0.52 (9) | 0.77 (13) | 5.72 | 0 | 2.30 | 0 | | | |
| Geo. | 19 | F | 0 | 0.38 (89) | 0 | 0.025 (11) | 0.43 | 0 | 0.45 | 0 | 0.26 | 0.12 | 2.2 |
| Adult Caucasoid normals | | | 9.4 ±1.9 (66 ±8) | 3.2 ±1.3 (23 ±8) | 1.0 ±0.45 (7.3 ±3.8) | 0.62 ±0.48 (4.2 ±2.6) | 14.1 ±2.5 | 1.94 ±0.58 | 0.53 ±0.20 | 0.030 (<0.002–0.6) | | | |
| Controls matched for age, sex, and race | | | 8.2 ±2.8 (69 ±9) | 2.3 ±1.2 (20 ±7) | 0.67 ±0.40 (5.5 ±2.4) | 0.64 ±0.47 (5.3 ±3.4) | 11.9 ±3.8 | 1.32 ±0.55 | 0.67 ±0.35 | 0.025 (<0.002–0.046) | | | |

* Normal value ±1 SD for adult Caucasoid population and matched controls.

‡ Values are estimated by difference; 0 = absence by direct measurement by hemagglutination inhibition.

subgroup locus (Table I). It was undetectable; and based on the sensitivity of the Gm(f) system, a reduction of Gm(f) of at least 1000-fold below normal titers was observed. The propositus and his father both showed a $Gm^{n-}$ $Gm^{-}$ $Gm^{za}$ gene complex which lacked the $Gm^{g}$ marker which is normally present with $Gm^{za}$. The titers of Gm(z) and Gm(a) were normal in the father but detectable and reduced 30-fold in the propositus. The titer of Gm(n) was normal in the mother but detectable and reduced 250-fold in the propositus. Both parents and the sister showed normal levels of total γG and normal percentages of the γG3 subgroup although the level of γG in the mother was in the low normal range (8.4 mg/ml). Both parents had normal γG1 and γG2 subgroup concentrations. Normal titers were present for Gm(z), Gm(a), and Gm(f) in the father, Gm(z), Gm(x), and Gm(a) in the mother, and Gm(n) in both parents.

Quantitative determinations (28) of Gm(a) and Gm(f) were performed by Dr. S. D. Litwin on the sera of the Hx. family. The father had levels of Gm(a) (1.28 mg/ml) and Gm(f) (3.5 mg/ml) which fall within the normal range for caucasoid heterozygotes. The sister had a normal homozygous level of Gm(f) (5.75 mg/ml). The mother, who lacked the expected γG1 marker Gm(f), had a level of Gm(a) of 2.42 mg/ml which is below the normal range for homozygotes (2.92–7.72 mg/ml) and is clearly in the heterozygous range. Careful quantitative analysis of γG1 in the mother gave

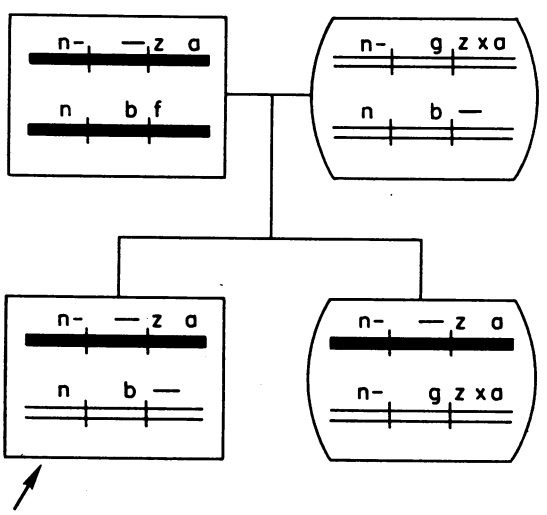


FIGURE 1 Gene complexes, family Hx. A Caucasian family in which the hypogammaglobulinemic propositus inherited from both parents abnormal Gm gene complexes with deletions of genetic markers. The propositus and his mother had Gm(b) and Gm(n) molecules but lacked the normally associated Gm(f) marker which is indicated as (—). The propositus, sister, and father had the Gm(az) markers but lacked the normally associated Gm(g) marker.

