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The Interaction of Normal Lymphocytes and Cells from Lymphoid Cell Lines

III. STUDIES ON ACTIVATION IN AN AUTOCHTHONOUS SYSTEM

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Summary. Activation of fresh blood lymphocytes has been measured by uptake of tritiated thymidine, following exposure to irradiated cells from autochthonous or allogeneic cell lines (LCL cells). Nine autochthonous combinations were studied. In every case activation was observed and in one the rate and peak level of activation were comparable to those found in control allogeneic mixtures. Neither foetal calf serum nor antibiotics appeared to be important factors in the activation process. There was no correlation between the number of identified HL-A histoincompatibilities in a given allogeneic mixture and the rate or peak level of activation achieved. Activation appears to be influenced by the metabolic state of the LCL cells and the rate of the reaction is sensitive to the precise culture conditions under which it proceeds. Some distinction can be drawn between the antigens responsible for activation in autochthonous and in allogeneic mixtures but the former may prove to be modified histocompatibility determinants.

INTRODUCTION

Heavily X-irradiated cells from cultured lymphoblastoid cell lines ('LCL' cells) have proved to be very potent activators of allogeneic fresh blood lymphocytes (Hardy and Ling, 1969; Hardy, Ling and Knight, 1969). We have shown (Steel and Hardy, 1970), using cultured cells from two patients with infectious mononucleosis, that LCL cells also provoke a mixed lymphocyte type reaction in an autochthonous system. This observation has since been confirmed by several other groups (Green and Sell, 1970; Junge, Hoekstra and Dienhardt, 1970, 1971; Knight, Moore and Clarkson, 1971; Han, Moore and Sokal, 1971), although in one study (Flier, Glade, Broder and Hirschhorn, 1970) inconclusive results were obtained. The antigens responsible for activation in this system have yet to be identified and a number of possibilities must be considered.

First, in association with the capacity for apparently unlimited proliferation *in vitro*, LCL cells may have developed surface neo-antigens comparable to those of virus-transformed cells of other species (for review and references see Habel, 1969). If this is the case,

activation of fresh lymphocytes *in vitro* by autochthonous LCL cells may represent one part of an immunological surveillance mechanism for defence against deviant cells *in vivo* (Burnet, 1970). It is consistent with this view that lymphocytes activated by autochthonous LCL cells simultaneously acquire cytotoxic potential directed against such cells (Hardy and Steel, 1971; and paper in preparation).

Secondly, it is probable that foreign macromolecules and haptenic groups are adsorbed from the culture medium on to the surface of LCL cells. It has been shown, in the case of cultured HeLa cells, that such molecules contribute to their antigenic make-up (Hamburger, Pious and Mills, 1963). Foetal calf serum antigens have also been shown, by immunization studies, to be present on LCL cells (Eng and Landon, 1970).

Thirdly, in the classical mixed lymphocyte reaction, activation can be related to disparities between the two cell populations in respect of their histocompatibility antigens, in man principally those of the HL-A system (Amos and Bach, 1968; Schellekens *et al.*, 1970), and it has been found, using serological techniques, that the HL-A phenotype of cultured LCL cells frequently differs from that of the original donor's fresh blood lymphocytes by the addition of one or more new specificities (Rogentine and Gerber, 1969; Moore and Woods, 1970; Bernoco *et al.*, 1969; Dick, Steel and Crichton, 1972; Mackintosh *et al.*, 1972). Such new or altered specificities may therefore contribute to the activation in an autochthonous system. It must be borne in mind, however, that these apparent changes may be a consequence of interaction between the HL-A determinants and either adsorbed constituents of the medium or an unspecified neoantigen.

In an attempt to deduce the nature of the stimulatory antigens, we have now extended our observations to a larger group of subjects and have investigated the kinetics of activation in autochthonous and allogeneic systems in which the HL-A phenotypes of the cell populations were known. The effect of eliminating some potential antigens from the culture medium has also been assessed.

MATERIALS AND METHODS

Mixed cell cultures

In principle, the experimental system consists of a mixture of lymphocytes, freshly separated from defibrinated whole blood, and heavily X-irradiated (3000 rads) cells from a lymphoblastoid cell line. These are incubated together in a total volume of 1 ml of culture medium consisting of Eagles' MEM (Glasgow modification) and 15 per cent human serum-gelatin. 0·5 μCi of thymidine-(methyl-^{3}H), 150 mCi/mmol. (Radiochemical Centre, Amersham,) was added 24 hours before harvesting. Two types of control culture were included in all experiments, namely fresh lymphocytes without irradiated LCL cells and irradiated LCL cells mixed with fresh red blood cells from the appropriate donor. Radioactivity in the TCA-insoluble material (DNA) was estimated by liquid scintillation counting. Full details of the techniques employed have been reported elsewhere (Hardy, Knight and Ling, 1970; Steel and Hardy, 1970).

Donors of fresh lymphocytes

These included nine young adults from whom lymphoblastoid cell lines had been established 6 months to 2 years earlier (Steel and Edmond, 1971). Eight of these had suffered from infectious mononucleosis at the time of initiating the cell lines; the ninth

was a healthy volunteer from whom a cell line had been grown by a co-cultivation technique (Steel, 1972). Three other healthy adults, from whom lymphoblastoid lines had not been established, also acted as donors of fresh blood lymphocytes.

