Clinical Significance of the Lewis Red Blood Cell System to Transfusion Practice in Thailand*

Dasnayanee Chandanayingyong, M.D.
Sudji Udee, B.Ed. Sci.

SUMMARY Serum samples, which were known to contain Lewis antibodies, were taken from 150 blood donors and 150 patients in order to investigate their serological properties.

It was found that there was no statistically significant difference in the serological properties of the Lewis antibodies of patients and blood donors. More than 75 per cent of anti-Le^b, and 90 per cent of anti-Le^a and anti-Le^a + Le^b antibodies were active at 37°C. Some of these Lewis antibodies were also tested for lymphocytotoxic activity; a non-specific pattern of reaction was found. About 30 per cent had lymphocytotoxic activity against T lymphocytes and 50 per cent had lymphocytotoxic activity against B lymphocytes at 37°C. Details of the serological reactions, *in vitro*, are presented in this paper.

The results suggest that Lewis antibodies should be given clinical importance in blood components therapy, especially in the transfusion of red blood cells and platelets.

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It is generally accepted that the main factors determining the relative clinical importance of red blood cell groups are the frequency of occurrence of specific antibodies taken in conjunction with the frequency of occurrence of the corresponding antigens, and the potency and thermal range of activity of the specific antibodies.

It has been revealed in previous studies[1-3] that the most frequent unexpected red blood cell antibodies detected in both patients and blood donors in Thailand are Lewis antibodies, i.e., anti-Le^a, anti-Le^b and anti-Le^a + Le^b. These antibodies are found in persons of the Lewis phenotype Le (a-b-). The incidence of this phenotype among Thai people is close to 30 per cent;[4,5] the remaining 70 per cent have the corresponding Lewis antigen.

Most Lewis antibodies agglutinate at room temperature saline-suspended red blood cells of the appropriate phenotype. In some cases, agglutination may be observed after incubation at 37°C. A positive indirect antiglobulin reaction may be seen if complement is present in the mixture and also if the antiglobulin reagent has sufficient anticomplement activity. Haemolysis is more often seen with enzyme-treated cells than with the standard saline suspension.

In 1959, Mollison[6] showed that there is a close relationship between the thermal range of red blood cell antibodies *in vitro* and their ability to destroy red blood cells *in vivo*. Antibodies which are not active *in vitro* above 30°C do not cause destruction of incompatible cells at 37°C *in vivo*. On the other hand, those antibodies which bind complement *in vitro* at 37°C can cause rapid destruction of at least a proportion of transfused incompatible red blood cells. Clinical problems may arise and these antibodies must be regarded as potentially dangerous in clinical transfusion practice.

The present study was carried out to obtain more information about the serological properties of Lewis antibodies found in both patients and blood donors. We hope that the result of this study will help to clarify the clinical importance of the Lewis blood group system with regard to transfusion practice in Thailand.

*From the Blood Bank, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

MATERIALS AND METHODS

Fresh, randomly selected serum samples (150 from blood donors and 150 from patients at Siriraj Hospital), which were known by routine screening procedures to contain Lewis antibodies, were investigated for following serological properties:

1. Specific cold (below 30°C) saline agglutinin
2. Specific agglutinin at 37°C.
3. Specific haemolysin at 37°C.

Known fresh 0 cells were taken from three samples of Le (a+b-), three of Le (a-b+) and two of Le (a-b-). These panels of cells were collected in C.P.D. and used within five days of collection.

The identification of Lewis antibodies was carried out using three conventional techniques:

1. Saline technique at room temperature for 60 minutes. Room temperature was considered to be around 25°C.
2. Saline technique at 37°C for 60 minutes, after which antihuman globulin technique was performed.
3. Two-stage enzyme (Papain) technique at 37°C for 30 mintues. Results were read for haemolysis only.

These serum samples were also tested against 20 random samples of B lymphocytes at 37°C. T and B lymphocytes were isolated by Thrombin-Nylon Wool technique[7] and tested by the lymphocyte microcytotoxicity test, a two-stage procedure.[8] A panel of T and B lymphocytes separated from the peripheral blood of six Le (a+b-), nine Le (a-b+) and five Le (a-b-) individuals was also used to study lymphocytotoxic activity.