levels that corresponded closely with the value for Gm(a) indicating that it was highly unlikely that a new allele was operative in replacing the missing Gm(f) marker. For the propositus calculated levels of γG1 were 0.93 mg/ml and levels of Gm(a) were 0.90 mg/ml on the same specimen. The possibility of a Negroid $Gm^{n-}$ $Gm^{b}$ $Gm^{za}$ gene complex in the father is ruled out by the presence of $Gm^{n-}$ $Gm^{-}$ $Gm^{za}$ in the sister of the propositus. A Negroid $Gm^{n-}$ $Gm^{b}$ $Gm^{za}$ gene complex in the father would require that the sister of the propositus was illegitimate. Red cell genotypes for ABO, Rh, MS, Jk, Ns, K, Lewis, and Duffy were studied and offered support for the family relationships illustrated in Fig. 1. The possibility of a $Gm^{n}$ $Gm^{b}$ $Gm^{za}$ gene complex in the mother and propositus is highly unlikely for two reasons: first, because $Gm^{n}$ has never been found associated with $Gm^{b}$ $Gm^{za}$ in any previous studies, and second, because this would necessitate that the mother be homoxygous Gm(az) while the quantitative studies indicated heterozygous levels.

Another unusual finding for the genetic antigens was in two male siblings of family Tu. (Table III). Both showed a preponderance of γG3 molecules with marked diminution of γG1 subgroup and absence of the γG4 subgroup. Gm($a^{+}$) markers were readily detected but with markedly reduced titer. Gm(f) was markedly diminished but detectable in both siblings. However, it was of special interest that, along with the marked relative increase in γG3 level, the two γG3 genetic markers, Gm(b) and Gm(g), showed a wide difference in level (Table IV). The propositi were clearly heterozygous but the Gm(g) was reduced approximately 100-fold although still detectable while Gm(b) was not reduced at all. Both siblings showed this unusual differential in products of allelic genes in exactly the same fashion. For the γG1 markers in J. Tu., Gm(f) was in excess of Gm(a) but no asynchrony was seen in G. Tu. The father was homozygous Gm(f) and no reduction was observed. However, it is of special interest that the Gm(n) titer was reduced approximately 8-fold. The latter finding is strongly indicative of a gene defect in the father. The mother could not be studied.

The family of Tia., a patient demonstrating selective reduction of the γG1 subgroup and moderate diminution of γA and γM, was informative. The propositus was heterozygous at the γG3 locus with asynchronous synthesis of the associated genetic markers; Gm(b) was minimally reduced but Gm(g) was more markedly reduced in titer. There was a striking absence of the Gm(z) and Gm(a) markers normally linked to Gm(g) in Caucasoids. Gm(z) and Gm(a) are detectable in normal persons at titers of 1/640. Gm(g, z, and a), all of maternal origin, were present in normal titers in the mother and in two female siblings of the propositus.

TABLE IV

*Predominance of Gm(b) vs. Gm(g) Allelic Product in Heterozygous Hypogammaglobulinemic Siblings in Hemagglutination Inhibition Systems for Gm(b) and Gm(g). Agglutination Is Designated 0 (Complete Inhibition) to 3 (No Inhibition)*

| Inhibitor | whole | 1/2 | 1/4 | 1/10 | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 | 1/640 | 1/1280 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gm(b) | | | | | | | | | | | |
| Normal serum b; b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 |
| Normal serum b; g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 |
| Normal serum g; g | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Patient J. Tu. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 |
| Patient G. Tu. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 |
| Gm(g) | | | | | | | | | | | |
| Normal serum b; b | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Normal serum b; g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 |
| Normal serum g; g | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Patient J. Tu. | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Patient G. Tu. | 0 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Despite the normal levels of Gm(b) in the propositus, the associated markers Gm(f) at the γG1 locus and Gm(n) at the γG2 locus were both markedly reduced (250- and 320-fold respectively). The Gm(n, b, and f) markers, of paternal origin, were present in normal titers in the father and two sisters of the propositus. The parents and two siblings all had normal levels of immunoglobulin classes and γG subgroups. The concentration of γG3 subgroup molecules in one brother was at the upper limit of normal (1.95 mg/ml). The propositus therefore exhibited extreme γG1 suppression along with more moderate decreases in the other immunoglobulins with selective retention of ability to synthesize γG3, Gm($b^+$) molecules.

