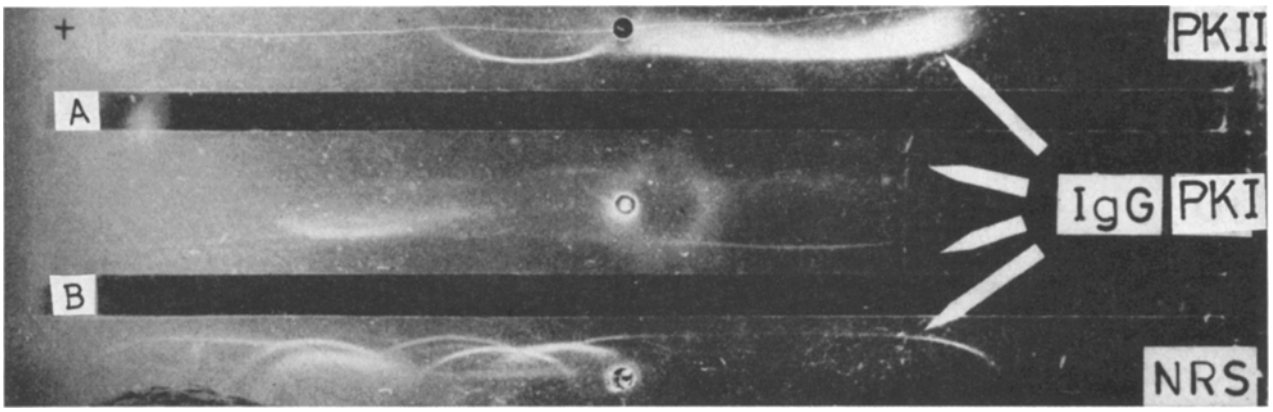

Fig. 4. Immunoelectrophoretic analyses of normal (NRS) rabbit serum and peaks I (PK I) and II (PK II) obtained with Sephadex G-200 column. A, goat anti-rabbit IgG; B, goat anti-rabbit whole serum.

A small degree of cross-reaction was observed between *E. histolytica* and *E. invadens*, an observation in agreement with that of ZAMAN[6]. *E. moshkovskii*, the only free-living species tested, show no antigenic similarity to the other 2 species.

Résumé. Les antisérums de lapin utilisées contre les trophozoïtes de l'*Entamboeba histolytica, E. moshkovskii* et *E. invadens* furent fractionnés par la cellulose DEAE et le Sephadex G-200. Les essais d'immobilisation avec ces antisérums fractionnés montrent que la fraction IgG est la plus active à cet effet. En usant les fractions actives, des essais démontrent l'existence d'une réaction croisée entre *E. invadens* et *E. histolytica.*

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Interaction Between Peripheral Blood Leucocytes and X-Irradiated Cells from Lymphoid Cell Lines

Peripheral lymphocytes from unrelated individuals when mixed in vitro are activated to blast cells which go on to synthesise DNA and enter mitosis[1]. Similar reactions occur in homologous mixtures of animal lymphoid cells[2]. The immunological significance of the reaction and its potentialities as a tissue typing test have been intensively studied in recent years[3]. The reaction is normally a two-way reaction, i.e. each population of cells stimulates and is also stimulated. Since this is obviously an undesirable complication in the interpretation of mixed cell tests there have been a number of attempts to make the reaction 'one-way' by making one donor's cells unresponsive while retaining stimulating capacity, e.g. by freezing and thawing[3,4], treatment with drugs[5,6], or X-irradiation[5,7,8]. None of these methods is ideal. However, X-irradiation appears to be the most effective way for obtaining a one-way reaction[9]. Lymphoid cells from various patients and from some normal individuals can now be maintained in continuous culture[10]. We have found that mixtures of freshly isolated human blood lymphocytes and X-irradiated lymphoid cell line cells give unusually intense interactions.

Established cell lines used in this work were kindly donated by Professor M. A. EPSTEIN[11,12], Professor G. MOORE[10] and Dr. P. GERBER[13]. The derivation of these cell lines and results of tests for the presence of herpes-like virus (HLV) are summarised in Table I. The cells were maintained in static suspension cultures in Eagle's medium supplemented with 20% human, pig, calf or foetal calf serum. Prior to irradiation the cells were suspended in fresh Eagle's medium containing 20% human serum, at a concentration of 2×10^6/ml. Cells were exposed to 6000 r of X-rays (200 kV; 12.5 mA; Filter aluminium, 1 mm; dose rate 150 r/min) and were normally used 3 h later. Preserved lymphoma cells were treated with pyruvic aldehyde or glutaraldehyde as described previously[14]. Lymphocytes were separated from

