Selective Deficiency of Immunoglobulin A2

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ABSTRACT A case of familial selective IgA2 deficiency is described. The mother had no detectable IgA2, but a low level of IgA1. She had anti-α2 antibodies of the IgG class. One of her daughters also lacked IgA2 with a normal level of IgA1.

The analysis of the immunoglobulin haplotypes of the family suggested the deletion of the α2-gene. In addition, the analysis of B lymphocytes of mother and daughter showed the absence of IgA2-bearing cells. Upon stimulation with pokeweed mitogen, the B cells differentiated into IgA1-containing plasma cells, but IgA2-containing cells were not found. The results suggest a defect in the generation of intraclonal B cell isotype diversity. The molecular basis of this phenomenon is unknown.

INTRODUCTION

In man, primary immunoglobulin (Ig) deficiencies are known in which either all Ig classes or distinct Ig classes or subclasses are severely decreased or completely lacking (1, 2). Several reports have been published on the familial occurrence of Ig deficiencies (1–6).

The increased knowledge of the ontogeny of the B cell system in man has led to the discovery that patients with Ig deficiency may nevertheless have the appropriate precursor cells of the B cell lineage (7–9). This indicates that differentiation defects of B cells or B cell precursors, rather than structural Ig-gene defects, may be the basis of disorders of humoral immunodeficiency. Examples of these types of humoral immunodeficiency are common variable hypogammaglobulinemia, congenital agammaglobulinemia, agammaglobulinemia based on deficiency of transcobalamine II, and some, if not all, cases of selective IgA deficiency (2, 5, 10–12).

Received for publication 29 March 1983 and in revised form 18 August 1983.

The typing of genetic markers of human IgG, IgA, and kappa chains provides possibilities to investigate defects or deletions of structural Ig genes in selective humoral immunodeficiency. In this approach, use is made of the fact that the heavy-chain genes are closely linked on the chromosome and that the known heavy-chain allotypes (Table I) are inherited in fixed combinations called haplotypes (13–15). Individuals homozygous for haplotypes lacking one of the heavy-chain loci, will not exhibit any of the allotypes or isotypes of the locus concerned. The composition of a haplotype can be deduced from its segregation in a family. Application of Gm and Am typing in two families with selective IgA deficiency (for both subclasses) has brought indirect evidence that the deficiency observed in these families was not due to a structural gene defect (5). On the contrary, in studies of the family of individuals with selective subclass deficiency of IgG1, IgG2, or IgG3, a deletion of IgG-subclass structural genes has probably occurred (16, 17). In one case of kappa-type Ig deficiency (18), evidence was obtained that the C-kappa gene is deleted from B cell lines obtained from the patient, whereas the J-kappa segments are present (J. Stavnezer, personal communication).

We now present a family with IgA2-subclass deficiency occurring in two members. The analysis of the

TABLE I
Genetic Markers of Human Immunoglobulins

Light chains	Heavy chains				
κ	γ^1	γ^2	γ^3	α^2	ϵ
Km	G1m	G2m	G3m	A2m	Em
1	f,a	n	b0, g5	1	1
2	z, x		b1, b4, g1	2	
3			b3, c3		
			b5, c5, v		
			s, t, u		

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/83/12/1918/06 $1.00*
Volume 72 December 1983 1918–1923

immunoglobulin haplotypes suggested that members of the family lack the structural gene coding for $\alpha 2$-chains. Analysis of the B cells of the family disclosed the absence of IgA2-bearing cells in two members.

METHODS

Case report. A 35-yr-old mother (Fig. 1, I-2) of three healthy daughters, born in 1966, 1969, and 1972, respectively, underwent hysterectomy in December 1978 and appendectomy in June 1979. After the latter operation, she developed a severe anaphylactoid reaction following a whole-blood transfusion. Further medical history revealed very mild, but recurrent infection of the upper respiratory tract since the last few years. The patient had suffered from sarcoidosis 7 yr before. Physical examination revealed no abnormality. The transfusion reaction was due to the presence of anti-IgA antibodies (see below). Serum protein electrophoresis showed no abnormality. Despite the presence of anti-IgA antibodies, a low level of serum IgA was found, i.e., 0.04 g/dl; serum levels of IgM and IgG were normal. These findings prompted us to analyze the serum IgA subclasses, the specificity of the anti-IgA antibodies as well as the genetic markers of the patient, her husband (who, as far as known, was not related) and her three daughters.

