Acta Dermatovener (Stockholm) 55: 131–133, 1975

EXAMINATION OF HL-A ANTIGENS AND LYMPHOCYTOTOXIC ANTIBODIES IN DISCOID LUPUS ERYTHEMATOSUS

V. Stenszky, E. Nagy and P. Szerze

From the Blood Transfusion Centre of the Hajdu-Bihar County Hospital and the Department of Dermatology, the Medical University School, Debrecen, Hungary

Abstract. The distribution of HL-A antigens and lymphocytotoxic antibodies has been studied in the sera of patients suffering from discoid lupus erythematosus (DLE). A significant deviation in HL-A5 antigen has been found in the 33 cases investigated, while the lymphocytotoxic antibodies were present in only 8 sera. The possible cause of these findings are discussed.

Key words: HL-A antigens; Lupus erythematosus, discoid; Antibodies, lymphocytotoxic

In recent years, more and more authors have dealt with the relation between tissue antigens (HL-A) and certain diseases. It has been discovered by means of animal experiments that susceptibility to the investigated disease develops via the common effect of different genes, i.e. on their simultaneous occurrence. Thus, for example, it has been stated by Lilly (8, 9, 10, 11), Mühlbock & Dux (13), Tennant & Snell (17, 18), Boyse et al. (2), based on their experiments performed with various mouse strains, that the occurrence and development of mouse leukaemia produced by Gross, Tennant, or Friend virus was obviously influenced by the murine histocompatibility system (H-2). These findings have drawn attention to the fact that there may well be an interrelation between the occurrence of different diseases, and certain histocompatibility antigens. It is known that antibodies against certain antigen systems can be detected in several immune diseases, e.g. antibody produced against the P antigen system can be found in paroxysmal haemoglobulinuria; autoimmunisation against tissue antigens can be detected in patients suffering from autoimmune diseases. The results of investigations on the subject, published in the literature, are not unanimous, either regarding the incidence of tissue antigens occurring significantly more frequently or more rarely in the various diseases, or regarding the development of cytotoxic antibodies in certain disease entities. From the autoimmune group of diseases, SLE has been investigated from this aspect by many authors. It is common knowledge that there exists a relation between discoid lupus erythematosus (DLE) and SLE (15). Immunological abnormalities can be found in DLE, too.

The more frequent occurrence of W15 antigen was found by Waters et al. (22), that of W15 and HL-A8 antigens was more frequently found by Grumet et al. (5) and that of HL-A7 and W5 antigens by Bitter et al. (1) in their patients suffering from SLE. By examining the tissue antigens of 90 patients suffering from SLE, Stenszky & Szegedi (16) found that the occurrence of HL-A2 and HL-A8 antigens was significantly more frequent and that of W10 was significantly more rare, than those antigens found among the normal population.

Antibodies in the sera of such patients have been detected by several authors. Terasaki et al. (20), Kreisler et al. (7), Stenszky & Szegedi (16) have found lymphocytotoxic antibodies in the sera of patients suffering from SLE, which presumably did not originate from either pregnancy or polytransfusion.

In our own work we examined the occurrence of HL-A antigens among 33 patients suffering from DLE as well as the occurrence of the occasionally produced lymphocytotoxic antibodies in their sera.

MATERIALS AND METHODS

The determination of HL-A antigens was performed by means of the micro-lymphocytotoxicity test described by Terasaki & McClelland (19). The lymphocytes were isolated according to Böyum's method (3), employing Ficoll-Isopaque mixture. The principle of the method is the following: the cytotoxic antibody in the serum causes damage to the lymphocytes in the cell suspension, in the presence of complement. As a consequence of the antigen–antibody reaction the cell membrane will be injured and become penetrable to the stain—which is unable to penetrate any unharmed cell —and thus the cell will be stained. The percentage of cells stained in this way denotes the positivity of the test. The sera

