THE INTERACTION OF HUMAN MONOCYTES AND LYMPHOCYTES*

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Several lines of evidence indicate that phagocytic mononuclear cells are involved in the initial recognition and processing of antigen. The two most cogent arguments for this thesis are as follows. First, lymphocytes in populations of greater than 98% purity fail to respond to specific antigen in vitro, but when phagocytes are added to such populations, transformation occurs in the usual manner (1). Similar results have been obtained in studies on two populations of cells derived from mouse spleen (2). The second argument is based on the ability of an "immunogenic RNA" extractable from antigen-sensitized macrophages to induce specific immunoglobulin synthesis by lymphocytes. Following the initial observations by Fishman and Adler (3), Askonas and Rhodes (4) demonstrated that an RNA extracted from the peritoneal cells of mice immunized with ^{131}I-labeled hemocyanin had the capacity to induce specific antibody production when injected into unimmunized mice. Such RNA preparations contained small amounts of the immunizing protein, and probably other proteins as well. Similar contamination of immunogenic RNA preparations by immunizing antigen has been reported in studies of phage T2 (5). Whether the trace contamination is critical to the immunogenicity of such RNA preparations from macrophages is not definitely known (6–8). It is unclear whether the production of specific immunoglobulins by lymphoid cells is dictated solely by a species of macrophage RNA or whether the RNA acts primarily as an adjuvant to enhance the immunogenicity of associated antigen proteins. The changes in the metabolism of RNA of macrophages after exposure to antigen are as yet unknown. Changes in the properties and composition of macrophage RNA have been described after exposure of the phagocytic cells to red cells or albumin from other species (9, 10). These studies, however, have not taken into account the effect of particle ingestion per se on the metabolism of RNA by phagocytes (11, 12).

Antibody induction by macrophage RNA appears to be biphasic, with an early IgM component and a later IgG component. The IgM antibody produced by lymph node cells in vitro has the allotype specificity of the macrophage from which the RNA is derived, whereas the IgG antibody possesses the allotype of the lymph node donor (13).

Few studies have appeared on the role of macrophages in the processing of antigen and interaction with lymphocytes in man. The paucity of studies no

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doubt reflects the difficulty of obtaining a large number of human macrophages under standardized conditions. In 1966, however, Bennett and Cohn (14) described a method for isolating peripheral blood monocytes in good yield and high purity, thus paving the way for experiments on the activity of human mononuclear phagocytes. The studies of these investigators and their collaborators (15, 16) and the early histologic studies of Maximow (17), Aschoff (18), and Metchnikoff (19) defined the morphologic and metabolic continuum between the peripheral blood monocyte and the larger macrophages. On the basis of these observations, we undertook the present investigations of the interaction of human monocytes and lymphocytes in the response to an antigen.

Materials and Methods

Leukocyte Preparations.—The blood donors were hematologically normal tuberculin-positive or -negative subjects or patients receiving treatment for active tuberculosis.

Monocytes were isolated from 70 ml of heparinized venous blood by a minor modification (12) of the method of Bennett and Cohn (14). This technique utilizes the differences in buoyancy of various leukocytes in albumin gradients and the ability of monocytes to adhere to glass. Purity of the monocyte preparations varied between 70 and 99.6%. In experiments in which a high purity of monocytes was critical, populations of at least 98% purity were used. Differentiation of monocytes from morphologically similar large lymphocytes was made in two ways: by the ability of monocytes to phagocytize polystyrene particles 1.3 μ in diameter (12), and by their failure to incorporate thymidine-^{14}C after culture for 72–96 hr with phytohemagglutinin. In most experiments lymphocyte contamination was less than 5%, and when necessary less than 1%.

Lymphocytes were isolated from 70 ml of heparinized venous blood by a minor modification of the method of Johnson and Garvin (20). After incubation on glass wool columns at 37°C for 30 min, whole blood was collected and added to ½ volume of 3% Dextran (mole wt 100,000–200,000) in saline. After 30 min the leukocyte-rich supernatant was collected and centrifuged at 150 *g* for 7 min at 20°C. The leukocyte pellet was washed twice in Hanks' balanced salt solution containing 10% fetal calf serum and suspended at a concentration of 1×10^6 cells/ml in the culture medium (Eagle's minimal essential medium containing 25% fetal calf serum, penicillin, 100 units/ml, and streptomycin, 50 μg/ml). Lymphocyte purity varied between 70 and 100% (mean 93%).

Conditions of Culture.—

Direct contact between monocytes and lymphocytes: Sterile conditions were used throughout. Monocytes were cultured in TC-199 containing 30% fetal calf serum, penicillin, 100 units/ml, and streptomycin, 50 μg/ml, at a concentration of 1×10^6 cells/ml. After a 90 min interval to allow the monocytes to adhere to glass, either saline or purified protein derivative (PPD, Parke, Davis & Co., Detroit, Mich.) in saline was added, usually in a final concentration of 2.5 μg/ml. Incubation was allowed to continue for 1.5–24 hr (usually 4 hr); the cells were then thoroughly washed by decantation six times with Hanks' solution at 37°C. In a typical experiment, 3 ml of the lymphocyte suspension was added to each tube, and incubation was continued for 6 days. The degree of lymphocyte transformation was determined morphologically and by incorporation of radioisotope. Cultures were performed in triplicate or quadruplicate.

