120. *Studies on Lewis Blood Group System.*

I. Le^c Blood Group Factor

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In 1939, Furuhashi and Ueyama,¹⁾ and Ueyama,²⁾ using anti-T precipitin in normal chicken serum or in anti-non-secretor human saliva immune serum, discovered a specific antigenic substance T in non-secretor human saliva. Recently, Tōyama,³⁾ and Tsuganezawa⁴⁾ succeeded in obtaining anti-T agglutinin, the former by immunizing the rabbit with ovarian cyst fluid, and the latter by immunizing the goat with non-secretor human saliva.

On the other hand, Mourant⁵⁾ found in 1946 a new antibody in human serum, and reported on Lewis blood group which could be determined with this antibody. Then Andresen⁶⁾ found another antibody related to this blood group system. The former is now called anti-Le^a antibody, and the latter anti-Le^b antibody.⁷⁾ And according to the presence or absence of agglutination against these antibodies, three groups of human blood are distinguished from each other: Le(a+b-), Le(a-b+), and Le(a-b-).

Then Grubb,⁸⁾ and Grubb and Morgan⁹⁾ made clear that there is a close relationship between Lewis blood group and the secretor or non-secretor type; and they reported that those with Le(a+) belong to non-secretors while those with Le(a-) belong almost always to secretors only with rare exceptions to the non-secretor type.

In view of these reports it is now clear that blood group system T which was developed in Japan first by means of precipitation is related to Lewis blood group, and that T substance is nearly identical with Le^a substance. Also J blood group factor which was previously reported by our Department was confirmed to be Le^a blood group factor on account of the fact that those with J belonged to non-secretors.¹⁰⁾ Further, anti-Le^a precipitin which was prepared by Glynn, Glynn, and Holborow¹¹⁾ by immunizing the rabbit with group-A type-4 haemolytic streptococcal vaccines coated with Le^a substance from ovarian cyst fluid is known to be the anti-T precipitin which was discovered by Furuhashi and Ueyama.¹⁾

We succeeded in preparing anti-Le^a, anti-Le^b, and further anti-Le^c antibody by immunizing the rabbit with human saliva of Le(a+b-), Le(a-b+), and Le(a-b-) groups, respectively.

Grubb and Morgan⁹⁾ recognized the presence of Le^a substance in a secretion, and Annison and Morgan,¹²⁾ who carried out chemical studies of Le^a substance purified from ovarian cyst fluid, demonstrated that this, like other blood group substances, was composed of L-fucose, D-galactose, N-acetylglucosamine, and N-acetylgalactosamine. Further Watkins¹³⁾ reported that a liquid extract of *Trichomonas foetus* decomposed Le^a and Le^b substances as well as A, B, and H substances. Also Iseki and Masaki¹⁴⁾ demonstrated, by means of precipitation, that the O(H)-decomposing enzyme derived from *Bacillus fulminans* decomposed not only O(H) substance but also T(Le^a) substance. In the present experiments we investigated this enzyme for the ability to decompose Le^a, Le^b, or Le^c substance, and confirmed that it decomposed all of them.

Materials and methods. 1) Enzyme-treated red cells. To red cells, 1/10 its volume of a 1% papain solution was added, and the whole was incubated at 37°C for 45 minutes. Before use, these were washed with saline solution.

2) Immune antibody. Human saliva was boiled in a water bath at 100°C for 30 minutes, and centrifuged. To the supernatant was added 5–6 times its volume of 95% alcohol, to cause precipitation. The precipitate was dissolved in saline solution of 1/10 the initial volume of the saliva, and the solution was dialyzed overnight in a medium of saline solution. The non-dialyzed fraction was injected in a single dose of 4–6 cc into an ear vein of the rabbit every other day 5–6 times in total, and a week after the last injection, blood was taken, from which serum was separated.

3) Determination of secretor or non-secretor type. Secretor or non-secretor type was determined by the group specific precipitation and the group specific agglutination inhibition test against anti-O(H), anti-A, anti-B, and anti-T(Le^a) antibodies.

