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Study of a French Family with a New Variant of Blood Group A: A_{lae}

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INTRODUCTION

Although A_1, A_2, and A_3 are well-established subgroups of human blood group A, several weaker variants have been described; they have been grouped by Race and Sanger into two major phenotypes, A_x and A_m [1]. The weaker forms such as A_{end} and A_{el} do not fit into this classification. Form A_{el} is considered to be the weakest variant of A and has been detected in at least 10 families [2–4]. The present report describes a French family with a new inherited variant of blood group A. The variant is indistinguishable from normal group O cells when standard cell typing and serum typing (reverse grouping) are performed. However, the red cells absorb anti-A from sera of groups B and O, and the eluates react with A_1 and A_2 cells. The unique feature of the variant is its detection by lectin or phythohemagglutinin from *Dolichos biflorus* with anti-A_1 specificity (hereafter designated DBL). The new allele is designated A_{lae}; the letter *l* in the subscript designates detection by lectin, and the letters *ae* designate its detection by absorption and elution of anti-A. The survival of ^{51}Cr-labeled A_{lae}O cells in a group-O normal volunteer was investigated to assess the clinical implications of this type.

MATERIALS AND METHODS

Initial tests on all members of the pedigree were performed in Strasbourg using commercially obtained anti-A, anti-B, anti–A+B, absorbed human anti-A (hereafter designated human anti-A_1), DBL, and lectin anti-H (*Ulex europeus*). Plain clotted blood and saliva specimens treated at 100° C for 30 min were collected in Strasbourg and shipped by air to San Francisco in insulated containers with adequate coolant. Upon arrival, the serum and clot of each specimen were separated, and the clot was chopped and mixed with saline

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to obtain a fresh cell suspension. The cell suspension was washed twice before use in the blood grouping tests using standard tube techniques [5]. Commercial typing reagents were used in accordance with the manufacturer's directions. A high-titered immune anti-A (1:8000 by antiglobulin reaction) was obtained from a woman who had given birth to a baby with hemolytic disease of the newborn caused by anti-A. In addition, a panel of sera from unselected pilot tubes from 50 group-B and 50 group-O donors was used for testing the $A_{lae}O$ cells. The $A_{lae}O$ cells were tested with lectin and human anti-H (obtained from a "Bombay" donor) using serial twofold dilutions of each reagent. The cells which did not react with anti-A, anti–A+B, immune anti-A, and human anti-A_1 in a hemagglutination reaction carried out at room temperature were incubated at 37° C and tested by an indirect antiglobulin test using anti-human serum (Ortho); similarly, the cells after papain treatment [5] were also tested with the same reagents and carried through the antiglobulin reaction. Serum from each member of the family was tested with commercially obtained reagent cells of group A and group B; in addition, blood was freshly collected from staff members having blood types A_1, A_2, B, and O for use in serum grouping and as controls. The secretion of ABH-group-specific substances in saliva was tested by standard hemagglutination inhibition assay [5]. For absorption-elution experiments, the red cells incubated with anti-A, anti–A+B, and human anti-A_1 were washed eight times with saline, mixed with equal volumes of saline, heated at 56° C for 10 min, and centrifuged at 56° C. The supernatant elutes were tested with cells of group $A_{lae}O$, A_1, A_2, B, and O. All hemagglutination reactions were scored at 4+, 3+, 2+, and + [5]. The red cells of subject I-5 were labeled with 80 μc of ^{51}Cr and injected into a normal group-O male volunteer. Similarly, the red cells of subject II-5 were labeled with ^{51}Cr and injected into her own circulation to obtain autologous survival time. The half-life of ^{51}Cr-labeled red cells was determined by the rate of disappearance of the label from the circulation over a period of 3 weeks.

