Immunologically Specific Activation of Macrophages Armed with the Specific Macrophage Arming Factor (SMAF)[1] (37298)

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(Introduced by J. S. Remington)
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Macrophages from suitably immunized mice or from unimmunized mice treated with sensitized lymphoid cells *in vitro* inhibit growth of lymphoma and sarcoma cells in an immunologically specific way (1, 2). This growth inhibitory effect may be directed against both tumor specific antigens or normal transplantation antigens (3, 4, 5). When such immunologically reactive macrophages are exposed to specific target cells, the macrophages undergo a change referred to as activation, as a result of which they acquire the capacity to inhibit growth of all murine lymphoma and sarcoma cells tested in an immunologically nonspecific way (6). Macrophages from mice immunized with BCG or normal macrophages incubated with lymphoid cells from mice immunized with BCG are not growth inhibitory to tumor cells but become so after exposure to the "purified protein derivative" (PPD) of tubercle bacilli. A further method used to obtain specifically growth inhibitory macrophages is the exposure of macrophages (7) or suspensions of bone marrow cells (8) to a soluble factor referred to as the specific macrophage arming factor (SMAF) (3). SMAF is obtained when sensitized lymphoid cells are cultured with the specific antigen used for immunization, and is a product of immune T-cells (9). This report shows that SMAF-treated macrophages after exposure to the specific antigen acquire the capacity to inhibit growth of target cells in a nonspecific manner, *i.e.*, become activated in the sense defined above.

Materials and Methods. Mice. Pure-line CBA mice 8–10 wk of age were used throughout.

Cells. (a) Macrophages were obtained from the peritoneal cavity of mice, (b) lymphoma cells designated SL2 and TLX9 (10), syngeneic for DBA/2 and C57Bl mice, respectively, were harvested from the peritoneal cavity of ascites-bearing mice 7 days after implantation, (c) sheep red blood cells (SRBC), (d) BCG (Glaxo percutaneous vaccine).

Culture systems. Monolayers of macrophages from normal mice were prepared as described previously (1) and were maintained for 24 hr before use in experiments in Fischer's medium for leukemia cells (Grand Island Biological Co.) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (growth medium). Lymphoma cells obtained from ascites-bearing mice were washed $\times 3$ by centrifugation and finally suspended in growth medium at a concentration of 10^5 cells/ml. SL2 and TLX9 lymphoma cells in the present experiments divided every 13 and 24 hr respectively.

Production of specific macrophage arming factor (SMAF). CBA mice were immunized with two injections of 5×10^6 lymphoma cells separated by 10 days, or with two injections of BCG (1 ampoule of vaccine was diluted to 1 ml in saline and 0.1 ml was injected ip into each mouse). Mice were also injected with SRBC in complete Freund's adjuvant [(25% suspension SRBC mixed with an equal volume of complete Freund's adjuvant and 0.1 ml injected ip (Weissmann, personal communication)]. Spleens were removed 10 days after the second injection of lymphoma cells or BCG or 14 days after a single injection of SRBC. The spleens were

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minced in medium, fragments were allowed to sediment and the cell suspension was removed and centrifuged. After resuspension in growth medium, 2×10^7 spleen cells were mixed with 10^5 lymphoma cells/ml of growth medium, 20 ml containing 10^7/ml were mixed with 0.1 ml BCG suspension, and 10^7 cells were mixed with 10^7 SRBC/ml of growth medium. After incubation for 24 hr, the supernatants were separated from the cells by centrifugation and tested on macrophage monolayers for SMAF activity. The test of whether SMAF has bound to macrophages relies on the ability of SMAF to recognize in an immunologically specific way the antigen used to immunize the host. After such recognition the macrophage undergoes a change referred to as "activation" (6) whereby it obtains the capacity to inhibit the growth of a range of lymphoma and fibrosarcoma cells in a totally nonspecific manner. To effect this change to the activated state SMAF-armed macrophages were incubated with specific antigen, *viz*, 10^6 SL2 or TLX9 cells, 20 μg PPD or a 0.1% suspension of SRBC for 4 hr at 37°. The monolayers were then thoroughly washed to remove antigen followed by the addition of 10^5 SL2 or TLX9 cells in growth medium. Since these cells grow in suspension their numbers can be assessed at intervals by counting the cells in a hemacytometer. Growth inhibition refers to the difference between growth in test cultures and control or normal cultures and is usually expressed as a percentage.

Results. The experimental findings sum-

TABLE I. *In Vitro* Growth Inhibition of Lymphoma Cells by SMAF-Armed and Activated Macrophages.

