A New Leukocyte Isoantigen System in Man

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INTRODUCTION

The leukocyte antigens provide a rich new source of material for the study of genetic polymorphism in human populations. The inheritance of these antigens has been discussed previously by Dausset and Brecy (1957), Payne and Rolfs (1958), Lalezari and Spaet (1959), van Rood, van Leeuwen, and Eernisse (1959), Payne and Hackel (1961). The antigenic determinants identified by leukocyte agglutinins are distinct from those of the red blood cells, and have been detected in the granulocytes, lymphocytes, platelets and other tissues (van Rood 1962; Shulman, Marder, Aledort, and Hiller 1962a; Payne 1963).

Isoagglutinins for the human leukocyte were first observed in 1952 during a search for an immunologic etiology of leukopenia (Dausset and Nenna, 1952). Not long after discovery of the leukocyte agglutinins, their formation was demonstrated to be primarily the consequence of multiple transfusions or repeated pregnancies, and not related to an immunoleukopenia (Dausset 1954; Payne 1957, 1962; Payne and Rolfs, 1958; Brittingham 1957; van Loghem, van der Hart, and Borstel, 1957; Killman 1958; André, Dreyfus, and Salmon 1958; van Rood, Eernisse, and van Leeuwen 1958; Jensen 1962a). Their presence is prejudicial to transfusion therapy, for the receipt of incompatible leukocytes by a patient with these antibodies will induce a febrile transfusion reaction (Brittingham and Chaplin 1957; Payne 1957, 1960; Dausset, Fonseca, and Brecy 1957). These unpleasant, but not ordinarily life-endangering sequelae, are currently prevented by removal of the leukocytes from blood which is to be transfused. There is as yet no established disease of the newborn, comparable to erythroblastosis fetalis, which is due to isoimmunization of the mother by fetal leukocyte antigens. If such a disease does occur, it must be a rare event (Payne 1964a; Jensen 1962b; Hitzig and Gitzelmann 1959; Lalezari, Nussbaum, and Gelman 1960; Braun, Buckwold, Emson, and Russell, 1960).

A considerable amount of information on the significance of the leukocyte agglutinins and their corresponding antigenic determinants has accumulated over the past decade (for reviews see Walford 1960; Killman 1960; van Rood 1962). What is required at this stage of development is a comprehensive genetic classification of the leukocyte antigen systems. They are likely to provide important markers for a variety of biological phenomena. In man, apart from their significance as new genetic polymorphisms, the ability to type leukocytes would find immediate application for transfusion practice and for transplantation research. Until recently, attempts to classify the leukocyte antigens into allelic systems proved unrewarding. In earlier work antisera were employed which were obtained from multitransfused persons. These studies were unsuccessful, largely because these reagents were multispecific. The leukocyte agglutinins from women sensitized only during pregnancy are less complex and so more suitable as reagents for the establishment of genetic groups. The first leukocyte antigen system, Group 4 was reported by van Rood, van Leeuwen, and Bosch (1961) and van Rood (1963) on the basis of an analysis of the reactions with such antisera. In this paper, evidence for the existence of a new leukocyte isoantigen system in man, which defines another locus independent of Group 4, will be presented.

METHODS

The leukocyte agglutination test has been described elsewhere (Payne and Rolfs 1958; Payne 1964b). In brief, heat inactivated test sera are combined with leukocyte suspensions prepared from defibrinated blood. The mixture is incubated at 37°C for 90 minutes following which the contaminating red cells are lysed with acetic acid. The leukocytes are examined for agglutination microscopically (100×). The tests are always carried out in duplicate and confirmed with a second sample of leukocytes.

REPRODUCIBILITY OF AGGLUTINATION ASSAY

A comparison of the results obtained using nine different sera with two samples of leukocytes from the same individual collected at different times is

shown in Table 1. The sera are grouped according to the reproducibility with which they agglutinate. Those sera which agglutinate most samples of leukocytes proved to be the most reliable, but are the least informative for the establishment of groups. Other sera give a relatively high proportion of discordant results, and thus also cannot conveniently be used for grouping. However, sera which react with intermediate frequencies and give no more than 10–15% discordant results on repeated tests can be found and have been effectively used in our work. A considerable proportion of the discordant results obtained with these more reliable sera have recognizable causes, most commonly a general unreactivity of the sample of leukocytes used. This occurs, for example, when the cells come from young infants or people with infections.

Table 1. Reproducibility of Agglutination Assay

			1st Test +	1st Test ?	1st Test −	% Discordant Reactions
Reliable Sera (14 28 18 31)	2nd Test	+	73	4	4	13.6
		?	6	2	1	
		−	6		58	
Poor Sera (13 25)	2nd Test	+	23	2	5	27.7
		?	3	—	—	
		−	14	2	45	
Reliable Very Frequently Reacting Sera (1 3 4)	2nd Test	+	178	—	2	3.6
		?	—	—	—	
		−	4	1	9	

+ = Positive Reaction ? = Questionable Reaction − = Negative Reaction.

Analysis of 2 × 2 Associations

The use of 2 × 2 tables in the analysis of blood group relationships was emphasized by Race and Sanger (1962), in their discussion of the MNSs. This also formed the basis for van Rood's (1962) elucidation of leukocyte Group 4. A similar approach has been followed in the analysis of the relationships shown by our sera. This seems to be the only way of establishing any order out of information so complex as that provided by the predominantly multispecific leukocyte agglutinins.

Two sera are considered identical if cells are always agglutinated either by both of the sera or by neither. Two multispecific sera may

(1) be identical; in other words contain identical antibodies;

(2) be such that one contains the other; in other words, has the other's antibodies as well as one or more unshared antibodies;

(3) share some antibodies but not others;

(4) have no antibodies in common.

Following the notation of Table 2, these possibilities imply

(1) $b = c = 0$.

(2) either $b = 0$, and serum 2 is contained within 1, or $c = 0$ and serum 1 is contained within 2.

(3) A tendency toward an excess of like reactions which should be reflected by a significant positive association ($ad - bc > 0$, or $ad/bc > 1$).

(4) In general, no significant positive association.

Consider, for example, the relationship between two dispecific sera sharing one antibody, anti-X.