RESULTS

The results of ABO blood grouping and ABH secretor status are shown in table 1, and the inheritance of the A_{lae} allele in the family is illustrated in figure 1. The serological reactions of the $A_{lae}O$ genotype of the propositus and six other members of the pedigree (I-5, I-7, II-5, II-9, III-3, and III-6) were unique and remarkably uniform. The characteristic features were: (1) Although the cells failed to react with typing reagents anti-A, anti-B, anti–A+B, and a high-titered immune anti-A (1:8000, from group-O mother) at room temperature (24° C) and 37° C in spite of papain treatment and testing by the indirect antiglobulin technique, they consistently reacted with six different preparations of DBL giving a 3+ to 2+ agglutination reaction. Normal anti-A from the panel of 100 group-O and group-B donors failed to agglutinate the $A_{lae}O$ cells. Four human anti-A_1 reagents failed to react with $A_{lae}O$ cells, but two human anti-A_1 (Dade Reagents, lot AA-50AB and lot AA-48AV gave a hemagglutination reaction of 2+ to + at 24° C with a mixed field appearance on slide tests when subjects I-5 and II-5 were tested with it. The reactions were inconsistent and equivocal using $A_{lae}O$ cells from other members of the family. (2) The direct antiglobulin test was negative, and no antibody could be eluted from the cells. (3) When serial dilutions of human and lectin anti-H were concurrently tested against $A_{lae}O$ and normal group O, the reactions were similar, indicating no apparent difference in the content of the H substance. (4) Although there was no direct agglutination caused by anti-A or anti–A + B, the antibody was absorbed from both reagents and eluted

TABLE 1

RESULTS OF BLOOD GROUPING, SERUM GROUPING, AND SECRETION OF ABH-GROUP-SPECIFIC SUBSTANCE IN SALIVA OF VARIOUS MEMBERS OF A FRENCH FAMILY

Subject	Reaction of Cells with Anti-				Reaction of Serum with Cells			Saliva Dilution Inhibiting	
	A	B	A + B	A_1 Lectin	A_1	A_2	B	A	H
I-1	0	0	0	0	4+	3+	4+	0	128
I-4	4+	0	4+	0	0	0	4+	0	0
I-5	0	0	0	3+	4+	3+	4+	0	256
I-6	0	0	0	0	4+	3+	4+	0	128
I-7	0	0	0	3+	4+	3+	4+	0	128
I-8	0	0	0	0	3+	2+	3+	0	256
II-1	0	0	0	0	3+	2+	3+	0	128
II-2	0	0	0	0	3+	2+	3+	0	0
II-3	4+	0	3+	3+*	0	0	3+	0	0
II-4	4+	0	4+	4+†	0	0	4+	256	128
II-5	0	0	0	3+	3+	2+	4+	0	0
II-6	4+	0	4+	4+*	0	0	4+	16	128
II-7	0	0	0	0	4+	3+	4+	0	128
II-8	4+	0	4+	4+	0	0	4+	0	0
II-9	0	0	0	3+	4+	3+	3+	0	128
III-1	0	0	0	0	4+	3+	4+	0	128
III-2	0	0	0	3+	3+	2+	3+	0	128
III-3	0	0	0	4+	4+	2+	3+	0	128
III-4	0	0	0	0	4+	3+	4+	0	64
III-5	4+	0	4+	4+†	0	0	3+	128	64
III-6	0	0	0	3+	3+	2+	3+	‡	‡

NOTE.—The cells of all members reacted with anti-H, and their sera failed to react with O cells.

* No reaction with any of absorbed human anti-A (anti-A_1). The cells of subjects I-5 and II-5 gave equivocal reactions with absorbed human anti-A.

† Strong reaction with human anti-A_1.

‡ Not tested.