In all experiments the degree of direct lymphocyte transformation by PPD was also determined by incubation of the lymphocyte population with PPD (2.5 μg/ml) continuously for a 6 day period under similar conditions to those used for the lymphocyte-monocyte cultures.

Conditioned medium: Monocyte cultures were incubated in T flasks with saline or PPD (2.5 μg/ml) for 4–24 hr. The cells were washed thoroughly by decantation with warm Hanks' solution containing 20% fetal calf serum to remove extracellular PPD. Fresh culture medium was added, and the incubation was continued for an additional 24–72 hr. The "conditioned medium" was collected by centrifugation and Millipore filtration. Lymphocytes from the same monocyte donor (autologous) or from a different donor (homologous) were then cultured for 6 days in a mixture of equal parts of conditioned and fresh culture media, and the extent of lymphocyte transformation was then determined.

Millipore chambers: Monocytes (1×10^6 cells in 1 ml of culture medium) were introduced into one chamber of a vessel separated into two parts by a Millipore filter (pore size 0.45 μ). After the cells had been incubated with PPD, 2.5 μg/ml, for 4 hr the extracellular PPD was

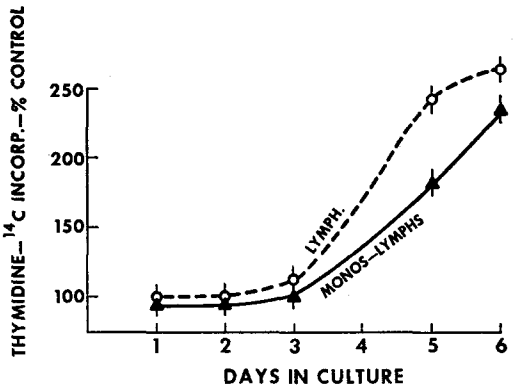

FIG. 1. Time course of thymidine-^{14}C incorporation by lymphocytes in culture. ◊ - - - - - ◊, a population of 95% lymphocytes and 5% phagocytes exposed continuously to PPD; and ▲——▲, monocytes exposed to PPD for 4 hr, washed extensively, and incubated with lymphocytes.

removed by washing and fresh medium was added. Control specimens incubated with saline as the only addition were handled similarly. Lymphocytes (1×10^6 cells in 1 ml of medium) were added to the second chamber, and the incubation was continued for 6 days. Transformation was then determined morphologically. In these experiments controls were included which demonstrated the passage of PPD from one chamber to another and eliminated the possibility that significant amounts of PPD were adhering to the filter or glass wall of the chamber despite washing.

Measurement of Transformation.—Thymidine-2-^{14}C (30 mc/mmole, 0.2 μc/3 ml of culture) was added after 120 hr of leukocyte culture. The incorporation of radioisotope was determined 18 hr later by liquid scintillation counting as previously described (21). Incorporation of radioisotope in PPD-stimulated cultures was expressed as the percentage of incorporation in control cultures per cent control values). Control cultures containing saline as the only additive showed a low level of thymidine uptake on the 6th day of culture, with a mean uptake in 40 experiments of 0.5% (SD ± 0.2%) of the total thymidine-^{14}C added. Isotope incorporation in triplicate or quadruplicate cultures in general varied no more than ±30% from the mean. Incorporation of thymidine-^{14}C of greater than 150% of control values was considered to represent significant stimulation.

The degree of lymphocyte transformation to "blast-like" cells was determined by counting

at least 600 cells. Uniform, well spaced cell preparations were prepared with a Shandon cytocentrifuge (Shandon Sci. Co., London, England) and stained with Wright's or Giemsa. Replicate samples varied no more than ±3% from the mean when 10% or less of the cells were transformed and no more than ±6% when more than 15% were transformed. The results were expressed as the increment in per cent transformation (Δ % transformation) in PPD-stimulated cell cultures above that in saline controls. An increment in morphologic transformation of greater than 4% (mean) was considered to represent significant stimulation.

RESULTS

"Direct" Transformation of Lymphocytes

When PPD is added to populations of leukocytes from tuberculin-sensitive donors under standardized conditions, the lymphocytes undergo a series of characteristic biochemical and morphologic transformations (21–24). These changes include morphologic transformation to blast-like cells and greatly increased DNA synthesis as measured by enhanced incorporation of radioactive thymidine into acid-precipitable macromolecules. Fig. 1 shows the time course of incorporation of thymidine-^{14}C in a population of 95% lymphocytes and 5% phagocytes exposed continuously to PPD during 5 days of culture. A progressive increase in thymidine uptake occurred after the 3rd day, reaching a peak about the 6th day of culture.

The incorporation of thymidine was dependent on the concentration of PPD in the medium; little enhancement of incorporation was observed at concentrations less than 0.1 μg/ml. Maximal stimulation was achieved at concentrations between 1.0 and 2.5 μg/ml. Similar results were obtained in previous studies (21).

These experiments, in which mixed leukocyte populations rich in lymphocytes were exposed directly and continuously to PPD, served as the methodologic controls for monocyte-lymphocyte interaction. Similar controls were included in all subsequent experiments.