Table 2. The 2 × 2 Table

		1st Serum +	1st Serum −
2nd Serum	+	a	b
	−	c	d

a, b, c, and d represent the number of observations of the four possible types of reactions.

(1) If $b = c = 0$, the sera are identical.

(2) If $b = 0$, serum 2 is contained within serum 1, and vice versa if $c = 0$.

(3) If the sera show a significant positive association ($ad - bc > 0$, or $ad/bc > 1$), they may share one or more antibodies.

Let anti-Y and anti-Z represent the unshared components and suppose that the corresponding antigens X, Y, and Z occur in proportions p_1, p_2, and p_3 of the population respectively. The frequencies of the four types of reactions on the assumption that the antibodies are independent, are shown in Table 3. A measure of the association between the sera is given by

$$\rho = \frac{p_1}{(1 - p_1)p_2p_3}$$

The association cannot be negative since ρ is always greater than (or equal) to 0. When p_1 is small, and the shared antigen X is rare, ρ is near 0 and the association is small. As either p_2 or p_3 decreases, ρ increases until eventually, when $p_2 = 0$ or $p_3 = 0$, the sera are such that serum 2 is contained within serum 1, or vice versa. When both p_2 and p_3 tend to 0, the sera approach identity. When p_1 tends to 1, the situation becomes indeterminate because almost all individuals carry the antigen X. Thus, in a limited body of data, the chance of detecting an association between two sera due to a shared antibody increases as the frequency of reactions to this shared antibody increases but decreases with increasing frequencies of reactions to the unshared antibodies. Given a number of sera with one shared antibody, any pair of them should show an association whose extent

TABLE 3. RELATIONSHIP BETWEEN TWO DISPECIFIC SERA WITH A SHARED ANTIBODY

Serum 1 contains anti-X and anti-Y Serum 2 contains anti-X and anti-Z				
Phenotypes	1+ 2+	1+ 2−	1− 2+	1− 2−
Antigenic Constitution	X, XY, XZ, XYZ, or YZ	Y	Z	None
Frequency	$p_1 + p_2p_3(1 - p_1)$	$p_2(1 - p_1)(1 - p_3)$	$p_3(1 - p_1)(1 - p_2)$	$(1 - p_1)(1 - p_2)(1 - p_3)$

p_1, p_2, p_3 are the respective frequencies with which antigens X, Y, and Z occur in the population. Only the antigens recognized by their respective antibodies are listed under antigenic constitution. The antigens are assumed to occur independently of each other in the population.

A measure of the association between the sera is given by

$$\rho = \frac{'ad'}{bc} - 1 = \frac{[p_1 + p_2p_3(1 - p_1)][(1 - p_1)(1 - p_2)(1 - p_3)]}{[p_2(1 - p_1)(1 - p_3)][p_3(1 - p_1)(1 - p_2)]} = \frac{p_1}{(1 - p_1)p_2p_3}$$

$\rho \geqslant 0$ and so the association cannot be negative.

will, however, depend on the frequencies of the particular unshared components. The chance that an individual does not carry the antigen recognized by the shared antibody but does react with all the sera will, in general, decrease rapidly as the number of sera, and so of unshared antibodies, increases (e.g. see Table 7).

Two genetically determined traits may be associated in a population for one or more of the following reasons:

(1) multiple effects of a single gene,
(2) selective or physiological interactions,
(3) inbreeding of the population,
(4) stratification,
(5) allelism (or very close linkage) of the genes determining the traits.

Points (1) and (2) are not likely to be relevant in the case of antigenic traits, which generally are closely related to primary gene products. The association caused by inbreeding is proportional to the inbreeding coefficient, which is small in human populations. Stratification is not likely to go unrecognized unless it is the cause of only minor associations. Allelism is thus the one remaining factor which may be a major cause of associations between antigenic traits. Allelism always results in a negative association. This is obvious for two codominant alleles, in which case there will be no individuals lacking both antigens. The situation for three alleles, in which only two of the antigens are identifiable, is illustrated in Table 4 in terms of the ABO blood groups. The association is negative and ρ is always less than $-\frac{1}{2}$. This is, in fact, the way that allelism of the genes determining the ABO blood groups was first demonstrated. No such association is expected, save in exceptional circumstances, for two antigenic traits controlled by genes at different loci, even if these are linked, so long as the genes are at their equilibrium frequencies.

TABLE 4. ASSOCIATION OF TWO ANTIGENS IN A THREE ALLELE SYSTEM

	A	not A
B	$2pq$	$q^2 + 2qr$
not B	$p^2 + 2pr$	r^2

A and B refer to ABO blood type. p, q, and r are the frequencies of the A, B, and O alleles respectively. The frequencies of the four antigenic types are given on the assumption of random mating. The measure of association is

$$\rho = \frac{2pqr^2}{(p^2 + 2pr)(q^2 + 2qr)} - 1 = -\frac{pq + 2r(p + q)}{2(p + 2r)(q + 2r)} - \frac{1}{2}$$

which is always less that $-\frac{1}{2}$, so that the association is always negative.

IDENTIFICATION OF LEUKOCYTE GROUPS

The reactions of 35 sera with up to 150–200 people were summarized in the form of 2×2 tables. Individual reactions were recorded on punch cards and an IBM 7090 computer was used to sort the data, calculate a 2×2 chisquare (with Yates' continuity correction) for each association and indicate whether the association was positive or negative. There were no sera which were unequivocally identical, though some pairs showed rare − + and + − reactions. There were a few pairs where one serum was contained within the other. (See appendix Tables A and B.) The limited number of people who were tested, and the possibility of misclassification, make it essential to judge an association between two sera on the basis of the significance of the 2×2 chisquare.

