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LEUKOCYTE GROUPING. A METHOD AND ITS APPLICATION *

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Leukocytes carry group specific characteristics, as a number of observations have demonstrated. Dausset was the first to demonstrate the existence of such a leukocyte isoantigen, which he designated as the leukocyte group Mac (2). That these antigens are genetically controlled has been shown in studies on twins (3, 4), in family studies (1, 5–8), and in the appearance of maternal isoantibodies during pregnancy (5, 7, 8). Though the existence of leukocyte groups is now generally accepted, the exact characterization and description of leukocyte groups has been hampered by difficulties in the serological procedures and by the multiplicity of antibodies with different specificities found in the sera usually employed for these studies, namely sera from recipients of repeated blood transfusions.

The data to be presented are based on work that sought to establish a testing procedure of acceptable reliability. The basic testing procedure was a leukocyte agglutination test using leukocytes from blood made unclottable by EDTA. These leukocytes are to be preferred for leukocyte grouping work to leukocytes from defibrinated blood, since it has been shown that the test with leukocytes from EDTA blood has a better reproducibility than the test using leukocytes from defibrinated blood (80% compared to 50 to 60%) (9). These percentages refer to the reproducibility as determined by testing the same random sera against leukocyte samples from the same donors on different days. When only those sera that gave good strong positive results were used, reproducibility was much better (over 90%). Maternal sera containing leukocyte isoagglutinins rather than sera from recipients of multiple blood transfusions were employed to keep the number of isoagglutinins of different specificity present in any one reagent at a minimum. Cross-absorption procedures were used to select sera with leukocyte agglutinins of a single specificity. In the course of such a cross-absorption study, it was found that some leukocyte samples which were not agglutinable in a given serum nevertheless specifically removed antibody from that serum. This potential source of error has been designated the agglutination-negative, absorption-positive phenomenon (10).

The study to be reported yielded data that revealed the existence of a leukocyte isoantigen locus, designated as 4, which may be occupied by one of two alleles, 4^a or 4^b, with a gene frequency of 0.38 and 0.62, respectively.

METHODS

Sera. Specimens of venous blood, obtained by venipuncture, were collected in clean dry glassware. After at least 1-hour incubation at 37° C, the serum was harvested by centrifugation. Sera were stored at −20° C after the addition of sodium azide (1 drop, 10% solution per 8 ml serum). When necessary, the sera were cleared by centrifugation at 16,000 × *g* for 10 minutes. Before use, the sera were inactivated by heating at 56° C for 30 minutes.

Leukocyte suspensions for the agglutination tests. Leukocytes were obtained from the following persons: *1*) 100 volunteers associated with the University Hospital, all of blood group O—this group will be referred to as the panel; *2*) donors of the Blood Transfusion Service, whose cells were used for screening purposes and for estimation of gene frequencies; *3*) mothers whose sera were found to contain leukocyte isoagglutinins, and cells from their husbands and children; and *4*) members of 23 randomly selected families.

Blood was obtained by clean venipuncture. About 8 ml was collected in a siliconized centrifuge tube containing 1 ml 5% EDTA in physiological saline. After mixing, the EDTA blood was poured into a siliconized tube containing 2.5 ml of 5% dextran [1] (mean molecular weight 200,000) in buffered physiological saline. The tube was incubated at 37° C for 30 minutes at a 45° angle. After incubation, the upper four-fifths of the su-

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pernatant plasma was transferred to a siliconized centrifuge tube with a siliconized pipette. This part of the plasma, which contained 3,000 to 9,000 leukocytes per mm^3, was used for the agglutination reaction.

Leukocytes from defibrinated blood were obtained in the same manner, except that 1 ml 2% Tween 80, in buffered physiological saline, was added to 2 ml of the dextran solution.