Lymphoblastoid cell lines

Cells from a total of fifteen lines have been used. These were routinely maintained in Ham's F10 medium supplemented with 20 per cent foetal calf serum and 10 per cent tryptose phosphate broth. Detailed information on lymphocyte donors and cell lines is given in Table 1.

RESULTS

1. STIMULATION OF FRESH LYMPHOCYTES BY AUTOCHTHONOUS LCL CELLS

Table 2 lists the data from experiments using fresh lymphocytes from each of the nine donors from whom autochthonous cell lines were available. In every case there was marked stimulation by irradiated cells from both autochthonous and allogeneic lines.

2. EFFECT OF ELIMINATING POTENTIAL ANTIGENS FROM THE CULTURE MEDIUM

Samples from a number of cell lines were cultured in medium supplemented with 10

TABLE 1

DONORS OF FRESH LYMPHOCYTES AND LYMPHOBLASTOID CELL LINES USED IN MIXED CULTURE EXPERIMENTS

Donor			Corresponding cell line	
Initials	Sex	HL-A type (fresh lymphocytes)*	Name	Most consistent HL-A specificities†
E.D.	F	2,7,?3	DEW_1	2,7,3(?FJH)
D.D.	M	‡8,11,?5	DUN_1	‡1,8,?9
I.G.	F	N.D.	GOL_1	1,10,8,?13
K.F.	M	1,7,8	FLE_1	1,7,8
E.B.	F	1,2,8,W5/17	KAT_1	1,2,8,W18(?Maki)
V.B.	F	2,7,10	LIZ_1	2,7,10
A.M.	M	1,3,7,8	MAR_1	1,3,7
M.O.	F	1,2,8,12	ORI_1	1,2,8,12,?3,?7,?13
K.Y.	F	1,10,?5,?9	YUD_1	1,10,?8,?W5
D.H.	M	1,10,12,LND	None	—
D.V.	M	1,8,9	None	—
M.S.	M	1,2,8,12	None	—
Not available	F	N.D.	CLA_4	?2,W28,12,W19
Not available	M	3,7	HUN_1	1,3,7,8
Not available	M	N.D.	SAD_1	2,3,7,4c* Walland
Not available	F	N.D.	$G\text{-}S_1$	7,13
Not available	F	N.D.	LAM_2	3,8
Not available	M	N.D.	F137	1,3,LA-W7

* Most results confirmed independently by Dr A. R. Sanderson, Dr W. M. Dick and Mrs P. Mackintosh, but in a few cases, lymphocytes have been typed on only one occasion.

† This column records only those specificities which have been consistently detected on repeated typing by Dr Dick and Mrs Mackintosh. It excludes reactions detected with sera showing anomalous (i.e. unrelated to HL-A) cytotoxicity

‡ HL-A 11 detected on fresh lymphocytes of DD and HL-A 1 detected on corresponding LCL cells DUN_1 may in fact be represented more accurately as a variant of HL-A 1 which cross-reacts strongly with HL-A 11.

N.D. = not done.

TABLE 2

STIMULATION OF FRESH LYMPHOCYTES BY AUTOCHTHONOUS OR ALLOGENEIC LCL CELLS

Fresh lympho-cyte donor	Time in culture (days)	Radioactivity (Dpm) Control (no LCL cells)	Autochthonous	Allogeneic (Line used)	No. of histo-incompats.
I.G.	5	5773	33,607	122,860 (DEW_1)	N.D.
A.M.	5	2124	12,333	82,120 (DEW_1)	1
*D.D.	6	5084	80,214	59,295 (DEW_1)	3
*K.Y.	7	1511	47,727	38,281 (GOL_1)	3
*K.F.	7	2916	16,562	22,841 (YUD_1)	1
*E.D.	7	6134	23,526	42,887 (FLE_1)	2
M.O.	6	6710	40,564	35,612 (DUN_1)	0
V.B.	5	4059	88,438	68,139 (GOL_1)	2
E.B.	6	7065	24,374	49,352 (CLA_4)	3

Data are means of triplicate cultures. In different experiments different pools of human sera were used to supplement the medium and the uptake of ^{3}H-thymidine was measured on different days as shown in column 2.

Each tube contained 10^6 viable fresh lymphocytes and 10^5 irradiated LCL cells except in the case of experiments with M.O. fresh cells in which each tube contained 1×10^6 irradiated LCL cells.

Figures in 'control' column refer to tubes containing donor fresh lymphocytes, without added LCL cells. In the additional control system (irradiated LCL cells + donor fresh red cells) counts never exceeded 500 dpm.

* No antibiotics in the culture medium during the 6–7 days of these experiments.

Dpm = Disintegrations per minute.