Nineteen of the sera were resolved into three groups which were such that all (or nearly all) of the sera within a group showed significant positive pairwise associations. The 2×2 chisquare for these sera are shown in Tables 5 and 6, and the summarized data on the pairwise reactions is given in appendix Tables A and B. Following the analysis

TABLE 5. X^2 RELATIONSHIPS OF LA1 AND LA2 SERA

	Serum Number	2	8	14	15	25	30	32	6	7	9	17	28	22
LA1	2													
	8	**43**												
	14	**69**	**37**											
	15	**42**	**39**	**47**										
	25	**6**	**12**	**10**	**9**									
	30	**24**	**20**	**20**	**25**	**8**								
	32	**18**	**12**	**22**	**8**	2	**15**							
LA2	6	**9***	**11***	**18***	**10***	**9***	**1***	0.2*						
	7	**11***	**1***	**12***	**4***	**2***	**0***	**0***	**28**					
	9	**5***	**5***	**14***	**10***	**9***	**0***	0	**40**	**32**				
	17	**1***	**1***	**3***	**6***	0.2*	0.2	**0***	**18**	**19**	**17**			
	28	**4***	**4***	**3***	**13***	**10***	0.6*	3	**40**	**15**	**20**	**16**		
	22	**3***	**12***	**9***	**4***	0.6*	0	0.2	**24**	**12**	**9**	**19**	**41**	
	Serum Number	2	8	14	15	25	30	32	6	7	9	17	28	22
				LA1							LA2			

bold type $X^2 \geqslant 5$ * negative association

described in the previous section, these three groups define three different antibodies, and hence three corresponding antigens, one common to each group.

Almost all the pairs of sera consisting of one from the first group (LA1), and one from the second (LA2) show a negative association, which is significant about half the time (Table 5). The chisquares are rounded to the first decimal place. The small values involving sera numbers 30 and 32 are in large part due to the small number of tests which have so far been carried out with these sera. The negative association implies that the antigens LA1 and LA2, defined by the two corresponding shared antibodies, are determined by allelic (or very closely linked) genes. Since there is an appreciable number of people who do not react to sera from either of these groups, there must be at least one more allele in this system. Some effort has been devoted to identifying a serum containing the antibody corresponding to the third allele, but so far without success. There are no significant associations between the LA groups, and the third group of sera depicted in Table 6.

The antibody shared by the sera of the third group (Table 6) has been shown to correspond to van Rood's anti-4*b*, by matching reactions with anti-4*b* sera kindly made available by Dr. van Rood. Anti-4*a* has been provisionally identified in three further sera (4, 31, 33) (Table 6). Serum number 4 was shown by van Rood (personal communication) to contain anti-4*a*. Sera 31 and 33 show a significant positive association with each other, although not with 4, probably because the latter is a high frequency reacting multispecific serum. All three sera, however, show significant negative associations with the sera of the 4*b* group, as would be

TABLE 6. X^2 RELATIONSHIPS OF 4b AND PUTATIVE 4a SERA

	Serum Numbers	3	10	11	18	19	26	4	31	33
4b	3									
	10	**28**								
	11	**19**	**36**							
	18	**11**	**34**	**35**						
	19	4	**35**	**29**	**56**					
	26	2	4	3	**11**	**26**				
Putative 4a	4	**0***	**2***	**2***	**5***	**5***	**0.5***			
	31	**0.1***	**2***	**1***	**4***	**10***	**3***	1		
	33	0.2	**0.1***	**3***	**6***	**6***	**5***	0.5	**9**	
	Serum Numbers	3	10	11	18	19	26	4	31	33
					4b				Putative 4a	

bold type $X^2 \geqslant 5$ * negative association

TABLE 7. NUMBER OF SERA IN LA1, LA2 AND 4b GROUPS TO WHICH INDIVIDUALS REACTED

	Number of Sera to Which Individuals Reacted	Individuals Not Classified as Having Antigen Common to Group	Individuals Classified as Having Antigen Common to Group
Group LA1			
	0	17	—
	1	21	—
	2	13	—
	3	2	—
	4	1	—
	5	—	4
	All		28
Group LA2			
	0	10	—
	1	21	—
	2	15	—
	3	3	—
	4	—	—
	5	1	—
	All	—	30
Group 4b			
	0	2	—
	1	10	—
	2	6	—
	3	2	—
	4	1	—
	5	—	15
	All	—	42

expected if they were allelic. However, the reactions of serum 33 with the 4*b* group are not compatible with it being a simple component of a 2 allele system. Serum 31 shows a positive association with sera of the second group (LA2), and probably contains a mixture of the antibodies anti-4a and anti-LA2.

The practical possibility of using reactions to sera of these various groups for classifying individuals as to whether they carry the antigens LA1, LA2, and 4b is demonstrated by the data shown in Table 7. This table shows the number of sera in the groups to which the individuals reacted. There are, for each group of sera, clearly two categories of people:

(1) Those that react to all, or all but one of the sera in a group.

(2) Those that react to 0, 1, 2, and infrequently 3 or more of the sera in a group.

The first category identifies individuals with the antigen corresponding to the shared antibody. The second category consists of people who do not carry this antigen but may be reacting to one or more of the unshared antibodies. As discussed above, the reliability of a classification based on reactions to a group of sera sharing an antibody will in general increase as the number of sera in the group increases.

Further evidence for the existence of a shared antibody identifying the LA2 antigen comes from absorption studies, the results of which are shown in Table 8. Two of the anti-LA2 containing sera, 17 and 22, were absorbed with a number of samples of leukocytes which were 17+ or 22+, but did not react to all members of the group, and so were LA2(−). The absorbed sera agglutinated in all

TABLE 8. DEMONSTRATION BY ABSORPTION OF SHARED ANTIBODY IN SERA OF THE LA2 GROUP

	Absorbing	Test WBC: Contain LA2 Antigen												Test WBC: Lack LA2 Antigen											
Sera	WBC	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
(a) Anti-LA2 #17	13							+	+	+				−		−	−			−					
	15	+	+	+	+	+	+									−	−			−					
	19	+	+	−?		+	+										−			−					
	20	+	+	+	+	+	+			+	+	+	+		−						−				
(b) Anti-LA2 #22	13	+	+	+	+	+	+							−			−	−	−				−		
	16	+	+	+	+	+	+										−	−	−				−		
	17	+	+	+	+	−	+					+	+				−	−	−				−		
	21	+		+	+			+	+	+	+			−			−	−				−			
(c) Anti-LA2 #28	3		−	−	−	−																			
	4	−	−	−	−	−	−	−																	
	5	−	−	−	−	−	−	−																	
	6			−	−	−	−																		
	7	−	−	−	−	−	−	−																	
(d) Anti-LA2 #28	13			+	+			+						−											
	17		+	+		+												−							
	19	+			+	+	+													−					
	23	+	+	+	+	−	+	+																−	
	24	+		+	+	+		+																	−

(a) Serum 17 was absorbed with leukocytes (WBC) that were 17(+) but LA2(−). These should absorb out any antibody other than anti-LA2, leaving behind the major LA2 component.