Leukocyte suspensions for cross-absorption experiments. These suspensions were prepared as described in the preceding paragraph, with leukocytes obtained from EDTA blood. "Packed' leukocytes were used for the absorption, i.e., the leukocyte suspension was centrifuged for 30 minutes at 3,000 rpm, and the plasma was removed. The cell suspensions contained not only leukocytes, but also erythrocytes and platelets. The erythrocytes do not contain the leukocyte isoantigens under study, in contrast to the platelets which do (7, 11). As this antigen is only present on the platelets if it is also carried by the leukocytes, the contamination of the cell suspension with platelets did not introduce a complication. The average suspension contained 100 to 300 erythrocytes and 2,000 to 10,000 platelets per 100 leukocytes. An average of 2.5×10^8 leukocytes could be isolated from 70 ml of blood.

Agglutination reaction (12). Two drops (approximately 0.10 ml) of a leukocyte suspension were mixed in nonsiliconized, round-bottomed tubes (50×7 mm) with two drops of inactivated serum or serum dilution. The tubes were then incubated for 120 minutes at 37° C. After the incubation, the supernatant plasma was removed, and one drop of 6% acetic acid was added to the sediment to lyse the red cells. The sediments were transferred to a nonsiliconized slide without spreading and observed microscopically (magnification 50 to 100 times).

Titration of the serum was performed by stepwise twofold dilution in saline when leukocytes from EDTA blood were used.

The test was done in duplicate, and the result was recorded as the mean of the two observations. The reactions performed during the screening of the sera for the presence of leukocyte agglutinins were, however, carried out only once with each leukocyte sample. When leukocytes from defibrinated blood were used (13), essentially the same procedure was employed, with the single difference that normal inactivated AB serum was used as the diluent.

Cross-absorption tests. Samples containing 2.5×10^8 packed leukocytes were obtained from at least 10 donors. Each sample was incubated for 30 minutes at 37° C with 1.0 ml of inactivated serum containing antibodies against leukocytes. After centrifugation of the mixture at 3,000 rpm for 45 minutes, two drops of the absorbed serum were tested against leukocytes of the donor whose cell suspension had been used for the absorption and further against the leukocytes from the other donors. To exclude the possibility that the absorption of the agglutinin was caused by a nonspecific effect, the serum was absorbed not only with cells that carried the corresponding antigen, but also with cells that did not carry this antigen.

Calculations. As will be described below, 66 sera were tested against the leukocytes from the panel. It was necessary to compare the results of each serum with those of all other sera. Hence $(66 \times 65)/2 = 2{,}145$ comparisons, each consisting of comparing the reactions of the panel of 100 samples of white cells with two different sera, had to be made.

The results were originally classified as positive, negative, or doubtful. To facilitate the calculations, the dubious results were changed to "positive" or "negative" according to the following rule: the reaction was recorded as positive if the same serum-leukocyte combination gave a positive reaction with leukocytes from defibrinated blood as well, and as negative if a negative reaction with leukocytes from defibrinated blood was obtained. The reaction was recorded as negative if both tests yielded doubtful results. To compare the results of the agglutination tests of two sera against the 100 cell preparations of the panel, these cell preparations were divided into four groups: Group A consisted of those leukocyte samples that gave negative results with the two sera (the number of leukocyte samples belonging to group A is called a). Group B consisted of those leukocyte samples that gave a negative result with the first serum and a positive result with the second serum (= b). Group C consisted of those leukocyte samples that gave a positive result with the first serum and a negative result with the second serum (= c), while group D consisted of the leukocyte samples that were agglutinated by both sera (= d). This classification was done with the aid of the statistical sorter IBM 101. This can be summarized schematically as follows:

		Serum II		
		−	+	
Serum I	−	a	b	a + b
	+	c	d	c + d
		a + c	b + d	a + b + c + d = T

To investigate to what extent the distribution of the leukocyte samples in the above-mentioned groups could be due to chance, Fisher's 2×2 test was used (14). The necessary calculations were made by the electronic calculator IBM 604 using the following formula:

$$X^2 = \frac{(ad - bc)^2 \ (a + b + c + d)}{(a + b) \ (c + d) \ (a + c) \ (b + d)}$$

The resulting X^2-values were tabulated in two written records: *1)* in the order of the serum numbers used in the comparison, e.g., serum 1 with sera 2, 3, 4, 5, etc. (record A), and *2)* in the order of decreasing magnitude of the X^2-values (record B).