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defibrinated normal human blood after sedimentation of the red cells with gelatin, and made up to 2×10^6 viable cells/ml in 20% pooled human serum in Eagle's medium. Cell suspensions consisting of irradiated lymphoid cells (1 ml, varying cell concentrations), peripheral cells (1 ml) or mixtures of the 2 (0.5 ml + 0.5 ml) were incubated in capped $3 \times 1/2$ inch tubes in an atmosphere of 5% carbon dioxide in air. Tritiated thymidine ([H^3]-methyl-thymidine 0.5 μc, 150 mc/m*M*, Radiochemical Centre, Amersham) was added to each tube 24 h before harvesting. The activity in the trichloracetic acid insoluble fraction was estimated by liquid scintillation counting[15]. X-irradiation of lymphoid cells markedly decreased cell survival as determined by trypan blue dye-exclusion. There was a corresponding reduction in their capacity to incorporate tritiated thymidine (Figure 1). Mixtures of normal peripheral and irradiated EB cells gave a marked stimulation of DNA synthesis as compared with non-mixed controls (Table II). So far we have tested the cells of 20 individuals with irradiated EB cells and have always obtained a marked activation. The timing of this response varies slightly with the donor and the cell line but a typical result is shown in Figure 2. The best response for irradiated EB2 and EB5 was obtained at a cell concentration of 2×10^5/ml whereas with irradiated EB4 cells the optimal concentration was in the region of 2×10^6/ml (Table III). Preserved EB cells gave no stimulation when mixed with peripheral cells (Table IV). Irradiated cells of the NHDL cell lines have been tested against the lymphocytes of 6 individuals and marked thymidine incorporation has again always been obtained, even when the irradiated cells were from a line in which virus has not been demonstrated (Table V).

The increased DNA synthesis observed when irradiated and normal lymphoid cells are mixed in culture is open to a number of possible explanations.

(1) *Viral transfer.* A herpes-like virus has been found to be so consistently associated with Burkitt lymphoma cell lines and most of the lymphoid cell lines derived from blood cells of normal individuals that it has been suggested

Table I. Origins of lymphoid cell lines

Cell line	Originator	Source	Herpes-like virus demonstrated	References
EB-2	Epstein	Lymphoma	Yes	11
EB-4	Epstein	Lymphoma	?	12
EB-5	Epstein	Lymphoma	?	12
NHDL-1	Gerber	Normal	Yes	13
NHDL-2	Gerber	Normal	Yes	13
NHDL-3	Gerber	Normal	No	13
NHDL-4	Gerber	Normal	Yes	13

Table II. Stimulation obtained by mixing X-irradiated lymphoma cells with human peripheral blood lymphocytes

Composition of culture	72 h of culture DPM	Increment	120 h of culture DPM	Increment
Irrad. EB-2 (2×10^5)	767	–	132	–
Irrad. EB-4 (2×10^5)	4,189	–	147	–
Irrad. EB-5 (2×10^5)	941	–	357	–
RT (2×10^6)	2,092	–	8,251	–
RT (10^6) + Irrad. EB-2 (10^5)	33,989	32,559	98,131	93,949
RT (10^6) + Irrad. EB-4 (10^5)	8,508	5,367	22,452	18,252
RT (10^6) + Irrad. EB-5 (10^5)	4,149	2,633	31,812	27,000
NM (2×10^6)	5,482	–	14,276	–
NM (10^6) + Irrad. EB-2 (10^5)	38,430	35,228	101,681	94,477
NM (10^6) + Irrad. EB-4 (10^5)	21,386	16,500	75,618	68,400
NM (10^6) + Irrad. EB-5 (10^5)	9,654	6,393	36,105	28,789

RT and NM, normal human donors; Irrad. EB, irradiated EB lymphoma cells; DPM, disintegrations per min of tritiated thymidine incorporated. Mean of triplicate cultures; increments, test values minus half the sum of the control values. Cell numbers are in parentheses.

Table III. Variation of mixing increment with number of irradiated EB cells

Composition of culture	Increment over controls DPM Cell concentration 10^6	10^5	10^4
MOC (10^6) + Irrad. EB-2	52,251	92,935	26,014
MOC (10^6) + Irrad. EB-4	118,160	18,350	0
MOC (10^6) + Irrad. EB-5	–ve	23,562	9,262

Cells were incubated for a total of five days. MOC, normal human donor. DPM see Table II.

Table IV. Lack of stimulation with 'preserved' EB-4 cells

Composition of culture	DPM
ST (2×10^6)	1,735
Irrad. EB-4 (2×10^6)	2,303
ST (10^6) + irrad. EB-4 (10^6)	12,516
ST (10^6) + EB-4 (pyr) (10^6)	1,371
ST (10^6) + EB-4 (glut) (10^6)	425
EB-4 (pyr) (2×10^6)	230
EB-4 (glut) (2×10^6)	215

Cells were incubated for a total of 6 days. Numbers in parenthesis refer to number of cells in tubes. ST, normal human donor; EB (pyr) cells, pyruvic aldehyde-preserved EB cells; EB (glut), cells are preserved with glutaraldehyde. DPM see Table II.