Procedures. Serum IgM, IgG, and IgA, as well as the serum IgG subclasses, were quantitatively determined, as described earlier in detail (19, 20). Radioimmunoassay (RIA) of IgA was done by a two-site sandwich RIA, following the general procedure as described for that of IgG (21). Anti-IgA was coupled to CNBr-activated Sepharose-4B (Pharmacia Fine Chemicals, Piscataway, NJ) and incubated with sample. After a washing, ^{125}I-anti-IgA2 was added and bound ^{125}I-anti-IgA2 was measured. The ^{125}I-anti-IgA2 antibodies were isolated from monkey anti-human IgA2 (monkey VI) with the use of Sepharose-bound IgA2. In this assay, 0.18 ng purified monoclonal IgA2 resulted in significant binding above the blank values. The classical hemagglutination-inhibition test in microtiter plates was used for allotype and IgA-subclass determination and to investigate the specificity of anti-Ig antibodies (5).

Determination of homozygosity or heterozygosity for G2m(n) of patient II-2 was done by affinity chromatography. Samples of known zygosity were used as controls. First, purified monoclonal IgG2 protein (positive for G2m[n]) was coupled to CNBr-activated Sepharose CL-4B in ratios of 10 mg IgG2 to 1 g Sepharose. A column of 0.9 × 15 cm, bed volume 10 ml, was equilibrated with 0.01 M phosphate-buffered saline (PBS) at pH 7.4. 30 ml of a monkey anti-G2m(n) (monkey P) serum was applied and then the column was washed with PBS. The adsorbed antibodies were eluted with McIlvain's citrate-phosphate buffer in physiological salt solution at pH 3.0. After neutralization and concentration to 1 ml (content 2.2 mg protein), the purified antibodies were bound to a second batch of CNBr-activated Sepharose CL-4B; this anti-G2m(n) column was used to deplete the sera of G2m(n).

Ficoll-Hypaque-isolated peripheral blood lymphocytes (PBL) were studied for the expression of IgA1 and IgA2 on the cell surface. IgA subclass-specific monoclonal antibodies (Becton, Dickinson & Co., Mountain View, CA) derived from the same cell clones as described in detail by Conley et al. (22) were used. Indirect immunofluorescence with fluorescein isothiocyanate-labeled antibodies against mouse IgG2b (for IgA2) and mouse IgG3 (for IgA1) have been applied (22). The total number of IgA-bearing B cells was determined (in combination with the subclass-specific staining) using an affinity-purified, tetramethyl-rhodamine-isothiocyanate-labeled anti-IgA conjugate (23). In this study, ~75–100 cells bearing surface IgA were counted in each preparation. B cell activation of PBL was performed by using pokeweed mitogen (PWM)[1] as described earlier (24). Cytocentrifuge preparations of the cultures were studied for the presence of Ig-containing cells; IgA-class and IgA-subclass expression were studied in combination staining with the antibodies mentioned above.

RESULTS

Serum studies. The serum of the patient (Fig. 1, I-2) agglutinated IgA2-coated cells (of the A2m[1] as well as of the A2m[2] allotype), but not cells passively coated by IgA1, IgM, or IgG. The reaction with A2m(1)-coated, as well as that with A2m(2)-coated cells could exclusively be inhibited by IgA2 proteins (equally well with either allotype). The specificity of the antibodies present in the serum of the patient was therefore anti-$\alpha 2$. The titer was ~1:100.

Table II shows the serum IgM-, IgA-, and IgG-subclass levels in the family members. The serum of the patient contained some IgA. By analysis in hemagglutination inhibition and in the RIA and confirmed indirectly by the lack of the antithetical allotypes A2m(1) and A2m(2), this IgA proved to be IgA1 only. Another approach to test for IgA subclasses was done by fractionation of the serum of the patient on an ultrogel-ACA34 column (Fig. 2). Fractions 45–70, containing both IgA and IgM, and fractions 75–90, containing IgG, were pooled. The first pool contained IgA1 but not IgA2, the second contained anti-$\alpha 2$-antibodies. Hemagglutination-inhibition experiments, aimed to test for the presence of IgA1 and IgA2 in the sera of the husband and the daughters, showed that the serum of the second daughter (Fig. 1, II-2) did not inhibit a system for the detection of IgA2 nor one for the allotypes A2m(1) or A2m(2). This child (11 yr of age) had a normal level of serum IgA (Table II), which proved to be IgA1. The lack of IgA2 could be confirmed by RIA. The father (I-1) and the daughters (II-1 and II-3) had normal levels of IgA2. With respect to the other serum Ig-isotype levels, only the father showed a lower serum IgG1, which is in the lowest range of control values.