The gene frequencies were calculated as described by Race and Sanger for MN blood groups (15).

TABLE I

Data from computer (record B)

First serum: / Second serum:		A Neg. Neg.	B Neg. Pos.	C Pos. Neg.	D Pos. Pos.	
First serum Serum no.	Second serum Serum no.	Number of leukocyte suspensions				
		a	b	c	d	X^2*
13	55	37	5	4	54	66
27	38	75	1	8	16	55
16	55	31	3	10	56	54
14	46	11	5	2	82	52
22	36	30	5	8	57	52
8	28	37	12	5	46	44
22	34	32	3	15	50	43
54	63	93	0	4	3	41
12	18	14	2	11	73	40
13	16	29	13	5	53	40
31	41	84	1	8	7	36
12	48	15	1	16	68	35
26	40	72	4	10	14	35
34	35	46	1	23	30	35
11	38	80	8	3	9	33
13	67	33	9	12	46	33
6	11	77	2	11	10	32
28	55	31	11	10	48	32
35	57	62	7	11	20	32
12	15	12	4	10	74	31

* Arrangement of X^2-values in decreasing magnitude obtained after comparison of the results of the agglutination test of the various sera with the 100 leukocyte suspensions of the panel.

RESULTS

Selection of the sera. The results of tests of the 66 sera from pregnant women (known to contain isoagglutinins for leukocytes on the basis of previous tests) against the 100 cell suspensions of the panel cannot be reproduced here because of space limitations. The analysis of the results obtained through the methods described in the previous section is best demonstrated by reference to Table I. Shown there are the reactions of pairs of sera with leukocyte suspensions of the panel. In columns A and D are recorded the numbers of samples that gave negative results (a) or positive results (d) with both sera of the pair. In columns B and C are recorded the numbers of samples of panel cells that were agglutinated by one but not by the other serum of the pair. The last column of the table (X^2) gives a statistical measure, which denotes to what degree the distribution of positive and negative results of the two sera could be due to chance. The table as shown is the top part of record B as described in the Methods section. The first row shows that sera 13 and 55 are related (X^2-value = 66). In the third row, evidence is given that sera 16 and 55 are related. The tenth row demonstrates a relation between sera 13 and 16. By working down the list in this way, the sera could be subdivided into four unrelated groups.

One group consisted of sera that could not be placed into one of the following groups. These sera represented a heterogenous group and were for that reason not further studied in detail.

Each of the other three groups consisted of a battery of sera that were either similar in their specificity or were thought capable of recognizing the products of alleles. Two sera were considered as potentially recognizing alleles, if one of the two agglutinated all or almost all leukocyte samples that were not agglutinated by the other serum. To find such sera record A was used.

The second group of sera consisted of two possibly identical sera, while the third serum of the group seemed to recognize the allele of the gene

TABLE II

X^2-values of fourth group of sera

Second serum, serum no:	12	15	18	19	22	23	34	35	36	48	50	59
12												
15	31											
18	40	17										
19	20	17										
22	7*	4*		15*								
23	24	19	13	16								
34	6*			11*	43	6*						
35	6*			6*	20		35					
36	8*		5*	15*	52	9*	29	19				
48	35	18	21	31	16*	19	14*	6*	12*			
50					27		24	16	27	6*		
59	13		21							17		

* Corresponding sera might recognize the products of allelic genes.

recognized by the two identical sera (sera 4, 14, and 46). Cross-absorption showed that serum 46 recognized probably only one leukocyte antigen, but that serum 4 contained antibodies against at least two different antigens. For that reason, the sera from this group were not further studied.

Of the third group of sera, twelve were similar (sera 6, 8, 11, 13, 16, 20, 27, 28, 38, 55, 56, and 67), while one other serum (serum 51) was thought to possibly recognize the product of the allelic gene. These sera appeared to be less suitable for further study because most of them had a low titer and only moderate avidity.