"Indirect" Transformation of Lymphocytes by Monocytes Plus Antigen

Because of the evidence implicating mononuclear phagocytes in the preliminary processing of antigens, we undertook to define the conditions under which human peripheral blood monocytes and antigen could induce lymphocyte transformation. The basic experimental design was to expose monocyte populations to specific antigen (PPD) and, after a period of incubation, to remove as completely as possible any antigen not bound to or taken up by monocytes. The primed monocytes were then incubated with responsive lymphocytes in the absence of extracellular antigen, and the degree of lymphocyte transformation was assessed. Under these conditions the primed monocytes provided the only source of antigen in the culture tubes.

In all initial studies, a series of control preparations were included to demon-

strate that PPD was removed completely from the walls of the culture vessels by the washing procedure. These studies also included controls to eliminate the possibility that the slight contamination of the monocyte population by lymphocytes contributed to the observed results.

Optimal Conditions for Transformation.—The optimal conditions for lymphocyte transformation were established in preliminary experiments as follows: Monocytes from tuberculin-sensitive donors were incubated with PPD for 1.5–72 hr, washed to remove unbound antigen, and incubated with autologous

TABLE I

Effect of Duration of Exposure of Monocytes to PPD and to Lymphocytes on Incorporation of Thymidine-^{14}C by Transforming Lymphocytes

Exp. No.	Duration of incubation of monocytes with PPD	Duration of incubation of PPD-primed monocytes with lymphocytes	Thymidine-^{14}C incorporation by lymphocytes exposed to PPD-primed monocytes
	hr	*hr*	*% saline control*
1	2	4	222
	18	4	240
2	4	144	145
	24	144	141
3	2	2	180
	2	24	210
4	2	2	142
	2	144	215

lymphocytes for 1.5–144 hr; the degree of transformation was then assessed. As shown by the representative experiments in Table I, incubation of monocytes with PPD for periods between 2 and 24 hr gave similar results. The data for incubations of less than 2 hr or greater than 24 hr were inconsistent and are not shown. In general, monocytes incubated with PPD for more than 48 hr before exposure to lymphocytes failed to induce transformation. By that time loss of monocytes from the glass surface and changes in cell morphology were grossly apparent.

We next determined the optimal duration of contact between monocytes and lymphocytes. This could be ascertained by taking advantage of the fact that monocytes adhere to glass and lymphocytes do not. For periods up to 4 hr there was no detectable loss of monocytes from the walls of the incubation vessels into the supernatant medium, and the two cell types could thus be conveniently separated. As shown in Table I, exposure of lymphocytes to autologous PPD-primed monocytes for as little as 2 hr was usually sufficient to

induce transformation. Exposure for longer periods produced higher levels of transformation, whereas exposure for shorter periods gave inconsistent results. Thus, in the subsequent experiments, unless otherwise specified, monocytes were exposed to PPD for 4 hr, washed and incubated with lymphocytes for 144 hr.

Further experiments showed that the concentration of PPD to which the monocytes was exposed was critical to the subsequent transformation of lymphocytes. At concentrations below approximately 0.1 μg/ml, the degree of transformation as measured by thymidine-^{14}C incorporation was no different

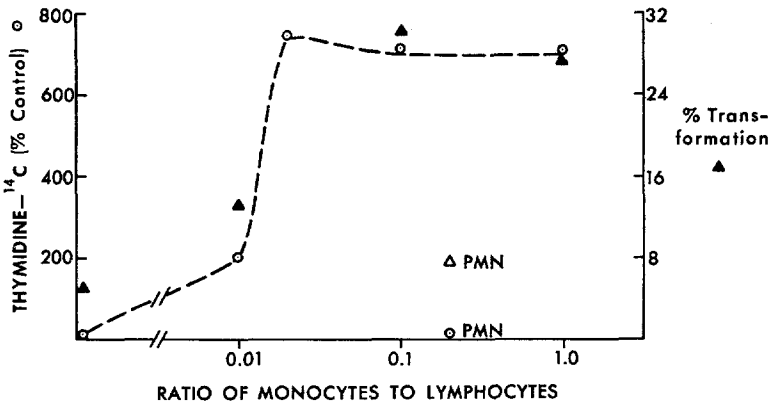

FIG. 2. Effect of varying ratios of monocytes to lymphocytes on the induction of lymphocyte transformation by PPD-sensitized monocytes. ▲, morphologic transformation; and ◉, thymidine-^{14}C incorporation. The effect of substituting neutrophils for monocytes is indicated by the symbols △ and ⊙ PMN.

from control values. At concentrations between 1 and 2.5 μg/ml, similar high levels of transformation were obtained. Therefore, unless otherwise stated, a concentration of 2.5 μg of PPD/ml was used in subsequent experiments.

