The Effect of Antimacrophage Serum on Macrophage–Lymphocyte Interaction *in Vitro*[1] (34767)

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Macrophages are thought to mediate the primary immune response to certain antigens by binding antigen to RNA and transferring this product to lymphocytes (1, 2). This has been shown for macrophages obtained from lymph nodes (3) and peritoneal exudate (4) and recently for leukocytes from peripheral blood as well (5). The secondary immune response to specific antigens such as tuberculin, Candida extract, and streptokinase as measured by lymphoblastic proliferation of small lymphocytes *in vitro* is also thought to be mediated by macrophages (5), although the *in vivo* secondary immune response resulting in antibody production may not be (6). Nonspecific stimulants such as phytohemagglutinin and antilymphocyte serum probably induce lymphoblastic proliferation without mediation by macrophages (5, 7).

In studying the effect of antimacrophage serum on blast transformation of lymphocytes from peripheral blood of guinea pigs we have found evidence that macrophages are necessary for a secondary immune response to specific antigens but do not play a role in the response to nonspecific stimulants.

Material and Methods. A potent antiserum to guinea pig macrophages (AMS) was prepared in rabbits according to the method of Unanue (8). This AMS inhibited the attachment of macrophages to glass at a dilution of 1:1280. AMS was repeatedly adsorbed on highly purified preparations of lymphocytes; leukagglutination titer (9) following adsorption was negative.

Outbred adult male guinea pigs were sensitized 2 or 3 times at weekly intervals with 0.1 ml of reconstituted BCG vaccine (Lilly) subcutaneously. Other guinea pigs received either 0.5 ml of streptokinase-streptodornase (SKSD, Lederle) or 0.5 ml of *Monilia albicans* extract (Candida, Hollister-Stier) in incomplete Freund adjuvant subcutaneously. Skin tests with the appropriate antigen were positive 2 weeks after the last booster injection.

Heparinized peripheral blood was obtained from the sensitized animals by heart puncture. Leukocytes were separated by sedimentation in 3% dextran and further purified as described by Bach and Hirschhorn (10). Cell suspensions (1.5×10^6 cells/1.5 ml of tissue culture medium, MEM-Difco) consisting of 85 to 90% mononuclear cells were cultured for 3 or 6 days. Phytohemagglutinin-M (PHA, 0.01 U; Difco) was added to the 3 day cultures; SKSD (100 U), Candida (10 U), or PPD (10 μg) was added to the 6-day cultures. AMS (0.2 ml) or an equivalent amount of normal rabbit serum (NRS) was added to both 3- and 6-day cultures. Three hr before termination of the cultures, cells were exposed to ^{3}H-thymidine (sp act 6.7 Ci/mmole, 1 μCi/1.5 ml). DNA was extracted with 5% trichloracetic acid, washed with absolute methanol, dissolved in 0.1 *N* NaOH, added to Bray's solution and counted in a liquid scintillation counter.

Results. AMS, in doses ranging from 0.05 to 0.8 ml added to the cultures had no effect on the ^{3}H-thymidine uptake by lymphocytes at 3 or 6 days of culture (Table I). Phase-

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TABLE I. ^{3}H-Thymidine Uptake (cpm) of Lymphocytes following Exposure to Varying Qualities of AMS.

The values indicate that AMS had no effect on lymphocytes compared with the unstimulated control cultures.

Culture (day)	Unstim. control	AMS ml: 0.05	0.1	0.4	0.8
3	1485	2910	1767	1166	1833
6	1625	1764	2598	1811	1909

contrast microscopy showed marked vacuolization and agglutination of macrophages without visible changes in lymphocytes. Trypan blue stained macrophages at the concentration of AMS used in these studies.

PHA increased the ^{3}H-thymidine uptake of cultured lymphocytes 90-fold. This effect was not altered by the addition of AMS. PPD, SKSD, and Candida stimulated lymphocytes to a lesser extent than did PHA. The addition of AMS to cultures containing specific antigens reduced ^{3}H-thymidine uptake to control levels. NRS did not produce any consistent change in the effect of specific or nonspecific antigens as demonstrated in control cultures (Table II).