Leukocyte group four. The sera that constituted the fourth group are listed in Table II with their respective X^2-values. Only X^2-values higher than 11 for the sera of similar specificity and higher than 4 for those that might recognize the products of allelic genes are given. It appeared that sera 12, 15, 18, 19, 23, 48, and 59 might recognize the same antigen, while sera 22, 34, 35, 36, and 50 might possibly recognize the product of an allelic gene.

The table shows that most of the sera with similar agglutination patterns were related to each other by X^2-values of 11 or higher (27 out of 31). On the other hand, generally lower X^2-values were found between sera that might recognize the products of two allelic genes (only 17 out of 35 were 4 or above).

Cross-absorption of three of the sera (12, 34, and 36) demonstrated that in all probability these sera contained antibodies that recognized only one leukocyte antigen. Table III shows the results of a cross-absorption experiment with a serum of this group. The table demonstrates that absorption of the serum with any of the cells, which were agglutinated, removed the leukocyte agglutinin for all the other agglutinable cells. Cells which were not agglutinated by the serum failed to absorb the agglutinin out of the serum. The antigen recognized by serum 34 has previously been designated 4^a (10).

Figure 1 depicts the agglutination pattern obtained with eight of the twelve sera listed in Table II. Sera 19, 35, 50, and 59 were not used for further study, because they either had a low titer or proved, when examined by cross-absorption, to contain antibodies against at least two leukocyte antigens. Every vertical row shows the results obtained with one serum against the 100 different leukocyte suspensions of the panel. There is one group of three sera similar to each other called anti-4^a and a second group of five sera called anti-4^b with a pattern of reactivity which suggested that they might recognize the product of the gene allelic to 4^a. Although there are a number of exceptions, the over-all results can be divided into three groups. These groups could correspond to the genotypes 4^a4^a, 4^a4^b, and

TABLE III

*Cross-absorption experiment with serum no. 34**

		Agglutination reaction of the leukocytes from donor no.													
		25	26	28	27	22	19	70	51	23	17	21	18	24	14
Serum no. 34 before absorption:		++	++	++	+	+	+	++	++	++	−	−	−	−	−
Serum no. 34 after absorption with leukocytes from donor no.:	25	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	26	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	28	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	27	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	22	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	19	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	70	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	51	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	23	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	17	+	+	+	+	+	++	+	++	++	−	−	−	−	−
	21	++	++	++	+	++	+++	++	+++	+++	−	−	−	−	−
	18	++	++	+	+	+	++	++	+++	++	−	−	−	−	−
	24	++	+	++	+	++	++	++	+++	++	−	−	−	−	−
	14	++	++	+	+	+	++	++	++	++	−	−	−	−	−

* Symbols: − = negative reaction; (+) = weakly positive reaction; + = one plus positive reaction; ++ = two plus positive reaction; +++ = three plus positive reaction; ++++ = four plus positive reaction; *** = agglutination reaction negative, absorption positive (see text).