Table V. DNA synthesis in cultures of normal peripheral blood cells and irradiated NDHL cells

Composition of culture	DPM
CD (2×10^6)	4,230
Irrad. NDHL-1 (2×10^5)	52
Irrad. NDHL-2 (2×10^5)	290
Irrad. NDHL-3 (2×10^5)	62
Irrad. NDHL-4 (2×10^5)	372
CD (10^6) + Irrad. NDHL-1 (10^5)	44,417
CD (10^6) + Irrad. NDHL-2 (10^5)	48,054
CD (10^6) + Irrad. NDHL-3 (10^5)	60,632
CD (10^6) + Irrad. NDHL-4 (10^5)	54,796

Cells were incubated for a total of 5 days. CD, normal human doner; Irrad. NDHL, irradiated cells from lines derived from normal human doners (Table I); cell numbers are in parentheses. DPM see Table II.

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that it has some vital role in the proliferation[16]. A lymphocyte activatory role for such a virus would also be consistent with evidence supporting its incrimination in the pathogenesis of infectious mononucleosis[17,18]. On the other hand, MOORE and MCLIMANS[10] do not think there is any evidence that virus is required for successful establishment of a cell line. In experiments very similar in design to our own HENLE et al.[19] reported transfer of virus from irradiated lymphoma cells to normal peripheral lymphocytes leading to continued proliferation of the cells of the normal lymphocyte donor. Because, under the conditions used in our short term cultures, no continuous proliferation of normal cells has been observed and because stimulation of normal lymphocytes also occurs after the addition of irradiated cells from a cell line reported to be free of virus, it seems unlikely that the activation effect reported here is viral dependent.

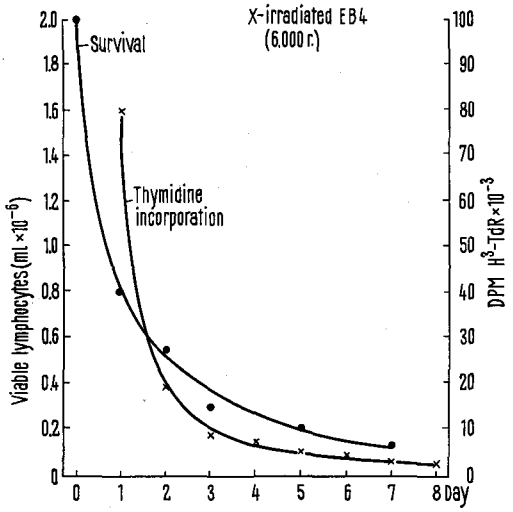

Fig. 1. The effect of X-irradiation on survival of and thymidine incorporation by EB-4 lymphoma cells.

DPM, see Table II; H^3TdR, tritiated thymidine.

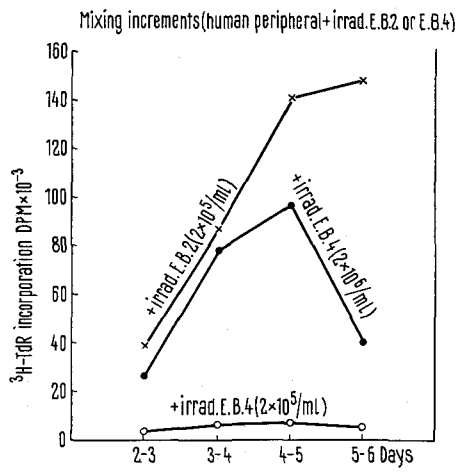

Fig. 2. 10^6 human peripheral cells were cultured with irradiated EB cells.

DPM, see Table II; H^3TdR, see Figure 1; numbers of cells in parentheses.

The involvement of a virus or virus product, however, cannot be completely ruled out.

(2) *Contagious activation.* DNA synthesis in the repressed lymphocytes may be initiated by the transfer of some activating substance. This contagious activation might be effected by any of the 3 mechanisms proposed by FRIEDMANN et al.[20] to explain the phenomenon of metabolic cooperation between cells. The activating substance could be a cytoplasmic factor similar to that reported by THOMPSON and MCCARTHY[21]. Direct cell to cell contact is necessary (Millipore membrane experiments) but the fact that, in rabbits, mitotically active thymus cells do not stimulate autologous peripheral lymphocytes suggests that antigenic differences are essential.

(3) *Delayed death of irradiated cells.* Although the lymphoid cells used to produce the activation are lethally irradiated and their death following arrest of cell division is inevitable and irreversible, it is conceivable that the presence of normal cells could enhance survival and temporary regeneration of the irradiated cells. They might then be able to incorporate thymidine in spite of their inability to divide[22]. This possibility was disproved by the total inactivity found in mixtures of irradiated normal blood lymphocytes and irradiated lymphoid cell line cells.

(4) *Mixed lymphocyte-like reaction.* Results with a range of donors indicate that although there are few qualitative differences between the cell lines, quantitative differences are often quite marked. The reaction further parallels the mixed lymphocyte reaction in that frozen and thawed cells[23] and preserved cells give no stimulation.

The reaction appears to depend upon a recognition of foreign antigens on the continuously cultured lymphoid cells by the normal blood lymphocytes. Antigens other than normal lymphocyte antigens may be involved and the reaction may be augmented by cell-cell interactions of a more general nature[24].

Résumé. Une interaction stimulante très marquée s'observe dans les cultures de lymphocytes frais du sang humain mêlées à des cellules lymphoïdes de plusieurs lignées continues exposées aux rayons X.

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