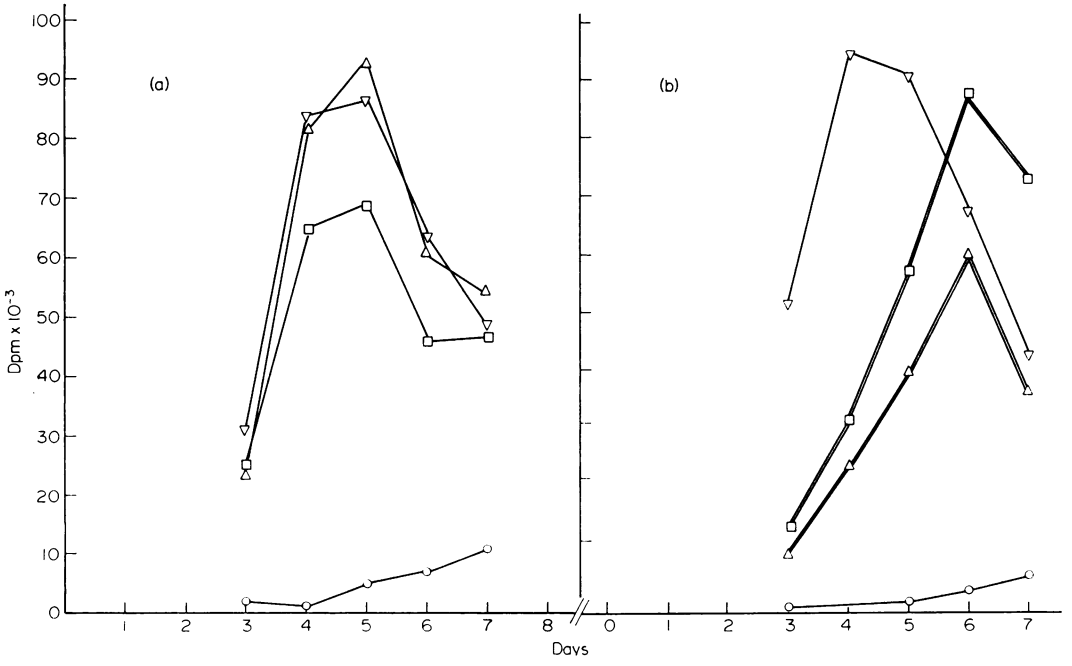

FIG. 1. Response of fresh blood lymphocytes from donors (a) D.V. and (b) V.B. to irradiated LCL cells from LIZ_1 and GOL_1.

One sample of LIZ_1 had been grown in medium supplemented with human serum, in place of foetal calf serum, for 6 weeks before this experiment. LIZ_1 is derived from donor V.B.: △, LIZ_1 (human serum); □, LIZ_1 (foetal calf serum); ▽, GOL_1; ○, control; =, Autochthonous mix; —, allogeneic mix.

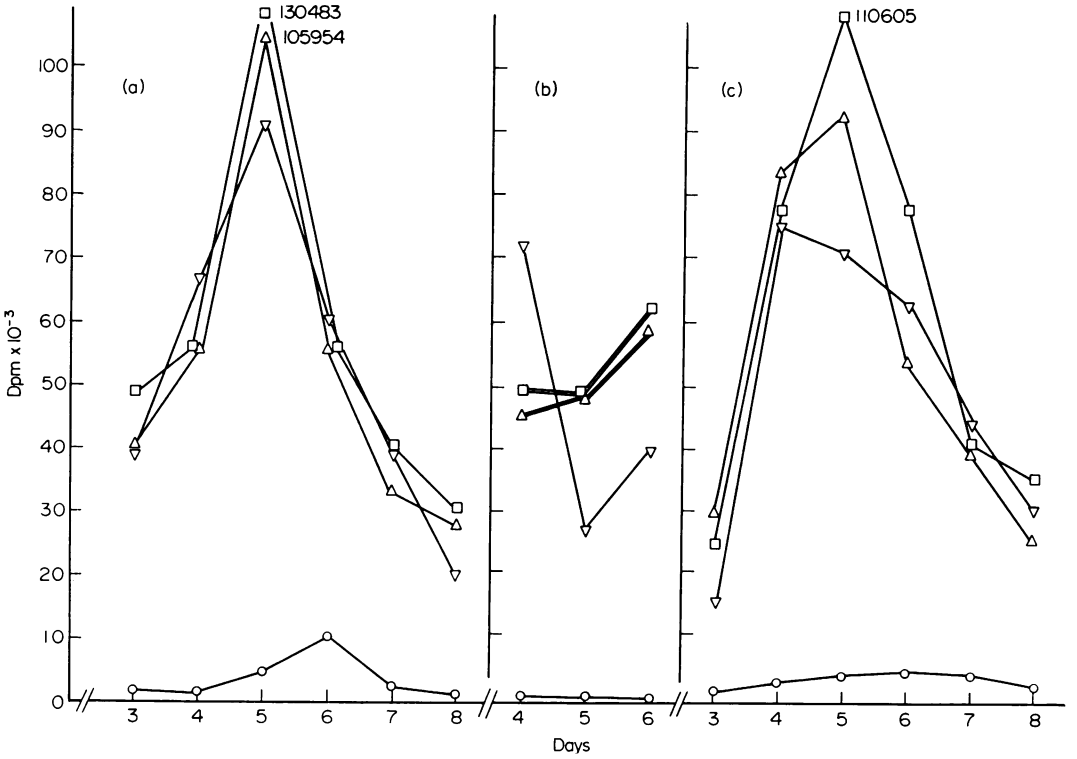

FIG. 2. Response of fresh blood lymphocytes from donors (a) D.M., (b) M.O. (frozen) and (c) M.S. to irradiated cells from ORI_1 and HUN_1. One sample of ORI_1 had been grown in medium supplemented with human serum, in place of foetal calf serum, for 6 weeks before this experiment.