Origin of SMAF used to arm macrophages	Exposure of armed macrophage to antigen	% Growth inhibition at 48 hr	
		SL2	TLX9
No SMAF; culture medium alone	None	<10[a]	<10[a]
	SL2	<10	<10
	TLX9	<10	<10
	SRBC	<10	<10
	BCG	<10	<10
Spleen cells from mice immunized with SL2 cells cultured with SL2 cells for 24 hr	None	79 ± 6	3 ± 2
	SL2	86 ± 4	74 ± 6
	TLX9	78 ± 6	4 ± 3
	SRBC	79 ± 3	7 ± 1
	BCG	83 ± 8	3 ± 2
Spleen cells from mice immunized with TLX9 cells cultured with TLX9 cells for 24 hr	None	3 ± 1	78 ± 2
	TLX9	82 ± 8	92 ± 4
	SL2	3 ± 1	83 ± 6
	SRBC	6 ± 2	89 ± 9
	BCG	7 ± 3	82 ± 7
Spleen cells from mice immunized with SRBC and cultured with SRBC for 24 hr	None	1 ± 1	NT[b]
	SRBC	78 ± 8	NT
	SL2	4 ± 1	NT
	TLX9	2 ± 3	NT
	BCG	6 ± 2	NT
Spleen cells from mice immunized with BCG cultured with BCG for 24 hr	None	69 ± 7	72 ± 3
	BCG	78 ± 6	84 ± 5
	SL2	73 ± 2	72 ± 4
	TLX9	82 ± 2	79 ± 6
	SRBC	69 ± 6	81 ± 2

[a] Growth of target cells on macrophage monolayers is compared with growth in cultures devoid of macrophages and expressed as a percentage.

[b] NT = not tested.

marized in Table I confirm that growth inhibition by SMAF-armed macrophages was immunologically specific in that growth of SL2 cells was inhibited only by SL2-SMAF-armed macrophages and not by TLX9- or SRBC-SMAF-armed macrophages. Similarly, growth of TLX9 cells was inhibited only by TLX9-SMAF-armed macrophages. However, after exposure of the specifically armed macrophages to the appropriate antigen the macrophages become activated and inhibited growth nonspecifically when SL2 and TLX9 cells were added. Macrophages armed with SRBC-SMAF did not inhibit the growth of SL2 lymphoma cells prior to "activation" by the specific antigen, following which they inhibited the growth of the lymphoma cells.

In contrast, macrophages armed with the BCG-SMAF were immediately growth inhibitory to both SL2 and TLX9 lymphoma cells; that is the armed macrophages did not require pretreatment with PPD. In this respect macrophages armed with BCG-SMAF differed from macrophages obtained from mice immunized with BCG or armed by direct contact with spleen cells from mice immunized with BCG since they required exposure to PPD (6) or BCG to become activated. We interpret this finding on the basis that the supernatant of the spleen cell/BCG cultures probably contains both SMAF and soluble antigen which has the same or similar properties to PPD, and which activates the macrophages armed with SMAF. Accordingly treatment of macrophages with a mixture of SMAF plus specific antigen resulted in activation taking place in a one-step reaction. Even after centrifugation at 100,000*g* for 2 hr BCG-SMAF activated macrophages without the addition of more antigen.

Discussion. Activated macrophages can be obtained directly from mice under certain conditions. Hibbs, Lambert and Remington (11) showed that macrophages obtained from mice chronically infected with Toxoplasma inhibited growth of transformed cells *in vitro* but not of normal cells.

An explanation based on the data presented in this paper is that the macrophages from such mice were perhaps armed with Toxoplasma-SMAF *in vivo* and as a result of continuous exposure to Toxoplasma antigen *in vivo* the macrophages became activated and retained this state *in vitro*. In a related situation (6) following injection with BCG the peritoneal macrophages were "activated" if they were taken within 3 days after a second challenge with BCG. Thereafter the macrophages were only "armed" and required exposure to PPD to become activated. BCG infection probably differs from infection with Toxoplasma in that after a state of immunity has been attained the level of BCG organisms or of antigen is too low and not sufficient to activate the armed macrophages. The change to the activated state which occurs when macrophages treated with SMAF are exposed to the specific antigen has a formal similarity to the changes induced in mast cells resulting in degranulation which occurs when mast cells coated with IgE come into contact with specific antigen (12). Furthermore, activation of macrophages and degranulation of mast cells can be brought about nonimmunologically by exposure to specific polyelectrolytes; for example the polycationic 48/80 causes degranulation of mast cells (12) and double-stranded RNA activates macrophages (13). However, the relationship of SMAF to cytophilic immunoglobulins such as IgE remains to be elucidated. Following gel chromatography SMAF activity has been found in two molecular weight ranges. The lower of these is in the region of 50,000 daltons, and this suggests that this material, which is a product of T-cells (9) does not belong to one of the known classes of immunoglobulins. The possibility that SMAF contains subunits of known immunoglobulins is a possibility that cannot be excluded.

Summary. A specific macrophage arming factor (SMAF) is produced in the culture supernatant of sensitized spleen cells incubated with the specific antigen. Macrophages armed with a SMAF directed to a transplantation or tumor specific antigen specifically inhibit the growth of tumor cells bearing this antigen. This study shows that if SMAF-armed macrophages are exposed to the specific antigen *in vitro* they become capable of growth inhibiting lymphoma cells in a nonspecific way. This change induced

by the specific antigen is referred to as activation. Activation is also observed if mouse macrophages are armed with SMAF directed against sheep red cells. However, the culture supernatant obtained by incubating BCG-sensitized spleen cells with BCG organisms activates macrophages directly possibly because it contains both SMAF and the specific antigen.

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