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Chronic Lymphocytic Leukaemia and the Mixed Lymphocyte Reaction

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Summary

The lymphocytes from patients with chronic lymphocytic leukaemia (CLL) have been studied in the mixed lymphocyte reaction (MLR) using both the two-way (both populations viable) and one-way (only one viable population) systems. The ABO antigens were matched but the pairs were selected for at least two antigenic differences of the HL-A system. The lymphocytes of the patients were as capable as normal cells of invoking a response in allogeneic normal lymphocytes. Conversely, barely any response was produced when the lymphocytes of the patients were exposed to non-viable normal allogeneic cells. The failure of the leukaemic lymphocytes to respond adequately in the MLR is correlated with a very poor response to phytohaemagglutinin and may be contrasted with the definite positive responses obtained with specific antigens.

CLL and the MLR

When peripheral blood lymphocytes are cultured in the presence of mitogens, metabolic and morphological changes take place; this process is termed "transformation". When lymphocytes from patients with chronic lymphocytic leukaemia (CLL) are cultured in the presence of phytohaemagglutinin (PHA) for 72 h, they respond feebly in comparison with normal human cells, but given a longer time in culture these cells eventually exhibit some response (1, 2). This process of transformation can also be initiated in the presence of specific antigens such as tetanus toxoid, although a longer time is required to produce a smaller response. Furthermore, the subjects must have been exposed to these antigens previously for transformation to occur, so that in this case the responses are secondary (3, 4).

The capacity of the small lymphocyte to transform has been exploited in the mixed lymphocyte reaction (MLR) in which the lymphocytes from two individuals are cultured together. It used to be thought that the response of the lymphocytes depends on antigenic differences between the two populations, as defined by the HL-A system (5). However, it has been shown that the correlation between the extent of the HL-A differences between two populations of cells and the degree of response is not straightforward (6). There is experimental evidence supporting the view that the MLR and the HL-A systems are not controlled by the same genetic region but by separate closely linked loci (7). Furthermore, it is suggested that the MLR system may be separable into two entities – the stimulatory factor and the response mechanism. By treating one population of cells with Mitomycin C, which prevents responses without impairing antigenicity, each component of a mixed cell population can be studied separately (8).

The purpose of the present study was to see whether:

(a) lymphocytes from patients with CLL would respond normally in culture to another population of differing HL-A constitution

(b) the patient's lymphocytes could evoke a response in the normal lymphocytes of unrelated individuals.

Materials and Methods

Source of lymphocytes

Nine pairs of normal lymphocyte populations, eleven pairs of normal + CLL and three pairs of CLL + CLL populations (in all, twenty normal people and thirteen leukaemic patients) were studied, some from each group being included in more than one experiment. The pairs were matched for the ABO system and each differed by at least two recognised HL-A antigens.

Reaction mixtures

20 ml of blood was taken in each case into phenol-free heparin (Weddel Pharmaceuticals Ltd., London – 250 μ) and allowed to stand upright for 1-2 h, at room temperature. Half of each white-cell-rich plasma layer was treated by incubation for 20 min at 37°C with 25 μg/ml of Mitomycin C (Kyowa Hakko Kogyo Co. Ltd., Tokyo). Both treated and untreated cells were washed thrice in medium TC 199 (Wellcome Research Laboratories, Beckenham) before being suspended in human group AB serum (kindly supplied by the South London Blood Transfusion Service) to give a concentration of 5000 lymphocytes per μl. One batch of AB serum was used throughout these experiments. No attempt was made to remove white cells other than lymphocytes from the suspensions, since it has been established that the polymorphonuclear leucocytes in culture play a contributory part in the MLR response (9).

Design of experiments

Twelve culture tubes were set up for each experiment and in each case the suspension or mixed suspensions were cultured in TC 199 in the ratio of 4 parts 199 to 1 part suspension(s). The contents of each tube are given in Table 1a. The experiment was designed to assess the efficacy of the Mitomycin C and the hazard of spontaneous or Mitomycin C-induced transformation.

The cultures in tubes 1-6 were terminated at 72 h and the cells in cultures 7-12 were washed, resuspended in fresh media and incubated for a further 96 h.

After harvesting, the cell button was spread onto slides, fixed in methyl-alcohol and stained with May-Grünwald and Giemsa (George T. Gurr Ltd., Chadwell Heath, Essex). In each case 4000 cells were surveyed and transformation was expressed as a percentage (10).

Radioactive assay

In three of the experiments, each involving a different pair of normal cell populations, parallel cultures were assessed in terms of uptake of tritiated thymidine (Radiochemical Centre, Amersham). The technique was based on a modification of that of *Marshall* et al. (11).

Table 1a. Format of Culture Tubes of each Paired Experiment

Tube No.	Contents
1	Lymphocyte population A. No stimulant.
2	Lymphocyte population A + PHA* (0.05 ml per 10 ml culture).
3	Mitomycin-treated lymphocyte population A + PHA* (0.05 ml per 10 ml culture).
4	Lymphocyte population B. No stimulant.
5	Lymphocyte population B + PHA* (0.05 ml per 10 ml culture).
6	Mitomycin-treated lymphocyte population B + PHA* (0.05 ml per 10 ml culture).
7	Lymphocyte populations A and B in equal proportions.
8	Lymphocyte population A and mitomycin-treated population B in equal proportions.
9	Lymphocyte population B and mitomycin-treated population A in equal proportions.
10	Mitomycin-treated lymphocyte population A + mitomycin-treated lymphocyte population B in equal proportions.
11	Lymphocyte population A + mitomycin-treated lymphocyte population A in equal proportions.
12	Lymphocyte population B + mitomycin-treated lymphocyte population B in equal proportions.