Lymphocytes from M.O. had been frozen down in liquid nitrogen for 2 weeks before use in this experiment. ORI_1 is derived from donor M.O. △, ORI_1 (human serum); □, ORI_1 (foetal calf serum); ▽, HUN_1; ○, control. = autochthonous mix; —, allogeneic mix.

TABLE 3

STIMULATION OF FRESH LYMPHOCYTES BY LCL CELLS GROWN FOR 6 WEEKS IN MEDIUM SUPPLEMENTED BY HUMAN AB Rh + ve SERUM OR IN FOETAL CALF SERUM

Fresh lymphocyte donor	Time in culture (days)	Radioactivity (Dpm)				
		Control (no LCL cells)	Autochthonous		Allogeneic (in FCS)	(Foreign line used)
			in HS	in FCS		
D.D.	7	1486	29,629	69,881	21,474	(RAJI)
D.D.	7	4003	82,299	71,379	68,711	(CLA_4)
M.O.	6	2497	58,157	63,735	40,308	(HUN_1)
V.B.	5	4059	61,161	88,438	68,139	(GOL_1)
E.B.	6	7065	18,018	24,374	49,352	(CLA_4)

Each culture contained 10^6 fresh blood lymphocytes and (except in controls) 10^5 X-irradiated LCL cells.

Results are mean values of triplicate cultures. HS = human AB Rh + ve serum; FCS = foetal calf serum.

per cent human AB Rh+ve serum in place of foetal calf serum for at least 6 weeks (twelve to twenty cell cycles) before use in mixed culture experiments. The stimulating capacity of these cells was then examined in an autochthonous system. The results (Table 3 and Figs 1–4) demonstrate that removal of foetal calf serum had no profound effect on the levels of stimulation recorded.

In routine culture of cell lines, penicillin 100 μg/ml and streptomycin 100 μg/ml are always added to the medium. These antibiotics were normally present, in the same concentration, in the medium used for mixed cultures but in four cases they were omitted entirely throughout the stimulation experiments. These four experiments are indicated by an asterisk in Table 2. It should be noted that none of the lymphocyte donors was known to display delayed hypersensitivity to either drug.

3. POSSIBLE ANTIGENIC CHANGES INDUCED BY IRRADIATION

To exclude the possibility that X-irradiation of LCL cells might induce surface changes resulting in recognition of these cells as 'foreign' in an autochthonous system, fresh blood lymphocytes from four donors were subjected to the standard irradiation procedure, then used in place of LCL cells in mixed culture against both autochthonous and allogeneic unirradiated lymphocytes. The results (Table 4) demonstrate clearly that activation occurred only in allogeneic mixtures.

TABLE 4

LEVEL OF STIMULATION BY X-IRRADIATED AUTOCHTHONOUS SMALL LYMPHOCYTES

Autochthonous cultures				Allogeneic cultures			
Control		Mixture		Control		Mixture	
Cells	Dpm	Cells	Dpm	Cells	Dpm	Cells	Dpm
DD	5084	DD+XDD	7962	MS	2605	MS+XDD	12385
KY	1511	KY+XKY	815	DM	1785	DH+XKY	9500
KF	2916	KF+XKF	2261	MS	1117	MS+XKF	4213
ED	6134	ED+XED	3243	DH	1522	DH+XED	3506

DD, KY, KF and ED; four patients who had recovered from infectious mononucleosis 6–12 months before these experiments. MS, DH; two healthy adult subjects. Same initials refer to viable fresh lymphocytes from these donors. XDD etc. refers to X-irradiated (3000 rads) fresh lymphocytes from these donors. Control cultures consisted of 10^6 viable fresh lymphocytes only. Mixed cultures contained 10^6 viable fresh lymphocytes + 10^5 X-irradiated lymphocytes. Values are means of triplicate cultures. All cultures were maintained for 7 days before harvesting.

Dpm = Disintegrations per minute.

4. KINETICS OF LYMPHOCYTE ACTIVATION

Although estimation of tritiated thymidine incorporation into fresh lymphocytes over a single 24-hour period some days after mixing with irradiated LCL cells always demonstrates that activation has occurred (Tables 2 and 3), variation in the level of activation in different situations (autochthonous compared with allogeneic mixtures, LCL cells cultured in human serum compared with those in foetal calf serum or allogeneic mixtures with different degrees of HL-A incompatibility) appears to follow no consistent pattern. A series of experiments was therefore undertaken in which the time course of activation was

