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Role of Monocytes in the Mixed Leukocyte Culture Reaction* (32613)

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The mixed leukocyte culture reaction (MLCR) refers to, and is detected by, the transformation and mitosis of some of the lymphocytes, when suspensions of peripheral white blood cells from two genetically different donors are allowed to interact in culture (1). In this reaction the part played by white blood cells other than lymphocytes is not known. Experiments reported in this communication show that lymphocytes isolated from suspensions of peripheral leukocytes from two donors do not transform when cultured together. The reactivity of these purified lymphocytes in mixed cultures can be revealed by the addition of purified monocytes or suspensions of peripheral leukocytes in which the lymphocytes had been rendered unreactive by 2000 R X-irradiation (2) or mitomycin (3). Furthermore, reactions will also occur between purified lymphocytes of one donor and monocyte preparations, devoid of reactive lymphocytes, of another.

Heparinized human peripheral leukocytes were fractionated on columns of glass powder (4) and cotton wool. Leukocytes, 50–200 $\times$ 10^6, in autologous plasma were applied on columns of glass powder. The first fraction eluted with plasma contained predominantly lymphocytes (lymphocytes from glass). A second fraction, eluted with a solution of ethylenediaminetetraacetate (EDTA), contained monocytes and neutrophils and no lymphocytes (monocytes–neutrophils from glass). In two experiments, this second fraction was incubated for 4–7 days at 37°C to allow degeneration of the neutrophils. Cells recovered from these cultures were essentially pure macrophages. The lymphocytes from glass were further purified by passage on a column (1 $\times$ 25 cm) loosely packed with cotton wool. The first fraction, eluted with medium 199 (Microbiological Associates, Bethesda, Md.) contained pure lymphocytes (lymphocytes from cotton wool), whereas a second fraction, eluted with EDTA, contained monocytes and neutrophils and trapped lymphocytes (leukocytes from cotton). In an alternative procedure the lymphocytes were purified directly on cotton wool. Leukocytes

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TABLE I. Incorporation of Thymidine-H³ by Lymphocytes in Mixed Cultures.

In each experiment cells from two donors were mixed (columns 1–6); the lymphocyte concentration in each culture was 500/mm³. Entries in column 1 represent incorporation in mixtures of unfractionated leukocytes. Leukocytes from each donor were first purified on glass powder (column 2) followed by further fractionation on cotton wool to yield pure lymphocytes (column 3) and a mixture of monocytes, neutrophils and lymphocytes (column 4). In column 5 monocytes and neutrophils from one or the other reactant (I and II) were added to mixtures of lymphocytes purified on cotton wool (Expts. 1–4) or glass powder (Expts. 5–6). The monocyte fraction used in the last two experiments was devoid of neutrophils. Column 6 represents mixed cultures of monocytes and neutrophils from the two reactants.

Col. no.:	(1)	(2)		(3)		(4)		(5)				(6)	
								Monocytes, neutrophils and purified lymphocytes					
	Total leukocyte suspension	Lymphocytes from glass powder		Lymphocytes from cotton wool		Leukocytes eluted from cotton with EDTA		I		II		Monocytes + neutrophils from glass powder	
Expt. no.	cpm[a]	cpm	% of total	cpm	% of total	cpm	% of total	cpm	% of total	cpm	% of total	cpm	% of total
1	2500	1224	49	16	1	reaction[b]		480	19	270	10	30	1
2	460	30	6	24	5	no reaction[b]		290	63	150	32	5	1
3	2470	270	11	20	1	770	41	233	9	283	11	32	1
4	3130	900	28	66	2	reaction[b]		1300	41	ns	ns	200	6
5	785	210	26	ns[c]	ns	ns	ns	2200	280	1650	210	10	1
6	2240	1040	45	ns	ns	ns	ns	1540	68	3540	158	0	0

[a] Counts for background and single control cultures have been deducted.
[b] Insufficient cells were eluted to carry out incorporation; the reactions were evaluated only on smears.
[c] ns = not studied.

in plasma, from approximately 50 ml blood, were diluted threefold with medium 199 and were applied to 100-cm columns of cotton wool. The lymphocytes were eluted with medium 199.

All the cell preparations were cultured in single or mixed cultures; each was adjusted to contain 500 lymphocytes per mm³ in a total volume of 4–6 ml of medium 199 containing 12% plasma from either one or both of the donors. Cultures were terminated after 5 days; the reactions were evaluated from smears and by the incorporation of thymidine-H³ (specific activity 10 C/mmole during the last 1–3 hours of culture. All determinations were done in triplicate. Count rates (cpm) were determined in a liquid-scintillation counter, using Bray's solution (5).

In 16 of 18 experiments lymphocytes purified on cotton wool gave no reaction in mixed cultures. Smears prepared from cultures at 5 days showed typical lymphocytes and complete absence of macrophages and transformed cells. Incorporation of thymidine-H³ in these cultures was not significantly different from that found in single cultures of purified lymphocytes. There was evidence of incomplete removal of monocytes in the two experiments in which residual activity persisted. Differential counts performed on 8 of the 16 lymphocyte preparations showed no monocytes and 0–0.7% polymorphs over 138–500 cells counted.

In 5 of 6 experiments (Table I) lymphocytes partially purified on glass powder displayed significant reactivity in mixed cultures (column 2). This reactivity could be removed by further purification on cotton wool (column 3) and could be eluted subsequently from the cotton by EDTA (column 4).

The reactivity in mixed cultures of lymphocytes from cotton wool could be restored by the addition of monocyte–neutrophil fractions purified on glass powder (Expts. 1–4, column 5); the reactions obtained were 9–63% of those given by the corresponding unfractionated leukocyte suspensions (column 1). In the last 2 experiments shown in Table

TABLE II. Reactions in Mixed Cultures of Cells from Two Donors.