Table I. *The antigen frequency of 33 patients suffering from DLE*

Antigens	410 controls		33 patients suffering from DLE		
	%	gene frequency	%	gene frequency	*P* value
Locus 1					
HL-A 1	27.3	0.1475	21.2	0.1124	
HL-A 2	50.8	0.2989	42.4	0.2412	
HL-A 3	23.8	0.1271	21.2	0.1124	
HL-A 9	22.8	0.1215	27.3	0.1472	
HL-A 10	17.6	0.0923	12.1	0.0626	
HL-A 11	13.5	0.0701	18.2	0.0955	
W 28	7.6	0.0388	3.0	0.0153	
W 32	6.2	0.0315	3.0	0.0153	
Locus 2					
HL-A 5	10.1	0.0521	24.2	0.1296	0.0106
HL-A 7	18.0	0.0946	15.2	0.0789	
HL-A 8	19.3	0.1016	18.2	0.0955	
HL-A 12	21.4	0.1135	15.2	0.0789	
HL-A 13	9.7	0.0498	15.2	0.0789	
W 5	16.3	0.0853	18.2	0.0955	
W 10	16.3	0.0853	12.1	0.0626	
W 14	7.0	0.0359	9.1	0.0465	
W 15	8.5	0.0432	7.6	0.0381	
W 17	10.0	0.0513	9.1	0.0465	
W 21	7.0	0.0359	8.4	0.0428	
W 22	3.9	0.0199	12.1	0.0626	0.0237
W 27	9.9	0.0506	15.2	0.0789	

containing antibodies were obtained from multigravida. As complement, rabbit complement was used. The incubations were performed at 22°C. Our patients, as well as normal persons serving as controls, were examined for 22 officially accepted HL-A and W antigens. Antibody-containing sera originating from 80 multigravida were used for the typing; more than two sera containing antibodies served for the examination of each antigen. The antigen distribution of the normal population agreed with the data for Caucasian population, as known from the literature.

The same micro-lymphocytotoxicity test was adopted for the detection of cytotoxic antibodies occasionally occurring in the sera of our patients.

The determinations were performed on lymphocytes from 30 healthy donors, typed for HL-A antigen, at room temperature and at 15°C. The lymphocytes of the patients were also examined with their own sera under the same circumstances, in order to look for the presence of autocytotoxic antibodies.

RESULTS

On examining the tissue antigens of 33 patients suffering from DLE, a significant difference was only found in the case of HL-A5 ($P = 0.0106$) and W22 ($P = 0.0237$), on comparison with the antigen frequency of 410 normal persons. Apart from this significant rise, the other 21 tissue antigens did not differ significantly from those of the controls (Table I).

When examining the sera of patients with lymphocytotoxic antibody, and performing the incubation at 22°C, positive results were obtained in 8 cases (23%). Each serum was examined with the cells of 30 healthy persons typed for HL-A antigens. Only the sera of the above-mentioned 8 patients were positive, with 8–15 cells, i.e. the sera contained lymphocytotoxic antibodies against these cells. The sera were of polyspecific character.

None of these 8 patients had previously received a blood transfusion, but 2 of them had pregnancy in their medical history. The lymphocytotoxic antibody detected in these 2 patients was evaluated with caution because of possible allo-immunisation.

When performing the examination at 15°C, no positivity was detected in other sera; there was merely a stronger positivity with the same lymphocytes, than was found at 22°C. When searching for autocytotoxins, no positive results were seen.

DISCUSSION

On examining the frequency of tissue antigens in our 33 patients suffering from DLE, a significant rise was found in the case of HL-A5 and W22 antigens. In our earlier investigations Stenszky & Szegedi (16) concerning the tissue antigens of 90 patients, the frequency of HL-A5, HL-A2, and 8 antigens was significantly increased. It may be worth mentioning that both disease groups were typed with the same five different sera containing anti-HL-A5. The five sera containing antibodies always showed a strong positivity, both in the case of SLE and of DLE patients. Two of these sera had a narrow specificity when the normal population was investigated. The latter observation is striking on account of the close connection existing between the two diseases.

A search for lymphocytotoxic antibody in the sera of the patients disclosed positivity in 8 of them. The 23% positivity—which was detected at 22°C—did not change at 15°C; only the intensity of the reaction increased, in inverse proportion to the decrease in temperature. From the data of Naito et al. (14) it is known that the heat optimum of lymphocytotoxic antibodies found in several diseases differs from that of antibodies occurring in pregnancy or after polytransfusion.

Hitherto, few attempts have been made to explain the role of antibodies occurring in the above-

mentioned diseases. According to several hypotheses, they are identical—mainly in the autoimmune diseases—with autoantibodies produced against histocompatibility antigens (4, 12, 21).

In our opinion, and as DLE is a chronic disease, it might be expected that antibody would be produced first of all in the case of extensive skin inflammation.

As regards the sex of the patients, the group producing antibodies consisted of 1 man and 7 women, while the other group consisted of 18 men and 7 women.

A remarkable correlation has been found between the duration of the disease and the occurrence of antibodies. In the group producing antibodies, the disease has persisted for 5–35 years, while in the case of non-producers, it has lasted for 1–17 years. This relationship is impressive in both groups, mainly in the lower limits of the disease course. It is an open question whether the relation would be more manifest if we were to examine a larger patient population.