*Wellcome Research Laboratories, Beckenham, Kent.

Results

In the normal controls, the lymphocyte responses to PHA were generally within the range previously found in a larger number of normal subjects in this laboratory (36-60%). The exception was a girl aged 24 whose lymphocytes gave a response of 4% only. The not infrequent finding of low scores in young people, especially females, has been discussed fully elsewhere (12).

The responses of leukaemic lymphocytes to mitogenic stimulation were below the normal range except for a single atypical case in which a score of 45% was achieved.

In the three experiments in which the activity of the cells was assessed by uptake of tritiated thymidine, the results corresponded with those obtained by scoring morphological transformation. The details of the results are shown in Table 1b, the tube numbering corresponds to that given in Table 1a; the lymphocytes were given by healthy donors A, B, C, D, E and F.

The results of all the experiments are given in Table 2. In column 2 under 'stimulus', the word 'normal' in parenthesis indicates that the leukaemic population has been treated with Mitomycin C. Conversely the letters 'CLL' in parenthesis indicate that the normal cell population has been so treated.

Turning now to the MLR results the two-way responses in normal pairs ranged from 1-18% and the one-way from 1-11%.

Comparing this with the situation in which normal populations were mixed with leukaemic ones, it is evident that leukaemic lymphocytes gave a feeble response to normal allogeneic lymphocytes in culture, but were as effective as normal lymphocytes in evoking responses. The mean one-way response in terms of normal-versus-normal was identical to that of normal-versus-leukaemic, namely 5.3%.

Table 1b. CLL and the MLR. Correspondence between tritiated thymidine uptake and morphological transformation in three mixed lymphocyte experiments

Tube No. (see Table 1a)	Counts per min.	% morphological transformation	
1	224	<1	
2	34000	58	
3	333	2	
4	182	<1	Experiment
5	5950	4	1
6	179	<1	
7	967	6	Donors A + B
8	847	4	
9	380	1	
10	202	<1	
11	199	<1	
12	265	<1	
1	118	<1	
2	21839	52	
3	182	1	
4	216	<1	Experiment
5	18579	37	2
6	172	<1	
7	593	1	Donors C + D
8	255	<1	
9	384	<1	
10	94	<1	
11	101	<1	
12	141	<1	
1	159	2	
2	32971	55	
3	202	<1	
4	114	<1	Experiment
5	16243	36	3
6	204	<1	
7	7744	15	Donors E + F
8	3154	5	
9	2180	7	
10	139	<1	
11	116	1	
12	105	<1	

Where two leukaemic cell populations were mixed, the responses were consistently weak.

Discussion

The results on normal paired cultures indicated generally an ability for lymphocytes to respond to others of different HL-A constitution. However, exceptions were found, the first being a young girl whose lymphocytes had failed also to respond to PHA.

The other exception was a normal pair whose lymphocytes, despite adequate responses to PHA, failed to react against each other, although there was a marked disparity in HL-A constitution between these people (13).

Table 2. Responses of Lymphocytes from Normal Donors and from Patients with Chronic Lymphocytic Leukaemia

	Stimulus	Transformation range %	Transformation mean %
Normals + normals	PHA	4-58	45.6
	2-way MLR	1-18	10.9
9 pairs	1-way MLR	1-11	5.3
Normal + CLL	PHA normal	40-59	49.1
	PHA CLL	< 1-45	8.5
	2-way MLR	< 1-18	8.5
	1-way (normal) MLR	< 1-13	5.3
11 pairs	1-way (CLL) MLR	< 1- 8	1.5
CLL + CLL	PHA	< 1- 4	2.2
	2-way MLR	< 1- 1	< 1
3 pairs	1-way MLR	< 1	< 1

It is evident from the results that in general lymphocytes from patients with CLL barely respond in the MLR. Since the lymphocytes from patients respond very poorly to mitogens, it is clear that the capacity to respond in the MLR correlates with the responses to mitogenic stimulation.

On the other hand it appears that the responses of lymphocytes of previously sensitised CLL patients to specific antigens approach normal limits (4), although secondary responses in CLL patients in terms of antibody production is weak in comparison with normal people and those having non-lymphomatous reticuloses (14).

The MLR is not regarded as a secondary response unless there have been previous transfusion and/or pregnancies.

In terms of evoking responses, leukaemic lymphocytes are comparable with normal ones, in other words their antigenic 'foreignness' is being adequately expressed.

The one patient whose lymphocytes more nearly resembled normal lymphocytes throughout poses a special problem. It has been suggested (15, 16) that the preponderant cells in CLL are bone marrow derived and concerned with humoral immunity ('B' cells) and that this would explain the lack of response of CLL lymphocytes to stimulation in tissue culture. It is possible that this patient has a preponderance of 'T' cells (Thymus dependent) and the suggestion has indeed been made that such a situation could exist (17).

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