Additional studies defined the optimal ratio of monocytes to lymphocytes for induction of transformation and examined the ability of other phagocytes, in particular the neutrophil, to substitute for the monocyte in inducing lymphocyte transformation. Varying numbers of monocytes were incubated with PPD, washed, and cultured with a fixed number of lymphocytes (3×10^6). In three experiments populations of leukocytes enriched with granulocytes (95% mature neutrophils or metamyelocytes) were substituted for monocytes. As shown in Fig. 2, ratios of monocytes to lymphocytes as low as 1:100 induced morphologic transformation and enhanced incorporation of thymidine-^{14}C. Ratios of 1:30 were more effective, but increasing the ratio to 1:10 or 1:1 had little additional effect. In most subsequent experiments a ratio of one monocyte to three lymphocytes was used. In the three experiments with leukocyte populations comprised

principally of neutrophils, little effect was observed, even at ratios of one cell to four lymphocytes, and smaller ratios had no detectable effect.

The optimal time for the addition of thymidine-^{14}C and measurements of morphologic transformation and incorporation of radioisotope was determined in a series of experiments of different durations. As seen in Fig. 1, the uptake of thymidine-^{14}C was similar in experiments involving monocyte-lymphocyte interaction and in experiments in which PPD was added directly to leukocyte populations consisting primarily of lymphocytes (95%). Peak uptake occurred

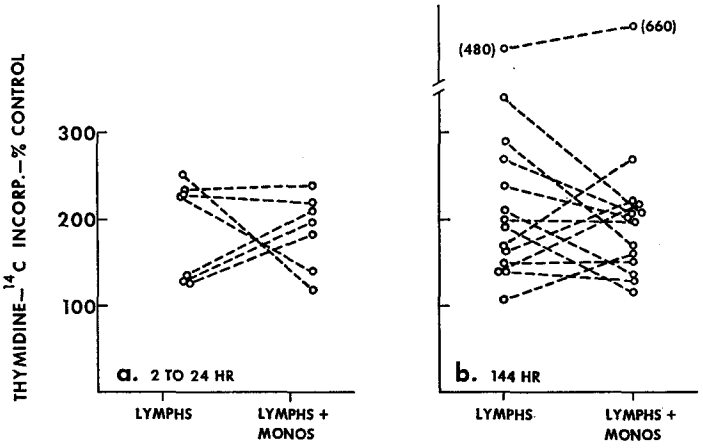

FIG. 3. Comparison of thymidine-^{14}C incorporation induced by the direct addition of PPD to lymphocyte cultures (lymphs) and by the addition of PPD-sensitized monocytes to similar cultures of lymphocytes (lymphs + monos). The period of incubation of monocytes with lymphocytes was (*a*), 2–24 hr, and (*b*) 144 hr.

between 5 and 6 days of culture, although increased incorporation was sometimes observed by day 3 and was seen consistently by day 4. Transformation assessed morphologically was usually detectable by day 3 and was maximal between days 4 and 5.

Efficiency and Replicability of Monocyte-Lymphocyte Interaction.—In 21 separate experiments the degree of transformation induced by direct and continuous exposure of lymphocytes to PPD was compared with that induced by PPD-primed monocytes. The periods of exposure of monocytes to lymphocytes were 2–24 hr and 144 hr. As shown in Fig. 3, neither method of inducing lymphocyte transformation was consistently more efficient than the other. In eight studies monocyte-lymphocyte interaction resulted in a higher level of isotope incorporation, in eight studies direct addition of PPD to lymphocytes was more efficient, and in five studies no difference in the results of the two methods was detectable.

To determine the consistency of behavior of leukocytes from a single donor

over a period of time, cells from each of two donors were studied on six or seven occasions during an 8 month period. The ranges of thymidine-^{14}C incorporation into lymphocytes induced by PPD-primed monocytes above saline-monocyte control values were 150–280% and 145–251%. These ranges of values were comparable to those observed with the direct addition of PPD to lymphocyte cultures.

Metabolic requirements: A series of studies examined the question of whether living and metabolically intact monocytes are necessary for the induction of lymphocyte transformation. Monocytes were incubated with either potassium cyanide, 1×10^{-3} M, iodoacetate, 2×10^{-3} M, or actinomycin D, 0.5 to 10 μg/ml, for 1–3 hr after a 120 min exposure to PPD. After being washed free of antigen and metabolic inhibitor, the monocytes were incubated for 6 days with autologous PPD-positive lymphocytes. The degree of transformation in the experimental and control cultures containing no inhibitor was then assessed. As shown in Table II, both potassium cyanide and iodoacetate inhibited lymphocyte transformation. The results with actinomycin D are not shown, since the antibiotic consistently resulted in death of the cultured lymphocytes, although low concentrations (0.5 μg/ml) were used and great pains were taken to wash out all the drug before adding the lymphocytes. The effects of these drugs on the phagocytic capacity of short-term cultures of human monocytes have been reported previously (12).

TABLE II

Effect of Metabolic Inhibitors on the Induction of Lymphocyte Transformation by PPD-Primed Monocytes

Exp. No.	Conditions	Morphologic transformation	Thymidine-^{14}C incorporation
		Δ %	*% saline control*
1	Control	+4.5	215
	Potassium cyanide	−2.3	170
	Iodoacetate	−4.0	113
	56°C	+1.0	91
2	Control	+13.5	142
	Potassium cyanide	+1.0	91
	Iodoacetate	+1.0	92
	56°C	+2.0	61

As another approach to the requirement for monocyte viability, these cells were killed by heating at 56°C for 30 min after PPD-uptake was complete. The heat-killed monocytes were incapable of inducing lymphocyte transformation (Table II).

