*Am(1), THE FIRST GENETIC MARKER OF HUMAN IMMUNOGLOBULIN A**

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Abstract.—The genetic markers of human immunoglobulins have significantly contributed to the understanding of the molecular biology of antibody synthesis. Like the Gm markers of IgG, the first genetic marker of serum IgA, a major immunoglubulin of exocrine secretions, has now been defined and termed Am(1). It is inherited as a Mendelian dominant trait and is independent of the Gm and Inv allotypes. Am(1) is localized in the α-chains of the γA_2 subclass and is independent of the serum γA_2 levels. Its polymorphism makes it suitable for studies in population genetics and the molecular biology of IgA globulin.

More than 25 isoantigens ("allotypes," the Gm and Inv groups) of human immunoglobulins have been delineated by serological methods of passive hemagglutination. The Gm groups are associated with the heavy polypeptide chains of IgG (the γ-chains) and the Inv groups with the light chains common to the three classes, IgG, IgA, and IgM. Their genetic, immunologic, and biochemical analyses have contributed immensely to the current concepts of molecular biology and genetic control of antibody synthesis. The genetic polymorphism of these antigens has influenced such diverse fields of biology as evolution, population genetics, and forensic medicine.[1]

Recognition of similar allotypes of human α- and μ-chains (heavy chains of IgA and IgM) was limited by unavailability of suitable serological assays. However, the new passive hemagglutination assay, using IgA paraproteins (purified myeloma proteins) for detection of nonprecipitating human antibodies to human IgA, has opened the possibility of defining the allotypes of the α- and μ-chains.[2] Since IgA is the major immunoglobulin in all exocrine secretions responsible for what is now recognized as the local immunity of mucous membranes,[3] identification of genetic isoantigens of IgA is essential to the study of the immunobiology of human IgA.

Human IgA globulin has been differentiated into two subclasses (called γA_1-γA_2, Le-He, or Major-minor) based on the capacity of certain rabbit antisera raised against γA_1 paraproteins to show antigenic deficiency of γA_2 proteins relative to γA_1 in agar-gel diffusion analysis.[4–6] Grey *et al.* have observed that paraproteins of γA_2 subclass have a unique characteristic of noncovalently bound light chains which dissociate and migrate as a fast-moving band on acid-urea-starch gel electrophoresis.[6] They further observed that the γA_1 and γA_2 proteins are present in the sera and secretions of normal persons of various populations and do not represent genetically determined differences in IgA globulin.

The first genetically determined isoantigen Am(1) has now been defined, using as an agglutinator the serum of a woman having anti-IgA antibodies causing

serious anaphylactoid reactions to transfusion of blood with biologically incompatible IgA. This paper reports our studies defining this genetic marker, establishing its inheritance, demonstrating its molecular localization in the α-chains and its association with only the γA_2 subclass of human IgA. This allotype, in keeping with the established Gm nomenclature for γ-chain, has been termed Am(1) (A denoting α-chains, and m denoting genetic marker).

Materials and Methods.—Serum was obtained from a Caucasian female (W. F.) before and three weeks after transfusion of blood. Neither specimens contained atypical isoantibodies to any of the formed elements of blood except an anti-IgA of limited specificity (titer 1:256) reacting with paraproteins IgA(2), IgA(6), and IgA(12) from a panel of 20 proteins used in the assay. Details of the procedure of coating proteins by the $CrCl_3$ method and assay for the detection of anti-IgA and its serological specificity have been published elsewhere.[2] The IgA(6) coat was selected for further studies with W. F. serum. It was observed that agglutination of the IgA(6) coat by her anti-IgA antibodies was inhibited by certain normal human sera but not by others. Therefore, this polymorphic inhibititory ability of normal sera was studied further in an agglutination inhibition assay. Anti-Am(1) agglutinator was made up by mixing one part of serum W. F. with one part of a noninhibitory serum (C. S.) and 38 parts of TAP buffer (Tween, bovine serum albumin, polyvinyl pyrollidone).[2] The test serum samples were diluted 10-, 20-, and 40-fold for testing their inhibitory ability (i.e., Am typing) using appropriate controls as is done for the Gm typing.[7] Samples of saliva from selected donors were concentrated 10-fold and tested concurrently with their respective sera for Am(1) typing. An agglutination inhibition assay for relative levels of γA_2 proteins in normal sera was set up with an anti-γA_2 antiserum raised in monkeys and paraprotein IgA(2) as an indicator coat. The heavy and light polypeptide chains of IgA proteins were prepared by reduction, alkylation, acid dissociation, and Sephadex G-200 gel filtration.

Results—The discrimination between inhibitory and noninhibitory ability of normal human sera tested in the Am(1) system was more than 128-fold. An Am(−1) serum was noninhibitory when tested undiluted, whereas the inhibitory titers of representative Am(1) sera were 1:128–256, which is comparable with the sensitivity of the Gm and Inv systems. The results of Am(1) typing on the 621 sera, showing the phenotype frequencies in Caucasians, Negroes, Japanese, and Chinese, are listed in Table 1. All but three of the inhibitory samples showed inhibition at a 1:40 dilution. The three samples which inhibited only at a dilution of 1:20 were interpreted as Am(1). The gene frequencies in various populations show the polymorphism of Am(1).