	Donor K		
Donor W	Untreated leukocytes[a] (cpm)	Lymphocytes purified on cotton wool (cpm)	Mitomycin-treated leukocytes (cpm)
Untreated leukocytes	2360	720	2380
Lymphocytes purified on cotton wool	2220	280[b]	2380
Mitomycin-treated leukocytes	1320	940	200[b]
Irradiated leukocytes	1500	520	150[b]

[a] Thymidine-H^3 incorporated in mixed cultures.

[b] No reaction (less than control values).

I, cultured monocyte preparations were added to glass powder purified lymphocytes: the increase in reactivity (close to 3-fold) over that given by the corresponding unfractionated leukocyte suspensions is due, in part, to the virtual absence of neutrophils, which preliminary experiments show inhibit the reaction. In control experiments there was no incorporation of thymidine-H^3 when the monocyte and neutrophil preparations were cultured together (column 6).

Leukocyte suspensions subjected to X-irradiation or to treatment with mitomycin do not react in mixed cultures (2, 3). Leukocyte suspensions so treated were used in the present investigation to restore the reactivity of lymphocytes purified on cotton wool. As can be seen in Table II, mitomycin-treated or irradiated cells of one donor did not react with mitomycin-treated or irradiated cells of the other. Similarly, there was no reaction between the purified lymphocytes of the two donors. By contrast, reactions occurred in mixtures containing purified lymphocytes of one donor and mitomycin treated or irradiated cells of the other. Reaction between purified lymphocytes and irradiated cells was obtained in 7 out of 10 experiments and between purified lymphocytes and mitomycin-treated cells in 4 out of 4 experiments.

Jones (6) and McFarland *et al.* (7) concluded in their studies that macrophages play a role in the mixed leukocyte culture reaction. The findings presented in this communication support this view and suggest that monocytes or macrophages into which monocytes transform in culture (4, 8) are essential to the MLCR. It is unlikely that damage incurred during the preparative procedures rendered the purified lymphocytes unreactive. Cells processed through the entire procedure, i.e., including elution from cotton wool with EDTA, retained reactivity in mixed cultures. The purified lymphocytes appeared microscopically healthy after 5 days' culture. They could be fully stimulated by phytohemagglutinin (not shown) and could stimulate untreated cells from another donor. Finally, the reactivity of the lymphocytes could be restored by the addition of purified monocytes and mitomycin-treated or irradiated cells. Mitomycin and X-irradiation render the lymphocytes unreactive in the MLCR, but in the dosages used these procedures did not appear to damage monocytes, as judged by their ability to phagocytose polystyrene-latex particles (not shown). In these preparations presumably the monocytes are responsible for the activation of purified lymphocytes.

Whatever the role of the macrophage in the MLCR, or the mechanism by which it facilitates the reaction of lymphocytes, the mixed leukocyte culture represents a system in which reaction of one cell type requires the participation of another. The reaction can take place when the two cell types are derived from two different donors. Recently we presented evidence which suggests that the MLCR is an immune reaction (9). On the basis of that evidence and the results presented in this report, and in view of the demonstrated role of macrophages in antibody synthesis (10), it is suggested that the role of the macrophage in the MLCR is to process the antigen for the lymphocyte. If this view is correct, then the role of the macrophage is

nonspecific, i.e., it can process autologous antigens for allogeneic lymphocytes.

Summary. Suspensions of pure lymphocytes did not interact in mixed cultures; the reaction could be restored by the addition of purified monocytes or of unfractionated leukocyte suspensions in which lymphocytes had been rendered unreactive by X-irradiation or mitomycin. These findings suggest that the monocyte is essential to the mixed leukocyte culture reaction.

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Catabolism of Adenine Nucleotides by the Isolated Perfused Rat Heart* (32614)

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Some evidence exists that adenosine, a known coronary dilator, is involved in the regulation of coronary blood flow. Berne (1) found that perfused cat hearts released inosine and hypoxanthine, the degradation products of adenosine, into the perfusion medium under hypoxic conditions. From such findings he suggested that during periods of decreased oxygen tension or decreased coronary blood flow, heart muscle nucleotides were broken down to adenosine which diffused out of the cardiac cell, reached the coronaries via the interstitial fluid, and produced arteriolar dilation. Following this, deamination of adenosine by adenosine deaminase would yield inactive inosine. Since that time, considerable evidence has accumulated that adenosine is produced by cardiac tissue during anoxia. Richman and Wyborny (2) found that adenosine could be detected in perfusates of rabbit hearts treated with 8-azaguanine to inhibit adenosine deaminase and with dinitrophenol to uncouple oxidative phosphorylation. Moreover adenosine has been shown to appear in rat and rabbit ventricular tissue after short periods of ischemia (3, 4) and is present in coronary sinus blood of the ischemic dog heart (5). Baer *et al.* (6) have isolated a 5′-nucleotidase from heart muscle which dephosphorylates adenosine 5′-phosphate (5′-AMP) to adenosine. Adenosine deaminase was also isolated from ventricular tissue (6), and thus the enzymatic machinery for formation and degradation of adenosine is present in the myocardium. In the model of Berne (1), it was suggested that adenosine was produced in the myocardial cells and reached the coronary arterioles via the interstitial fluid. We have considered the possibility that 5′-AMP may be dephosphorylated to adenosine directly within the smooth muscle cells of the coronary vasculature. Some evidence that this might occur has been provided by Williamson and Di Pietro (7), who found that various 5′-nucleotides and other phosphate esters were dephosphorylated when continuously recycled through perfused rat hearts. Similar findings were reported by Hoffman and Okita (8)

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