The Gm phenotypes of the family are shown in Fig. 1. I-1, II-1, and II-3 were A2m(1+2−). The Em(1) allotype was present in I-2, the other family members were not tested for Em(1). In I-2 and II-2, no A2m allotypes were present. The A2m phenotypes are not informative for segregation of α-genes in this family, because the father is seemingly homozygous for A2m(1), whereas the mother lacks IgA2 and, therefore, does not

[1] *Abbreviation used in this paper:* PWM, pokeweed mitogen.

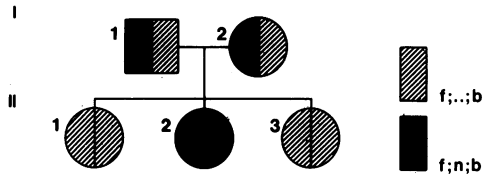

FIGURE 1 Pedigree of family B. Inheritance of Gm haplotypes. I-1, II-1, and II-3 are A2m(1+2−); I-2 and II-2 are A2m(1−2−).

express an A2m phenotype. However, the polymorphism on Gm allotypes offers an indirect way to study the inheritance of α-genes in families, because of the close linkage of α- and γ-genes (15). The Gm phenotype of II-1 and II-3 shows that one of the haplotypes in each parent must be *Gm(f;..;b)*. Therefore, both parents must be heterozygous *Gm(f;n;b/f;..;b)*. We postulate a deletion of the α2-gene in both the haplotypes of the mother. If the father is heterozygous for a haplotype with a deletion of the α2-gene, then his normal haplotype must be *Gm(f;..;b)A2m(1)*, which follows from the presence of IgA2 in the daughters II-1 and II-3, who have inherited the paternal haplotype without G2m(n). The α2-deleted paternal haplotype would then be *Gm(f;n;b)A2m(−)*. The daughter II-2 may have inherited this haplotype and thus be homozygous for a Gm-Am haplotype with deletion of the α2-locus. This homozygosity could definitely be proven as follows. Serum samples from known G2m(n) homozygous, heterozygous, and negative individuals and from II-2 were applied to an anti-G2m(n) column. In the filtrate, concentrations of IgG2 and G2m(n) were determined: simultaneous loss of IgG2 and G2m(n) was found for the G2m(n) homozygote and for II-2, whereas the heterozygote control showed disappearance of G2m(n) with partial loss of IgG2 (Table III).

Lymphocyte studies. IgA-bearing cells were present in all family members studied (Table IV). The percentages in the mother (I-2) and the IgA2-deficient daughter (II-2) were low; the normal range in our laboratory is 0.5–3.5%. All IgA-bearing B cells in I-2 and II-2 exclusively carried IgA1, whereas in II-3 the normal predominance of IgA1 over IgA2 was found as reported by Conley et al. (22). B cell activation with PWM resulted in the generation of IgA-containing blasts in all family members studied (Table IV); mother (I-2) and daughter (II-2) showed only IgA1-containing blasts. In contrast, the plasmablasts of II-3 included IgA1- as well as IgA2-containing cells in a ratio as previously described (22, 25).

DISCUSSION

A selective deficiency of IgA may be associated with a number of diseases, such as ataxia telangiectasia, coeliac disease, autoimmune diseases, etc., but can also occur in healthy individuals and clustering in families is observed. The frequency reported varies between 1:300 and 1:3,000, depending on the method used for screening and on the population tested. When very sensitive methods were used, no IgA could be demonstrated in individuals with anti-IgA antibodies, whereas in cases without antibodies, trace amounts of IgA were often detectable (6).