The family of Gau., another patient with predominant γG3, was informative in that the propositus, who was Gm($f^+b^+n^-a^-g^-$), had three children who were Gm ($f^+b^+n^-a^+g^+$) with Gm($a^+g^+$) derived from the mother. Although the Gm(f) titer was reduced 64-fold in the propositus, Gm(f) titers were normal in the children as were the γG globulin and γG subgroup concentrations. The propositus therefore transmitted the capacity to synthesize γG1 Gm(f) molecules normally. The mother of the propositus was uninformative from a genetic standpoint but had a low level of γG globulin (5.3 mg/ml) which was proportionate for reductions of all subgroups. The oldest of the patient's three children, a 13 yr old daughter, totally lacked γA molecules (less than 0.020 mg/ml).

The other families of patients with subgroup imbalance were not informative and genetic defects could not be ruled in or out. However, absence of γA was found in two first degree relatives and two others showed striking depressions of γA. Thus in the entire group of patients with subgroup imbalance, 5 of 22 first degree relatives showed absence or marked depression of γA. The parents and another sibling of the hypogammaglobulinemic Mes. siblings with predominant γG3 were Gm($b^+f^+g^-a^-$) and therefore uninformative from a genetic standpoint. Of 18 other relatives 8 were heterozygous and normal both for genetic markers and γG3 concentrations. 10 additional relatives were homozygous with normal concentrations of γG3, but an 11 yr old paternal male cousin had very low levels of γA (0.040 mg/ml) and polycylonal elevation of γG. A 2 yr old male cousin had low levels of γG for his age group (4.1 mg/ml). The family of patient Feld. with selective absence of γG2 and γA was uninformative regarding genetic markers. The γG level of the father was low (6.2 mg/ml) with proportionate reduction of γG subgroups. The propositus and two female siblings also manifested lymphopenia and impaired delayed hypersensitivity. One of the female siblings lacked γA as well; the other had mild proportionate reduction of all subgroups. The mother and other siblings all had normal levels of γG2 and other subgroups. The mother, a brother, and sister of patient Geo. with predominant γG2 and absent γG1 and γG3 were also uninformative regarding Gm typing. The brother lacked γA. The levels of immunoglobulin classes, subgroups, and Gm markers were otherwise normal. 18 family members of a patient with selective absence of γG2 subgroup have revealed a 2½ yr old sibling with significant reduction in concentration of γG2 (0.105 mg/ml) and γM (0.093 mg/ml), a maternal uncle with γG2 significantly diminished (0.38 mg/ml) and significant diminution of γG3 as well (0.078 mg/ml), and low levels of γG2 in a 12 yr old maternal aunt (0.89 mg/ml).

Families from 18 patients without subgroup imbalance were also studied. The type of hypogammaglobulinemia in the propositus included the following: infantile

sex-linked, 4 patients; non sex-linked, 7 patients; selective inability to produce γA, 3 patients; immune deficiency with thrombopenia and eczema, 1 patient; focal nodular hyperplasia, 1 patient; and unclassified, 2 patients. Of 52 first degree relatives, 27 were heterozygous and no abnormal gene complexes or quantitative abnormalities of γG, γA, or γM were encountered except for a low level of γG in the mother of one patient and a polyclonal elevation of γA in the male sibling of another.