4) O(H)-decomposing enzyme. Autolysate of *Bacillus fulminans* was subjected to the following procedures: Centrifugation; precipitation of the supernatant by adding the same volume of cold acetone; dissolution of the precipitate in neutral phosphate buffer solution; addition of ammonium sulphate to 0.6 saturation, again dissolution of the precipitate in neutral phosphate buffer solution, 48 hours dialysis in an ice-box; addition of the same amount of methanol; dissolution of the precipitate in distilled water; and finally freezing and drying. When used, the powder was dissolved in neutral phosphate buffer solution, and this enzymic solution was added to saliva. The whole was adjusted to pH 6.3–6.5, and allowed to stand at 37°C for 48 hours. Then the solution was heated at 100°C for 5 minutes, centrifuged, and the supernatant was investigated for the decomposition of Lewis blood

group substances by the agglutination inhibition test.

Results. 1. Lewis antibodies. Anti-Le^a antibody was obtained when the rabbit was immunized with non-secretor human saliva of Le(a+b-) group containing a large amount of Le^a substance, and then the immune serum was absorbed at low temperature with the enzyme-treated red cells of Le(a-b+) group. This was a cold incomplete antibody which agglutinated at low temperature the enzyme-treated red cells of Le(a+b-) group, at titer 16-32. It did not show any reaction at 37°C. In the immune serum was also produced a precipitin against human saliva. When this serum was adequately absorbed with secretor human saliva of Le(a-b+) group which scarcely contained Le^a substance, then anti-T (Le^a) precipitin which reacted only with non-secretor human saliva could be obtained. The precipitin titer (antigen titer) of this antibody was 32-64.

Anti-Le^b antibody was obtained by immunizing the rabbit with secretor human saliva of Le(a-b+) group, which contains a large amount of Le^b substance and then by absorbing the antiserum at low temperature with the enzyme-treated red cells of Le(a+b-) group. This was a cold incomplete antibody which agglutinated at low temperature the enzyme-treated red cells of Le(a-b+) group, but did not react at 37°C. Its agglutinin titer against groups O and B red cells was 4-8, but against group A₁ red cells it was very low, as mentioned in the first report by Andresen.⁶⁾

When this immune serum was absorbed with non-secretor human saliva, anti-O(H) precipitin which reacted only with secretor human saliva and which had precipitin titer (antigen titer) of 64-128 was obtained. If this was further absorbed with secretor human saliva of Le(a-b-) group which scarcely contained Le^b substance, anti-Le^b precipitin which reacted only with secretor human saliva of Le(a-b+) group at precipitin titer (antigen titer) of 8-16 was produced.

These anti-Le^a and anti-Le^b agglutinins prepared by immunizing the rabbits with human saliva were compared with anti-Le^a and anti-Le^b agglutinins from human sera, which were kindly supplied by Dr. Mourant of Lister Institute, and agreements between their reactions to human red cells were confirmed as seen in Table I.

We immunized the rabbit with human saliva of Le(a-b-) group which reacted with neither of the two antibodies, and then absorbed the immune serum at low temperature with the enzyme-treated red cells of Le(a+b-) and Le(a-b+) groups. And we obtained anti-Le^c cold incomplete antibody which agglutinated at low temperature only the enzyme-treated red cells of Le(a-b-) group. The agglutinin titer of this antibody was 2-4, and it did not react at 37°C.

When the rabbit was immunized with secretor human saliva of

Table I. Comparison of saliva-immunized anti-Le^a and anti-Le^b antibodies with Lewis antibodies from Lister Institute

Cases	K.S.	F.T.	K.A.	T.I.	K.F.	S.A.
Blood groups	OMNq Le(a+b-)	ONq Le(a+b-)	OMNq Le(a-b+)	OMNQ Le(a-b+)	OMq Le(a-b+)	ONq Le(a-b-)
Saliva types	sT	sT	St	St	St	St
Saliva immunized anti-Le ^a	+	+	-	-	-	-
Saliva immunized anti-Le ^b	-	-	+	+	+	-
Lister Institute anti-Le ^a	+	+	-	-	-	-
Lister Institute anti-Le ^b	-	-	+	+	+	-

Le(a-b-) group containing O(H) substance, and the immune serum was absorbed with non-secretor human saliva, anti-O(H) precipitin was obtained. If this was further absorbed with secretor human saliva of Le(a-b+) group, anti-Le^c precipitin which reacted only with Le(a-b-) human saliva at precipitin titer (antigen titer) 4 was produced.

Each of these Lewis antibodies was made to react with human red cells and saliva, and as a result it was found out that Lewis blood group system was divided into three groups, Le(a+b-c-), Le(a-b+c-), and Le(a-b-c+) as shown in Table II, and that Le(a-b-c+) group was, though infrequently, found also in those of non-secretor type, whose saliva slightly reacted with anti-T(Le^a) precipitin besides anti-Le^c.

