Cell Transfer Studies in Immunosuppressed Mice: The Role of the Macrophage (34144)[1]

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Evidence is accumulating that macrophages play an active role in the initial processing of antigen during the inductive phase of the primary immune response. The product (mRNA, RNA-antigen complex, or processed antigen) released through cell interaction, stimulates competent cells of the lymphoid series to secrete antibody. Much of the pertinent literature has been recently reviewed by Sulitzeanu (1). The work of Gallily and Feldman (2) was of special interest due to their utilization of a cell transfer system in which the recipients were immunosuppressed by irradiation. Macrophages from normal mice which had engulfed *Shigella* antigen *in vitro* were capable of eliciting a specific agglutinin response in X-irradiated recipients.

The cell transfer technique was used for studying the hemagglutinin and splenic plaque response of mice given immunosuppressive drugs. Cycloleucine (1-aminocyclopentane-carboxylic acid) was selected since it, like X-irradiation (3, 4), was known to be inhibitory when administered up to 12 days before antigenic stimulation. A cyclophosphamide, Cytoxan (Mead-Johnson Laboratories), was chosen as the contrasting drug; it was most effective during the 18–36-hr period after administration of antigen (5). Additional data suggested that the inhibitory activity of cycloleucine was directed against an early inductive event while Cytoxan appeared to influence antibody synthesis (5, 6). The following experiments were designed to test the capacity of macrophages from normal or drug-treated animals to elicit antibody synthesis in cycloleucine or Cytoxan-treated recipients. The results provide additional evidence in favor of a distinctive antigen-processing role for the macrophage during the primary immune response.

Materials and Methods. Animals. Initially, C57B1/6 male mice, 10 weeks old, were selected (2). Normal serum from this strain characteristically hemagglutinated sheep erythrocytes to a high titer but hemolytic plaque-forming cells were absent from their spleens. Therefore, in the irradiation experiment, testing was limited to the plaque assay procedure. Ten-week-old C_3H mice were substituted as both donors and recipients in the drug experiments.

Immunosuppression. A dose of 350 mg/kg of cycloleucine or 200 mg/kg of Cytoxan was given intraperitoneally in H_2O. The former drug was injected 2 days before antigen, the latter 1 day before antigen. These schedules insured that inhibitory concentrations of both drugs would be available to target cells before and after contact with antigen (5, 6). Mice were exposed in partitioned Lucite containers to total body X-irradiation of 550 R (277 kV, 68 r/min, 15.8 mA) at a distance of 50 cm.

Cells. Peritoneal macrophages were obtained by a modification of the method of Bang (7). Each animal was injected intraperitoneally with 3 ml of thioglycollate medium (Difco). Four days later the mice were killed by cervical dislocation, the peritoneum was opened and a total of 5 ml of iced medium (Hanks' BSS with 1% calf serum, penicillin, streptomycin, and heparin; 100 U, 50 g, and 10 USP units, respectively) was injected. The medium was aspirated with a plastic syringe fitted with a blunt needle. Cells were

[1] Supported by Research Grant AI 02661 and Training Grant AI 00139, from the United States Public Health Service.

[2] In partial fulfillment of the requirements for the Master of Science Degree.

TABLE I. Antibody Production by Irradiated C57Bl/6 Mice Following Inoculation of Macrophages or Spleen Cells.

Recipient	Plaque-forming units /10^6 spleen cells[a]
Irradiated	
Normal macrophages + sheep RBC	157
Normal spleen cells + sheep RBC	4
Sheep RBC alone	8
Nonirradiated	
Sheep RBC	492

[a] Each value represents triplicate counts from a pool of three spleens.

counted in a hemocytometer, centrifuged in the cold at 1000 rpm for 10 min and resuspended in medium 199 at a concentration of 30×10^6 cells/ml. Each test recipient was injected intraperitoneally with a mixture of 0.5 ml of exudate cells and 0.5 ml of 2% sensitized sheep erythrocytes (8). Differential counts indicated that at least 90% of the peritoneal cells were macrophages. Viability was checked by the trypan blue dye exclusion test. Spleens were removed and a suspension was made using a Snell cytosieve and cold medium 199. The cells were handled in the same manner as the macrophages from this point on. The antigen consisted of sheep erythrocytes preserved in Alsever's solution, washed in 3 vol of saline, and administered intraperitoneally as a 2% solution in medium 199.

Antibody assay. Antisheep red blood cell agglutinins were determined as previously reported (9). Jerne plaque assays were done according to the method of Jerne *et al.* (10). Each splenic suspension was plated in triplicate and the mean number of plaques/million cells was recorded.

Results. The first experiment was an attempt to elicit an immune response with normal C57Bl/6 macrophages injected together with antigen into previously irradiated animals. Measurement of antibody was limited to the plaque assay procedure. One set of irradiated mice received normal macrophages plus antigen; one set was given a similar number of spleen cells and antigen; a third group was injected with sheep erythrocytes alone. The nonirradiated controls also received sheep erythrocytes. The assays, recorded in Table I, were done 5 days after antigen administration. A 20-fold increase in the number of hemolytic plaques was observed in the mice given macrophages and antigen as compared with the irradiated controls. The results confirmed those of Gallily and Feldman (2) and encouraged us to proceed with the drug studies.