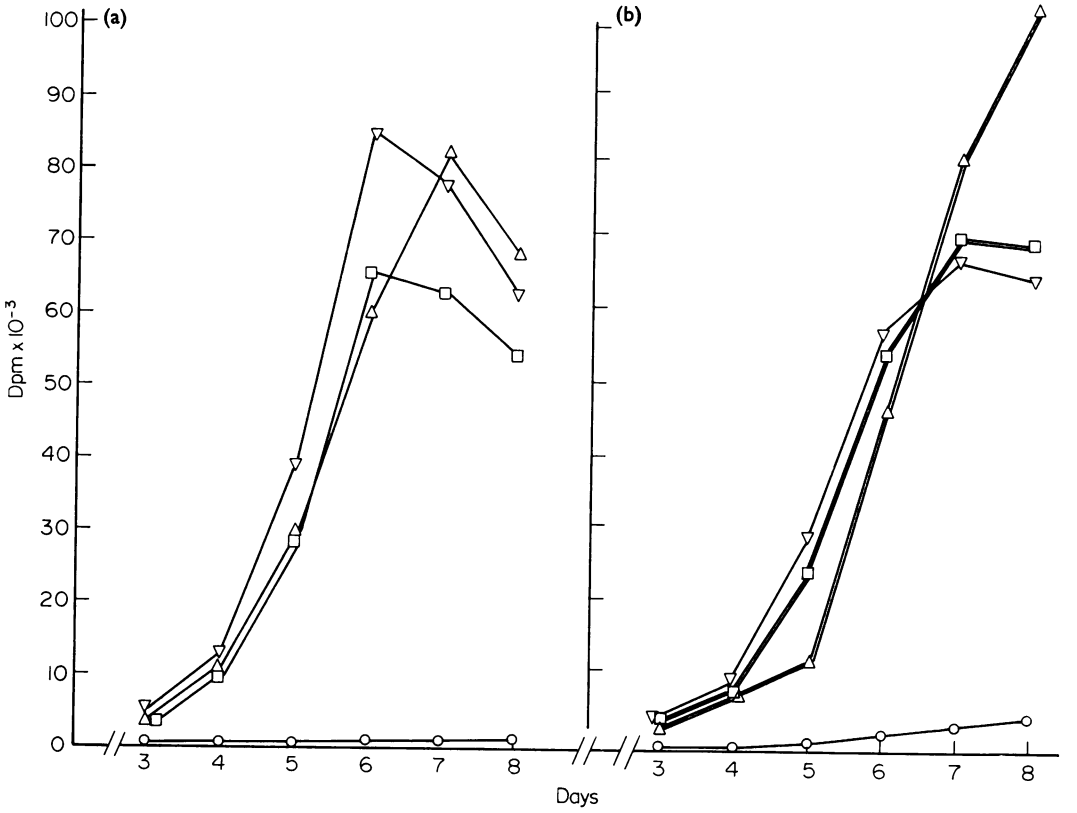

FIG. 3. Response of fresh blood lymphocytes from donors (a) D.H. and (b) D.D. to irradiated LCL cells from DUN_1 and CLA_4.

One sample of DUN_1 had been grown in medium supplemented with human serum, in place of foetal serum, for 6 weeks before this experiment.

DUN_1 is derived from donor (b) D.D.: △, DUN_1 (human serum); □, DUN_1 (foetal calf serum); ▽, CLA_4; ○, control =, autochthonous mix; —, allogeneic mix.

studied. In two of the same experiments, the response to different numbers of irradiated LCL cells was measured. The results are illustrated in Figs 1–7.

Each point in Figs 1–7 represents a mean value of triplicate cultures.

Except where indicated in the individual figures, each mixed culture contained 10^6 fresh blood lymphocytes and 10^5 irradiated LCL cells.

Control cultures contained the same number of fresh blood lymphocytes as corresponding mixed cultures, but no LCL cells.

Alternative control cultures containing X-irradiated LCL cells and donor erythrocytes but no fresh blood lymphocytes, yielded counts of less than 500 dpm.

DISCUSSION

It is essential to establish that the cell lines, particularly those used in autochthonous mixtures, are not contaminated with cells from another culture. Precautions are taken to minimize this risk and the consistency of the results argues against such an accidental explanation for the observed stimulation in an autochthonous system. Nevertheless,

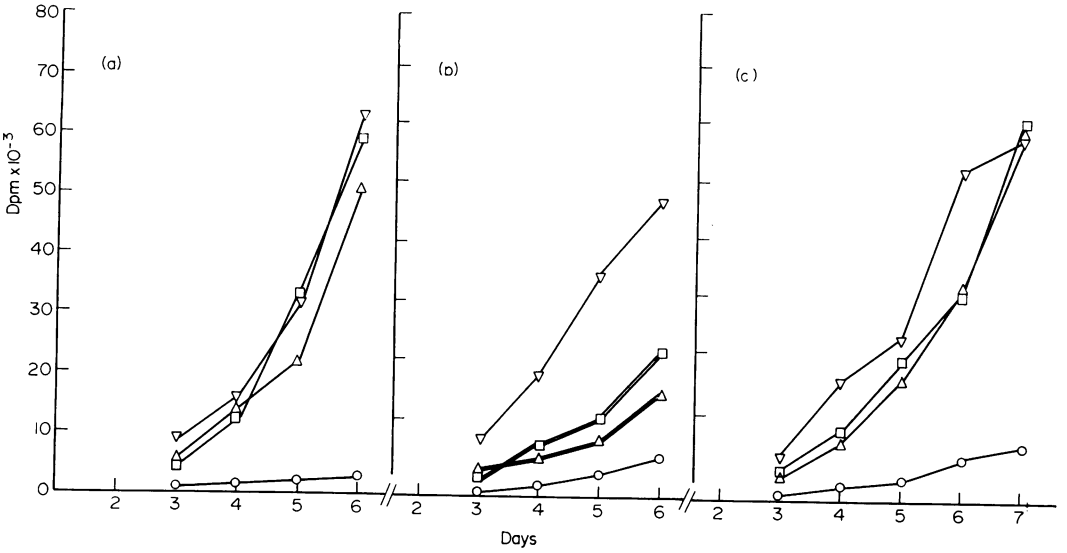

FIG. 4. Response of lymphocytes from donors (a) D.H., (b) E.B., and (c) M.S. to irradiated LCL cells from KAT_1 and CLA_1.