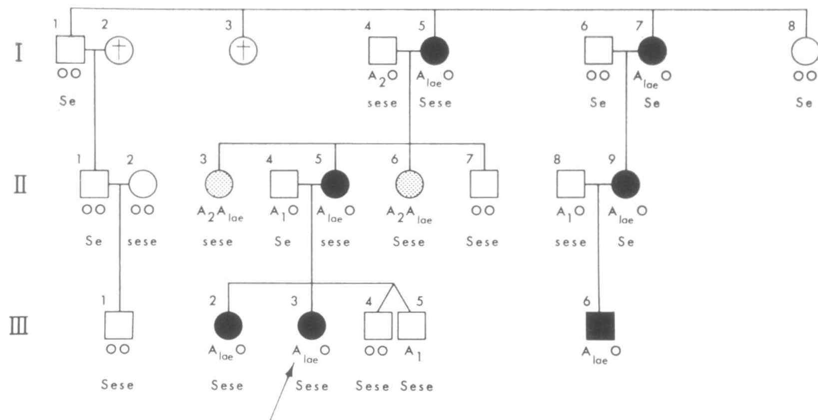

FIG. 1.—French pedigree with A_{lae}

by heating at 56° C. The eluate agglutinated A_1, $A_{1,2}$ (weak A_1), and A_2 cells but failed to agglutinate $A_{lae}O$, B, or O cells. The absorption and elution of anti-A from each of the three normal group-B sera and five normal group-O sera showed essentially similar results. The eluates in each case showed no significant difference in their reactivity when tested against the cells of five normal group-A controls. A typical reaction of the eluate is presented in table 2. (5) The absorption of anti-

TABLE 2

TEST OF ELUATES FROM TYPICAL $A_{lae}O$ CELLS (SUBJECT I-5) AFTER REACTION WITH ANTI-A

ELUTION FROM $A_{lae}O$ REACTED WITH	ELUATES TESTED WITH CELLS OF					
	A_1	$A_{1,2}$	A_2	B	O	A_2B
Anti-A	4+	4+	3+	0	0	0
Anti-A + B	4+	4+	2+	0	0	0
Human anti-A_1	2+	+	0	0	0	0
DBL (*Dolichos*)	2+	2+	0	0	0	0
AB serum	0	0	0	0	0	0
Anti-B	0	0	0	0	0	0

A with $A_{lae}O$ cells did not accomplish complete removal of anti-A. However, the results in table 3 suggest that anti-A_1 reactivity from DBL could be absorbed out, but total absorption of anti-A from group B and O sera was not practical. (6) The serum of $A_{lae}O$ persons reacted with A_1, A_2, B, A_1B, and A_2B cells and was indistinguishable from normal group-O serum in titer range or avidity of reaction. The serum failed to react with autologous or other $A_{lae}O$ cells and normal group-O cells. (7) Whenever $A_{lae}O$ persons were secretors, blood group substance A was not detectable, but only H substance was demonstrable in saliva. The inhibitory titers

TABLE 3

RESULTS OF ABSORPTION OF THREE POOLED B SERA AND FIVE POOLED O SERA WITH EQUAL VOLUMES OF A_1, $A_{lae}O$, AND O CELLS

SERUM ABSORBED WITH CELLS	ABSORBED SERUM TITRATED WITH		
	A_1	$A_{lae}O$	A_2B
Group B:			
A_1	8	0	0
$A_{lae}O$*	64	0	0
O	128	0	2
Group O:			
A_1	16	0	0
$A_{lae}O$*	128	0	16
O	256	0	32
DBL (*Dolichos*):			
A_1	0	0	0
$A_{lae}O$†	2	0	0
O	4	2	0

NOTE.—The numbers are reciprocals of the highest dilution showing agglutination.

* After eight repeated absorptions, the titer with A_1 cells came down to 16–32, and the reactivity with A_2B was not detectable.

† Two repeated absorptions removed the reactivity with A_1 cells.

of H substance ranged between 64 and 256. The heterozygote A_2A_{lae} secretor (subject II-6) secreted H and A substances with inhibitory titers of 128 and 16, respectively. Further, the saliva from $A_{lae}O$ persons secreting H substance did not inhibit the reactivity of DBL with A_1 or $A_{lae}O$ cells. (8) Although normal A_1 will not permit detection of the A_{lae} allele in a single dose, its presence in a heterozygote form with A_2 was detectable in subjects II-3 and II-6 because of the strong reactions with DBL anti-A_1 and failure to react with all absorbed human anti-A (anti-A_1) reagents. (9) The half-life of ^{51}Cr-labeled $A_{lae}O$ red cells in a group-O recipient was 29 days in comparison with an autologous half-life of 28 days.

