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Requirement of Macrophage Metabolic Activity for T-Lymphocyte Activation

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It has been recently demonstrated that macrophages are necessary accessory cells for T-lymphocyte activation by antigens, mitogens and allogenic cells. This paper shows the function of metabolically inactive macrophages in the activation of T-lymphocytes by phytohemagglutinin (PHA). Macrophages inactivated by mitomycin C are unable to collaborate with T-lymphocytes in the development of the proliferative response to PHA indicating that the role of the macrophage in T-lymphocyte activation by mitogens goes beyond insolubilizing it or rendering it more stimulatory by another passive mechanism.

Research in the past few years has demonstrated that macrophages are necessary accessory cells for T-lymphocyte activation by antigens (10, 14, 15), mitogens (2, 12, 13), and allogenic cells (1), however, the mechanism by which macrophages collaborate in these processes have not been established yet.

In order to further clarify how the macrophages collaborate in T-lymphocyte activation, we have studied the function of metabolically inactive macrophages in the stimulation of these lymphocytes by phytohemagglutinin (PHA).

Materials and Methods

Animals. 2 to 3 month old mice of the CBA/H strain were used. They came from the London Medical Hospital Research Laboratories and were bred in our laboratory.

Culture medium. All cultures were performed in RPMI (Difco) containing added ampicillin (100 μ/ml), cloxacillin (100 μg/ml), and gentamycin (5 μg/ml), as well as AB+human serum, previously heat inactivated to a final concentration of 5 %. The pH was adjusted between 7.2-7.4 with 4.4 % sodium bicarbonate (Wellcome).

Cell preparation. The macrophages were obtained from the peritoneum of mice sacrificed by cervical dislocation. They were injected i.p. with 4 ml of the above culture medium. After massage of

the abdomen, the medium was removed and the cell concentration adjusted to 5×10^5 macrophages/ml. The lymphocytes were obtained from the spleen of singenic animals also killed by cervical dislocation. The spleen was removed aseptically, freed of fat, minced with scissors and gently pressed through a mesh screen to produce a single cell suspension. This cellular suspension was centrifuged in a gradient of Urograph-Ficoll with a density of 1076 (3). The halo was resuspended in the medium and washed twice in PBS. These cells were partially purified by passage over an adherence column of nylon wool. The eluted spleen lymphocytes were then further purified by incubation (45 min, 37° C) in a packed nylon-wool adherence column. The non adherent cells were obtained by slow elution without compressing the wool (4, 12).

Macrophage culture. Macrophages were prepared in «Limbro multi-disposal» plates in aliquots of 0.1 ml at a concentration of 5×10^5 macrophages/ml.

After 2 hours incubation in an atmosphere of 5 % CO_2 at 37° C, the non-adherent cells were removed by several washings with culture medium leaving behind the attached macrophage monolayers. 100 μl of culture medium and 10 μg of ^{14}C-uridine (57.8) μCi/mMol; 2 μCi/ml) were added to the wells, incubated for another 16 h as described above and then washed three times with PBS. The plates were left overnight to dry, the wells cut, transferred into vials containing 10 ml of scintillation fluid (5 g of PPO per liter of toluene) and then counted on a liquid scintillation counter (LKB 1215).

Where indicated the macrophages were labelled with 10 μl of ^{51}Cr (sodium chromate; 0.1 mCi/ml) and counted on an gamma counter (LKB 1580).

Mitomycin C treatment. Mitomycin C was dissolved in medium to a final concentration of 40 μg/ml. The adherent cells were incubated for 0, 1, 2, 4, 8 and 18 h in this suspension, then washed 5 times with the culture medium and further incubated for another 16 h, as indicated above.

Lymphocyte culture. The lymphocytes were dispensed in a Limbro plate where the treated and non-treated macrophages had previously been added to. The number of cells were 2×10^4 macrophages/well and 2×10^5 lymphocytes/well stimulated with the phytomitogen PHA (0.24 μg/ml) and then incubated for 48 h in a 5 % CO_2 atmosphere at 37° C. At the end of the incubation period, they were pulsed with 20 μl ^{3}H-thymidine (28 μCi/mmol; 8 μCi/ml) for 16 h, harvested in a semi-automatic harvester and counted in a LKB 1215 scintillation counter.

Results

Effect of mitomycin C on the ^{14}C-uridine uptake by the macrophages. ^{14}C-uridine uptake by the macrophages decreased as the incubation period with mitomycin C

Table I. *Effect of Mitomycin C treatment on ^{14}C-uridine and ^{51}Cr uptake by peritoneal macrophages (5×10^4 macrophages/well).*

Incubation period (h)	Mitomycin C treatment (40 μg/ml)	^{14}C-uridine uptake * (c.p.m.)	^{51}Cr uptake * (c.p.m.)
0	—	1735 ± 133	9454 ± 363
1	—	1739 ± 237	8337 ± 1287
	+	1613 ± 139	9104 ± 871
2	—	1776 ± 193	8597 ± 781
	+	1300 ± 83	8294 ± 1332
4	—	2026 ± 217	8220 ± 782
	+	1044 ± 189	8487 ± 406
8	—	1613 ± 128	7224 ± 892
	+	411 ± 85	7990 ± 1177
18	—	2058 ± 157	7891 ± 523
	+	175 ± 58	7426 ± 851

* Each value is the mean ± S.D. from 5 experiments.

increased, while this uptake was not affected at all when the macrophages were left untreated (table I). On the other hand, the amount of cell in the culture did not significantly vary, as shown by their labeling with ^{51}Cr, an unspecific cellular tracer. Their uptake did not show any significant variation whether treated or not with mitomycin C.