One sample of KAT_1 had been grown in medium supplemented with human serum, in place of foetal calf serum, for 6 weeks before this experiment.

KAT_1 is derived from donor E.B. △, KAT_1 (human serum); □, KAT_1 (foetal calf serum); ▽, CLA_4; ○, control. =, autochthonous mix; —, allogeneic mix.

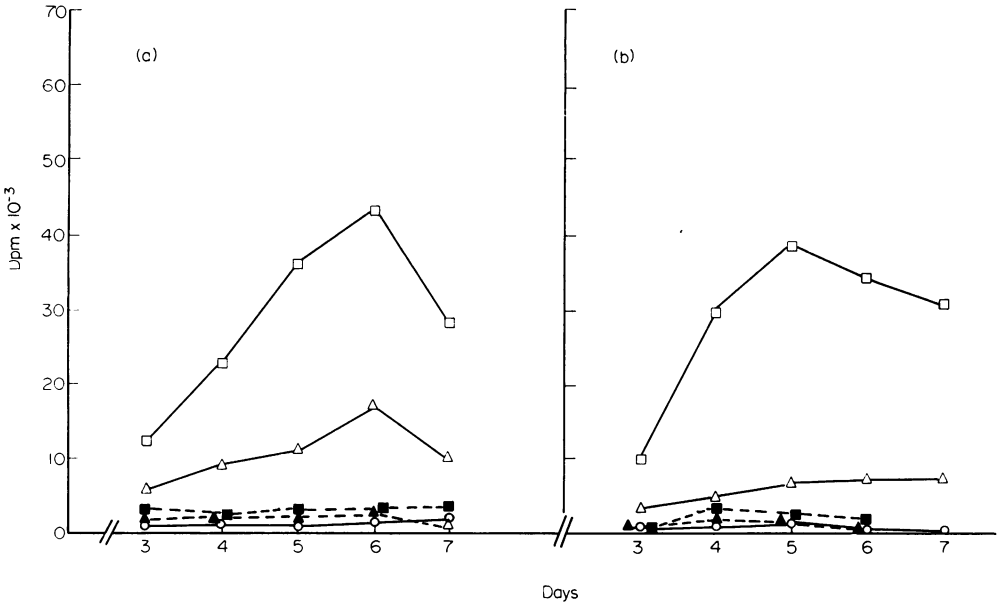

FIG. 5. Response of lymphocytes from donors (a) D.H. and (b) M.S. to two different doses of irradiated LCL cells from DEW_1 and DUN_1.

The LCL cells had been growing rather slowly during the few weeks before this experiment. △, DEW_1 3×10^5; ▲, DEW_1 3×10^4; □, DUN_1 3×10^5; ■, DUN_1 3×10^4; ○, control.

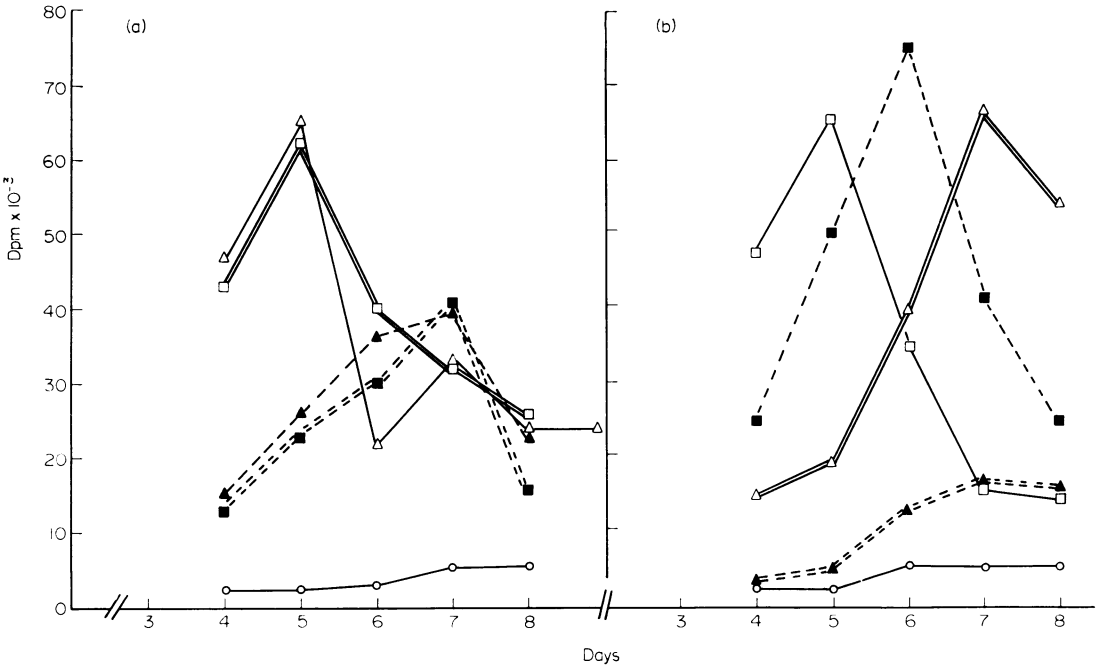

FIG. 6. Response of lymphocytes from donors (a) D.D. and (b) M.O. to two different doses of irradiated cells from DUN_1 and ORI_1.