RESULTS

The serological properties of the Lewis antibodies of 300 serum samples (150 from patients and 150 from blood donors) were compared with a panel of three Le (a+b-), three Le (a-b+) and two Le (a-b-) cells. Table 1 shows the comparison of serological properties of Lewis antibodies detected in the patients and blood donors. A specific pattern of reaction can be demonstrated. Most of the Lewis antibodies in this study reacted at 37°C, using anti-human globulin technique and most haemolysed enzyme-treated cells of Lewis incompatible cells. Seven of the anti-Le^a and four of the anti-Le^b antibodies showed specific haemolysis with incompatible, saline-suspended cells at 37°C. It is worth noting that there is no single technique that can detect all known Lewis antibodies.

A comparison of the lymphocytotoxic activity of Lewis antibodies againt 20 random samples of B lymphocytes in patients and donors is shown in Table 2. There was no statistically significant difference between the lymphocytotoxic activity of the patients and that of the donors.

A panel of T and B lymphocytes separated from the peripheral blood of six Le (a+b-), nine Le (a-b+) and five Le (a-b-) individuals was tested with a total of 58 Lewis antibodies: anti-Le^a = 15, anti-Le^b = 12, and anti-Le^a + Le^b = 31. Of that total, 31 per cent were positive with T lymphocytes and 50 per cent were positive with B lymphocytes at 37°C. No specific pattern of reaction was observed. T and B lymphocytes from Le (a-b-) individuals also reacted to these Lewis antibodies (Table 3).

DISCUSSION

Giblett[9] believed that only a small proportion of anti-Le^a can cause severe red blood cell destruction *in vivo* and that no haemolytic transfusion reaction has been proven to be caused by anti-Le^b.[10] On the other hand, Zmijewski[11] mentioned that the high frequency of Lewis antibodies, their haemolytic activity and poor reactivity *in vitro*, make them extremely dangerous. Kaczmarski and Wilson[12] also placed Lewis antibodies on the "most feared antibody" list because

Table 1 Comparison of the serological proporties found in patients and blood donors.

Techniques	Anti-Le^a				Anti-Le^b				Anti-Le^a + Le^b			
	No. tested	No. positive Saline R.T.	No. positive Saline 37°C* I.C.T.	No. positive Two-stage enzyme	No. tested	No. positive Saline R.T.	No. positive Saline 37°C* I.C.T.	No. positive Two-stage enzyme	No. tested	No. positive Saline R.T.	No. positive Saline 37°C* I.C.T.	No. positive Two-stage enzyme
Patient	65	52	59	61	37	30	32	34	48	47	46	46
		80%	91%	94%		81%	86%	92%		96%	98%	96%
Donor	72	55	67	69	41	31	32	38	37	34	35	34
		76%	93%	96%		76%	78%	93%		92%	95%	92%
X^2		0.26	0.24	0.28		0.34	0.94	0.02		0.59	0.68	0.59

*Some showed specific haemolysin at 37°C.

Table 2 Comparison of the lymphocytotoxic activity of Lewis antibodies against 20 random samples of B lymphocytes in patients and blood donors.

	Anti-Le^a			Anti-Le^b			Anti-Le^a + Le^b		
	No. tested	No. positive	%	No. tested	No. positive	%	No. tested	No. positive	%
Patient	30	14	46.67	18	10	55.56	32	22	66.00
Donor	16	9	56.25	11	7	63.64	36	18	50.00
X^2		0.38			0.18			2.46	

Table 3 Lymphocytotoxic activity against T and B lymphocytes from six Le (a+b-), nine Le (a-b+) and five Le (a-b-).

	Anti-Le^a			Anti-Le^b			Anti-Le^a + Le^b		
	No. tested	No. positive	%	No. tested	No. positive	%	No. tested	No. positive	%
T lymphocytes	15	3	20.00	12	1	8.33	31	14	45.16
B lymphocytes	15	8	8.33	12	4	33.33	31	17	54.84
X^2		3.59			2.27			0.58	

of their ability to induce *in vivo* haemolysis. Acute haemolytic transfusion reaction[13-21] and delayed haemolytic transfusion reaction[22] due to Lewis incompatibility have been reported.

From the results of this study, it may be seen that most Lewis antibodies are active *in vitro* at 37°C. More than 90 per cent of anti-Le^a, anti-Le^b and anti-Le^a + Le^b haemolysed enzyme-treated Lewis incompatible cells. It was also shown that more than 90 per cent of anti-Le^a and anti-Le^a + Le^b antibodies, and 75 per cent of the anti-Le^b antibodies can be demonstrated by anti-human globulin technique at 37°C. Few of them showed specific haemolysis with saline-suspended cells at 37°C. Therefore, these Lewis antibodies must be regarded as potentially dangerous antibodies in clinical transfusion practices. (*In vivo* study has not been performed).