The serologic and genetic study of 21 members of three generations in this French family clearly shows that the variant A_{lae} is genetically determined as a Mendelian character in seven family members with genotype $A_{lae}O$ and two with genotype A_2A_{lae}. It is not possible to say if subject III-5 has genotype A_1A_{lae} or A_1O. Subject II-6 is unmarried. Subject II-3 has been married, but her spouse and one daughter were not available for testing; their second daughter is normal group A_2 (not shown in pedigree).

DISCUSSION

The unique serologic feature of the $A_{lae}O$ blood type, otherwise indistinguishable from normal group-O blood, is its detection by DBL and absorption and elution of anti-A from group-B and group-O sera. It may be noted that although the eluates reacted with A_1 and A_2 cells, they failed to react with $A_{lae}O$, B, or O cells.

The serum has normal anti-B and anti-A (reacting with both A_1 and A_2 cells). The antibodies are indistinguishable in titers and avidity from normal type-O serum samples of the same family. The antigen produced by the A_{lae} gene reacts strongly with DBL but not with human anti-A_1. This feature helps in detecting not only the $A_{lae}O$ genotype but also the heterozygote A_2A_{lae}. An interesting observation was that two members with the A_2A_{lae} genotype were typed A_1 and A_2 by different laboratories in Europe depending upon whether the laboratory used lectin or human anti-A_1 reagents. Consistent with the scheme of Watkins and Morgan [6], one can say that the A_{lae} gene defines the weakest variant of A different from A_{end} and A_{el} [2, 3]. The presence of naturally occurring anti-A in $A_{lae}O$ serum reacting with both A_1 and A_2 may well be the result of a genetic lack of a majority of the antigenic determinants comprising the group-A mosaic. The residual mosaic of the A antigen on A_{lae} cells may be too weak to be detected by direct agglutination. The fact that ^{51}Cr survival of $A_{lae}O$ red cells was not reduced in a normal group-O volunteer is provocative and suggests that the quantitative aspects of the red cell antigenic mosaic are important in immunologically mediated red cell interaction with antibody and its consequent destruction. To our knowledge, red cell survival studies on most weak variants of A have not been performed.

The inherited polyagglutinability of Cad antigen described by Cazal et al. [7] has no relation to the $A_{lae}O$ phenotype because $A_{lae}O$ cells are not agglutinated by any of the typing or normal human sera. However, in a brief addendum to their paper, Cazal et al. have noted a subject of group O whose cells were agglutinated by *Dolichos biflorus* without the characteristic polyagglutinability of Cad red cells [7]. Further, from a recent study by Bird and Wingham [8], it becomes apparent that the serological properties of Cad receptors are different from the serological characteristics of $A_{lae}O$.

The $A_{lae}O$ phenotype differs from A_m in that the A antigen is not detected in saliva or on the red cells. Although the possibility of a new sex-linked modifier gene or an autosomal modifier gene controlling the development of A antigen both on the red cells and in saliva cannot be excluded, it is our inclination to instead view A_{lae} as another allele of A producing the weakest variant of A; this variant is undetectable in saliva and detectable on red cells only by absorption-elution of anti-A. Presumably the occurrence of apparently normal anti-A in the serum results from a genetic lack of a part of the mosaic of the A antigen in the organism.

SUMMARY

A new allele of blood group A, A_{lac}, was detected in nine members of a French family. Seven persons had the $A_{lae}O$ genotype, which reacted like normal group O when tested with anti-A, anti-B, anti-A + B, and anti-H; their sera contained both anti-A and anti-B antibodies. The $A_{lae}O$ red cells reacted strongly with anti-A_1 lectin from *Dolichos biflorus* but equivocally or not at all with human anti-A_1. Because of this unique reaction, we could determine the genotype of two heterozygote members of the family (A_2A_{lae}). The red cells absorb anti-A from group-B

and group-O sera, and the eluates agglutinate A_1 and A_2 cells but not B and O cells. The secretor members of the pedigree secrete only the H substance but not A. Since all members having the A_{lae} gene were in good health, it is presumed that no disease is associated with the A_{lae} allele. The survival of ^{51}Cr-labeled $A_{lae}O$ cells in a group-O volunteer was 29 days as compared with an autologous survival of 28 days.

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