Specificity of Monocyte-Lymphocyte Interaction

PPD-Positive and -Negative Monocytes.—After establishing standardized conditions for the measurement of lymphocyte transformation by antigen-sensitized monocytes, we were able to explore several aspects of the specificity of the cellular interaction. Are monocytes from tuberculin-positive and -negative subjects equally effective in inducing transformation? Can monocytes from a

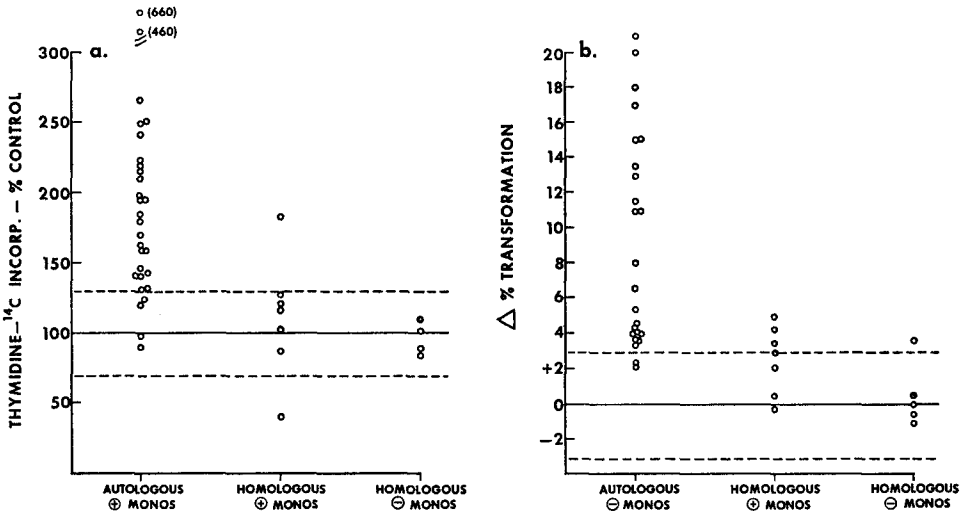

FIG. 4 *a* and *b*. Transformation of tuberculin-positive lymphocytes induced by compatible PPD-sensitized autologous and homologous monocytes. (*a*) Thymidine-^{14}C incorporation; and (*b*) morphologic transformation. ⊕ indicates a tuberculin-positive donor; ⊖ indicates a tuberculin-negative donor. The limits of the range of control values are shown.

tuberculin-negative subject induce tranformation of lymphocytes from a tuberculin-positive subject? These questions were difficult to answer since they required mixed leukocyte cultures from genetically dissimilar individuals. Such mixed cultures usually showed a high level of lymphocyte transformation in the absence of added antigen. In about a third of the experiments with monocytes and lymphocytes from different donors, however, such transformation did not occur, and these cultures could be used to investigate the specificity of the interaction of monocytes and antigen with lymphocytes. The experimental design was as follows: Monocytes from subject A were incubated with PPD, thoroughly washed, then added either to autologous lymphocytes from A or to homologous lymphocytes from subject B for measurement of transformation. The indicator lymphocytes in these experiments were always from tuberculin-positive donors; the monocytes were either from positive or negative donors. The first studies employed cultures of autologous cells or of homologous mono-

cytes and lymphocytes which did not induce lymphocyte transformation in the absence of added antigen (i.e., compatible cultures). As shown in Fig. 4, when autologous PPD-positive monocytes and lymphocytes were used, a significant level of transformation occurred with exposure of monocytes to antigen in 19 of 29 studies with thymidine-^{14}C (incorporation greater than 150% of control

TABLE III

Transformation of Lymphocytes from Tuberculin-Positive Donors by Incompatible Homologous Monocytes

Exp. No.*	Morphologic transformation of lymphocytes incubated with				Thymidine-^{14}C incorporation by lymphocytes incubated with			
	Saline	PPD	Unprimed monocytes	PPD-primed monocytes	Saline	PPD	Unprimed monocytes	PPD-primed monocytes
	% transformed	*% transformed*	*% transformed*	*% transformed*	*μμmoles/3 × 10⁶ cells*	*μμmoles/3 × 10⁶ cells*	*μμmoles/3 × 10⁶ cells*	*μμmoles/3 × 10⁶ cells*
1	3.5	10.2	10.5	15.0	22.0	49.2	28.1	45.4
2	6.0	12.3	11.2	9.0	20.4	33.3	29.1	37.1
3	2.7	8.6	8.5	6.0	12.8	29.2	67.0	62.4
4	1.8	9.0	—	—	21.9	31.8	156.2	35.7
5	1.8	9.0	5.3	8.5	21.9	31.8	51.4	52.5
6	4.1	7.3	12.8	16.0	35.2	95.8	116.1	154.5
7	3.8	8.8	28.0	35.0	—	—	27.9	27.7
8	7.0	20.1	16.2	21.0	19.3	87.2	84.3	81.4
9	3.5	10.2	9.6	6.4	22.0	49.2	27.5	39.5
10	3.5	10.2	14.0	10.0	22.0	49.2	31.1	33.3
11	4.1	7.3	14.1	18.5	35.2	95.8	256.1	147.2
12	4.1	7.3	10.0	11.5	35.2	95.8	122.3	108.2

* In Experiments 1–6, the homologous monocytes were from *tuberculin-positive* donors, and in Experiments 7–12, from *tuberculin-negative* donors.

values) or 16 of 24 morphologic studies (increment in transformation greater than 4%). In stimulated cultures, thymidine-^{14}C uptake ranged from 155 to 660% of control values, and the increment in morphologic transformation above control levels from 4.2 to 21%.