Restoration of PHA induced lymphoblastic response by untreated and mitomycin C treated macrophages. When the lymphocytes are macrophage depleted there is no response to PHA. When normal macrophages are added to these lymphocytes, there is a significant increase in the ^{3}H-thymidine uptake. However, macrophages treated with mitomycin C were unable to restore PHA induced lymphoblastic response (fig. 1, *A*).

The response became positive once again as fresh macrophages were added to the combination of lymphocytes and mitomycin C treated macrophages. The response of the lymphocytes and untreated macrophages was not significantly affected by the addition of these fresh macrophages (fig. 1, *B*).

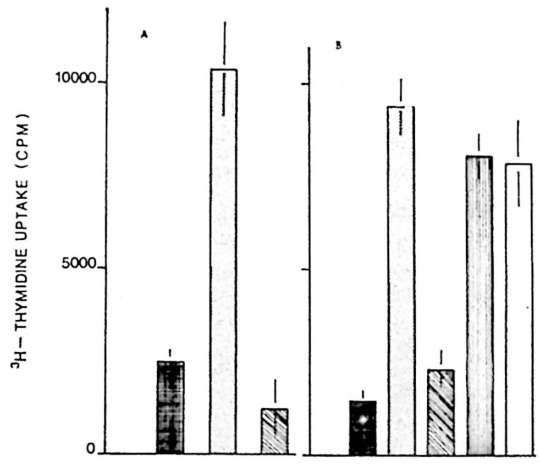

Fig. 1. *^{3}H-Thymidine uptake by purified spleen T-lymphocytes (2×10^{5}/well) stimulated with PHA (0.28 μg/ml) and incubated alone (squared column) or in the presence of untreated (dotted column) or mitomycin C treated (inclined lines column) macrophages.*
A. Macrophages (2×10^{4}/well). *B*. Macrophages (10^{4}/well). Additional fresh macrophages (10^{4}/well) were added to the purified lymphocytes incubated with the untreated macrophages (vertical lines column) or with the mitomycin C treated macrophages (empty column). The ^{3}H-Thymidine uptake by untreated or mitomycin C treated macrophages was always less than 1,000 c.p.m. Each value is the mean ± S.D. from 3 experiments.

Discussion

Effect of mitomycin C on the uptake of ^{14}C-uridine by the macrophages. Our results confirm previous experiments which show that the macrophages actively incorporate ^{14}C-uridine (9), mostly in RNA (5). This uridine uptake decreases as the incubated period with mitomycin C increases. This does not happen when the macrophages remain untreated. The decrease in the uridine uptake is not due to a diminution in the number of cells, as is demonstrated by the uptake of ^{51}Cr. Thus, the decrease in the uridine uptake may be due to the action of mitomycin C which blocks the transcription from DNA to RNA (6).

Restoration of PHA induced lymphoblastic response by untreated and mitomycin C treated macrophages. Lymphocytes previously depleted of macrophages did not proliferate in response to PHA. When the macrophages were added this proliferation appeared. However, mitomycin C treated macrophages were unable to restore the response.

In order to see if these results were due to a contaminating effect of mitomycin C left after washing, non treated macrophages were added to the cultures and studied again. The response became positive as these macrophages were added

to the group of lymphocytes and mitomycin C treated macrophages. The response of the group containing lymphocytes and untreated macrophages was not significantly altered (fig. 1, *B*).

Metabolically inactivated macrophages lose their capacity to collaborate with T lymphocytes in PHA induced proliferation response, suggesting that the function of the macrophages in the lymphocyte activation by the mitogens involve more than merely insolubilizing it or somehow rendering it more stimulatory by passive mechanisms. From our results we can suppose that metabolically active macrophages may be necessary for the synthesis and release of factors which activate the lymphoblastic process. These factors may act upon the mitosis of the lymphocytes sensitized to respond because of their previous binding to the mitogens, as has been recently suggested (7, 8, 11). Thus, the macrophages with a blocked DNA dependent RNA synthesis would be unable to provoke these factors, resulting in an incapacity to reconstitute the proliferation response induced by PHA, as is supported by our results.

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Resumen

Se estudia la capacidad de los macrófagos para colaborar con los linfocitos en el desarrollo de su respuesta inducida por la fitohemaglutinina (PHA).

Los macrófagos inactivados metabólicamente por tratamiento con mitomicina C son incapaces de colaborar con los linfocitos T en su respuesta proliferativa a la PHA. Esto apoya la hipótesis de que el papel del macrófago en la estimulación de los linfocitos T por mitógenos es algo más que insobulizarlos o volverlos inmunógenos por un mecanismo pasivo.

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