has an Fc receptor. The interaction of that cell with the antibody molecule causes injury or death to the target cell. Thus antibody dependent cell mediated cytotoxicity (ADCC). A cell which kills only in the presence of antibody is called the K cell; it does not rosette with red cells (so it is not a T cell) and in most instances it is not immunoglobulin bearing (so by convention it is not a B cell). I would suggest that K cells are not a single cell type, however, but a group of cells which have Fc receptors and meet the criteria I have described. It can be shown, for instance, that activated T cells acquire Fc receptors and both macrophages and mononuclear cells, neither of which rosette with erythrocytes or bear immunoglobulin, have Fc receptors.

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Lymphocytotoxic Antibodies in Disease

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Since early reports demonstrating lymphocytotoxic antibodies in the sera of patients with various diseases[1, 2], there has been considerable interest in the possible significances of these antibodies.[3, 4, 5] In spite of many attempts, however, there is still difficulty with definition and identification of such antibodies. It is important to distinguish those antibodies which apparently arise spontaneously from those antibodies which are induced by alloimmunisation.

Definition

Lymphocytotoxins can be defined as serum factors which produce lysis of lymphocytes in the presence of complement. Although there is considerable indirect evidence to suggest that these factors are true antibodies, caution is necessary until the antigen specificity can be demonstrated. Further characterisation of lymphocytotoxins requires consideration of the differences between those antibodies occurring in diseases such as systemic lupus erythematosus and those antibodies which clearly follow alloimmunisation.

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Classification

Table 1 illustrates the major differences between these two categories of lymphocytotoxin.

Autoreactivity

Since the early work of Terasaki[1] and Stastny[6] and others, it has been confirmed that cytotoxins found in patients with systemic lupus erythematosus (SLE) are autoreactive. For this reason, SLE associated lymphocytotoxins can be considered to be autoantibodies. In contrast, lymphocytotoxic antibodies appearing after alloimmunisation, such as pregnancy, blood transfusion or renal transplantation, will not react with autochthonous lymphocytes. In practice, however, difficulty can be experienced. For example, we and others[7, 8] have detected factors in the sera of renal transplant recipients which are cytotoxic for autochthonous lymphocytes as well as lymphocytes of a normal panel. In such cases, it may be difficult to distinguish between these "autoantibodies" and antibodies due to alloimmunisation.

Characteristics

Recent reports attempting to characterise disease associated autolymphocytotoxin have emphasised that maximum activity is seen in the

cold (15°C or less).[9] In contrast lymphocytotoxic antibodies induced by alloimmunisation appear to be most active at room temperature or 37°C.[10] Again difficulties arise, however. Patients with SLE may have both warm and cold reactive antibodies and it is well known that temperature of incubation affects the apparent reactivity of antibodies apparently induced by transplantation.[10]

Immunoglobulin Class

There can be little doubt that cold reactive lymphocytotoxins found in SLE are predominantly or wholly of the IgM class. Supporting evidence has been obtained by means of chromatography, class specific inhibition by antibody, immunofluorescence and 2-mercaptoethanol reduction. However, none of these techniques entirely exclude the possibility of lymphocyte reactive IgG antibodies. By contrast, HLA typing sera are generally assumed to be IgG although it is clear that IgM antibody can be induced by alloimmunization.

Specificity

The lymphocytotoxic antibody which follow alloimmunisation often have readily demonstrable specificity for HLA-A and B. More recently it has been found that these antibodies may react with HLA-C and D antigens.[11] In the case of HLA-A, B and C antibodies, both T and B lymphocytes are lysed in the presence of complement. HLA-D antibodies, on the other hand, react predominantly with B lymphocytes. For antibodies of proven HLA specificity, tissue reactions are in accord with the known distribution of HLA-A, B and C or HLA-D antigens. However, in the case of lymphocytotoxins occurring in SLE, most workers have failed to demonstrate specificity for HLA-A or B.[9, 12] As yet there has been inadequate examination or reactivity with HLA-C or D. There is some controversy as to reactivity with T and/or B cells[9, 12, 13] and it seems unlikely that this can be resolved until antibodies can be separated according to their antigenic specificity. The situation must be complicated by the presence of at least two categories of antibody in some SLE sera. Unfortunately, it can not be assumed that sera lacking HLA-A and B specificity are autoreactive. Many transplantation recipients develop multi-specific antibody, possibly indicating reactivity with multiple antigens or with an antigen which is cross reactive.