(b) As for (a), using serum 22.

(c) Serum 28 was absorbed with a random sample of 28(+) (and LA2[+]) WBC.

(d) Serum 28 was absorbed with a sample of 28(−) (and LA2[−]) cells as a control.

The results show that sera 17, 22 and 28 share an agglutinin. 17 and 22 are multispecific whereas 28 is monospecific.

but 2 out of 49 tests involving samples of cells carrying the LA2 antigen, but did not agglutinate any of the test samples of LA2(−) cells. Thus the second unshared components of these sera were successfully absorbed out, leaving behind the major shared component anti-LA2. A third LA2 serum, 28, was absorbed with LA2(+) leukocytes, and also with some of the LA2(−) leukocytes used to absorb sera 17 and 22. The latter did not remove the anti-LA2 specificity whereas all the former did. Thus, nonspecific absorption was excluded and, within the limits of the tests, serum 28 was shown to be a pure serum. If it contains a second component other than anti-LA2 this must detect a rare antigen. Current studies with LA1 sera suggest that serum 14 is also pure.

TABLE 9. RANGE OF NUMBERS OF CELLS FROM VARIOUS DONORS REQUIRED TO ABSORB 1 ml OF LEUKOCYTE ANTI-SERUM.

Group	Serum #	Cells × 10^7 WBC	Lymphocytes	Platelets
LA1	25	8	10	80
	8	8–12	18	60–200
LA2	28	1–3	2	60
	22	6–10	16	200–600
	17	4–12	12–32	180–540
4a	4	4–15	14–15	300–620
4b	18	8–15	6–10	60–120

WBC = Mixed white cells as they occur in peripheral blood, i.e., about 60% (or more) granulocytes and 40% (or less) lymphocytes.

Neither WBC or lymphocyte preparations contain platelets.

Platelet preparations contain on the average about 1WBC/1000 platelets.

It should be mentioned that in all matings involving women who have produced leukocyte agglutinating sera, the combined phenotypes of husband and wife fit in with the specificity of the serum produced. Thus, for example, all women producing anti-LA1 containing sera are LA1(−) and their husbands LA1(+). The pattern of mate differences indicating potential antibody stimulations can be a useful guide to the grouping of sera. However, it has been found that not all potential antigenic differences give rise to all expected antibodies.

A comparison of the absorption of representative sera by mixed leukocytes, purified lymphocytes, and platelets is shown in Table 9. The agglutination reaction in the main depends on the presence of granulocytes. These data, however, show that the antigens recognized by our serum groups are shared by granulocytes, lymphocytes and platelets. The antibodies of these systems could not be absorbed by red blood cells. Further details of this work will be published elsewhere.

GENETICS OF THE LEUKOCYTE GROUPS

A summary of the LA1, LA2 segregation in families is shown in Table 10. All but one of the ten possible mating types has been observed. The segregation in $+ - \times - -$, and $- + \times - -$ matings, indicates dominant inheritance of these antigens. The lack of any recombinants ($+ +$ or $- -$) in $+ + \times - -$ matings is expected on the assumption that the genes for LA1 and LA2 are allelic or closely linked. The probability of finding only parental types among the nine observed offspring from these matings, on the assumption of no linkage, is

$$\left(\frac{1}{2}\right)^9 = \frac{1}{512}.$$

The 95% upper limit for the recombination fraction p, is given by

$$(1 - p)^9 = \frac{1}{20} \text{ or } p = 28.3\%.$$

The occurrence of $+ +$ and $- -$ in $+ - \times - +$ matings shows that the genes determining the antigens LA1 and LA2 are codominant, as is generally found for antigenic markers.

The population frequencies of the four types of reactions to anti-LA1 and anti-LA2 are shown in Table 11. Only data from unrelated individuals has been included. There is a highly significant negative association between the frequency of reactions to the two sera. This association is such that it is not possible to fit the data by a genetic model involving two loci unless these are involved

TABLE 10. FAMILIAL SEGREGATION OF LA1 AND LA2

Parental Types				Number of Families	Total Offspring Typed	Offspring Types			
LA1	LA2	LA1	LA2			LA1 + LA2 +	LA1 + LA2 −	LA1 − LA2 +	LA1 − LA2 −
+	+	+	+	1	—	—	—	—	—
+	+	+	−	—	—	—	—	—	—
+	+	−	+	2	1	0	0	1	0
+	+	−	−	2	9	0	6	3	0
+	−	−	+	10	11	6	4	0	1
+	−	+	−	1	5	0	4	0	1
+	−	−	−	5	10	0	7	0	3
−	+	−	+	7	9	0	0	9	0
−	−	−	+	7	20	0	0	9	11
−	−	−	−	5	5	0	0	0	5

in a very unusual type of selective interaction. The situation is once again reminiscent of that for the ABO blood groups as analyzed by Bernstein in 1925. A model involving three alleles, *LA1*, and *LA2* corresponding to antigens LA1 and LA2, respectively, and *LA3* as the "silent" allele fits the data perfectly. The gene frequencies of 16.2%, 28.1% and 55.7%, respectively, were calculated using the method of Bernstein (see e.g. Neel and Schull, 1954).

TABLE 11. LA PHENOTYPE AND GENE FREQUENCIES

Phenotype LA1	Phenotype LA2	Observed	Expected	
				Gene Frequencies
+	+	11	12.3	(and standard errors)
+	−	29	27.8	LA1 = 0.1617 ± 0.03
−	+	54	53.0	LA2 = 0.2811 ± 0.023
−	−	41	41.9	LA3 = 0.5572 ± 0.033
Total....		135	135.0	

Contingency X_1^2 testing (negative) association between LA1 and LA2 reactions = 9.7 ($P < 0.5\%$)
X_1^2 testing goodness of fit of expected phenotype frequencies = 0.227 ($P > 10\%$)

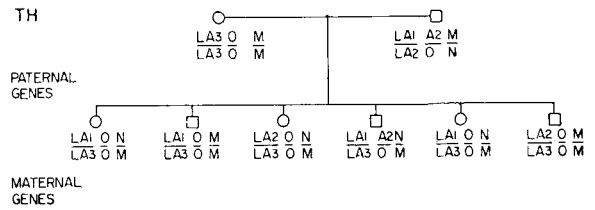

FIGURE 1. Segregation of LA with ABO and MN Red Cell Groups. The mating is a triple backcross. Close linkage between LA and the ABO or MN loci is excluded.