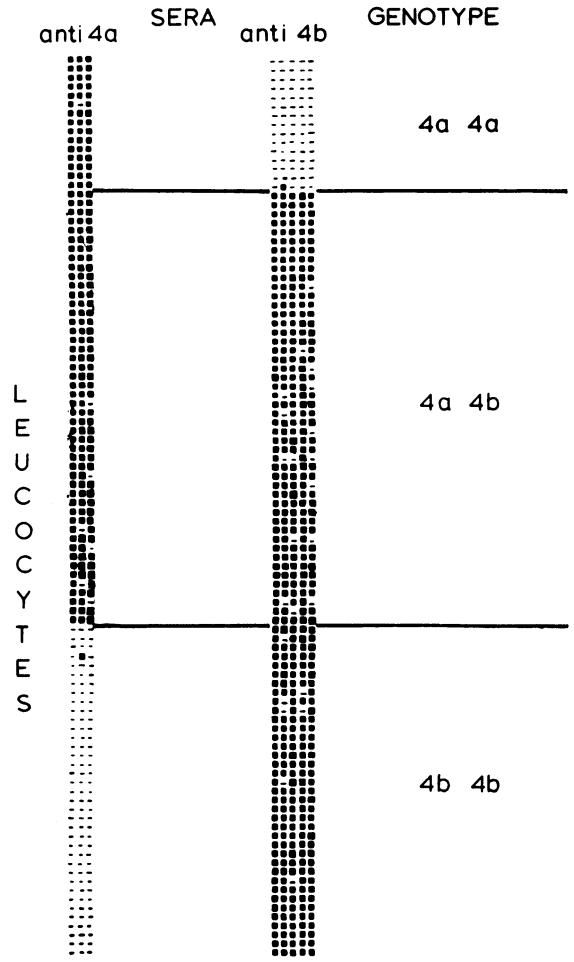

FIG. 1. AGGLUTINATION PATTERN OF PART OF THE SERA DEPICTED IN TABLE II WITH THE LEUKOCYTES OF THE PANEL. Every vertical row of squares and hyphens shows the results obtained with one serum against the panel; every horizontal row shows the results of the eight sera with one leukocyte sample. Black squares mean agglutination positive; hyphen means agglutination negative. Doubtful results were recorded as agglutination negative.

4^b4^b with a gene frequency for 4^a of 0.38 and for 4^b of 0.62. The results that do not follow the general pattern are probably due to the agglutination-negative, absorption-positive phenomenon.

The 100 leukocyte samples were tested twice with these eight sera with an interval of 6 months. Most of the original results were confirmed, but 9.5% of the leukocyte-sample–serum combinations gave divergent results (i.e., first positive, next negative, or vice versa; dubious results were always scored as divergent). As a consequence of this, two donors originally typed as 4^a4^b proved on retyping to be 4^b4^b.

In addition to the panel shown here, the white cells from another 347 unrelated persons were tested with the sera employed in the test shown in Figure 1. The observed phenotype frequencies show good agreement with those predicted on the basis of the Hardy-Weinberg law (Table IV).

Further support for the hypothesis that the sera were recognizing a leukocyte group was found in the observation that women who had produced the antibody anti-4^a all had leukocytes of type 4^b4^b, while the leukocytes of their husbands were of types 4^a4^a or 4^a4^b (Table V). On the other hand, the leukocytes of the women who had produced anti-4^b were of types 4^a4^a, and the leukocytes of their husbands were 4^b4^b or 4^a4^b. In these tests, as in prior ones, some leukocyte specimens that failed to be agglutinated by certain sera nevertheless could be shown to possess the antigen in question by proper absorption procedures. They are indicated in the tables with asterisks.

Additional evidence for the existence of leukocyte group four was found in the demonstration of a definite dosage effect (Table VI). Cells from homozygous individuals were more strongly agglutinated and by higher dilutions of the antisera than were the cells from heterozygous individuals.

As a final test, the distribution of leukocyte group four in 25 kindreds with 33 matings and

TABLE IV

Expected and observed frequencies of the phenotypes of group four in a group of 347 random donors

Observed	Numbers	Fraction of total
4^a4^a	46	0.1326
4^a4^b	175	0.5043
4^b4^b	126	0.3631
Total	347	
	Gene freq. 4^a = 0.3847	
	Gene freq. 4^b = 0.6153	

Expected	Numbers
$4^a4^a = 0.3847^2 \times 347$	= 51.35
$4^a4^b = 2 \times 0.3847 \times 0.6153 \times 347$	= 164.27
$4^b4^b = 0.6153^2 \times 347$	= 131.37
$X^2 = 1.478$ $\quad 0.20 < p < 0.30$	