Discussion. These results indicate that peripheral lymphocytes in tissue cultures react differently in the presence of AMS to stimulation by specific or nonspecific mitogens. Antigens such as PPD, SKSD, and Candida cause lymphocytes from a previously sensitized individual to undergo blast transformation; this effect is abolished by the presence of AMS. PHA, a nonspecific mitogen, stimulates transformation of lymphocytes without prior sensitization; this effect is not altered by AMS. Our findings suggest that viable macrophages must be present for the processing of specific antigens in the secondary immune response but need not be present for the induction of blast transformation by nonspecific mitogens. Whether macrophages process antigen and then transfer it by intimate contact to lymphocytes, or whether under antigenic stimulation they produce some substance which affects lymphocytes (11) has not been investigated in these studies.

The importance of macrophages in the development of a primary immune response *in vitro* has been demonstrated previously (1–4). Hersh and Harris have shown (5) that macrophages are necessary for the secondary immune response to PPD and SKSD *in vitro*. They used potentially damaging separation procedures (glass bead filters), a problem which has been avoided by using AMS. The recent demonstration by Pierce (6) that lymphocytes from previously sensitized mice respond to an antigen in the absence of mac-

TABLE II. ^{3}H-Thymidine Uptake by Lymphocytes (cpm) Following Stimulation with Nonspecific Mitogen and Specific Antigens in the Presence of NRS and AMS.

(A) shows that AMS did not alter the ^{3}H-thymidine uptake after stimulation with nonspecific mitogens (PHA). (B) shows that the stimulation effect of specific antigens was abolished when AMS was present.

A. Nonspecific mitogen (3-day culture)					
Unstim. cult.	PHA	AMS	PHA + AMS	NRS	PHA + NRS
1485	88,444	1166	86,102	2903	102,090
B. Specific antigens (6-day culture)					
Unstim. cult.	PPD	AMS	PPD + AMS	NRS	PPD + NRS
1559	8098[a]	1809	1999[a]	1285	9841
	SKSD		SKSD + AMS		SKSD + NRS
1764	6426[a]	1811	1046[a]	1286	7440
	Cand.		Cand. + AMS		Cand. + NRS
1634	3709[a]	1811	916[a]	1286	4303

[a] Differences are statistically significant ($p = 0.01$).

rophages relies upon a plaque-forming technique which is felt to measure an immune response associated with circulating antibodies rather than one associated with delayed hypersensitivity. Evidence that blast transformation and delayed hypersensitivity represent identical phenomena is still controversial.

In this study we have confirmed recent observations (5) that a macrophage–lymphocyte interaction occurs in cells obtained from peripheral blood. Previous reports had dealt with cells obtained from lymph nodes and peritoneal exudates. However, Bennett and Cohn (12) demonstrated that peripheral blood monocytes transform into macrophages *in vitro*, and other authors (13) showed that circulating monocytes are the source for macrophages which accumulate at an inflammatory site *in vivo*. Observations of Cline and Swett (14) have indicated an interaction between blood monocytes and lymphocytes in the induction of blast transformation after exposure to PPD.

The inhibition of antigenically induced blast transformation by AMS seems to result from destructive effects of the antiserum on macrophages or their precursors. Preliminary results have suggested that macrophages obtained from various sites in the body are antigenically similar despite their metabolic differences. Monocytes appear to be antigenically similar as well, and it is therefore not surprising for them to be attacked by AMS. Our findings add evidence that an interaction between macrophages or their precursor cells and antigen in the peripheral blood may be of prime importance in the cell-mediated secondary immune response *in vivo*.

Summary. Antimacrophage serum inhibited the ability of specific antigens to induce blast transformation of previously sensitized circulating lymphocytes, without altering their response to nonspecific mitogens.

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