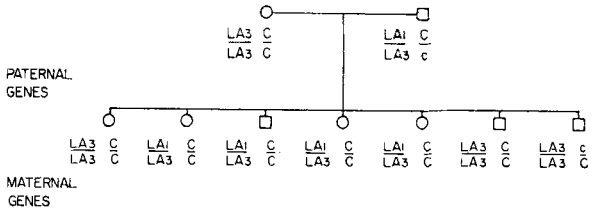

FIGURE 2. Segregation of LA and Rhesus Type C-c. Close linkage between LA and Rh is excluded.

The LA1 and LA2 types show no significant association with sex or with those red cell blood groups for which tests have been made (ABO, MNSs, Rhesus, P, Kidd, Kell and Duffy). Pedigrees illustrating the segregation of *LA1*, *LA2*, and *LA3* with ABO, MN, and Rhesus red cell blood groups are illustrated in Figs. 1 and 2. The first pedigree shows the joint segregation of *LA1*, *LA2* and *LA3* with A_2, *O*, *M*, and *N*. The mating is a triple backcross

$$\frac{LA1\ A_2\ M}{LA2\ O\ N} \times \frac{LA3\ O\ M}{LA3\ O\ M}$$

There are offspring which must be recombinant for LA, ABO, and MN loci, demonstrating the lack of close linkage between these loci. On the other hand, none of the offspring are recombinants for the LA types. The second pedigree demonstrates lack of close linkage between LA and the Rhesus antigens *C* and *c*.

The population frequencies of combined LA and 4*b* types are shown in Table 12. All possible combinations of LA and 4*b* types have, in fact, been observed, thus ruling out the possibility that antigenic determinants LA1, LA2, 4*a* and 4*b* are

TABLE 12. RANDOM ASSOCIATION OF 4 AND LA GROUPS

LA Phenotype LA1	LA Phenotype LA2	4*b* Phenotype 4*b*(+)	4*b* Phenotype 4*b*(−)	
+	+	7	4	
+	−	14	10	
−	+	32	16	
−	−	27	6	
		80	36	116

For overall association of 4*b* and LA, $X_3^2 = 4.08$ ($P > 10\%$)
For association of 4*b* and LA1, $X_1^2 = 1.88$ ($P > 10\%$)
For association of 4*b* and LA2, $X_1^2 = 0.46$ ($P > 10\%$)
Gene Frequency of 4*a* (calculated from 4*b* [−]) = 0.56 ± 0.039
Observed number of 4*a*4*b* heterozygotes = 57
Expected number of 4*a*4*b* heterozygotes = 57.2

variants of the same basic antigen which are controlled by alleles at the same locus. There is no significant association between 4*b* and either LA1 or LA2 types. The frequency of the *4a* gene calculated as the square root of the frequency of 4*b*(−) individuals is 56%. This is, in fact, just significantly greater at a 95% level than the frequency of 38.5% given by van Rood and van Leeuwen (1963) for the Dutch population. The expected number of 4*a*4*b* heterozygotes agrees very well with the observed number. This lends some weight to the reliability of our 4*a* classification based on the putative 4*a* sera recorded in Table 6. The lack of association between van Rood's group 4 and LA at the population level does not, of course, rule out linkage between these loci. Neither does it rule out the possibility that 4*a*, 4*b* and LA1, LA2 may be antigenic components of the same gene product. However, the lack of analogy with the highly significant association between the components of the MNSs and Rhesus red blood cell systems makes this somewhat unlikely. Appropriate pedigrees for conclusively demonstrating independence of the LA and 4 systems based only on a 4*b* classification, using our third group of sera (and not 4*a*), are very rare. However, a suitable pedigree, involving what seems to be a reliable 4*a* classification, is shown in Fig. 3. Classification for 4*a* in LA2(−) individuals was based on reactions

to sera 4, 31 and 33. Although 31 is a mixture of anti-LA2 and anti-4*a*, it can be used to classify LA2(−) individuals for 4*a*. The identification of offspring 5 as a *4a4b* heterozygote was based on the use of a sample of serum number 4 which had been absorbed with *4b4b* (31[−] and 33[−]) cells. This

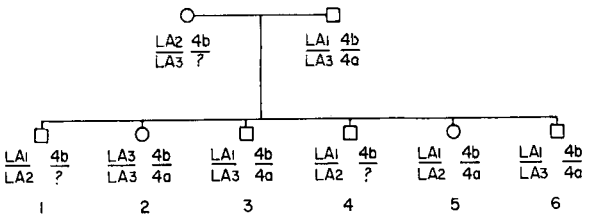

FIGURE 3. Independent Segregation of LA and 4 Loci. The parents are LA heterozygotes since offspring 2 is LA1(−) LA2(−). The 4*a* classifications of the mother and offspring 1 and 4 were not clearly established.

(a) If the mother is 4*b*/4*b*, then either 2 or 3, 5 and 6 are recombinants for LA and 4.

(b) If the mother is 4*b*/4*a*, the mating is one of four possible types, according to the putative linkage phases of the parental genotypes:

LA4 LA4

$\left.\begin{matrix} \frac{2b}{3a} \times \frac{1a}{3b} \\ \frac{2a}{3b} \times \frac{1b}{3a} \end{matrix}\right\}$ Offspring 3 and 6 are recombinants

$\left.\begin{matrix} \frac{2b}{3a} \times \frac{1b}{3a} \\ \frac{2a}{3b} \times \frac{1a}{3b} \end{matrix}\right\}$ Offspring 2 and 5 are recombinants

In any case, the group 4 and LA loci are shown not to be very closely linked.

was shown to give the same reactions as one of van Rood's 4*a* sera. If the mother is *4b4b*, either offspring 2 or offspring 3, 5, and 6 are recombinants. If the mother is a *4a4b* heterozygote, then offspring 3 and 6 or offspring 2 and 5 are recombinants. In either case the 4 and LA loci are shown not to be closely linked and so the possibility that they are antigenic components of the same gene product is ruled out.