Practical Methods for the Separation of Alloimmunisation and Disease Associated Lymphocytotoxins

It can be seen from the above that no single criterion can be accepted absolutely. Unless otherwise stated, the results given below are based on the assumption that disease associated lymphocytotoxins are cold reactive, IgM antibodies which are autoreactive and lack HLA-A and B specificity. In only some cases have all of these characteristics been proven. Lymphocytotoxin is presumed to be related to alloimmunisation if it has clear HLA-A or B specificity, fails to react with autochthonous cells and reacts best at room temperature or 37°C. Again, all of these characteristics have only been shown in some cases.

TABLE 1

Major features distinguishing post-alloimmunisation from disease associated lymphocytotoxins

Characteristic	Post-alloimmunisation	Disease associated
HLA specificity	+	−
Autoreactive	−	+
Maximal activity at 15°C	−	+
Ig class	IgG	IgM

Criteria for Positivity

A major problem in evaluation the results of different workers has been the lack of entirely acceptable methods for quantitating lymphocytotoxic activity. It would appear unlikely that adequate methods will be available until the various antibodies can be titred against their specific antigen. In the meanwhile, we have compared a number of methods. Two of the most acceptable are compared in Figure 1. The "mean score" refers to the average of the individual scores where 0 represents less than 10% lysis of the target cells and 4 represents greater than 70% killing. "Mean titre" is obtained by summing these scores at successive doubling dilutions, and averaging this figure by dividing by the number

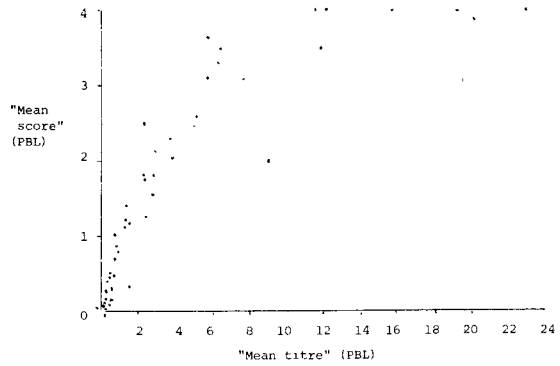

FIGURE 1. "Mean score" versus "Mean titre".

of different cells tested. It can be seen that there is a close relationship in the case of the weaker sera, but that the "mean score" fails to distinguish between strongly reactive sera. In Figure 2 "mean titre" is compared with the frequently used "percentage of panel reactive". Again it can be seen that "mean titre" is more precise for the stronger sera. It is clear that the results should be expressed quantitatively and that arbitrary classification of sera as positive or negative could be misleading. It would also seem that studies of the reactivity of different sera should be undertaken at dilutions which allow distinction between different cells. In general, high titre lymphocytotoxin reacts with all cells but only some when diluted.

Prevalence

From previous work it would appear that up to 16% of normal individuals may have lymphocytotoxic antibodies.[5, 14] Clearly, this figure

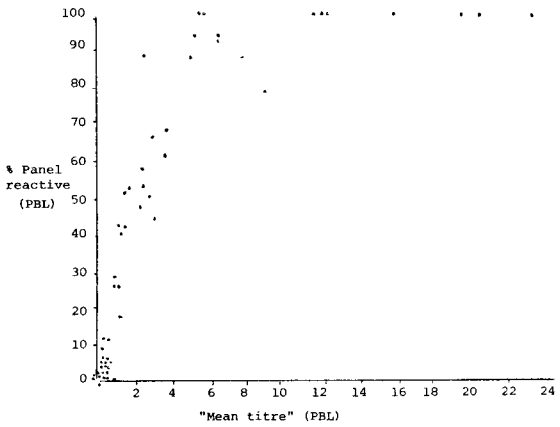

FIGURE 2. Per cent panel reactive versus "Mean titre".

