40. ABO and Lewis Blood Groups in Crab-eating Macaques

By Hachiro NAKAJIMA and Tadamitsu OGUSHI

Department of Legal Medicine, Tokyo Medical and Dental University

(Comm. by Tanemoto FURUHATA, M.J.A., Feb. 12, 1965)

Previously,¹⁾⁻⁴⁾ the present authors examined the blood of the Japanese apes, crab-eating macaques, and green monkeys for their Lewis blood groups and successfully divided the Japanese apes, as in man, into three groups, i.e. groups Le(a+b-), Le(a-b+), and Le(a-b-). All the Japanese apes tested contained Le^a, Le^b, and H substances in their saliva being independent of Lewis groups of the red cells. The Le^a substance was also detected in the blood of some individuals among crab-eating macaques, but not in green monkeys.

Subsequently, Wiener, Moor-Jankowski, and Gordon (1964)⁵⁾ tested the saliva from 10 species of non-human primates and found the presence of Lewis (Le^a) substance in 7 species, i.e. chimpanzee, orangutan, gibbon, gorilla, baboon, pig-tail monkey, and Celebes black ape.

The present paper gives the results of tests on the blood and submaxillary glands of crab-eating macaques not only for the Lewis groups but also for ABO groups.

Materials and Methods. Blood of macaque was collected by heart puncture, part of which was placed in a dry-clean test tube and part mixed with acid-citrate-dextrose solution. Immediately before use, the red cells were washed three times in normal saline. To test the Lewis substances in the serum, it was previously absorbed with human group 0 red cells to eliminate hetero-agglutinin for human red cells. The submaxillary gland was ground in a mortar and saline extract (10% w/v) was prepared. For Lewis group tests, a human anti-Le^a

Table I. Summary of ABO group tests on crab-eating macaques

Number observed	ABH substances in submaxillary gland	Group-specific agglutinins in serum
13	B and H	Anti-A (1:8-64)
7	A and H	Anti-B (1:4-128)
2		Anti-B (1:8-512) and weak anti-A (1:1-2)
4	A, B, and H	Weak anti-A (1:1-2)
4		—
Total 30		

serum and a rabbit anti-Le^b serum prepared by immunizing with human Le (a-b+) saliva were used. Human anti-A and anti-B sera and anti-H reagent prepared from *Ulex europaeus* seeds were used for ABO group tests. The agglutination, absorption, elution and agglutination-inhibition tests were performed according to the methods described by Nakajima and Miyagi (1964)⁴⁾ and by Nakajima and Hayashida (1963).⁵⁾

Results. 1) ABO groups. The saline extract of submaxillary gland of 30 macaques was tested for its ABO substances by the agglutination-inhibition technique using human anti-A and anti-B sera and *Ulex* anti-H reagent. The results obtained are summarized in table I. The extract of 9 of 30 macaques gave reactions corresponding to group A, 13 to group B, and the rest to group AB, and a rough reciprocal relation seemed to exist between the antigen of gland and the antibody of serum in crab-eating macaque, though it was not so perfect as in man and anthropoid apes.

2) Lewis groups. Thirty macaque blood and 14 submaxillary gland samples of them were examined for their Lewis groups by means of direct agglutination, absorption, elution, or agglutination-inhibition tests with human anti-Le^a and rabbit anti-Le^b sera.

The red cells of two of 30 macaques tested completely absorbed the anti-Le^a agglutinin adjusted to have a titre of 1:4 against human

Table II. Results of agglutination and absorption tests of the red cells of crab-eating macaques for Lewis grouping

Red cells of	Agglutination of red cells with		Titration scores of anti-Lewis sera after absorption with macaque red cells*)	
	anti-Le ^a (in saline)	anti-Le ^b (in bromelin)	anti-Le ^a	anti-Le ^b
Macaque				
No. 9	—	—	11	11
No. 10	—	++	10	8
No. 11	++	—	0	12
No. 13	—	—	10	11
No. 14	—	++	10	8
No. 19	++	—	0	11
No. 24	—	+	10	11
Human controls				
Le(a+b—)	+++	—	0	11
Le(a—b+)	—	+++	10	0
Le(a—b—)	—	—	11	12

Note: Anti-Lewis sera used for agglutination tests were previously absorbed with No. 9 macaque red cells.

*) Red cells vs. antiserum=1:1.