When monocytes from a tuberculin-positive subject were exposed to PPD, then added to compatible lymphocytes from another tuberculin-positive donor, a convincing level of transformation occurred only once in seven experiments (thymidine-^{14}C incorporation, 182% of control values). In two other experiments (Fig. 4) equivocal stimulation was measured morphologically. In five similar experiments with monocytes from a tuberculin-negative subject, no clear-cut evidence of transformation was obtained.

Such studies on mixed leukocyte populations that do not produce mutual stimulation may induce a bias toward selecting lymphocyte populations with

low capacity for transformation. Consequently, we examined our data on cultures of monocytes and incompatible homologous lymphocytes. The results, shown in Table III, demonstrate no consistent difference between the effects of unprimed and PPD-primed monocytes.

The preceding experimental design was used to determine whether tuberculin-positive monocytes exposed to PPD can induce tuberculin-negative lymphocytes to transform. In none of the six experiments was there evidence of lymphocyte transformation.

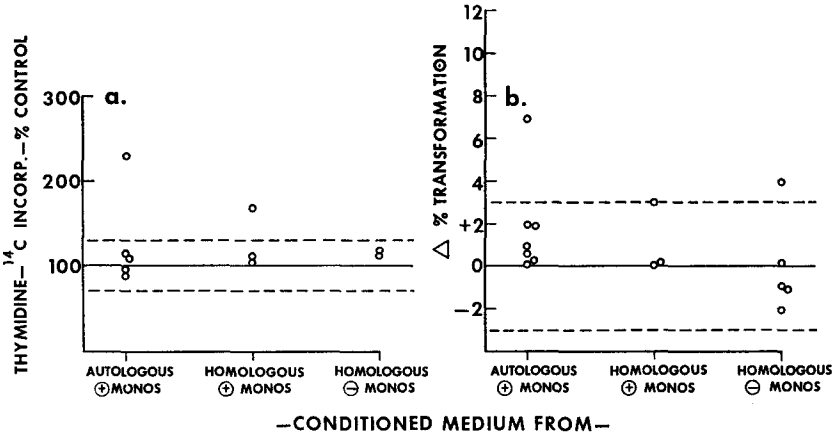

FIG. 5 *a* and *b*. Effect of conditioned medium derived from autologous and homologous monocytes on transformation of tuberculin-positive lymphocytes. (*a*) Thymidine-^{14}C incorporation; and (*b*) morphologic transformation. ⊕ indicates a tuberculin-positive donor; ⊖ indicates a tuberculin-negative donor. The limits of the range of control vaues are shown.

Cell-Cell Interaction and Conditioned Medium

When tuberculin-positive monocytes were exposed to PPD, washed and incubated with autologous lymphocytes for 5 or 6 days, the transformed lymphocytes were often seen as clusters or islands surrounding a central core of one or more monocytes (Fig. 6). At that time the cultured monocytes were easily identified by their characteristic morphology. They were large pale-staining cells, often with vacuolated cytoplasm, and lacked the prominent nucleoli and deep blue cytoplasm of transformed lymphocytes. The clustering of lymphocytes suggested that direct contact between monocytes and lymphocytes might be critical to the induction of transformation. To test this hypothesis, conditioned media were harvested at various intervals from preparations of autologous tuberculin-positive monocytes or homologous tuberculin-positive or -negative monocytes which had been incubated with either saline or antigen. The media were added to cultures of tuberculin-positive lymphocytes and tested for their ability to induce transformation. The results of 16 such studies are shown in Fig. 5.

In only two of these experiments was there clear evidence of lymphocyte transformation. The results were equally unsuccessful when the basic experiment was repeated with a variety of permutations, including variations in the duration of incubation of monocytes with PPD and with antigen-free medium and variations in the concentration of conditioned medium used for lymphocyte culture.

As an additional approach to the requirement for direct cell contact, a series of experiments were performed in which lymphocytes were separated from monocytes by a Millipore filter impermeable to cells. Simultaneous control studies consistently demonstrated that PPD placed in one chamber could transverse the membrane and stimulate the lymphocytes in the other chamber. However, PPD-primed monocytes in one chamber failed to stimulate autologous lymphocytes in the second chamber.