The remaining experiments were similar except that C_3H mice were used in combination with immunosuppressive drugs. The results of three separate experiments, compatible by *t* test, were pooled and summarized in Table II. The objective was to determine whether macrophages from normal or Cytoxan-treated donors could elicit antibody formation in cycloleucine-suppressed recipients. For this purpose one group was injected with normal macrophages and antigen; a second group received macrophages from Cytoxan-treated donors and antigen; the control group was given antigen alone. Each animal was bled from the orbital sinus at 5 and again at 8 days after antigen administration. Individual hemagglutinin titrations were performed and the mean value to the $\log_2$ was determined. The level of significance was calculated by *t* test. After 5 days, the hemagglutinin titer in mice given normal macrophages was significantly elevated over that observed in the control group given antigen alone. The Cytoxan-treated macrophages behaved in a similar manner. In both instances, titers in the experimental group were definitely increased but not to the level seen in nonsuppressed controls.

The third set of experiments tested the ability of normal macrophages or spleen cells to elicit a hemagglutinin response in Cytoxan-treated recipients. For this purpose 200 mg/kg of Cytoxan, a dose adequate to inhibit the immune response (6), was administered to all recipients 1 day prior to antigen. The first group, a pool of three separate experiments compatible by *t* test, was given normal macrophages plus sensitized antigen; the second received spleen cells and antigen;

TABLE II. Increased Antibody Production in Cycloleucine Suppressed C_3H Mice by Macrophage-Antigen Mixtures.

Recipients	No. of expts.	Hemagglutinin titer after 5 days: No. of mice	Mean titer $\log_2$	SE	Hemagglutinin titer after 8 days: No. of mice	Mean titer $\log_2$	SE
Suppressed							
Normal macrophages + sheep RBC	3	21	5[a]	0.27	18	5.4[c]	0.38
Cytoxan macrophages[e] + sheep RBC	1	5	4.8	0.66	2	7.5	0.5
Sheep RBC alone	3	20	2.3[b]	0.27	19	3.7[d]	0.26
Normal							
Sheep RBC alone	1	10	7.8	0.20	10	8	0.21

[a,b] Significant at .001 level.
[c,d] Significant at .01 level.
[e] From donors given 200 mg/kg of Cytoxan 1 day prior to transfer.

and the third, also a pool, was injected with antigen alone. The results in Table II indicate that hemagglutinin production in the Cytoxan-suppressed mice was not augmented by supplying normal macrophages. There were no significant differences between the control and the experimental groups by *t* test. Normal spleen cells, as previously reported by Santos for rats (11), did elicit an immune response in the Cytoxan-suppressed mice.

The purpose of the final experiment was to determine if macrophages from cycloleucine-treated animals were less effective than normal macrophages in stimulating an antibody response in cycloleucine-suppressed mice. The data, presented in Table IV, include both hemagglutinin titers and plaque assays carried out 5 days after antigen administration. Transfer of macrophages from cycloleucine-treated donors to cycloleucine-suppressed recipents failed to elicit the characteristic antibody rise produced by similarly treated normal macrophage–antigen mixtures. As expected, the normal spleen cell and antigen controls were ineffective. Direct observations revealed that normal and cycloleucine-treated macrophages were equally viable

TABLE III. The Effect of Macrophages and Spleen Cells from Normal Donors on the Production of Antibodies in Cytoxan Suppressed Recipients.

Suppressed recipients	No. of expts.	Hemagglutinin titer after 5 days: No. of mice	Mean titer $\log_2$	SE	Hemagglutinin titer after 8 days: No. of mice	Mean titer $\log_2$	SE
Normal macrophages + sheep RBC	3	17	1.9[a]	0.19	15	1.5[c]	0.25
Spleen cells + sheep RBC	1	5	4.4	0.21	4	4.3	0.25
Sheep RBC alone	3	18	1.5[b]	0.26	15	2.3[d]	0.29

[a,b] Not significant.
[c,d] Significant at 0.1 level.

TABLE IV. Antibody Production in Cycloleucine Suppressed Recipients; Effect of Macrophages from Cycloleucine Treated C_3H Mice.

Recipients	No. mice	Mean titer $\log_2$	SE	Plaque-forming units/10^6 spleen cells
Suppressed				
Cycloleucine macrophages[f] + sheep RBC	10	3.0[c]	0.36	2.66
Normal macrophages + sheep RBC	10	6.1[a]	0.23	11.33
Normal spleen cells + sheep RBC	10	3.1[d]	0.28	5.95
Sheep RBC alone	10	2.3[b]	0.36	0.58
Normal				
Sheep RBC alone	10	7.8[e]	0.20	87.5

[ab, ac, ad, ae] Significant at .001 level.

[be] Not significant, difference between *b* and *d* not significant.

[ce] Significant at .001 level.

[f] From donors given 350 mg/kg of cycloleucine 2 days prior to transfer.

and capable of engulfing large numbers of sensitized erythrocytes. The data indicate that an antigen-processing defect is induced by the drug and that X-rays (*2*, 12–14) and cycloleucine (5) act similarily on macrophages. Whether or not other cells are also affected (15), remains to be established.

Summary. In cell transfer experiments C_3H recipients, which had been immunosuppressed by cycloleucine, were capable of initiating a primary response to sheep erythrocytes if they were given macrophage–antigen mixtures from normal or from Cytoxan-treated donors. Similarly prepared macrophages from cycloleucine-treated donors and crude cell suspensions derived from normal spleens were ineffective. If the recipients were immunosuppressed with Cytoxan, the results were reversed, in that normal spleen cell–antigen mixtures stimulated antibody synthesis but normal macrophage–antigen mixtures did not. The data which were obtained with drug-treated and with X-irradiated recipients support the concept that antigen processing by the macrophage is necessary for initiating a primary type immune response. They also demonstrate the selective susceptibility of macrophages and spleen cells to immunosuppressive drugs.

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Received April 28, 1969. P.S.E.B.M., 1969, Vol. 132.