DUN_1 is derived from donor D.D., ORI_1 is derived from donor M.O.: △, ORI_1 2×10^5; ▲, ORI_1 2×10^4; □, DUN_1 2×10^5; ■, 2×10^5; ○, control; =, autochthonous mix; —, allogeneic mix.

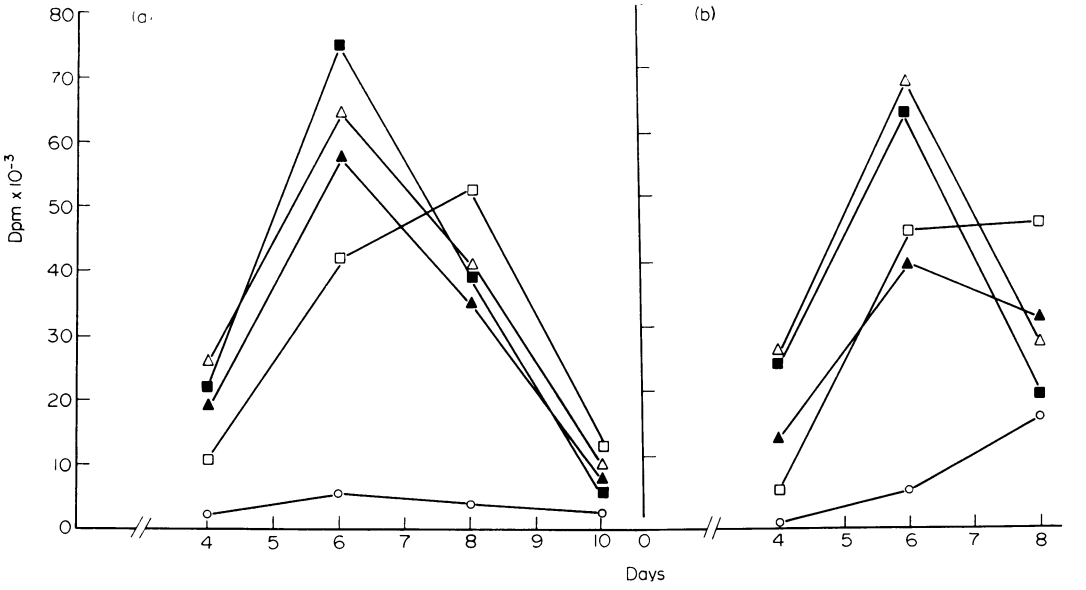

FIG. 7. Response of fresh lymphocytes from donors (a) D.H. and (b) M.S. to irradiated LCL cells from △ $G\text{-}S_1$; ▲ F137; □ ORI_1; ■ LAM_2 and ○ control.

These combinations represent varying degrees of histo-incompatibility as judged by HL-A typing results (see Table 1).

independent checks are desirable. These are provided by chromosome analysis and by HL-A typing both of which have been undertaken repeatedly on all the lines. In addition, the lines and fresh blood samples from the original donors have been screened for the electrophoretic pattern of from six to twenty cellular enzymes (Professor H. Harris, Galton Laboratories). Cross-contamination of cultures would undoubtedly have been detected by one or more of these methods. No such evidence has been obtained in the case of any of the lines used in the experiments described here.

Our preliminary report (Steel and Hardy, 1970) recorded findings in two patients who had recovered from infectious mononucleosis but we did not intend to suggest that stimulation of fresh blood lymphocytes by autochthonous cell line cells would be a specific characteristic of this disorder. Others have since shown that it is true of patients with malignant disease and of healthy subjects (Green and Sell, 1970; Knight, Moore and Clarkson, 1971; Han, Moore and Sokal, 1971). The present findings confirm that comparable results are obtained using fresh lymphocytes from a series of recovered infectious mononucleosis patients or from a healthy subject (M.O.). It is of interest that the capacity to respond to both allogeneic and autochthonous LCL cells was retained by the healthy subject's fresh lymphocytes after freezing and storage in liquid nitrogen (Fig. 2). This experiment has not yet been undertaken in the case of the other donors.

Our findings confirm those of Green and Sell (1970) and of Han, Moore and Sokal (1971), in showing that activation in an autochthonous situation cannot be attributed solely to antigens derived from foetal calf serum. However, the results of the experiments illustrated in Figs 1 and 4 indicate that LCL cells grown in foetal calf serum may provoke a slightly more vigorous activation than the corresponding line grown in human AB Rh+ve serum when tested in an autochthonous system. In the control allogeneic systems, the cells grown in human serum proved at least as antigenic as those grown in foetal calf serum. Thus, foreign molecules adsorbed from the culture medium may make some contribution to the antigenicity of LCL cells detectable when major histocompatibility differences are eliminated.

The experiments reported here provided no evidence that antibiotics in the medium act in this way though the possibility is not excluded that they may be incorporated into the cell walls over a prolonged period in culture.