TABLE 2

Prevalence of lymphocytotoxins in various diseases

Condition	No tested	15°C prevalence (%)	Reference
Reagin type allergy	46	37	Kreisler *et al* (1971)[23]
Tuberculosis	22	55	Kreisler *et al.* (1971)[23]
Rheumatoid arthritis	53	57	Terasaki *et al.* (1970)[1]
Multiple sclerosis	87	46	Schocket *et al.* (1977)[1]
MCTD	81	59	Diaz-Jouanen *et al* (1977)[25]
Infectious mononucleosis	100	80	Mottironi *et al.* (1970)[10]
Measles	64	85	Mottironi *et al.* (1970)[10]
Normals		2 5–16	Diaz-Jouanen *et al.* (1977)[25] and Ooi *et al.* (1973)

could be varied according to technique and criteria used for positivity. Comparisons between different studies must be accepted with caution. It does appear, however, that the prevalence of lymphocytotoxins is higher in some diseases than others. Although available information suggests that the highest prevalence is found in SLE, lower but abnormal frequencies of lymphocytotoxins are found in various diseases as illustrated in Table 2. Our own experience is shown in Table 3. Results obtained with 15°C and room temperature incubations are shown. It can be seen that in all cases the frequency at room temperature is reduced, suggesting that the antibodies detected are of the disease associated category. On the other hand, it should be emphasised that significant frequencies of lymphocytotoxins are found with room temperature incubation. Also shown in Table 3 is the prevalence of lymphocytotoxins in relatives of patients with SLE. Interestingly,

TABLE 3

Prevalence, thermal properties and specificity of lymphocytotoxins

Condition	No. tested	Prevalence (%) 15 C Anti-T	15 C Anti-B	RT Anti-T	RT Anti-B
SLE patients	14	86	93	36	29
Relatives of SLE patients	61	42	46	7	7
Male relatives	24	42	46	4	0
Female relatives	35	40	43	8·5	11·5
Consanguineous relatives	52	40	42	7·5	7·5
Non-consanguineous relatives	9	44	55	0	0
Myasthenia gravis patients	25	64	48	20	16
Multiparous women	301			14	
Blood donors with history of transfusion and/or pregnancy	131			2	

male and female relatives appear very similar in terms of cold reactive antibodies although females might be expected to have a higher incidence of alloimmunization (pregnancy) induced lymphocytotoxin. It can also be seen that the frequency amongst SLE relatives is not substantially affected by genetic factors, i.e. the frequency in 52 consanguineous relatives is similar to that seen in nine non-consanguineous relatives. Similar results have been found by some others[14, 15] but not all. From Table 3 it would appear that there is little difference in the frequency of lymphocytotoxins when sera are tested against predominantly T cells (peripheral blood lymphocytes) as opposed to predominantly B cells (CLL—chronic lymphatic leukaemia cells). It can be seen that lymphocytotoxins are also common in myasthenia gravis, although in this case there may be a preference for T rather than B cells. The prevalence of lymphocytotoxins in sera from females specifically chosen for multiparity was found to be 14% when screened at room temperature. Whether this figure would be significantly higher at 15°C is uncertain. In a series of 131 normal blood donors with a history of either transfusion or pregnancy, only 2% were found to be lymphocytotoxic at room temperature.

Specificity

With regard to alloimmunization induced lymphocytotoxins only a minority have a precise HLA-A, B or C specificity. In many instances the difficulty assigning a specificity would appear to be due to the presence of "extra" reactions against other unspecified antigens.

With regard to disease associated lymphocytotoxins the problems are even greater. From the data of others[9, 12, 13] and from our own experience, the following conclusions can be drawn.

(1) High titre sera react with essentially all cells (including autochthonous).