TABLE V

*Leukocyte groups of the women who had formed anti-4^a and of their husbands**

		Anti-4^a Serum no.					Anti-4^b Serum no.						
		34	36	22	35	50	12	15	18	23	48	59	19
Leukocytes from woman no.:	34	−	−	−	−	−	+++	++	+	++	+	***	***
	36	−	−	−	−	−	+++	++	++	+++	++	++	+
	22	−	−	−	−	−	+++	++	+++	++	++	+	+
	35	−	−	−	−	−	++++	+	+	+++	+	***	***
	50	−	−	−	−	−	++++	++	+	+++	++	+	+
Leukocytes from the husband of woman no.:	34	+++	+++	++++	++	+++	−	−	−	−	−	−	−
	36	++	+++	++++	***	++	−	−	−	−	−	−	−
	22	+++	++	++++	++	+++	+++	++	++	+	++	+	***
	35	+	++	++	+	++	+++	++	++	+++	++	++	+
	50	+	++	+	+	+++	+++	++	+++	+++	++	+	++

* For symbols used, see Table III.

140 children was studied. In the 4 matings of the $4^b4^b \times 4^b4^b$ type, all the 21 children were unmistakably of the 4^b4^b type. No matings of the type $4^a4^a \times 4^a4^a$ were encountered (expected frequency of this mating is 1/46). Very good agreement ($X^2 = 1.7$, $0.3 < p < 0.5$) was found between the expected and the observed distribution of the children's genotypes (Table VII).

Because 4^a has about the same frequency as Mac, the possibility was entertained that the two are identical. Dausset tested serum 22 and serum 12 against known Mac-positive and -negative leukocytes (Table VIII). The results show no significant correlation. It is not possible to state definitely whether anti-Mac and anti-4^a are identical or not, as no reliable anti-Mac serum is available and the number of leukocyte samples typed for Mac is rather small. As a definite conclusion cannot be reached, the name "leukocyte group four" will be retained.

TABLE VI

*Dosage effect**

Leukocytes from donor no.	Genotype	Dilution			
		Serum no. 34 (anti-4^a)			
		1/1	1/2	1/4	1/8
51	4^a4^b	++	+	−	−
28	4^a4^a	+++	++	+	−
		Serum no. 12 (anti-4^b)			
51	4^a4^b	++	+	−	−
9	4^b4^b	+++	++	+	−

* For symbols used, see Table III.

DISCUSSION

The evidence presented points to the existence of a di-allelic leukocyte group, and it shows that it is possible by the means used to classify leukocyte samples according to this group.

An essential feature of the method developed to recognize a leukocyte group was the use of statistical methods to overcome the shortcomings of an imperfect technique. To use Fisher's 2 × 2 test, it was necessary to record the doubtful results as either negative or positive. This was considered permissible for the following reasons: *1*) The effect of this method of recording will be proportional to the percentage of doubtful results obtained with a given serum. As sera with a high percentage of doubtful results will be useless in leukocyte grouping anyway, the above-mentioned effect will be of no consequence. *2*) The X^2-values obtained were only used as a means to

TABLE VII

*Distribution of leukocyte group four in family studies**

Mating		Children		
		4^a4^a	4^a4^b	4^b4^b
	no.	*no.*	*no.*	*no.*
$4^a4^b \times 4^a4^b$	9	10 [11¼]	24 [22½]	11 [11¼]
$4^a4^b \times 4^b4^b$	11	0 [0]	16 [18]	20 [18]
$4^a4^b \times 4^a4^a$	5	6 [9½]	13 [9½]	0 [0]
$4^a4^a \times 4^b4^b$	4	0 [0]	19 [19]	0 [0]
$4^a4^a \times 4^a4^a$	0	− [−]	− [−]	− [−]
$4^b4^b \times 4^b4^b$	4	0 [0]	0 [0]	21 [21]
Total	33	16	72	52
				140

* The expected values are given in brackets.

TABLE VIII

*Agglutination pattern of serum no. 34 (anti-4^a) and serum no. 12 (anti-4^b) with Mac-positive and Mac-negative leukocytes**

Leukocytes of donor:	a	b	c	d	e	f	g	h	i	j
Anti-Mac	+	+	+	+	+	−	−	−	−	−
Anti-4^a	(+)	−	++	++	++	−	−	(+)	−	++
Anti-4^b	+	+	+	+	+	++	++	−	++	++

* Courtesy of Dr. J. Dausset. For symbols used, see Table III.

select sera that possibly could recognize a leukocyte group, and not as definite proof of a positive correlation between two sera. The last argument can also be used to answer the possible cri icism that the formula used to calculate X^2-values gives reliable results only if the expected numbers are greater than 5.