The capacity of LCL cells to activate fresh lymphocytes from an autochthonous or an allogeneic source appears to be influenced by the condition of the cultured line immediately before irradiation, in the sense that if the cells are growing rapidly and the culture contains a very low proportion of dead cells it is likely to provoke a more vigorous activation than a similar number of cells from the same line taken during a phase of relatively poor growth. The difference is most obvious when small numbers of irradiated cells are used. (Compare, for example, the effect of 2×10^4 or 3×10^4 irradiated DUN_1 cells in the experiments illustrated in Figs 5 and 6.) The time course of activation also appears to be influenced by the precise conditions under which the experiments are carried out, the most obvious variable factor being the concentration of CO_2 in the gassed incubator and the resultant pH maintained in the medium. This probably accounts for the finding that the peak of activation varied from day 4 (Fig. 1) to day 7+ (Fig. 3) and it is emphasized that valid comparisons can only be made within single experiments in which indentical conditions prevailed for autochthonous and allogeneic mixtures.

The fact that activation is dependent upon the precise condition of the LCL cells is not incompatible with the view that the reaction has an immunological basis since the meta-

bolic activity of the cell line may influence the manner in which the antigen is 'presented' to the responding cells (Knight *et al.*, 1970; Lowe, 1971).

Although Junge *et al.* (Junge, Hoekstra and Dienhardt, 1970, 1971; Junge, 1970) have reported that cell-free supernatants from lymphoid cell lines will activate both allogeneic and autochthonous fresh lymphocytes, other workers have found that direct contact between the two cell populations is necessary for appreciable stimulation to occur (Hardy, Knight and Ling, 1970; Green and Sell, 1970; Han, Moore and Sokal, 1971) which suggests that the reaction does reflect some change in the surface of LCL cells following their establishment in culture. The subsequent cytotoxic phase of the response also requires direct cell-cell contact (Hardy, Ling, Walkin and Aviet, 1970) and shows a measure of specificity for the cell line used in the activation phase (Hardy and Steel, 1971 and paper in preparation), both of which observations support the above view.

The kinetic studies demonstrate the importance of following the activation of lymphocytes over a number of days. For example from the data illustrated in Fig. 6 is it clear that allogeneic LCL cells (2×10^5) evoked a more brisk and vigorous response in lymphocytes from donor M.O. than did the same number of autochthonous LCL cells. If the activation had been measured at only a single point in time, however, a quite different impression would have been gained depending on whether the measurement was made on day 5, day 6 or day 7.

When the results presented in Figs 1–7 are analysed in terms of the HL-A phenotypes of the cell populations involved (Table 1) there is no evidence of any relationship between rate or peak level of activation achieved and the number of identifiable histo-incompatibilities in a given allogeneic mix. For example M.S. and ORI both carry HL-A types 1, 2, 8, 12 while D.H. is typed 1, 10, 12, LND. Yet ORI_1 cells provoked virtually identical reactions in fresh blood lymphocytes from M.S. and D.H. (Fig. 2).

This finding is perhaps not surprising since it is recognized that activation frequently occurs in mixed cultures of small lymphocytes from HL-A identical unrelated individuals (Johnston and Bashir, 1971) though the level of activation may be low (van Rood and Eijsvoogel, 1970; Kissmeyer-Nielsen *et al.*, 1970).

The nature of the antigen or antigens responsible for stimulation in autochthonous cultures is unknown nor can it be established how great a part they play in allogeneic mixtures. Some distinction between the activating antigens in the two situations can be drawn, first because the rate and peak level of stimulation is generally greater in allogeneic than in autochthonous mixtures and secondly because in one experiment (Fig. 6) reduction in the dose of irradiated ORI_1 cells had a much more profound effect on the activation of autochthonous than of allogeneic lymphocytes.

On the other hand, differences between the response of autochthonous and of allogeneic lymphocytes to irradiated LCL cells are often relatively small and one autochthonous combination ($DD+X\text{-}DUN_1$) consistently proved as active as the control allogeneic mixtures. The simplest interpretation of our findings is that the factors responsible for activation in the autochthonous situation are additive in their effects with histocompatibility antigens in allogeneic mixtures, but that there is a maximal rate and level of activation which cannot be exceeded and which may be achieved even in an autochthonous mixture.

It is an attractive hypothesis that antigenic disparity between fresh lymphocytes and autochthonous LCL cells arises through modification of histocompatibility determinants. This could follow from interaction between histocompatibility antigens and new (possibly virus coded) determinants or with antigens 'unmasked' as the cells pass from a normal

non-dividing state into one of active division. Such interaction might account for the differences between LCL cells and fresh lymphocytes from the same donor in the patterns of reactivity with HL-A antisera though it is uncertain whether the discrepancies should be interpreted as evidence of new antigens on the cultured cells or of greatly increased sensitivity to antibody reactive, in lower titre, with fresh lymphocytes from the same donor. This problem is to be discussed further in a subsequent paper (Mackintosh *et al.*, 1972).

While further studies are required to establish the nature of the antigenic change observed in cultured LCL cells, our present findings suggest that it is not an artefact of the culture technique and hence that this *in vitro* system may be a valid model, for the immunological process whereby deviant cells are recognized and eliminated from the body

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p. 454: Line 25—three should read five

p. 455: Fig. 4c and d—P on the left should read $\frac{S}{P}$.

p. 458: Fig. 8b—XZ94 should read Z94.