Le(a+b-) red cells, and also reduced markedly the Le^a titration score as shown in table II. On the other hand, none of 30 macaque red cell samples significantly reduced the titration score of the anti-Le^b agglutinin for human Le(a-b+) red cells. The red cells of three of 30 macaques after contact with anti-Le^b serum, however, yielded eluates with anti-Le^b specificity, indicating the presence on the red cells of the Le^b substance similar to but not identical with that of man. The two macaque red cell samples, which absorbed the anti-Le^a agglutinin, and the three macaque red cell samples, which showed positive results in the elution tests with anti-Le^b serum, were agglutinated respectively by anti-Le^a and anti-Le^b sera absorbed with a macaque red cell sample (No. 9) which was confirmed by the absorption and elution tests to be lacking in either Le^a or Le^b substances. These agglutinations were specifically inhibited by the human saliva from group Le(a+b-) and/or group Le(a-b+) individuals but not by the saliva from group Le(a-b-) individuals.

Thus, of the 30 macaques tested, 2 or 7 per cent belonged to group Le(a+b-), 3 or 10 per cent to group Le(a-b+), and the rest (25 or 87 per cent) to group Le(a-b-). The frequency of Le(a+b-) observed in this study on crab-eating macaques was much lower than

Table III. Results of inhibition tests of anti-Lewis sera
by saline extracts (10% w/v) of submaxillary
glands of crab-eating macaques

Lewis groups of red cells	Macaque No.	Inhibition titres of the extract of gland for anti-		
		Le ^a	Le ^b	H
Le(a+b-)	11	1:2	8	256
	19	2	0	256
Le(a-b+)	10	2	8	128
	14	4	8	64
	24	4	4	128
Le(a-b-)	16	0	8	128
	17	0	1	128
	18	1	1	128
	20	2	8	256
	21	4	2	256
	22	0	0	256
	23	1	1	128
	29	8	1	64
	30	1	2	64

Note: The titres of the antisera used in the tests were previously adjusted to 1:4.

that of the Japanese apes (15 or 50 per cent of 30 blood samples; Nakajima, 1962).¹⁾

The serum of group Le(a+b-) macaques inhibited anti-Le^a, but the serum of group Le(a-b+) or Le(a-b-) macaques did not. The Le^a negative macaque red cells treated *in vitro* with the macaque serum containing the Le^a substance became Le^a positive, though the reaction of the red cells with anti-Le^a serum was weaker than that of the fresh red celled of Le(a+b-) macaques. This suggests that the Lewis substance on the red cells of macaques might be secondarily taken up from the serum, as in man.⁷⁾

Table III shows the results of inhibition tests of human anti-Le^a, rabbit anti-Le^b, and *Ulex* anti-H agglutinins by saline extract (10% w/v) of the submaxillary glands of 14 macaques. As illustrated in the table, most of the samples tested inhibited both anti-Le^a and anti-Le^b agglutinins being independent of Lewis groups of the red cells. All the 14 samples also inhibited the anti-H agglutinin, and there was no relation among the inhibition titres for anti-Le^a, anti-Le^b, and anti-H agglutinins.

Summary. Blood and submaxillary gland of crab-eating macaques were tested for their ABO and Lewis blood groups. All the macaques tested contained A and/or B substances in their glands and a rough reciprocal relation seemed to exist between the antigen of gland and the antibody of serum. In testing the red cells, crab-eating macaques could be divided into three groups in Lewis blood groups, as in man, and it was suggested that the Le^a substance on the red cells might be taken up from the serum. The submaxillary glands contained both Le^a and Le^b substances being independent of Lewis groups of the red cells.

Acknowledgements. We wish to thank Drs. H. Shimojo, M. Hara, and A. Hagiwara of Murayama Branch, National Institute of Health of Japan for supplying us with blood and gland of macaques.

References

- 1) Nakajima, H.: Jap. J. Legal Med., **16**, 143 (1962) (in Japanese).
- 2) Ogushi, T.: Jap. J. Legal Med., **16**, 140 (1962) (in Japanese).
- 3) Nakajima, H.: Jap. J. Legal Med., **17**, 210 (1963) (in Japanese).
- 4) Nakajima, H., and M. Miyagi: Jap. J. Legal Med., **18**, 76-80 (1964).
- 5) Wiener, A. S., J. Moor-Jankowski, and E. B. Gordon: J. Forensic Med., **11**, 67-83 (1964).
- 6) Nakajima, H., and Y. Hayashida: Jap. J. Legal Med., **17**, 341-346 (1963).
- 7) Sneath, J. S., and P. H. Sneath: Nature, **176**, 172 (1955).