(2) Sera with intermediate titres react against only a proportion of the panel cells.

(3) In the case of a panel of CLL cells and perhaps also with peripheral blood lymphocytes, some cells appear to be hyperreactive, i.e. they are always lysed with relatively high titre sera and generally lysed with low titre sera. Conversely, other cells appear to be relatively resistant (Table 4).

TABLE 4

Specificity of SLE lymphocytotoxins against CLL cells

Cell (CLL)	HLA antigens* A locus	B locus	DR locus	Mean titre† (mean of 13 SLE sera)	Group‡ mean
1 VE	2, 26	7, 18	NT	6·0	
2 SM	2, 9	7, 18	4, 8	3·0	
3 MA	2, 28	7, 40	3, 7	3 0	
4 MLC	2	7, 15	4, 7	10 0	7·0
5 T	2, 29	7, 12	NT	0·7	
6 LI	2, 23	7, 12	6, 7	4 2	
7 GR	28	7, 40	NT	15·0	
8 VI	2, 30	5, 7	NT	14 0	
9 WR	1, 10	7, 8	2	3·0	
10 Mc	1, 3	7, 8	3, 7	3 7	3·4
11 UN	1	21, 8	3	3·3	
12 BE	1,	18, 8	2, 4	2·5	
13 WH	1, 2	14, 8	2	1·9	3·5
14 LI	1, 11	35, 8	2	3·2	
15 MA	1, 9	5, 8	7	6·5	
16 --	Non-7/non-8 (6)				6·6

NT = Not tested.

*Apparent HLA-A, B, and D antigens present on chronic lymphatic leukaemia (CLL) cells Note: some cells appear to have more than two A locus antigens.

†"Mean titre" of 13 SLE sera against each target cell.

‡Group based on reactions of CLL cells with typing sera with specificity for (i) B7 (ii) B7 + B8 (iii) B8 (iv) neither

(4) When sera of moderate titre are compared, differences between their reaction patterns are seen. As illustrated in Table 5, some sera in this category are similar, but others differ substantially.

(5) Attempts to explain these different reaction patterns in terms of HLA-A and B antigens have not been rewarding.

(6) In our experience, different sera appear to show preference for either CLL cells or peripheral blood cells. In general, however, sera react similarly against both category of cell (see also Table 3).

In the light of these observations, the following tentative conclusions appear possible.

(1) Disease associated lymphocytotoxins are not specific for either T or B cells.

(2) Disease associated lymphocytotoxins are not reactive with a single HLA-A or B specificity.

(3) There may be a system of antigens with which these lymphocytotoxins react; apparently all cells must have some antigen and

TABLE 5

Reactivity of lymphocytotoxins with CLL cells

Cell (CLL)	HLA antigens			SLE sera*								
	A locus	B locus	DR locus	A	B	C	D	E	F	G	H	I
VEN	2, 26	7, 18	NT	NT	5	2	3	2	2	1		NT
LIN	2, 23	7, 12	6, 7	NT	NT	8	2	7	3			2
BRA	1, 9	12, 17	2, 3	2		4	2	12		2	1	NT
BEN	1, 9	15, 17	2	2	1	4	1	4				
STE	2, 11	15, 17	4, 6	NT		2	1	7	7			NT
PER	2, 29	12, 15	2, 7	NT	2	2		4			1	
MAR	1, 9	5, 8	7	10	8	7	3		4	2		
WIL	11, 29	12, 35	NT	NT	3	1	NT	2	2	5	1	
UNK	1	8, 21	3	3	4	1	2		NT			
BEA	1	8, 18	2, 4	3	1	2	2					
LID	1, 11	8, 35	1, 6	2	5	1	1		2		1	NT
MAC	2, 28	7, 40	3, 7	6	NT	3			1			
McM	1, 3	7, 8	3, 7	4	3							
SMI	2, 9,	7, 15	4, 8	2		3		1			4	
WRI	1, 10,	7, 8	2	NT	3		2					3
WHI	1, 2	8, 14	2	3	NT							1

*"Titre" obtained by adding scores at successive doubling dilutions of the serum.

yet some sera can distinguish between different cells. From these data it is not possible to distinguish between the presence of some as yet undefined, genetically determined, multi-allelic system and the presence of some environmental antigen which varies somewhat in concentration and cross reactivity.

