

Lymphocytotoxins in rheumatoid arthritis: prevalence, lymphocyte specificity, and HLA-DR antigens

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SUMMARY 37% (57/155) of sera from patients with rheumatoid arthritis contain cold-reacting lymphocytotoxic antibodies. Lymphocytotoxins were predominantly of the weakly reactive type and were more likely to be present in patients who were HLA-DR4 positive. By contrast, patients who were HLA-DR3 positive were more likely to have strongly reactive lymphocytotoxins. The lymphocyte subclass reactivity of the lymphocytotoxins was: 67% versus B lymphocytes, 27% against both T and B cells, and 7% for T cells.

Lymphocytotoxins are antibodies which bind on to the surfaces of lymphocytes and in the presence of complement cause cell lysis. Lymphocytotoxins (LCA) may be either IgM or IgG immunoglobulins; the former are cold reacting (optimum 15–17°C) while the latter are warm reacting (optimum 37°C).¹ LCAs are found in the serum of normal individuals following a variety of bacterial or viral infections and after vaccination and immunisation.² They are found also in many inflammatory and autoimmune diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).³ LCAs in juvenile chronic arthritis and SLE have been considered as possible candidates in the development of some of the disturbed immune function seen in these diseases, since they may inactivate or kill suppressor T cells and T cells responding to mitogens.^{4,5}

In rheumatoid arthritis (RA) some 35–50% of patients may have LCAs in their serum.^{3,6} The lymphocyte specificity of the LCA, HLA status of the patients and the functional consequences of the presence of LCA have not been studied in this disease. This study reports on the prevalence of LCAs, their lymphocyte specificity, and HLA DR status of 155 patients with RA.

Patients and methods

Patients. 155 patients with classical or definite RA according to the American Rheumatism Association

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criteria⁷ were tested for the presence of lymphocytotoxic antibodies. Serum samples were obtained at the time of tissue typing during previous genetic studies^{8,9} and stored at –70°C.

Normal panel. The lymphocytes from normal donors were used for the detection of lymphocytotoxins. Heparinised blood from laboratory staff or patients with back pain was obtained fresh, and lymphocytes separated by the method of Böyum.⁸ In testing for LCAs the panel of donor lymphocytes varied from 14 to 28 individuals.

Tissue typing. Patients were tissue typed as described previously^{8,9} for HLA, A, B, C, and DR locus antigens.

Detection of lymphocytotoxins. Lymphocytotoxins in the 155 RA sera were tested for on the lymphocytes from the normal donors by the standard microdroplet National Institutes of Health cytotoxicity test carried out at room temperature. The lymphocyte specificity of 74 of these sera was also investigated with separated T and B lymphocytes, prepared as described previously,⁹ in a similar microcytotoxicity test.

The percentage of cells killed by a test serum (strength of killing) was estimated to the nearest 5% above background cell death and grouped into the following categories: negative 0; + 20–35% death; ++ 40–45% death; +++ 60–75% death; +++++ 80–95% death. The frequency of cytotoxicity reactions against the panel of typing cells was also assessed as follows: negative 0; + <25%; ++ 25–50%; +++ 50–75%; +++++ 75–100%.

Results

PREVALENCE OF LYMPHOCYTOTOXINS

37% (57/155) of RA sera showed cytotoxicity against the lymphocytes of 2 or more members of the panel of normal donors (Table 1). When the results of prevalence were analysed according to the HLA DR phenotype of the patients contributing the serum, it was found that patients who were HLA DR4 had a significantly higher prevalence ($\chi^2 = 9.67$; $P < 0.005$) of LCAs in their serum. LCAs were predominantly of the weakly reactive type, 43 (75%) being of this nature.

FREQUENCY AND STRENGTH OF REACTIVITY

Table 2 shows the relationship between the number of lymphocytes of the panel killed by a particular serum (frequency) and the strength of killing. It can be seen that those sera which showed strong cytotoxicity also reacted with more members of the lymphocyte panel ($\chi^2 = 20.4$; $P < 0.001$). Having shown this relationship we then analysed strength of reactivity against the HLA DR antigen status of the serum donor. The relationship found was that between HLA DR3 and the presence of strongly reactive LCAs (Table 3(a), $\chi^2 = 5.77$, $P < 0.025$), and HLA DR4 with the presence of weakly reactive LCAs (Table 3(b), $\chi^2 = 5.00$, $P < 0.025$).