DISCUSSION

Monocytes and tissue macrophages appear to be part of a spectrum of a single cell type (15–19). Evidence for this concept is based on the demonstration of a continuum of cellular differentiation from the relatively small monocyte with low levels of hydrolytic enzymes and primarily glycolytic metabolism to the larger macrophage rich in acid hydrolases and containing abundant mitochondria. The present observations on the ability of monocytes to interact with lymphocytes in the induction of transformation by an antigen is consistent with the thesis of a close relationship between the monocyte and the macrophage. Most previously reported studies on the involvement of mononuclear phagocytes in the response to antigen have employed peritoneal macrophages from animal sources (2–10). Leukocytes derived from the peritoneal cavity often represent a considerable mixture of cell types, whereas peripheral blood monocytes have the advantage of being available in high purity (12, 14). A simple test for the purity of such preparations is their ability to phagocytize particulate matter. Most investigators would agree that such phagocytosis is not a property of the blood lymphocyte. Hence, these two key cellular elements in the immunologic response are readily distinguishable.

The present experiments were undertaken to define the in vitro conditions necessary for the induction of lymphocyte transformation by monocytes. The minimal concentration of PPD required to induce transformation of lymphocytes in mixed cell cultures (95% lymphocytes and 5% phagocytes) appears to be comparable to that needed to sensitize monocytes alone. Once the concentration of PPD in the medium reaches 1 μg/ml, further increases to 10 μg/ml produce no greater sensitization of monocytes.

The time of exposure to PPD required to sensitize monocytes so that they are subsequently capable of inducing lymphocyte transformation appears to be brief: 2 hr, and in some cases even less, suffices. This brief time period is consistent with the demonstration that immunogenic RNA may be extracted from

macrophages after a 30-min exposure to antigen (3, 6, 13) and the suggestion that the RNA species involved remains stable for 2–3 hr (6).

Our experiments indicate that living and metabolically active monocytes are necessary for optimal induction of lymphocyte transformation. We made several unsuccessful attempts to determine whether synthesis of new RNA by monocytes was critical for the subsequent stimulation of lymphocytes. Unfortunately, treatment of monocytes with actinomycin D resulted in the death of the lymphocytes subsequently added to the culture, despite vigorous efforts to remove any actinomycin not bound to the cells.

In our experiments, direct contact between monocytes and lymphocytes seemed requisite for consistent and maximal stimulation of the latter cells. Similar observations have been reported by other investigators (1). Direct contact was associated with islands of transforming lymphocytes around central monocytes. Attempts to demonstrate lymphocyte transformation by cell-free media derived from monocytes were in general unsuccessful. How this observation can be reconciled with the documented ability of cell-free macrophage RNA to induce immunoglobulin production by lymphocytes is not clear. Of several possible explanations, the most obvious is that immunoglobulin production and morphologic (and metabolic) transformation are not equivalent processes. Technical failure is another possible explanation. For example, destruction of unprotected monocyte RNA may occur in the extracellular media. Monocytes are rich in hydrolytic enzymes including ribonuclease, which in most circumstances is an exceedingly stable enzyme. Death of monocytes might therefore result in low levels of extracellular ribonuclease. The discrepancy between the ability of immunogenic RNA to induce lymphocyte immunoglobulin production and the inability of cell-free media from monocytes to induce transformation also raises the question of the relevance of these models to in vivo immunologic response. Are lymphoid cells in vivo likely to encounter the appropriate concentrations of macrophage RNA? If so, is direct cell contact a more efficient mode of transfer of the macromolecule? Unfortunately, the answers to these questions are not yet available.

An important problem is that of the specificity of monocyte interaction with antigen. There is general agreement that lymphocyte response to a given antigen is conditioned by the previous experience of the host. Is the same true for the macrophage? The evidence bearing on this point is indirect. Macrophages obtained from animals infected with certain facultative intracellular parasites behave differently from cells from uninfected animals (25–27). The altered response, however, lacks specificity and is directed against several microorganisms. Furthermore, it is not clear whether the altered response is attributable to serum factors or to a modified cell, for example, one with a greater content of hydrolases or peroxidases.

In studies of the specificity of monocyte interaction with antigen, we ex-

amined the behavior of monocytes from tuberculin-positive and tuberculin-negative donors. All these experiments suffered from the limitation that the cultures contained leukocytes from genetically dissimilar individuals. With this limitation in mind, we interpret our results as follows. Antigen-specific transformation of lymphocytes by monocytes occurs only when both cell types are derived from the same individual. When monocytes and lymphocytes are from different individuals, lymphocyte transformation cannot be shown to be dependent on PPD. Whether monocytes from tuberculin-positive and -negative subjects differ in their behavior toward PPD and lymphocytes therefore cannot be answered by this experimental technique. The answer presumably must await studies with cells from highly inbred animals or preferably identical twins.

In view of the preference of monocytes for autologous lymphocytes, the lack of response of tuberculin-negative lymphocytes to monocytes previously exposed to PPD is not surprising. The use of immunogenic RNA to stimulate previously uninstructed lymphocytes avoids the problem of direct cell contact.

From separate studies on the uptake of ^{125}I-labeled PPD by monocytes, we have been able to calculate that on the average each cell contains less than 1×10^{-9} μg of PPD after washing and at the time of exposure to lymphocytes. Therefore, under the usual conditions of our experiments, the lymphocytes in a culture tube would be exposed to less than 0.3×10^{-4} μg/ml of PPD. This amount is far less than the amount required to stimulate a population of lymphocytes containing few monocytes (5% phagocytes and approximately 0.4% monocytes). This observation has led us to the thesis, also advanced by others, that monocytes may function as amplifiers in that part of the immune response concerned with initial recognition and processing of antigen.