*Relationship of subgroup imbalance to imbalances of light-chain types and other immunoglobulin classes.* Quantitation of kappa and lambda light-chain determinants were carried out on sera from all the patients with hypogammaglobulinemia and some available family members. Isolated γG myeloma proteins or pools of γG proteins of a single light-chain type were employed as standards because the majority of remaining γ globulin in the patients was the γG class. The pooled and individual normal sera and fraction II gamma globulin consistently demonstrated expected ratios of kappa: lambda molecules of approximately 2:1, as did most of the patients with hypogammaglobulinemia. Three patients with hypogammaglobulinemia, one of whom had γG subgroup imbalance, showed reversal of the normal kappa: lambda ratio with lambda type molecules in excess of kappa (Table III). Patient Hx. from the family with structural gene abnormalities showed a consistent slight excess of lambda to kappa in specimens studied over a 2 yr interval. Six additional patients from the group with γG subgroup imbalance showed kappa: lambda ratios of 1.5 or less.

The possibility of "monoclonality" of γG3 in patients with predominance of γG3 subgroup was evaluated by study of the distribution of light-chain types among the γG3 molecules. Detailed studies of the relationship of the γG3 line on Ouchterlony plates with respect to kappa and lambda precipitin lines in patient Hx. and two additional patients with γG3 predominance indicated that the γG3 molecules clearly represented a mixture of kappa and lambda types. The precipitin line produced with γG3 antiserum penetrated through that produced by the kappa antiserum and the lambda antiserum. In addition, both the kappa and lambda lines penetrated through the γG3 line but each was decreased in intensity as a result. In two other patients with γG3 preponderance and marked excess of kappa chain (Rod. and Lind., Table III), technical difficulties prevented a similar analysis. The remaining patients without subgroup imbalances showed ratios of kappa: lambda molecules of approximately 2:1.

Among the patients with γG subgroup imbalance, low levels of γA were observed in all instances and normal levels of γM were noted in only three instances (Table III). Retained capacity to synthesize γM was associated with a variety of subgroup imbalances (predominant γG3, predominant γG2, low γG2). The selective absence of γA was not associated with γG subgroup imbalance in four asymptomatic propositi and in one patient with chronic respiratory infections or in two patients with ataxia telangiectasia. The capacity to synthesize γD molecules was preserved in both sibling pairs exhibiting γG subgroup imbalance (Table III).

## DISCUSSION

The chance mating of persons with structural or regulator gene abnormalities might be expected to result in individuals who lack the capacity to synthesize specific types of heavy chains and manifest as hypogammaglobulinemia. Disproportionate levels of γG subgroups would then be expected since the heavy chains are synthesized at separate although closely linked loci. Numerous instances of such alteration in subgroup concentration were noted in the present study. The most common finding was a relative increase in the γG3 subgroup, although a slight absolute increase was noted in three instances. The γG2 subgroup was also relatively increased in several instances, strikingly so in patient Geo. However, no example was encountered where only one subgroup was depressed without an associated abnormality in another immunoglobulin.

One family, in which the propositus, Hx., had a relative increase in γG3 concentration, clearly showed evidence of gene abnormalities. The mother lacked the expected γG1 marker, Gm(f), and the father lacked the expected γG3 marker, Gm(g). The accumulated evidence indicated that the propositus inherited the two abnormal gene complexes from his parents and was hypogammaglobulinemic. His sister only inherited one abnormal gene complex and was normal. The abnormalities in the two parents were of differing types although they involved closely linked genetic loci. In the patient both chromosomes involved in heavy-chain synthesis contained a defect that appeared to involve structural genes. Just how this defect related to the severe depression of his γG1 and γG2 globulin or to the decrease in γA and γM is not entirely clear. One might have expected the γG1 and γG3 levels to be reduced to one-half without effecting the γG2 subgroup or the other immunoglobulins. However, little is known about the regulation and interrelationships of such closely linked and partially similar structural genes as those involved in the synthesis of different heavy chains. Despite the fact that there appeared to be a complete absence of the specific gene products, the possibility of a regulator gene abnormality in at least one of the parents certainly remains. The associated abnormalities in the other immunoglobulins perhaps favor such an interpretation.

