

# A study of lymphocytotoxins in families of patients with systemic lupus erythematosus

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**SUMMARY** Lymphocytotoxic antibodies are a common feature in the sera of patients with systemic lupus erythematosus. This family study, however, has not confirmed the previous findings of an increased incidence in the sera of their relatives. Adsorption studies suggest that when lymphocytotoxins occur in the sera of normal subjects and relatives of patients they have a different specificity and thus significance.

Lymphocytotoxins have been found in a large number of conditions including viral illnesses,<sup>1</sup> malignancy,<sup>2</sup> multiple sclerosis,<sup>3</sup> organ transplantation, following pregnancy, and after a variety of immunising procedures including vaccination.<sup>4</sup> They are recognised to occur in high titre in patients with systemic lupus erythematosus (SLE) and to a less extent in rheumatoid arthritis.<sup>5</sup> Since lymphocytotoxins occur in viral illness, though only for a short time, the concept has arisen that a virus or viruses may be of aetiological importance in SLE.<sup>6</sup> Some family studies of patients with SLE examining the incidence of lymphocytotoxic antibodies in patients, and in consanguineous and non-consanguineous contacts have demonstrated a significant increase in lymphocytotoxins in the latter 2 groups compared with normal control subjects.<sup>7–12</sup> But other investigators have not confirmed these findings.<sup>13, 14</sup>

In order to elucidate further the incidence and nature of lymphocytotoxins in SLE, particularly with regard to their antigenic and temperature specificity, a family study has been undertaken.

## Subjects and methods

### SLE PATIENTS AND FAMILIES

Sixteen families in whom one individual met the ARA criteria<sup>15</sup> for classification of SLE were studied. In addition there were 15 other patients with SLE, whose families were not studied, making a total of 31 patients. All patients and their relatives came from

the same geographical area and belonged to the one ethnic group. The consanguineous relatives were parents, siblings, children, and cousins while the non-consanguineous relatives were spouses and parents-in-law. All families were visited in their homes, and over 85% of first-degree relatives were included in the study. Relatives were questioned and examined for the possibility of previously undetected rheumatic disease. All patients had clinical evidence of disease activity at the time of study and suffered from a wide variety of clinical manifestations.

Control subjects (22 female and 16 male) were selected because they had no contact with the patients and were not hospital staff. As a group the female controls were the same age and parity as the patients with SLE. All subjects were questioned on a history of previous blood transfusion and recent viral infection or vaccination.

### METHODS

*1. Lymphocytotoxicity assay.* The sera from each subject were stored at  $-20^{\circ}\text{C}$  in aliquots until used. The presence of cold lymphocytotoxic antibodies was investigated by means of the microdroplet cytotoxicity assay of Terasaki and McClelland.<sup>16</sup> The sera were coded and randomised so that they were assayed blind. An AB serum negative control was included in each assay. All sera were reacted in duplicate against lymphocytes from 33 normal panel subjects. Approximately 2000 lymphocytes were incubated with  $1\ \mu\text{l}$  of neat serum at either  $15^{\circ}\text{C}$  or  $4^{\circ}\text{C}$ . After the addition of  $5\ \mu\text{l}$  of rabbit complement (Buxted Rabbit Co., UK Ltd) the incubation continued for a further hour at room temperature (approximately  $20^{\circ}\text{C}$ ).

*2. HLA typing.* The lymphocytes from the 33 normal panel subjects were tissue typed by microdroplet

Accepted for publication 14 March 1983.

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lymphocytotoxicity assay.<sup>17</sup> HLA typing sera had specificities for HLA A, B, and C antigens.

3. *Adsorption assay.* Six different types of adsorbing tissue were used. Heterophile antibodies were tested for and are of 3 known types. Forsmann antibodies are characterised by adsorption by both sheep red cells and homogenised guinea-pig kidney tissue.<sup>18-20</sup> Paul-Bunnell heterophile antibodies are known to be adsorbed by sheep and ox red cells but not by guinea-pig kidney tissue.<sup>21,22</sup> The third heterophile antibody system originally described by Hanganutziu<sup>23</sup> and Deicher<sup>24</sup> is adsorbed by sheep and ox red cells and guinea-pig kidney tissue.<sup>25</sup> RhO +ve adult red cells are known to contain the I antigen system. In contrast fetal cord RhO -ve red cells are known to possess predominantly i as opposed to I antigen in adults.<sup>26</sup> Pooled date-expired platelets are known to possess not only ABO and Rh blood group substances but also the human leucocyte antigens belonging to the HLA A, B, and C series but not the HLA DR.<sup>17</sup>

Each adsorbing tissue was washed separately in phosphate buffered saline 3 times. The sera for adsorption were chosen because they were observed to be strongly lymphocytotoxic. Two separate aliquots of each sera were mixed with an equal volume of the washed adsorbing tissue, either cell or tissue homogenate, incubated at either 4°C or 15°C. This adsorbing step was repeated 3 times. The thrice adsorbed sera were then tested for lymphocytotoxicity. A full set of the 5 unadsorbed sera and an AB serum negative control were included in each assay with the adsorbed sera.