SUMMARY

Monocytes isolated from the peripheral blood of tuberculin-positive and tuberculin-negative donors were exposed to PPD, extensively washed, and incubated with autologous or homologous lymphocytes. Lymphocyte transformation was measured morphologically and by incorporation of ^{14}C-labeled thymidine.

Monocytes from tuberculin-positive subjects induced transformation of autologous lymphocytes in 19 of 29 experiments. Studies to define the optimal conditions of exposure to monocytes to PPD and to autologous lymphocytes showed that viable, metabolically intact monocytes are required. A ratio of only 1 monocyte to 100 lymphocytes sufficed to induce transformation; neutrophils were inactive. In general, PPD-sensitized monocytes failed to induce transformation of homologous lymphocytes from either tuberculin-positive or tuberculin-negative subjects. Direct contact between monocytes and lymphocytes was required for consistent transformation, and islands of transforming lymphocytes were observed around a central core of monocytes.

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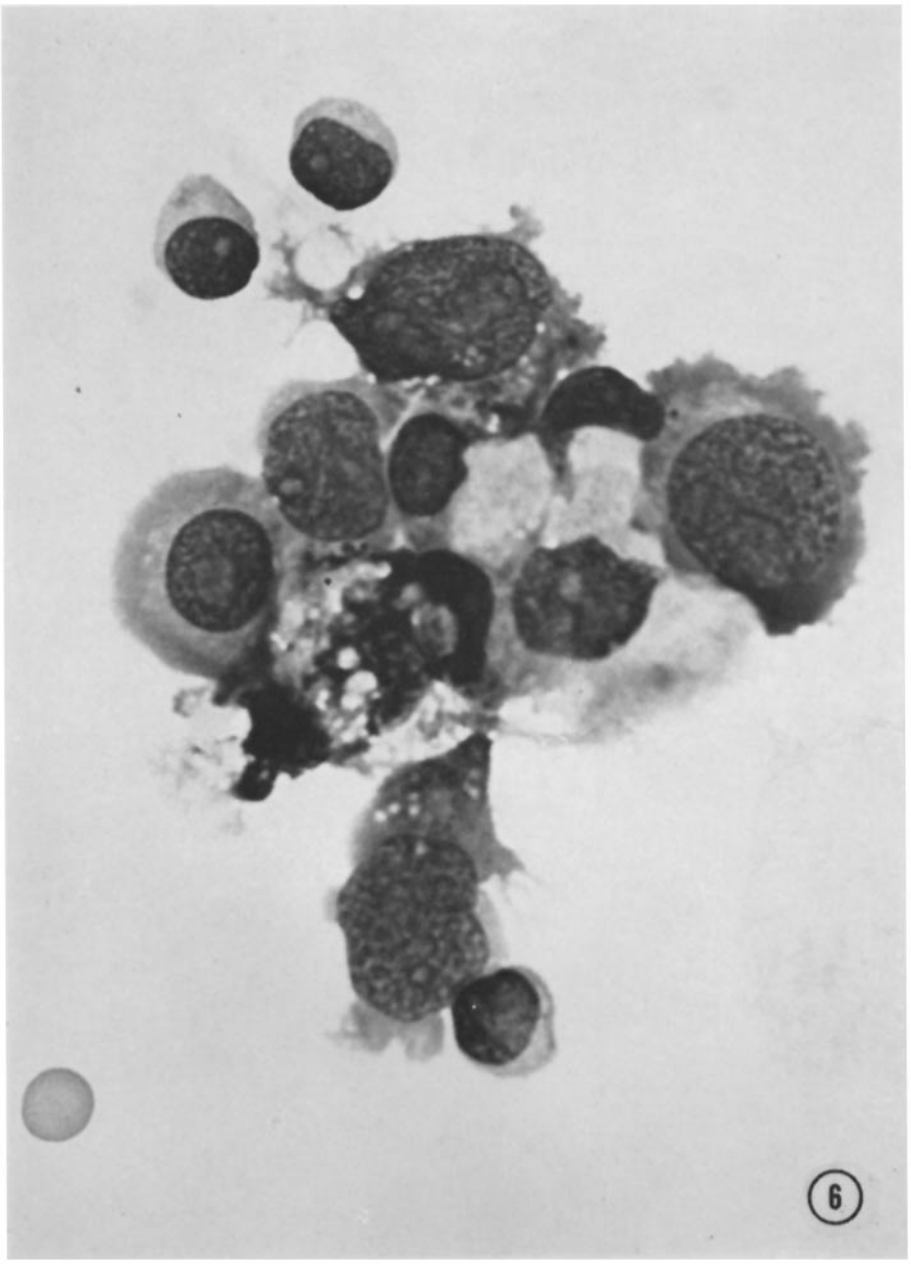

FIG. 6. Transforming lymphocytes surrounding central monocytes after 6 days of culture. The monocytes are identified by their lightly stained cytoplasm and absence of round nuclei containing prominent nucleoli. Wright's stain, × 1000.