To establish the inheritance of Am(1), serum samples from 264 members of 51 Japanese families were typed for Am(1). The parental combinations and the frequency of Am(1) and Am(−1) offspring are shown in Table 2. The inheritance of Am(1) was established by genetic analysis of the data as follows: The alleles are designed *Am* and *am* (hypothetical).

Gene frequencies (from Table 1): $Am = 0.5130$
$am = 0.4870$

Genotype frequencies: $AmAm = 0.2632$
$Amam = 0.4996$
$amam = 0.2372$

The expected genotype frequencies of various matings and their off-spring were calculated from these data. The calculated values were then used for deriving

TABLE 1. *Frequency of allotype of human IgA (Am(1)) in various races.*

Race	Number tested	Am(1) Phenotype: Positive No.	Positive %	Negative No.	Negative %	Gene Frequency: Am(1)	Hypothetical allele
Caucasian	351	344	98.00	7	2.00	0.9858	0.0142
Negro	108	56	51.85	52	48.15	0.3061	0.6939
Japanese	116	85	76.28	31	23.72	0.5130	0.4870
Chinese	50	28	56.00	22	44.00	0.3367	0.6633

TABLE 2. *Inheritance of Am(1) studied in 51 Japanese families.*

Matings	Number of Families Obs.	Number of Families Exp.	Number of Offspring Am(1) Obs.	Number of Offspring Am(1) Exp.	Number of Offspring Am(−1) Obs.	Number of Offspring Am(−1) Exp.
Am(1) X Am(1)	29	30	84	83	9	10
Am(1) X Am(−1)	19	18	44	43	20	21
Am(−1) × Am(−1)	3	3	0	0	5	5
Total	51		128		34	

expected phenotype frequencies of matings and the proportion of Am(1) and Am(−1) offspring from each mating. The observed and expected frequencies of matings and of Am(1) and Am(−1) offspring are compared in Table 2. These data provide convincing evidence of the inheritance of Am(1). The distribution of Am(1) in a family illustrated in Figure 1 and the observation of five Am(−1) children from three Am(−1) × Am(−1) matings (Table 2) indicate an autosomal dominant mode of inheritance.

An attempt was then made to determine whether a relationship exists between Am(1) and the Gm, ISf, and Inv allotypes. For this purpose a WHO reference panel of sera extensively typed for Gm, ISf, and Inv factors was tested in the Am(1) system. The results indicated that Am(1) is not related to Gm, ISf, and Inv phenotypes. The alleles Gm^1, $Gm^{1,2}$, and $Gm^{3,4,5,13,14}$ commonly present in Caucasians, $Gm^{1,5,13,14}$, $Gm^{1,5,14}$, and $Gm^{1,5,6}$ in Negroes and Gm^1, $Gm^{1,2}$, $Gm^{1,13}$, and $Gm^{1,3,4,5,13,14}$ in Asians were distinctly different from the Am^1 allele. In addition, a rare serum (2904) negative for all Gm factors[8] inhibited the Am(1) system to a titer of 1:256. Therefore, the locus for Am(1) is different from the loci for Gm, ISf, and Inv.

Since IgA is the major immunoglobulin of exocrine secretions,[3] we ran concurrent tests on samples of saliva and donor serum in the Am(1) system. The saliva of seven of the ten subjects tested was Am(1); the saliva of the other

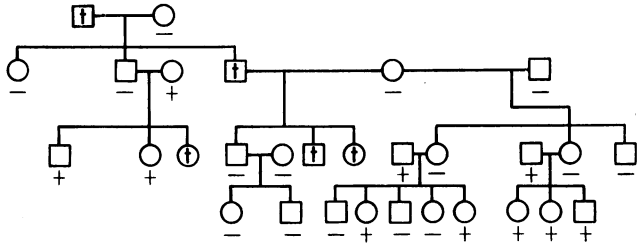

FIG. 1.—A Negro pedigree showing inheritance of the Am(1) antigen. Am(1) and Am(−1) are indicated by + and −, respectively.

three was Am(−1). The inhibitory titers ranged from 1:64 to 1:256. The results of Am(1) typing of saliva and serum from the same subject were in agreement and were independent of his secretor status for ABH blood group substances.

Thirty Caucasian cord sera were also tested in the Am(1) system. Only 1 of the 30 samples was inhibitory (at a dilution of 1:10). This serum was obtained from an infant who had received three intrauterine transfusions. The Am(1) antigen is fully developed, with inhibitory titers of 1:40 and higher, in infants at the age of one year.

