

PROLIFERATION OF B AND T CELLS IN MIXED LYMPHOCYTE CULTURES

BY LEIF C. ANDERSSON, STIG NORDLING, AND PEKKA HÄYRY

(From the Third Department of Pathology and the Fourth Department of Surgery, University of Helsinki, SF 00290 Helsinki 29, Finland)

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Thymus-dependent (T) lymphocytes alone are triggered to proliferation in the mixed lymphocyte culture (MLC), whereas thymus-independent (B) cells are not. Mouse lymphocyte populations depleted from T cells by anti- ϕ serum plus complement do not respond (1, 2). Lymphocytes from neonatally thymectomized rats (3, 4) or chicks (5) respond very weakly or not at all, whereas lymphocytes from neonatally bursectomized chicks respond (5).

When the MLC-responder cells were taken from karyotypically marked thymus/bone marrow cell chimeras most cells in mitosis carried the marker chromosomes of the thymus graft (6, 7). However, lymphocytes from athymic "nude" mice, known to have only few ϕ -antigen carrying cells (8), display a weak MLC response (9).

Piquet and Vassalli (10) prepared T/B radiation chimeras between CBA/Ca and CBA/H-T6T6 mice by transfer of anti- ϕ serum plus complement-treated bone marrow cells and thymocytes. When spleen cells from these chimeric mice were stimulated in one-way MLC with (C57BL/6 \times CBA/H-T6T6) F_1 spleen cells, most mitoses on day 2-3 of culture had the karyotype of the thymus graft, whereas later they had the karyotype of bone marrow graft. The authors interpreted these results as an indication of T cells recruiting B cells to proliferate in MLC.

We have reexamined this possibility with an in vitro chimeric technique: electrophoretically fractionated (11) syngeneic mouse T and B cells with distinct chromosome markers were stimulated in one-way MLC. The results demonstrate that B cells alone do not respond and when electrophoretically fractionated T and B cells, recombined in equal numbers, are used as responders no more than 5% of the dividing cells have the karyotype of the B cells.

Materials and Methods

Mice.—CBA/H-T6T6 strain was obtained from the Wistar Institute, Philadelphia, Pa., CBA/Ca strain, histocompatible with the T6 translocation stock (12), from the Medical

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Research Council, Mill Hill, London and the DBA/2 strain from the Jackson Laboratories, Bar Harbor, Maine. All mice were bred in our own colony. Blood lymphocytes of the two CBA strains did not stimulate each other in MLC.

Fractionation of Lymphocyte Subclasses.—Spleens were teased into a single cell suspension, which was incubated with iron powder, phagocytic cells removed with a magnet, and red cells lysed with 0.83% NH_4Cl (13). More than 85% of the cells were lymphocytes as judged from May-Grünwald-Giemsa (MGG) stained cytocentrifuged cell smears. T and B lymphocyte populations were obtained by fractionation in preparative free flow cell electrophoresis (14) as previously described (15).

Cultures.—In the MLC 1.5×10^6 electrophoretically fractionated CBA/Ca or CBA/H-T6T6 spleen T and/or B lymphocytes were used as responders, and 3×10^6 Mitomycin C-blocked DBA/2 spleen cells as stimulators in 2 ml of 5% fetal calf serum—minimal essential medium (FCS-MEM). The serum batch did not give any background proliferation. The response was quantitated as “blasts per culture” from MGG-stained cytocentrifuged cell smears (13).

Stimulation with mitogens: phytohemagglutinin-M (PHA, Difco Laboratories, Detroit, Mich.) was used at a final dilution of 1:150 of the reconstituted stock (13, 16), and *Escherichia coli* lipopolysaccharide (LPS, from Dr. G. Möller, Karolinska Institutet, Stockholm) at a concentration of 10 $\mu\text{g}/\text{ml}$ (16). 1.5×10^6 CBA/Ca spleen T or B cells were used as responders in these cultures.