Genetic abnormalities similar to those found in the family of patient Hx. have been encountered in rare instances through population screening (9, 14). However thus far they have only been observed singly in the heterozygous state, and no severe abnormality was noted. They resembled the parents and other relatives of patient Hx. The patient might be considered a double heterozygote for the two defects even though they involved separate heavy-chain subgroups. Detailed studies of the rare families encountered in population screening who showed γG3 gene defects in the heterozygous state (15) revealed that their γG3 levels averaged approximately one-half the normal level, although clear exceptions were encountered. The father of Hx. in the present study had normal levels of γG3 despite a missing γG3 marker. In the case of the families with γG1 abnormalities, the results were not as clear.[1] 2 individuals were encountered with γG1 levels that were strikingly low; however, 19 others, also heterozygotes, showed levels in the normal range. Compensatory synthesis by the other gene in these heterozygous individuals appears to be a common phenomenon in the γ globulin system. No cases either in the present study or in the earlier investigations have been encountered that showed any similar defects in the homozygous state. It can be predicted that such cases will be found. The absence of the expected Gm(f) marker in these families and in the Hx family might also result from the production of an altered γG1 molecule where the Gm(f) site is altered or deleted. The production of unusual proteins such as those found in Franklin's heavy-chain disease cases could account for the findings. Studies are underway in a search for such altered molecules.

The explanation for the hypogammaglobulinemia in the other patients with subgroup imbalance remains unclear. Most of the families were uninformative. Therefore, possible explanation similar to that for Hx. cannot be ruled in or out. However, two families were partially informative, and here such an explanation for the subgroup variations probably could be excluded. Studies on the first degree relatives of these patients indicated a number of defects with reduced γA levels being the most striking. Other abnormalities encountered in the relatives included selective depression of γG2 and γG3, low levels of Gm(f) and Gm(n), and levels of γG and γM which were slightly reduced or in the low normal range. Previous studies (29) had indicated that multiple abnormalities are found in the families of patients of this type including γA depression. Recently, Rivat, Burtin, and Ropartz (30) have noted alterations involving certain genetic markers in a few similar families. Future studies with genetic markers for γA and γM may well reveal relevant structural gene defects. However, at the present time the possibility of regulator gene abnormalities, as proposed some years ago by Parker and Bearn (31), would appear the more attractive explanation for the partial reductions observed in various family members.

The subgroup imbalances noted in the 13 patients of this study were found primarily in those cases showing imbalance of the γG, γA, and γM levels. This might to some extent be expected since the class and subgroup distinction is in part artificial. Current evidence indicates that the heavy chains which determine class and subgroup all are controlled by independent but closely linked genetic loci (11). These appear to have arisen by gene duplication with the closely related subgroup genes representing the most recent duplications. Variations in the distribution of γG subgroups have also been observed, although to a much lesser degree in patients with diffuse hyperglobulinemia, as in systemic lupus erythematosus (4), and in rare patients with normal levels of γG (32).

Subgroup imbalances may be suspected in patients with hypogammaglobulinemia by the configuration of the γG precipitin arc on immunoelectrophoresis. The γG2 and γG4 subgroups are more anodal, the γG1 and γG3 more cathodal. The relative paucity of anodally migrating proteins, which has been observed primarily in some patients with hypogammaglobulinemia (33), would be compatible with either γG1 or γG3 preponderance and requires subgroup specific antisera for further evaluation. Several of the quantitative systems employed in the present study require subgroup-specific antigens occurring in both the Fd and Fc portions of γG subgroup heavy chains. Spurious values might be obtained if some of these antigens are lacking. One instance of an apparent imbalance of γG subgroups, in a serum lacking Gm genetic markers (34), has been shown to be due to the presence of hybrid molecules of γG3-γG1, analogous to the delta-beta chain hybrids established for Lepore-type hemoglobins (14). An unequal homologous crossover involving mispairing of heavy-chain cistrons was postulated to explain the deletion of heavy-chain markers (14). Such aberrations were sought in the present study by hemagglutination-inhibition techniques but no such instance was encountered. Patients with preponderance of γG3 had increased levels of γG3 Fc and γG3 Fab antigens; absence of γG2 or γG1 was for antigens both on the Fab and Fc portions of these molecules.