DISCUSSION

The elucidation of this three-allele antigen system brings us one step further in the understanding of what Race and Sanger call the "so splendidly complicated antigens of the white cells" (Race and Sanger 1962, preface to the third edition). Undoubtedly, as more of the white cell antigen systems are worked out, the analysis of new systems not yet understood will proceed at an ever increasing rate.

Anti-LA1 and anti-LA2 can, in terms of the current analysis, be identified among the sera reported on by Payne and Hackel (1961). All but three of their sera are distinct from those reported in this paper (sera 3, 6, and 12 of Payne and Hackel [1961] are sera 9, 14, and 11, respectively, of the present paper). Many of them have, in addition, been tested on a common panel of leukocyte donors so that sera numbers 1 and 6 of the previous paper can be identified as containing anti-LA1, sera 2, 3, and 10 as containing anti-LA2, and 12 as containing anti-4*b*. Sera 1, 3, and 10 were almost certainly multispecific, so that the conclusions from the pedigrees analyzed by Payne and Hackel (1961) concerning the independence of 1 and 6 and 2, 3, and 10 are not strictly valid. The apparent independence is probably due to the independent segregation of the genes controlling the antigens corresponding to the unshared antibodies. The fact that the LA grouping was present, but not recognized, in the earlier studies is a reflection of the amount of data that was needed before the complex interactions of these sera could be at least partially understood.

In our LA system, van Rood's group 4 and also the platelet antigen systems analyzed by Shulman et al. (1962a), and Shulman, Aster, Pearson, and Hiller (1962b), there are the beginnings of a series of polymorphisms involving human tissues which were hitherto genetically unmarked. These new genetic groupings are of obvious clinical significance.

The evolutionary interest in these systems will be equally important. Represented here are further polymorphisms in humans whose selective basis challenges our understanding. It may, however, be some time before these systems achieve the prominence as anthropological markers which is enjoyed by some of the red blood cell systems. The development of techniques for more sensitive and reproducible but still simple assays for the antigens, for the storing of white cells in such a way as not to interfere with the assay, and for the selection and production of appropriate antisera, is still of overriding importance.

SUMMARY

The 2 times 2 associations of the agglutination reactions of a number of leukocyte antisera were analyzed using an electronic computer. 19 out of 35 sera could be assigned to one of three groups which were such that any pair of sera within a group showed a significant positive association. Each group defines an antibody shared by all of the sera of the group, and identifies a corresponding antigen. Individuals carry the antigen if they react to all the sera in a group. Absorption studies indicate that two of our sera may be pure

and that the antigens defined by the groups are shared by granulocytes, lymphocytes, and platelets, but not by the erythrocytes. There is a significant negative association between reactions to the first two groups, defining antigens LA1 and LA2, indicating allelism of the genes determining these antigens.

The population frequencies and familial segregation of the four types, LA1(+)LA2(+), LA1(+)LA2(−), LA1(−)LA2(+), and LA1(−)LA2(−), are interpreted on the basis of three autosomal codominant alleles *LA1*, *LA2*, and *LA3*, with frequencies of 0.16, 0.28 and 0.56, respectively.

The third group of sera identifies van Rood's antigen 4*b* and further sera of a possible fourth group contain anti-4*a*. Pedigree data and population frequencies indicate that these two systems are unrelated. The importance of extending the understanding of leukocyte groups is emphasized.

Acknowledgments

This research has been supported by research grants GM 10452-02 and HE 03365, and by Training Grant 5TI-GM-295-05 from the National Institutes of Health, U.S. Public Health Service.

APPENDIX ON NOMENCLATURE AND TERMINOLOGY

This appendix is included in the hope of stimulating discussion which might lead to the adoption of a uniform, rational notation during the earlier stages of the discovery of leukocyte antigen systems.

The antigens and respective antibodies of the LA system are designated LA1, anti-LA1, LA2, anti-LA2, etc. The alleles are designated *LA1*, *LA2*, etc. L stands for leukocyte, A for the locus, so that other loci may be defined by LB, LC, etc. The numbers following the letters LA, LB, etc., do not need to follow any particular sequence, so long as there are no ambiguities. It may, of course, often take some time before the relationship between new antigens found in different laboratories can be determined. Numbers are preferred to letters for the identification of alleles since the number of alleles possibly may exceed 26, whereas the number of loci is unlikely to do so. It does not seem desirable to use superscripts, which are cumbersome for the printer and incompatible with automatic data processing equipment.

The expression "allele" is used here for alternate states of a genetic region concerned with a particular function. This concept is not really well defined until the function is clearly understood at the level of the protein subunit or polypeptide