The distinction between low levels and total absence of an Ig isotype may be of importance for the consideration of the genetics of the deficiency. In the former case, regulatory genes (5) of even non-Ig nature may be involved, in the latter, a defect of a structural Ig gene may be considered. Gm haplotypes with a deletion of one IgG-subclass gene have been described (16), but such haplotypes must be rare. Recently, simultaneous absence of IgG1, IgG2, IgG4, and IgA1 subclasses was

TABLE II
Serum IgM-, IgA-, and IgG-Subclass Levels

	IgM	IgA	IgG1	IgG2	IgG3	IgG4
			g/liter			
Family B						
Father I-1	1.51	2.53	4.75	3.19	0.75	0.27
Mother I-2	1.00	0.04	11.0	1.10	0.38	0.12
Daughter II-1	1.47	1.73	10.28	3.08	0.80	0.28
Daughter II-2	1.83	0.61	10.04	2.97	0.50	0.26
Daughter II-3	1.42	1.04	9.34	1.87	0.90	0.14
Normal controls*						
4–13 yr	0.97 ± 0.37	0.85 ± 0.37	6.8 ± 1.9	1.8 ± 0.55	0.55 ± 0.25	0.4 ± 0.35
Adults	1.32 ± 0.46	1.44 ± 0.65	7.1 ± 1.7	3.8 ± 1.1	0.7 ± 0.35	0.6 ± 0.4

* See references 19 and 20.

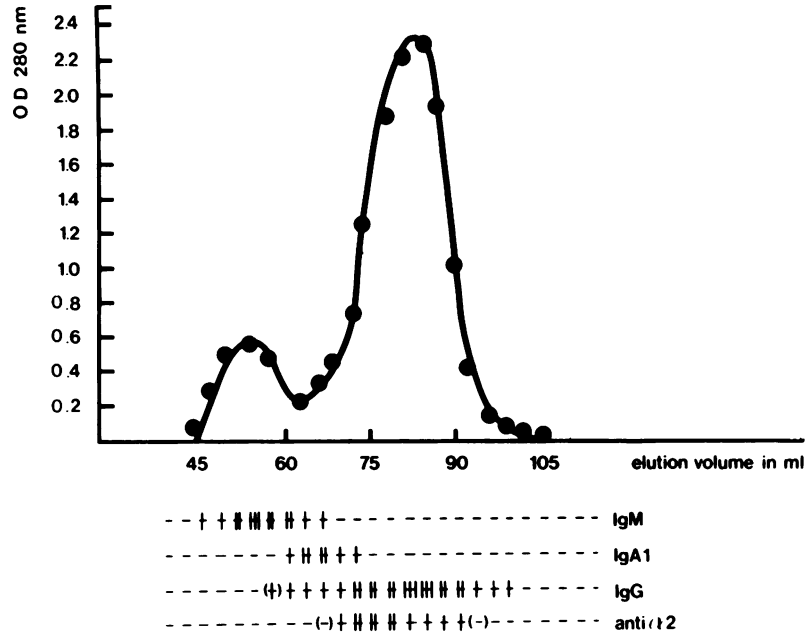

FIGURE 2 Elution pattern obtained after chromatography of serum of I-2 in PBS on ACA-34 column (3-ml fractions).

found, the deletion of a block of $\gamma_1 - \alpha_1 - \gamma_2 - \gamma_4$ structural genes was postulated and confirmed by DNA analysis (26, 27). As far as IgA deficiency is concerned, autosomal dominant and recessive modes of inheritance have been described (3–5). The finding of a normal percentage of lymphocytes carrying IgA in cases of selective IgA deficiency argues against a possible defect of structural α-genes (9). In families with selective deficiency of both IgA subclasses, it was proven by typing for Ig allotypes that the genes, regulating the synthesis of IgA in these families, are transmitted independently of structural Ig genes (5).