Table II. Reaction with Lewis antibodies

Cases	K.S.	F.T.	K.A.	K.F.	S.A.	E.K.
Blood groups	OMNq Le(a+b-c-)	ONq Le(a+b-c-)	OMNq Le(a-b+c-)	OMq Le(a-b+c-)	ONq Le(a-b+c-)	NOq Le(a-b+c+)
Saliva types	sT	sT	St	St	St	sT
Agglutination of red cells against	anti-Le ^a (anti-T)	+	+	-	-	-
	anti-Le ^b	-	-	+	+	-
	anti-Le ^c	-	-	-	-	+
Precipitation of saliva against	anti-Le ^a (anti-T)	+	+	-	-	±
	anti-Le ^b	-	-	+	+	-
	anti-Le ^c	-	-	-	-	+
	anti-O(H)	-	-	+	+	+

2. Lewis blood group substances in saliva. Lewis blood group substances in saliva could roughly be classified as shown in Table III.

Table III. Absorption test of Lewis antibodies with saliva of each type

Blood groups and saliva types	Antiserum	Saliva dilution					
		2	4	8	16	32	64
Le(a+b-c-) sT (non-secretor)	Anti-Le ^a	-	-	-	-	-	±
	Anti-Le ^b	+	+	+	+	+	+
	Anti-Le ^c	+	+	+	+	+	+
Le(a-b+c-) St (secretor)	Anti-Le ^a	-	-	±	+	+	+
	Anti-Le ^b	-	-	-	-	+	+
	Anti-Le ^c	+	+	+	+	+	+
Le(a-b-c+) sT (non-secretor) or St (secretor)	Anti-Le ^a	-	±	+	+	+	+
	Anti-Le ^b	±	+	+	+	+	+
	Anti-Le ^c	-	-	-	+	+	+
	Anti-Le ^a	±	±	+	+	+	+
	Anti-Le ^b	-	-	+	+	+	+
	Anti-Le ^c	-	-	+	+	+	+

Adults of Le(a+b-c-) group belonged to non-secretors, and their saliva contained a large amount of Le^a substance but not Le^b or Le^c substance. Those of Le(a-b+c-) group belonged to secretors, having principally Le^b substance, and generally a small amount of Le^a substance, too. Those of Le(a-b-c+) group belonged in general to secretors and infrequently to non-secretors. Some of them had in the saliva principally Le^a substance besides Le^c but scarcely any Le^b, while others had Le^b but an extremely small amount of Le^a besides Le^c.

On the above-mentioned behaviour of Le^b substance in the saliva of Le(a-b-) people, the same results were previously obtained by Grubb.¹⁵⁾ There was also a non-secretor case of Le(a-b-c+) group, in which similarly to the former of the above two cases Le^a substance was principally demonstrated in saliva besides Le^c.

As to the inheritance of Lewis blood group, details will be reported in our succeeding paper. Only it will be mentioned here that, judging from the relation of the Lewis substances to red cells and saliva, Le^c gene seems dominant over Le^b and Le^a, and Le^b gene over Le^a.

3. Action of O(H)-decomposing enzyme on Lewis substances. All of Le^a, Le^b, and Le^c substances in saliva were decomposed by the O(H)-decomposing enzyme derived from *Bacillus fulminans*.

Summary. Anti-Le^a(T), anti-Le^b, and anti-Le^c cold incomplete agglutinins and precipitins were produced by immunizing the rabbits with secretor and non-secretor human saliva. And according to the reaction against these antibodies, Lewis blood group system was divided into three groups, Le(a+b-c-), Le(a-b+c-), and Le(a-b-c+). Among adults, those of Le(a+b-c-) group belonged to non-secretors, and in their saliva only Le^a substance was demonstrated. Those of Le(a-b+c-) group belonged to secretors, and in their saliva, Le^a

substance was demonstrated besides Le^b . And those of $Le(a-b-c+)$ group belonged to secretors or infrequently to non-secretors, and in some of them Le^a substance, and in the others Le^b substance was principally demonstrated besides Le^c in saliva. All the Lewis substances from saliva were decomposed by the O(H)-decomposing enzyme derived from *Bacillus fulminans*.

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