Appendix Table A. LA1 and LA2 χ^2 Associations

Serum 1st	Pairs 2nd	++	+−	−+	−−	Total People Tested	χ^2
2	6	13	28	60	39	140	8.6*
2	7	11	23	63	30	127	11.4*
2	8	39	2	32	66	139	42.7
2	9	9	19	44	30	102	5.0*
2	14	29	4	5	73	111	68.7
2	15	29	4	17	66	116	42.0
2	17	17	13	47	19	96	1.4*
2	22	14	15	40	16	85	3.5*
2	25	24	4	26	20	74	5.5
2	28	10	16	30	16	72	3.8*
2	30	14	2	3	27	46	23.7
2	32	10	5	1	29	45	18.4
6	7	66	17	26	45	154	27.5
6	8	33	60	55	35	183	11.0*
6	9	44	9	10	42	105	40.3
6	14	13	65	39	39	156	18.0*
6	15	18	53	33	28	132	10.3*
6	17	66	9	34	29	138	18.2
6	22	55	10	21	32	118	23.9
6	25	29	26	44	10	109	8.9*
6	28	47	10	10	41	108	40.2
6	30	12	23	19	18	72	1.5*
6	32	8	25	12	26	71	0.2*
7	8	40	54	34	30	158	1.3*
7	9	47	15	8	36	106	32.0
7	14	15	68	25	27	135	12.4*
7	15	24	54	27	27	132	4.2*
7	17	60	7	20	20	107	18.7
7	22	46	12	14	20	92	12.1
7	25	31	17	23	5	76	1.9*
7	28	34	14	6	22	76	15.4
7	30	10	18	6	8	42	0.0*
7	32	6	18	4	10	38	0.0*
8	9	21	31	34	20	106	4.5*
8	14	45	32	12	80	169	36.6
8	15	42	20	9	61	132	39.5
8	17	45	23	63	21	152	1.0*
8	22	27	31	58	17	133	12.1*
8	25	49	10	34	32	125	12.5
8	28	21	35	39	29	124	4.1*
8	30	25	11	7	34	77	19.5
8	32	17	17	4	35	73	12.1
9	14	6	44	24	26	100	13.8*
9	15	12	42	25	20	99	10.3*
9	17	32	3	10	16	61	17.1
9	22	23	8	7	17	55	9.3
9	25	13	12	19	4	48	3.8*
9	28	22	4	3	18	47	20.3
9	30	3	14	3	8	28	0.0*
9	32	4	11	2	9	26	0.05
14	15	29	3	17	70	119	46.9
14	17	25	17	61	19	122	2.9*
14	22	17	26	48	20	111	9.2*
14	25	38	7	31	28	104	10.3
14	28	15	28	32	28	103	2.7*
14	30	20	8	6	35	69	20.5
14	32	16	9	3	38	66	21.7
15	17	19	13	47	8	87	6.2*
15	22	15	14	33	10	72	3.8*
15	25	23	2	17	16	58	9.1
15	28	6	17	27	8	58	12.7*
15	30	12	1	1	23	37	25.0
15	32	6	5	1	21	33	8.2
17	22	73	23	14	26	136	18.9
17	25	53	30	26	11	120	0.2*
17	28	54	31	8	28	121	15.7
17	30	22	29	9	17	77	0.2
17	32	14	32	6	21	73	0.2
22	25	44	27	34	14	119	0.6*
22	28	57	19	6	39	121	40.6
22	30	18	26	13	21	78	0.0
22	32	13	25	9	25	72	0.2
25	28	30	51	30	14	125	9.9*

APPENDIX TABLE A, CONTINUED

Serum 1st	Pairs 2nd	++	+−	−+	−−	Total People Tested	χ^2
25	30	27	23	6	25	81	8.1
25	32	15	31	5	26	77	1.8
28	30	15	29	18	22	84	0.6*
28	32	10	29	12	30	81	0.0
30	32	17	13	6	42	78	15.3

++ Positive to both sera
+− Positive to first serum and negative to second serum
−+ Negative to first serum and positive to second serum
−− Negative to both sera
* Negative association

APPENDIX TABLE B. 4b AND PUTATIVE 4a χ^2 ASSOCIATIONS

Serum 1st	Pairs 2nd	++	+−	−+	−−	Total People Tested	χ^2
3	4	154	17	12	1	184	0.1*
3	10	132	35	1	12	180	28.2
3	11	87	40	0	12	139	19.1
3	18	80	35	0	7	122	11.2
3	19	75	39	2	6	122	3.7
3	26	51	47	1	5	104	1.6
3	31	47	20	3	1	71	0.1*
3	33	16	39	1	3	59	0.2
4	10	118	45	15	1	179	2.5*
4	11	76	49	13	3	141	1.7*
4	18	71	48	13	1	133	4.6*
4	19	68	46	10	0	124	4.8*
4	26	48	55	8	5	116	0.5*
4	31	56	19	4	4	83	1.1
4	33	17	39	0	4	60	0.5
10	11	76	21	8	30	125	35.7
10	18	73	17	6	24	120	34.7
10	19	70	18	6	26	120	34.8
10	26	44	34	8	17	103	3.6
10	31	36	19	13	2	70	1.6*
10	33	14	35	3	7	59	0.1*
11	18	46	3	8	21	78	34.5
11	19	45	5	9	22	81	29.3
11	26	23	12	10	16	61	3.4
11	31	11	9	12	3	35	1.4*
11	33	4	15	6	4	29	2.8*
18	19	68	9	6	33	116	56.5
18	26	42	28	11	31	112	10.7
18	31	27	17	30	6	80	3.7*
18	33	6	31	11	11	59	6.1*
19	26	43	19	6	33	101	25.8
19	31	22	18	26	2	68	9.6*
19	33	6	30	10	9	55	6.1*
26	31	25	38	8	85	85	3.4*
26	33	4	25	13	16	58	5.3*
31	33	17	24	0	19	60	9.0

++ Positive to both sera
+− Positive to first serum and negative to second serum
−+ Negative to first serum and positive to second serum
−− Negative to both sera
* Negative association

chain. It is quite possible that some of the entities now called alleles will subsequently be shown to involve a genetic region defining two or more polypeptide chains controlled by adjacent cistrons. The classical use of allele refers to alternative states of a single mutant site which, by definition, are not separable by recombination. Even for mutants affecting the same amino acid in a polypeptide chain, it is not yet possible with data from human populations to achieve the genetic resolution required for such an understanding.

Many of the above complications may well be reflected in the future discovery of components for the human white cell systems such as arose, for example, in the MNSs and Rhesus systems, and the H2 system in mice. Thus, conceivably, a rare new allele might be described in the LA system which transmits both the LA1 and LA2 antigenic specificities. LA1 and LA2 would then be recognized as components rather than mutually exclusive alternatives of the LA system. The new allele might be informally designated *LA4*(1,2), to indicate that it carries components 1 and 2, the prefix LA being understood. Its formal designation would be, simply, *LA4*. There is no need for any relationship between the allele and component numbering. A system of nomenclature has to strike a compromise between the convenience and simplicity required for everyday use and the need for adequate definition of the concepts involved. The adoption of an unwieldy or inadequate notation may well retard advances in the understanding of a complex system. It is our hope that this may be avoided as the leukocyte antigen groups unfold.