A second essential feature in the approach to the recognition of a leukocyte group was the use of a panel of leukocytes from women who had formed agglutinins with group four specificity and from their husbands. When the specificity of an antibody against red cells has to be determined, a panel of donors of known group specificity is normally used. As the group specificity of the leukocytes was unknown, this was not possible. The use of the leukocytes of the couples overcame this difficulty.

These two points, combined with the circumstance that sera with leukocyte agglutinins formed during pregnancy often recognize only one leukocyte group, were the most important factors that made the recognition of a leukocyte group possible.

The fact that even with the precautions taken the results of the grouping of two leukocyte samples out of the 100 used in the panel could not be reproduced implies that leukocyte grouping is still not completely reliable. Nevertheless the reliability of the grouping with a number of strongly reacting sera of similar specificity is considerably better than the results obtained with a random serum, reproducibility in the series reported here being as good as 98%.

Even better reproducibility has been claimed by Shulman, who used the complement fixation test (16). Work in progress seeks to establish whether the same antigens are recognized by both methods and whether leukocyte group four is identical with one of the leukocyte antigens recently described by Shulman (17). The Coombs consumption test (12) has not been used in this study because, apart from being rather cumbersome, Engelfriet and van Loghem (18) and Jensen (19) have reported that this test, which is frequently positive after immunization by blood transfusions, is only rarely positive after immunization against leukocytes by pregnancy. As in this study only sera from women who had been pregnant were used, it was considered unlikely that the use of the Coombs consumption test would be advantageous.

The analysis of the X^2-values shows also that the technique employed here revealed antibodies against three leukocyte groups fairly often. This may imply that under our test conditions these groups are more reactive than other leukocyte groups, of whose existence we are as yet not aware.

Since it has been shown that platelets often carry the same isoantigens as leukocytes (8, 13), the question will arise whether leukocyte group four has any connection with the platelet groups described so far (20–24). Although leukocyte group four substance is present on platelets as can be demonstrated by absorption experiments, leukocyte group four is not identical with one of the platelet groups, because the isoantigens of these groups are not present on leukocytes, and furthermore their gene-frequencies are basically different from those of group four.

Absorption experiments with placental tissue (8) and kidney (25) show that leukocyte group four is also present in these organs. It seems also likely that both granulocytes and lymphocytes carry leukocyte group four substance, on account of agglutination tests with relatively pure granulocyte and lymphocyte suspensions isolated by albumin density gradient centrifugation and absorption tests with spleen and lymph node homogenates (25).

The fact that platelets often carry the same isoantigens as leukocytes implies that an insight into the leukocyte groups might be of importance in the clinical practice of platelet transfusions (26).

The leukocytes carry the transplantation antigens (27, 28). Whether or not the transplantation antigens are identical with or related to the isoantigens described in this paper is still unknown (1). To answer this question it will be necessary to "group" the leukocytes adequately. The present study is a first step toward this end.

Leukocyte group four fulfills the criteria of a gene-marker. Since there is no close linkage with one of the other gene-markers so far studied (25), the addition of leukocyte group four provides a first locus in a previously unmapped chromosome.

SUMMARY

By screening 66 sera containing leukocyte agglutinins formed during pregnancy against a panel of 100 random leukocyte samples and by taking into account the agglutination-negative, absorption-positive phenomenon, it was possible to select a number of sera that gave similar agglutination patterns. By cross-absorption it could be shown that some of these sera recognized only one antigen.

In this way it was possible to recognize two allelic leukocyte antigens 4^a and 4^b, with a gene frequency for 4^a of 0.38 and for 4^b of 0.62.

Family studies proved that 4^a and 4^b are inherited as simple Mendelian autosomal codominant alleles.

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