It is an open question whether the antibodies are identical with autoantibodies produced in the consequence of prolonged tissue damage. Several authors (6) detected virus-like structures in the skin of patients suffering from SLE and DLE. It is conceivable that these materials, as antigens, play a role in the production of antibodies. Our results suggest that—in spite of the fact that the DLE affects only the skin—some changes of the immuno-system result in the appearance of lymphocytotoxic antibodies.

REFERENCES

1. Bitter, T., Mottironi, W. D. & Terasaki, P.: HL-A antigens associated with lupus erythematosus. N Engl J Med *286:* 435, 1972.
2. Boyse, E. A., Stockert, E. & Old, L. J.: Isoantigens of the H-2 and T a loci of the mouse. Interactions affecting their representation on thymocytes. J Exp Med *128:* 85, 1968.
3. Böyum, A.: Separation of leukocytes from blood and bone marrow. Scand J Clin Invest *21:* 2, 1968.
4. Dausset, J.: Correlation between histocompatibility antigens and susceptibility to illness. *In:* Progress in Clinical Immunology (ed. R. Schwartz). Grune & Stratton, New York, 1971.
5. Grumet, F. C., Coukell, A., Bodmer, J. G., Bodmer, W. F. & McDevitt, H. O.: Histocompatibility (HL-A) antigens associated with systemic lupus erythematosus. A possible genetic predisposition to disease. N Engl J Med *285:* 193, 1971.
6. Hashimoto, K. & Thomson, D. F.: Discoid lupus erythematosus. Electron microscopic studies of paramyxovirus-like structures. Arch Dermatol *101:* 565, 1970.
7. Kreisler, M., Naito, S. & Terasaki, P. I.: Citotoxins in disease. Various disease. Transpl Proc *3:* 112, 1971.
8. Lilly, F., Boyse, E. A. & Old, L. J.: Genetic basis of susceptibility to viral leukaemogenesis. Lancet *II:* 1207, 1964.
9. Lilly, F.: The effect of histocompatibility-2 type on response to the Friend leukemia virus in mice. J Exp Med *127:* 465, 1968.
10. — Fv-2: Identification and location of a second gene governing the spleen focus-forming response to Friend leukemia virus infection in mice. J Nat Cancer Inst *45:* 163, 1970.
11. — The role of genetics in Gross virus leukemogenesis. *In:* Comparative Leukemia Research (ed. R. M. Dutscher). Karger, New York / Basel 1971.
12. Miescher, P. A.: Autoantibodies against thrombocytes and leukocytes. *In:* Textbook of Immunopathology (ed. P. A. Miescher & H. J. Müller-Eberherd), p. 500, 1969.
13. Mühlbock, O. & Dux, A.: Histocompatibility genes and susceptibility to mammary tumor virus (MTV) in mice. Transpl Proc *3:* 1247, 1971.
14. Naito, S., Hirata, A. A., Estrada, H. G. & Terasaki, P. I.: Lymphocytotoxins in disease. IV. Cold cytotoxins in pregnancy, sore throat and rheumatic heart disease. Tissue Antigens *1:* 219, 1971.
15. Petrányi, Gy. & Szodoray, L.: Collagen betegségek. Medicina, Budapest, 1963.
16. Stenszky, E., Szegedi, Gy., Aszódi, L. & Petrányi, Gy.: The HL-A and systemic lupus erythematosus. Haematologia *7:* 211, 1973.
17. Tennant, J. R. & Snell, G. D.: Some experimental evidence for the influence of genetic factors on vital leukemogenesis. Nat Cancer Inst Monogr *22:* 61, 1966.
18. — The H-2 locus and viral leukemogenesis as studied in congenic strains in mice. J Nat Cancer Inst *41:* 597, 1968.
19. Terasaki, P. I. & McLelland, J. D.: Microdroplet assay of human serum cytotoxins. Nature *204:* 1964, 998.
20. Terasaki, P. I., Mottironi, V. D. & Bernett, E. V.: Citotoxin in disease. Autocytotoxins in lupus. N Engl J Med *283:* 724, 1970.
21. Walford, R. L.: The isoantigenic systems of human leucocytes: medical and biological significance. Series Haemat. *II:* 2, 1969. Munksgaard, Copenhagen.
22. Waters, H., Konrad, P. & Walford, R. L.: The distribution of HL-A histocompatibility factors and genes in patients with systemic lupus erythematosus. Tissue Antigens *1:* 5, 1971.

Received May 14, 1974

E. Nagy, M.D.
Department of Dermatology
The Medical University School
4012 Debrecen
Hungary