Karyotype Analysis.—Karyotypes were analyzed in duplicate cultures after 3 h Colcemid-Ciba arrest as earlier described (17), except that the hypotonic treatment time was 20 min. In the tables, designation T6T6 refers to metaphases containing the translocation markers and T0T0 to metaphases which do not contain them.

RESULTS

Similar results were obtained in three experiments. The analysis of one of the experiments is described. Fig. 1 shows the electrophoretic distribution

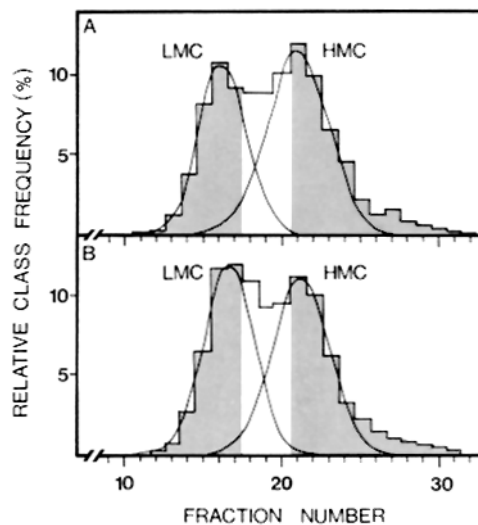


FIG. 1. Electrophoretic distribution of CBA/Ca (A) and CBA/H-T6T6 (B) spleen lymphocytes. LMC (low mobility cells) denote B cells and HMC (high mobility cells) T cells. Cells from shaded areas used for the experiment. The Gaussian distributions of the major lymphocyte subpopulations are shown.

patterns of CBA/Ca (Fig. 1 A) and CBA/H-T6T6 (Fig. 1 B) spleen lymphocytes, HMC (high mobility cells) denote T and LMC (low mobility cells) B cells. Only cells from the shaded areas were used for the experiment, intermediate fractions where there is an overlap of the HMC and LMC were discarded.

CBA/Ca T (HMC) or B (LMC) cells were stimulated with DBA/2(M), with PHA or with LPS. T cells alone responded to DBA/2(M) and to PHA, whereas no response to LPS was detected. B cells alone responded to LPS but did not respond to DBA/2(M) nor to PHA (Table I). 1.5×10^6 T cells responded better in the MLC than T plus B cells recombined in equal numbers ($0.75 \times 10^6 + 0.75 \times 10^6$) (Table I).

0.75×10^6 CBA/H-T6T6 spleen T (HMC) lymphocytes were combined with an equal number of CBA/Ca B (LMC) lymphocytes and vice versa. These mixtures of lymphocytes were stimulated with DBA/2(M) and daily karyotype analyses were made from the 4th to the 9th day in culture. Karyotype analysis

TABLE I

Quantitation of the Blast (BL) Response and the Number of Surviving Lymphoid Cells (SLC) in Cultures where Electrophoretically Fractionated CBA/Ca and/or CBA/H-T6T6 T and/or B Lymphocytes are Stimulated with Mitomycin-C Blocked DBA/2 Cells or with the Mitogens PHA or LPS

Responder cells*		Stimulator cells† or mitogen	Cell type	Surviving cells per culture ($\times 10^3$)‡							
T	B			2	3	4	5	6	7	8	9
T6T6	—	DBA/2 (M)	BL			190			480		
			SLC			570			610		
—	T6T6	DBA/2 (M)	BL			0			0		
			SLC			10			0		
T0T0	T6T6	DBA/2 (M)	BL	20	65	105	250	270	360	210	160
			SLC	690	420	395	340	345	445	290	260
T6T6	T0T0	DBA/2 (M)	BL	10	50	92	115	150	230	195	160
			SLC	650	390	245	225	325	325	295	270
T0T0	—	DBA/2 (M)	BL			180			450		
			SLC			590			600		
—	T0T0	DBA/2 (M)	BL			0			0		
			SLC			40			2		
—	—	DBA/2 (M)	BL			0			0		
			SLC			10			0		
T0T0	T0T0	—	BL			0			0		
			SLC			28			0		
T0T0	—	PHA	BL		320						
			SLC		570						
—	T0T0	PHA	BL		0				0		
			SLC		40				0		
T0T0	—	LPS	BL		0				0		
			SLC		30				0		
—	T0T0	LPS	BL		370				0		
			SLC		435				0		

* The responder cells are either CBA/Ca (T0T0) or CBA/H-T6T6 (T6T6) spleen T or B lymphocytes or mixtures of both. 1.5×10^6 responder cells per culture.