The association of the Am(1) antigen with α-chains was demonstrated by Am(1) typing of isolated polypeptide chains as shown in Table 3. Although

TABLE 3. *Inhibitory activity of intact molecules and isolated polypeptide chains of IgA (2), IgA (GNV), and IgA (C.S.) tested concurrently for Am 1).*

Test material (1 mg/ml)	Am(1) Detected at	
	Titer	mg/ml
IgA (2)		
Native	1:256	0.004
H chains	1:256	0.004
L chains	1:4*	0.250
IgA (GNV)		
Native	1:32	0.030
H chains	1:16	0.060
L chains	N.D.†	N.D.
IgA (C.S.)‡		
Native	N.D.	N.D.
H chains	N.D.	N.D.
L chains	N.D.	N.D.

* Possibly due to contaminating trace of H chains.
† N.D. = Not detectable. Failure to inhibit Am(1) system.
‡ C.S. is known to be Am(−1).

isolated heavy polypeptide chains of Am(1) proteins retained inhibitory activity, on a molar basis the isolated H chains had slightly reduced activity compared with the intact molecule; the light chains showed no significant Am(1) activity. Further studies on molecular localization, structural relationship and genetic linkage between Gm and Am loci will be reported later.

When tested for their Am(1) activity the paraproteins IgA(2), IgA(6) and IgA(12) were inhibitory up to 0.002 to 0.008 mg/ml protein concentration. However, IgA isolated from normal Am(1) serum was inhibitory only up to 0.062 mg/ml protein concentration. These results were consistent with the observation that the above three paraproteins belong to the γA_2 subclass with the characteristic noncovalently bound L chains[6] and this subclass represents only 6–7 per cent of the total serum IgA.[5] The association of the Am(1) allotype with the γA_2 subclass required evidence that an Am(−1) phenotype, indeed, did not represent a genetically determined selective absence of γA_2 protein in the serum. The estimates of the relative γA_2 levels in equal numbers of randomly selected Am(1) and Am(−1) normal Negro sera are shown in Table 4. Despite the wide variations in the relative levels of γA_2, their distribution was similar in Am(1) and Am(−1) sera. Therefore, the all or none of the inhibitory

ability of these sera in the Am(1) system was independent of a relatively wide dispersion of their inhibitory titers in the γA_2 system.

TABLE 4. *Comparison of quantitative levels of γA_2 in Am(1) and Am(−1) normal Negro sera.*

γA_2%	Am(1) (no.)	Am(−1) (no.)
1.56	1	1
3.12	4	6
6.25	9	6
12.50	3	5
25.00	1	0
Total	18	18
Mean γA_2%	7.4	6.6

Discussion.—Anti-IgA antibodies have been demonstrated in the serum of patients who experienced anaphylactoid reactions to blood transfusions.[9–11] Serum of one such patient has enabled us to define the first allotype of human IgA. The sensitivity of the serologic system for the detection of Am(1) is comparable to that for Gm(1), (4), and (5) using myeloma proteins.[7, 12] Our studies with the system showed that the Am(1) antigen is present only in the γA_2 subclass of IgA. Studies by other workers, however, have demonstrated both subclasses of IgA in normal serum, colostrum, and saliva specimens, indicating that they do not represent genetic variants.[5, 6] Many of our test serum samples containing normal amounts of IgA lacked the Am(1) antigen. The data obtained in the family studies (Table 2) show a Mendelian dominant autosomal mode of inheritance.

We have observed certain discrepancies yet unresolved in immunologic classification of some of our IgA paraproteins typed in the laboratories of three earlier investigators. This may have been due to more than three subclasses of IgA possibly discerned by the three groups. The suggestion by Vaerman *et al.*[5] that the subclasses of α-polypeptide chains produced by separate gene loci could be analogous to the subclasses of γ-polypeptide chains may well be true. We have confirmed the findings of Grey *et al.* that the fast moving band of noncovalently bound L chains in the γA_2 subclass is a consistent characteristic of these proteins when the purified proteins are subjected to acid-urea-starch gel electrophoresis.[6] Using specific precipitating anti-γA_2, Grey *et al.* have reported the presence of γA_2 protein in each of 200 sera from various population groups.[6] Since specific anti-γA_2 was not available, Vaerman *et al.* could not directly estimate γA_2,[5] but a value of 6.6 per cent was indirectly derived by testing 445 normal sera for their levels of total IgA and the γA_1 subclass. This is at variance with direct γA_2 quantitation of 20 to 36 per cent of total IgA in three normal sera reported by Grey *et al.*[6] Although the hemagglutination inhibition with serial double dilutions of serum has inherent limitations in providing absolute levels of γA_2, our data yielded a mean direct value of 6 to 7 mg/100 ml in normal human sera. However, the lack of relationship between levels of γA_2 and Am(1) phenotype (Table 4) is consistent with the fact that Am(1) is a genetically determined marker of the γA_2 subclass of IgA globulin.

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Note added in proof: Since completion of this work, Kunkel *et al.* have defined a genetic marker Am_2, associated with the γA_2 subclass (*Nature*, **223,** 1247 (1969)). Based on its gene frequency, Am_2 appears to be similar, if not identical, to the Am(1) described herein.

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