**Results**

1. *Lymphocytotoxicity.* The lymphocytotoxic data were analysed after allotting a value for cytotoxic index<sup>2</sup> for each sera at the 2 reacting temperatures of 15°C and 4°C. The cytotoxic index =

$$\frac{\text{average percentage lymphocytotoxicity of test serum} - \text{negative control}}{\text{average percentage cytotoxicity of known positive control} - \text{negative control}}$$

The positive control was the serum from an SLE patient with the greatest number and intensity of lymphocytotoxic reactions. The cytotoxic index makes allowances for batch to batch variation in results. Reactions of 5% or more were regarded as being positive, as 97.3% of normal controls had reactions less than this. By  $\chi^2$  analysis with Yates's correction patients with SLE were found to have significantly more reactions—48.4% at 15°C ( $p < 0.001$ ) and 41.9% at 4°C ( $p < 0.001$ )—than the normal controls (Table 1).

Table 1 Percentage positive lymphocytotoxicity (lymphocytotoxic index) of SLE patients, relatives, and normal controls

Subject group	Incubation temp.	Number of sera	Total percentage positive	0-4*	5-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-100	$\chi^2$ †	p value
All SLE patients	15°C	31	48.4	16	7	2	1			2	1		1	1	17.58	<0.001
	4°C	31	41.9	18	7	1		1		2					13.97	<0.001
Consanguineous Relatives	15°C	48	4.2	46				1							0.43	NS
	4°C	48	4.2	46				1							0.43	NS
Non-consanguineous Relatives	15°C	19	10.5	17	1	1									0.396	NS
	4°C	19	10.5	17	2										0.396	NS
Normal	15°C	38	2.6	37			1									
Controls	4°C	38	2.6	37												

All SLE contacts compared with normal controls,  $\chi^2$  0.068, NS.

\*Lymphocytotoxic index %.

† $\chi^2$  with Yates's correction compared with normal controls.

Table 2 Percentage of lymphocytotoxicity adsorbed

	Sheep		Ox		Guinea-pig		Adult RhO +ve red cells		Platelet		RhO -ve fetal cord red cells	
	4°C*	15°C	4°C	15°C	4°C	15°C	4°C	15°C	4°C	15°C	4°C	15°C
SLE	33	19	15	0	22	36	53	69	78	85	41	43
	53	16	41	64	54	29	54	0	62	18	55	66
	0	0	3	91	10	16	3	3	11	3	3	7
Sister of SLE patient	0	0	0	0	0	0	0	0	100	100	9	0
Control	13	20	0	57	11	14	100	100	100	70	13	41

\*Represents temperature at which serum was adsorbed.

Analysis of the data for consanguineous and non-consanguineous relatives of patients with SLE did not reveal any significant differences from the control. Three of the 4 patients with SLE whose relatives had lymphocytotoxins similarly were found to have lymphocytotoxins in their sera.

2. *HLA tissue typing.* Reactions of the sera were compared with those found with the HLA typing sera. There was no association between the cold lymphocytotoxins and any of the HLA specificities identified on the lymphocytes of the normal panel subjects.

3. *Adsorption study.* In one family both a patient and his sister had lymphocytotoxins in their sera. However, this patient's sister had her lymphocytotoxins adsorbed by platelets at both 4°C and 15°C. A control subject with lymphocytotoxins exhibited strong adsorption with platelets and adult RhO +ve red cells at both temperatures. Sera from patients with SLE tended to react with the majority of the adsorbing tissues (Table 2).

## Discussion

The results of this study have confirmed the significantly increased incidence of lymphocytotoxic reactions in the sera of patients with SLE. However, an increased incidence of lymphocytotoxic reactions was not found in consanguineous and non-consanguineous relatives of patients with SLE as reported by others.<sup>7-10 12</sup> A number of explanations could account for these differing results. In the present study the majority of patients had clinical evidence of disease activity when they were studied. Previous studies have given little indication of the type of clinical disease their patients suffered from as it has been reported that the highest cytotoxicity occurs in patients with CNS involvement.<sup>27</sup> At least one study reported a very high incidence of lymphocytotoxins in the control groups as well as relatives.<sup>10</sup> This would suggest that there may be a high background of lymphocytotoxins of whatever aetiology in the sera of that community.

The pathogenic role, if any, of lymphocytotoxins in the sera of patients with SLE is poorly understood. It has been suggested that those lymphocytotoxins which react with cerebral tissue may be of use in monitoring CNS involvement in this disease.<sup>28</sup>

Another study has reported a significant reduction in lymphocytotoxicity after adsorption with fetal cord red cells, and this was taken as evidence for predominantly i specificity in the sera of patients with SLE.<sup>29</sup> However, in the present study the 3 sera from patients with SLE gave strong reactions with many different tissue antigens, and there was no evidence for predominant i activity. There was no evidence that the lymphocytotoxins had anti-HLA activity as had been reported previously.<sup>11</sup>

The lymphocytotoxins in the serum of a relative of a patient with SLE were totally adsorbed by the platelet extract. This is evidence that these lymphocytotoxins were not of the same aetiology as those found in the sera of patients with SLE.

In summary, this study has not found an increased incidence of lymphocytotoxins in the sera of close contacts of patients with SLE. When they did occur in a consanguineous relative the adsorption pattern was consistent with their being active against some blood group substance and not having broad reactivity, as was the case of the lymphocytotoxins found in the sera of patients with SLE. This is strong evidence against the lymphocytotoxins in the sera of relatives and patients with SLE having a common aetiology.

I thank Professor Heather M. Dick and the staff of the Department of Clinical Immunology, Glasgow Royal Infirmary, Glasgow, and the many consultants who permitted me to study patients under their care. Thanks also to the patients and their relatives for co-operating in this study.

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