Recently we have obtained some evidence for the presence of an association between the antigen expressed on chronic lymphatic leukaemia cells and the presence of particular HLA specificities. When SLE sera are tested against a panel, it is found that sera tend to be less reactive against those CLL cells which react with conventional typing sera defining the B8 specificity. Those cells which react with anti-B7 sera appear to be relatively more reactive. Results are illustrated in Figure 3. From Table 4 it can be seen that this finding is not simply explained in terms of the presence of HLA-D antigens on the CLL cells.

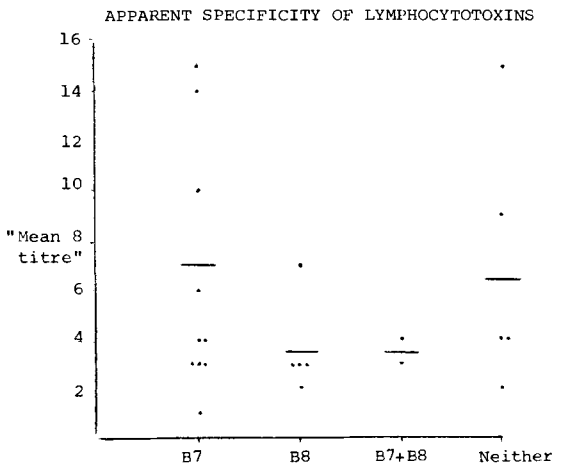

FIGURE 3. Groups based on reactions of CLL cells with B7 and B8 typing sera.

Significance

Until the specificity of lymphocytotoxic antibodies is defined it would be premature to speculate as to their significance. Any explanation should take account of the following:

(1) SLE is associated with particular HLA antigens, but this is impressive only if SLE is classified into severe and mild disease, i.e. HLA-A2-B7 is associated with mild, whereas HLA-A1-B8 is associated with severe disease.[16]

(2) Lymphocytotoxic antibodies are found in relatives including nonconsanguineous relatives suggesting the possibility of antibody directed against some environmental agent.

(3) Lymphocytotoxins found in SLE are generally, if not always, autoreactive.[1]

(4) Disease associated lymphocytotoxins are prominent in SLE, but are also frequent in many other diseases, some of which bear some immunological or clinical relationship to SLE. Some may be explained in terms of antiviral responses.

(5) Lymphocytotoxins from SLE sera are often concentrated in cryoprecipitates.[17, 18]

(6) SLE sera have been found to inhibit the response of lymphocytes to mitogens.[19]

(7) Lymphocytotoxic activity in SLE sera has been found to correlate with disease activity[20], lymphopaenia[3], and possibly CNS involvement.[21]

(8) Lymphocytotoxins are not found in 100% of SLE sera and therefore may not play an essential part in its pathogenesis.

(9) A similar antibody, found in New Zealand Black Mice has been shown to increase the rate at which lymphocytes are removed from the circulation.[22]

(10) The glomerulonephritis of SLE is thought to be associated with the switch from production of IgM to IgG anti-DNA. It would be necessary to explain why lymphocytotoxins of the IgM class appear to predominate in SLE.

Conclusions

Lymphocytotoxins are common in SLE but are by no means disease specific. Some evidence suggests that these antibodies reflect both environmental and genetic factors. The practical value of detecting lymphocytotoxins remains to be demonstrated. Further work is required to determine the antigen specificity of these antibodies and to evaluate the possibility that lymphocytotoxins react with a multiallelic system of antigens which affect susceptibility to unspecified viruses and severity of SLE.

Addendum

Further work has suggested that other B-locus alleles may be associated with variations in susceptibility to cold reactive lymphocytotoxins. HL-A B5 may be more important than B7 or B8.

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