REFERENCES

ANDRÉ, R., B. DREYFUS, and CH. SALMON. 1958. Isoimmunisation antileucocytes après transfusions, anticorps antileucocytes et leucopénies. Rev. Franç. d'Etudes Clin. Biol. *3:* 33–40.

BRAUN, E. H., A. E. BUCKWOLD, H. E. EMSON, and A. V. RUSSELL. 1960. Familial neonatal neutropenia with maternal leukocyte antibodies. Blood *16:* 1745–1752.

BRITTINGHAM, T. E. 1957. Immunologic studies on leukocytes. Vox Sang. (NS) *2:* 242–248.

BRITTINGHAM, T. E., and H. CHAPLIN, JR. 1957. Febrile transfusion reactions caused by sensitivity to donor leukocytes and platelets. J. Am. Med. Assoc. *165:* 819–825.

DAUSSET, J. 1954. Leuco-agglutinins. IV. Leuco-agglutinins and blood transfusion. Vox Sang. (OS) *4:* 190–198.

DAUSSET, J., and H. BRECY. 1957. Identical nature of the leukocyte antigens detectable in monozygotic twins by means of immune iso-leuco-agglutinins. Nature *180:* 1430.

DAUSSET, J., A. FONSECA, and H. BRECY. 1957. Elimination de certains chocs transfusionnels par l'utilisation de sang appauvri leucocytes. Vox Sang. (NS) *2:* 248–256.

DAUSSET, J., and A. NENNA. 1952. Présence d'une leucoagglutinine dans le sérum d'un cas d'agranulocytose chronique. Compt. Rend. Soc. Biol. *146:* 1539–1541.

HITZIG, W. H., and R. GITZELMANN. 1959. Transplacental transfer of leukocyte agglutinins. Vox Sang. (NS) *4:* 445–455.

JENSEN, K. G. 1962a. Leucocyte antibodies in serums of pregnant women. Vox Sang. (NS) *7:* 454–469.

———. 1962b. The influence of maternal leucocyte antibodies on infants. Proc. IX Congr. Internat. Soc. of Blood Tran. p. 759–762.

KILLMANN, S. A. 1958. Febrile transfusion reactions in patients with leukocyte agglutinins. Danish Med. Bull. *5:* 178–183.

——. 1960. Leukocyte agglutinins. Properties, occurrence and significance. Blackwell Scientific Publications, Oxford. 95 p.

LALEZARI, P., M. NUSSBAUM, S. GELMAN, and T. H. SPAET. 1960. Neonatal neutropenia due to maternal isoimmunization. Blood *15:* 236–243.

LALEZARI, P., and T. H. SPAET. 1959. Studies on the genetics of leukocyte antigens. Blood *14:* 748–758.

VAN LOGHEM, J. J. JR., M. VAN DER HART, and H. BORSTEL. 1957. The occurrence of complete and incomplete white cell antibodies. Vox Sang. (NS) *2:* 257–263.

NEEL, J. V., and W. J. SCHULL. 1954. Human Heredity. University of Chicago Press, Chicago. 361 p.

PAYNE, R. 1957. The association of febrile transfusion reactions with leuko-agglutinins. Vox Sang. (NS) *2:* 233–241.

——. 1960. Further observations on leukoagglutinin transfusion reactions with special reference to leukoagglutinin transfusion reactions in women. Am. J. Med. *29:* 449–458.

——. 1962. The development and persistence of leukoagglutinins in parous women. Blood *19:* 411–424.

——. 1963. The specificity of leukoagglutinins for lymphocytes. Proceedings IX Cong. Europ. Soc. Haemat. 1128–1131.

——. 1964a. Neonatal neutropenia and leukoagglutinins. Pediatrics *33:* 184–204.

——. 1964b. The agglutination technique for the demonstration of leukocyte isoantigens in man. Methods in Medical Research. Vol. 10, Yearbook, Med. Publ., Chicago. (In press.)

PAYNE, R., and E. HACKEL. 1961. Inheritance of human leukocyte antigens. Am. J. Hum. Genet. *13:* 306–319.

PAYNE, R., and M. R. ROLFS. 1958. Fetomaternal leukocyte incompatibility. J. Clin. Invest. *37:* 1756–1763.

RACE, R. R., and R. SANGER. 1962. Blood groups in Man. 4th Edition, Blackwell, Oxford. 456 p.

VAN ROOD, J. J. 1962. Leukocyte grouping. A method and its application. Ph.D. Dissertation, Univ. of Leyden.

VAN ROOD, J. J., J. G. EERNISSE, and A. VAN LEEUWEN. 1958. Leukocyte antibodies in sera from pregnant women. Nature *181:* 1735–1736.

VAN ROOD J. J. and A. VAN LEEUWEN. 1963. Leukocyte grouping. A method and its application, J. Clin. Invest. *42:* 1382–1390.

VAN ROOD, J. J., A. VAN LEEUWEN, and J. G. EERNISSE. 1959. Leukocyte antibodies in sera of pregnant women. Vox Sang. (NS). *4:* 427–444.

VAN ROOD, J. J., A. VAN LEEUWEN, and L. J. BOSCH. 1961. Leukocyte antigens and transplantation immunity. VIII Congr. Europ. Soc. for Haemat. *1:* 199.

SHULMAN, N. R., V. J. MARDER, L. M. ALEDORT, and M. C. HILLER, 1962a. Complement fixing isoantibodies against antigens common to platelets and leukocytes. Trans. Assoc. Am. Physicians *75:* 89–98.

SHULMAN, N. R., R. H. ASTER, H. A. PEARSON, M. C. HILLER. 1962b. Immunoreactions involving platelets. VI. Reactions of maternal isoantibodies responsible for neonatal purpura. Differentiation of a second platelet antigen system. J. Clin. Invest. *41:* 1059–1069.

WALFORD, R. L. 1960. Leukocyte Antigens and Antibodies. Grune and Stratton, New York. 182 p.

Cold Spring Harbor Symposia on Quantitative Biology

A New Leukocyte Isoantigen System in Man

Rose Payne, Walter Bodmer, Millie Tripp, et al.

Cold Spring Harb Symp Quant Biol 1964 29: 285-295
Access the most recent version at doi:10.1101/SQB.1964.029.01.031