† 3.0×10^6 spleen cells blocked with Mitomycin-C (M).

‡ Mean values from duplicate determinations.

TABLE II
Analysis of Karyotypes in Mixed Cultures, where Recombined Mixtures of Electrophoretically Fractionated CBA/Ca and CBA/H-T6T6 T and B Cells Were Stimulated with Mitomycin-C Blocked DBA/2 Spleen Cells

Days in culture	Responder cells*					
	T cell: CBA/H-T6T6 B cell: CBA/Ca			T cell: CBA/Ca B cell: CBA/H-T6T6		
	Metaphases			Metaphases		
	T6T6	T0T0	T6T6	T6T6	T0T0	T6T6
			%			%
4	54	4	91	5	41	11
5				8	140	5
6	125	6	96	6	104	5
7	112	3	97	5	129	4
8	36	1	97	4	104	4
9	16	0	100	2	73	3

* $0.75 + 0.75 \times 10^6$ responder cells of each type stimulated with 3.0×10^6 DBA/2(M) spleen cells. Controls (not shown) were performed to exclude proliferation of stimulator cells (Table I).

(Table II) of the MLC cultures revealed that during 5th–8th day in cultures only 3–5% of the mitoses were of B origin.

DISCUSSION

Preparative cell electrophoresis yields pure viable populations of mouse T and B cells (15). Such physically fractionated cells have not been subjected to immunological manipulation, nor have they been exposed to antisera plus complement. Lymphocytes fractionated from spleens of adult mice may be considered to represent mature T and B cells.

The results indicate that T cells alone respond in the MLC. This result is in agreement with most investigations (1–4). The fractionated cells are functionally viable since T cells respond to PHA and B cells to LPS.

Wagner has reported that spleen cells of nude mice respond in MLC (9). However, the stimulation index (response in counts per minute/background in counts per minute) in his experiment was only five to seven times background proliferation. It is possible that the response is of B cell origin although nude mice have a small (8), and variable (15) number of ϕ -antigen carrying cells. In our cultures 5% of the mitoses were of B type, and the average stimulation index was 200–300 times background. This would give an index of six times background for B cells, which is in agreement with the result obtained by Wagner.

Piquet and Vassalli (10) claimed a shift from the predominance of T cell mitoses to B cell mitoses when spleen cells of CBA/Ca-CBA/H-T6T6 T/B radiation chimeras were stimulated in one-way MLC. They do not give quan-

titative data on the amount of background proliferation. The stimulation indices in their cultures were very small (three to six times background on the 3rd day, declining to two to four times background on the 4th and two times background on the 5th day of culture).

The proliferation which takes place in the spleens of thymus-bone marrow radiation chimeras is largely a proliferation of bone marrow-derived (hematopoietic) and other precursor cells. This results in a high background proliferation when spleen cells are transferred to culture (unpublished). The relative increase of mitoses originating in the grafted bone marrow late in culture may therefore only reflect a decline of the specific T cell response. The possibility that T cells recruit B cells to divide in the MLC is very attractive. We did not find proof for such a phenomenon.

SUMMARY

Electrophoretically fractionated CBA/Ca spleen T cells alone respond to allogeneic cells in one-way MLC and to PHA. They do not respond to *E. coli* LPS. B cells alone do not respond to allogeneic cells nor to PHA, but do respond to LPS. When karyotypically distinguishable syngeneic mixtures of T and B lymphocytes are stimulated with allogeneic cells, at the most 5% of mitoses on 5-9th culture day are of B cell origin. This indicates that B cells are not substantially recruited to proliferate in the MLC.

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