With regard to the lymphocytotoxic activity of Lewis antibodies, it was demonstrated that at 37°C about 30 per cent and 50 per cent reacted with T and B lymphocytes respectively. These results may indicate some degree of clinical importance in the transfusion of platelets as well as the transplantation of organs.

Conclusion

The results of this study suggest that Lewis antibodies found in blood donors and patients should be given the same degree of clinical importance in blood components therapy, especially in the transfusion of red blood cells. However, further studies are needed to clarify the role of lymphocytotoxic activity of Lewis antibodies in the transfusion of platelets.

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REFERENCES

1. Chandanayingyong D, Paunpathom S, Ajarasirikul NG, Methaseta P. Incidence of atypical antibodies from routine cross-match procedure at Siriraj Hospital. J Med Assoc Thai 1968; 51:142-52.
2. Chandanayingyong D, Methaseta P. Incidence of blood group antibodies after blood transfusion. J Med Assoc Thai 1969; 52:640-3.
3. Bejrachandra S, Chandanayingyong D. Unexpected red cell antibodies in donors and patients at Siriraj Hospital. Southeast Asian J Trop Med Pub Hlth 1979; 10:204-6.
4. Chandanayingyong D, Sasaki TT, Greenwalt TJ. Blood groups of the Thais. Transfusion 1967; 7:267-76.
5. Chandanayingyong D, Bejrachandra S, Methaseta P, Pongsataporn S. Further study of Rh, Kell, Duffy, P, MN, Lewis and Gerbiech blood groups of the Thais. Southeast Asian J Trop Med Pub Hlth 1979; 10:209-11.
6. Mollison PL. Factor determining the relative clinical importance of different blood group antibodies. Brit Med Bull 1959; 15:92-8.

7. Danilovs JA, Ayoub G, Terasaki PI. In: Terasaki PI, ed, Histocompatibility testing. Los Angeles: UCLA Tissue Typing Laboratory, 1980:287-8.
8. Mittal KK. In: Workshop report, standardization of the HLA typing method and reagents. Vox Sang 1978; 34:58-63.
9. Giblett ER. Blood group alloantibodies: An assessment of some laboratory practices. Transfusion 1977; 17:299-308.
10. Miller WV, ed. The technical manual of the American Association of Blood Banks. Washington DC: 1977:116.
11. Zmijewski C. In: Immunohematology. 3rd ed. New York: Appleton, 1978:104-5.
12. Kaczmarski G, Wilson J. In: Blood group immunology: Theoretical and practical concepts. Miami, Florida: Dade Reagents 1976:76.
13. De Vries SI, Smitskamp HS. Haemolytic transfusion reaction due to an anti Lewis^a agglutinin. Brit Med J 1951; 1:280-1.
14. Brendemoen OJ, Aas K. Hemolytic transfusion reaction probably caused by anti Le^a. Acta Med Scand 1952; 141:458-60.
15. Van Lauer A, Claus J, Hoppe HH. Die Lewis Blutgruppen als Ursache von Transfusion Zwischenfallen. Dtsch Med Wochenschr 1954; 77:1869.
16. Matson GA, Coe J, Swanson J. Hemolytic transfusion reaction due to anti Le^a. Blood 1955; 10:1236-40.
17. Mollison PL, Cutbush M. The use of isotope labelled red cells to demonstrate incompatibility *in vivo*. Lancet 1955; 1: 1290-5.
18. Merrild Hansen B, Munk Andersen G. Haemolytic transfusion reaction caused by anti Le^a. Vox Sang 1957; 2:109-13.
19. Roy RB, Wesley RH, Fitzgeral JDL: Haemolytic transfusion reaction caused by anti Le^a. Vox Sang 1960; 5:545-50.
20. Chandanayingyong D, Paunpathom S, Ajarasirikul NG, Methaseta P. Hemolytic transfusion reaction: Report of two cases. Siriraj Hosp Gaz 1969; 21:423-32.
21. Hossaini AA, Shumaker J. Hemolytic transfusion reaction from minimal Lewis incompatible red cells with an unexpected immune response. (Abstract). Transfusion 1976; 16:520.
22. Pineda AA, Taswell HF, Brzica SMJR. Delayed hemolytic transfusion reaction. Transfusion 1978; 18:1-7.