Table 1 *The occurrence of lymphocytotoxins in the serum of 155 patients and the DR antigen phenotypes of the serum donors. There is a significant association between the presence of lymphocytotoxins and the presence of the DR4 haplotype*

DR	Lymphocytotoxins				χ^2
	Positive (57)		Negative (98)		
	n	%	n	%	
1	13	23	9	9	NS
2	7	12	15	15	NS
3	16	28	29	30	NS
4	43	75	49	50	9.67
					$P < 0.005$
5	3	5	6	6	NS
6	4	7	9	9	NS
7	7	12	26	26	NS
Blank	21	37	53	54	NS

NS=not significant.

Table 2 *Association in RA sera between strength of LCA and the frequency of positive reaction against cells of test panel. Sera with strong LCA react with significantly more cells*

Frequency of killing	Strength of killing	
	+ / + +	+ + + / + + + +
< 50%	41	5
> 50%	2	9

Table 3 *The relationship between the strength of lymphocytotoxicity of sera with respect to the HLA antigen of the patient. (a) Strongly cytotoxic sera occur most frequently in patients with HLA-DR3. ($\chi^2 = 5.77$, $P < 0.025$). (b) Weakly cytotoxic sera occur most frequently in patients with HLA-DR4. ($\chi^2 = 5.00$, $P < 0.025$)*

	HLA antigen		Lymphocytotoxins	
			Strong	Weak
(a)	DR3	+	9	7
		-	8	33
(b)	DR4	+	9	34
		-	8	6

Table 4 *Reaction pattern of LCAs in 45 sera when tested against separated human B and T lymphocytes*

Lymphocyte specificity of lymphocytotoxins		
Lymphocyte class	Number of sera (per cent)	Strength of cytotoxicity
T	3 (7)	High
B	30 (67)	Low
T + B	12 (27)	High

SPECIFICITY OF LYMPHOCYTOTOXINS

In a separate series 74 of these RA sera were screened against separated T and B lymphocytes from 12 normal panel members (Table 4). Twenty-nine sera did not have LCAs. Of the 45 sera with LCAs 30 (67%) showed cytotoxic activity specific for B cells, 3 sera (7%) were T cell specific, and 12 sera (27%) reacted equally against T and B cells. The overall frequency of lymphocytotoxins when sera were tested against separated cells was 61% and contrasted with the frequency of LCAs against unseparated cells of 46%. Performing the lymphocytotoxic assay on unseparated cells led to an underestimation of B lymphocyte specific LCAs, since 43% of sera with B cell specificity were detected only after using separated lymphocytes.

Discussion

In this study we have examined the frequency, strength, and specificity of lymphocytotoxic antibodies in RA. LCAs detectable by reactions against whole lymphocyte preparations were seen in 37% of RA sera. Highly cytotoxic sera reacted with a high frequency in the panel, while weak sera showed a low frequency. The latter group were the most frequent LCAs described in this study. Overall, the presence of LCAs in RA was significantly associated with HLA DR4, but it was of interest to note that

the strong/high frequency sera occurred in patients who were predominantly HLA DR3, while weak/low frequency sera were associated with DR4.

The results of LCA reactions against B and T cells indicate that the majority of sera in RA have higher reactivity with B lymphocytes. The increased detection of LCAs in RA with separated cells does indicate, however, that a large number of B specific sera are not detected by the cytotoxic test against whole lymphocytes.

These findings are in agreement with the description of LCAs in RA by Terasaki *et al.*³ with weak cytotoxic reactions in 57% of RA sera. The frequency of occurrence of LCAs in RA varies in the literature from 14 to 57%.^{3 10 11} This variation probably reflects the 'weakness' of the cytotoxicity of RA sera and minor differences in methodology. Research has concentrated on LCAs in systemic lupus erythematosus, which are more readily demonstrable, and occur in the majority of patients. Studies in other diseases have investigated lymphocyte specificities of LCA. Thus LCAs in lupus are thought to be T cell specific. However, the only published evidence of an HLA association with LCAs is in myasthenia gravis, where there is a weak association with DR3.¹²

The association of LCAs with HLA DR4 described in this study is remarkable, considering the high overall frequency of DR4 in the RA patients (59%). In view of the association of HLA DR4 with both RA itself and the occurrence of LCAs in the disease, it is particularly relevant to investigate LCAs in RA families. The role of these antibodies and their relationship to immune responsiveness and immunogenetic status may prove of particular importance in the rheumatic diseases.

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