The possibility was considered that the selective elevation of the γG3 subgroup might be explained in a fashion similar to that for fetal hemoglobin involving a type of nonspecific reversion. For this reason a panel of 5–7 month old infants was studied, a time when nadirs

[1] Litwin, S. D., W. J. Yount, L. Martensson, E. Van Loghem, and H. G. Kunkel. Unpublished observations.

of gamma globulin concentration have been reached and rising levels reflect autologous synthesis. Disproportions of γG subgroups, and particularly γG3 preponderance were not found. The relative preponderance of γG3 might in some instances reflect decreased catabolism. However, this mechanism would require increased survival of γG3 relative to other subgroups, a situation just the reverse of that found in normal individuals (35, 36). The rate of γG3 degradation has been found to exceed that of the remaining γG subgroups.

Another possible mechanism for subgroup imbalances might be the presence of small amounts of monoclonal bands in some patients. The serums of several of the patients in the present series have been shown previously to have limited electrophoretic distribution of gamma globulins when compared with normal individuals, which at times was bimodal (16, 17). The abnormal kappa to lambda ratios for the light chain types of both γG and γM, previously noted in some of these patients (17), were confirmed in the present study. Monoclonal bands have occasionally been described in other patients with primary immune deficiency (37). In the present study considerable attention was paid to this question and this was ruled out in those instances where sera could be tested. The chief evidence against such a possibility was that the subgroup selectively retained contained both kappa and lambda light chains. However, it was not possible to study all cases with regard to this question, and low concentrations of monoclonal bands remains a possibility in some instances. Another possible mechanism for subgroup or light-chain homogeneity is the proliferation of selective clones of immunocompetent cells either transplacentally or after therapeutic grafting. Such examples were not evident in the present series but have been shown to result in synthesis of monoclonal proteins (38).

Some cases of subgroup imbalance might relate to selective microbial or other antigenic stimulation of a limited number of heavy-chain cistrons. Such imbalances would be masked in normal individuals within the total gamma globulin spectrum. It has been recently shown with isolated human antibodies to certain carbohydrates that a single subgroup may be predominant (26). Thus, the minor γG2 class was the major heavy-chain type for antibodies to dextran, levan, and teichoic acid. Variations in light-chain type have also been noted in isolated human antibodies (26, 39), so that isolated antibodies may be enriched in kappa or lambda when fractionated on the basis of specificity. Such a mechanism might be involved in some of the altered light-chain ratios encountered in the present study.

It is perhaps of special interest that in most instances where γG3 subgroup retention was observed in patients with hypogammaglobulinemia it was the Gm(b) type that was selectively present. The Gm(g) type which is coded for by the gene allelic to Gm(b) was very markedly reduced although still detectable by hemagglutination-inhibition techniques. This was particularly striking in two siblings where the Gm(b) molecules showed a 100-fold greater concentration than Gm(g). The significance of this striking imbalance remains obscure. Levels of the γG3 subgroup in normal persons have been shown to relate to genetic type with significantly higher levels in homozygous Gm(b+) individuals, lower levels in homozygous Gm(g+) persons, and intermediate levels in heterozygotes (4).

None of the patients with infantile sex-linked agammaglobulinemia showed an imbalance of subgroup concentration and no gene defects were demonstrable in 18 first degree relatives. The structural genes involved in gamma globulin synthesis have not been found on the X-chromosome in any species studied so that similar gene defects in these cases would not be expected. Other explanations must be sought in these cases such as alterations involved in cell maturation and differentiation.

The relationship between genetic defects observed in the present series and the impaired capacity of lymphocytes isolated from first degree relatives of some patients with adult onset hypogammaglobulinemia to respond to phytohemagglutinin remains obscure (40). The latter suggests a defect in the thymus-derived cells which appear to be primarily involved in cellular immunity. Combined defects in both the antibody-forming and cellular immune systems are frequently encountered in these patients.

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