As far as we know, there are no data on the incidence of IgA-subclass deficiency. Quantitation of IgA1 and IgA2 usually is done only in selected cases. However, absence of IgA2 can be noticed when typing for A2m allotypes. There are two alleles of α2-genes, i.e., A2m(1) and A2m(2), which are antithetical markers: all IgA2 proteins carry either the one or the other allotype (28–30). Among >8,000 samples investigated in our laboratory for A2m, we found 17 samples negative for both

TABLE III
Inhibition Titer of γ^1- and γ^2-Isotype and G2m(n) Allotype Before and After Immune-Adsorbance Chromatography

	Gm genotype	γ^1	γ^2	G2m(n)
B.II-2	fnb/f?b			
Before		40,000	10,000	10,000
After		20,000	80	80
Donor H	fnb/fnb			
Before		10,000	2,500	2,500
After		5,000	40	40
Donor L	zaxg/fnb			
Before		20,000	10,000	10,000
After		10,000	1,280	320
Donor D	zaxg/zaxg			
Before		40,000	2,500	0
After		10,000	640	0

TABLE IV
IgA Subclasses on the Cell Surface of B Cells or in the Cytoplasm of Blasts, Generated In Vitro

Family B	Surface IgA* (α_1:α_2)	Cyt IgA‡ (α_1:α_2)
Mother I-2	0.1 (100;0)	2.1 (100;0)
Daughter II-2	0.5 (100;0)	3.7 (100;0)
Daughter II-3	1.2 (68;28)	4.0 (49;42)

* Percentage of IgA-bearing cells within the total of lymphoid cells; within brackets, the percentages of IgA1- and IgA2-bearing-cells are given, respectively.
‡ PWM-generated blasts were tested for cytoplasmic staining; between brackets, the percentages of IgA1- and IgA2-positive cells are given, respectively.

A2m(1) and A2m(2). These samples were quantitatively tested for α, $\alpha 1$, and $\alpha 2$. The detection limit is in the range of 0.2–0.5 μg/ml. All samples showed to be deficient in both subclasses (IgA1 and IgA2) except for two samples that were deficient for IgA2 only, one from a volunteer donor and the other from the proposita of family B (I-2), who had anti-IgA antibodies.

Anti-IgA antibodies in IgA-deficient individuals can cause severe transfusion reactions when IgA-containing products are administered (31–34). The agent that stimulates the generation of anti-IgA antibodies in deficient individuals is not known. Cross-reacting antigens in dairy products or infectious bacteria or yeasts have been suggested to serve as a trigger for antibody production. The anti-$\alpha 2$-antibodies in the proposita (I-2) could have been evoked as a result of blood transfusion during hysterectomy. These antibodies belonged to the IgG class and, therefore, may have passed the placenta. If such antibodies were already present before her pregnancies, these might have inactivated those clones of B cells that synthesize IgA2 of the newborn. Gene suppression mediated by either anti-isotype or anti-allotype antibodies has been reported in mice and rabbits (35). It is questionable if this mechanism acts in man in view of the fact that in the families of mothers with anti-IgA antibodies, which we investigated, IgA-deficient and -nondeficient children alternated (5).

The frequency of the haplotype carrying a deleted or injured gene must be rather low, but probably less rare than is usually supposed, because the presence of such a gene will be evident in homozygotes only. Therefore, it was interesting to know whether there was a family relationship between the father and the mother. No such relationship could be established for three generations. The finding that two different haplotypes occur with a structural gene deletion is not unusual. The pedigrees of families with members lacking IgG1 and IgG3 showed inheritance of more than one kind of deleted haplotype that carried different allotypes of other subclasses (16, 17).

The results obtained in family B suggest that a structural Ig-gene defect is the basis of the IgA2 deficiency. This view is supported by the observation that the mother and daughter lack IgA2-bearing lymphocytes; this was confirmed by the lack of appearance of IgA2-containing blasts after B cell activation by PWM and after B cell activation with formalinized *Staphylococcus aureus* (data not shown). These findings might be caused by the action of IgA2 subclass-specific suppressor T cells (11). However, the absence of IgA2 on the B cell surface makes the hypothesis of a structural Ig-gene defect the most likely explanation. The finding implies that the generation of intraclonal isotype diversity is impaired in the IgA2-deficient members of this family.

ACKNOWLEDGMENTS

We thank F. van Leeuwen, P. van Eede, and Ms. Annemarie Smulders for their excellent technical assistance. We are indebted to Mrs. Gerda de Lange and Drs. F. Gmelig Meyling, H. J. Schuurman, W. Kuis, T. A. Out, and G. C. de Gast for their help and stimulating discussions. Anti-mouse IgG-subclass antisera were kindly donated by Dr. M. Cooper, Birmingham, AL.

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