INTERACTION BETWEEN SENSITIZED LYMPHOCYTES AND ANTIGEN-CONTAINING TARGET CELLS

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Interaction between sensitized lymphocytes and antigen-containing target cells was studied in vitro in experiments on BALB/c mice (vaccinated with BCG, infected with strain $H^{37}Rv$, and intact). Depending on their source, the lymphocytes were found to differ in their cytotoxic activity.

Both in experimental tuberculosis and in animals vaccinated with BCG, a state of hypersensitivity of delayed type (HDT) develops. However, different tests with the cells of these animals were found to give different results as regards the actual criterion of late development characteristic of HDT (the blast-transformation test and the inhibition of migration test), depending on the intensity of immunity and the extent of spread of the specific process [2]. Tests revealing a state of hypersensitivity in vitro also include the cytotoxic action of lymphocytes on target cells [1, 3-5, 8, 10]. It was recently shown that lymphocytes, sensitized to mycobacterial antigens, destroy erythrocytes which have adsorbed PPD in vitro [9, 12], and that preincubation of sensitized lymphocytes with PPD induces their cytotoxic action on intact fibroblasts [11]. It has also been shown that the lymphocytes of mice vaccinated with BCG specifically destroy in vitro macrophages containing mycobacterial antigens.

The object of the investigation described below was to study the results of interaction between lymphocytes and antigen-containing macrophages in relation to the source of the lymphocytes and macrophages.

EXPERIMENTAL METHOD

Experiments were carried out on 60 BALB/c mice; 20 mice were infected 21 and 28 days before the investigation with 0.1 mg of a culture of strain $H^{37}Rv$, 20 mice were vaccinated 1.5 months before the experiment with 1 mg of BCG, and 20 animals were used as the control. Macrophages obtained from the infected, vaccinated, and control mice were grown for 48 h in flat-bottomed tubes. Each tube contained 200,000-250,000 macrophages in 1 ml medium No. 199 with 20% bovine serum and 10% lactalbumin. Lymphocytes, also from infected, vaccinated, and control animals were then added to the tubes in the proportion of 100 viable lymphocytes per macrophage (the lymphocytes were obtained from lymph glands by "extrusion," and their viablity was estimated by staining with 0.1% trypan blue and 0.1% eosin). Viable lymphocytes, 2×10^7 in number, were added to each tube with the macrophages in 1 ml medium No. 199 with 15% lactalbumin and the medium was changed after 24 h (for one with 2% inactivated bovine serum and 10% lactalbumin). The results of the test were estimated after 48 h by the formula:

$$\frac{a-b}{a} \times 100\%,$$

where a is the number of living macrophages (mean number per field of vision, magnification 200 ×) after incubation with the control lymphocytes, and b the number after incuvation with sensitized lymphocytes.

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TABLE 1. Interaction between Sensitized Lymphocytes and Antigen-Containing Target Cells

Source of macrophages	Lymphocytes of vaccinated animals				Lymphocytes of vaccinated animals				Lymphocytes of vaccinated animals			
	number of living macrophages		cytotoxic effect, %		number of living macrophages		cytotoxic effect, %		number of living macrophages		cytotoxic effect, %	
	1	2	1	2	1	2	1	2	1	2	1	2
Vaccinated animals	49,8	53,7	48,4[1]	47,1[1]	64,6	58,4	33,2[1]	42,4[1]	96,7	101,5	—	—
Infected animals	41,4	43,6	54,8[1]	53,5[1]	54,4	56,6	40,7[1]	41,4[1]	91,6	93,5	—	—
Control animals	98,7	94,5	—	—	96,6	93,4	—	—	102,6	101,8	—	—

[1] $P < 0.01$; in each of two experiments (at an interval of 1 week) the results of four identical specimens (tubes) were compared.

EXPERIMENTAL RESULTS

The results show (Table 1) that lymphocytes of vaccinated and infected mice have a cytotoxic action on antigen-containing (as shown by the immunofluorescence method) target cells, but not on the control target cells; lymphocytes of the control animals did not possess such an action. The lymphocytes of the vaccinated mice destroyed antigen-containing target cells more actively than lymphocytes of infected animals. It was also found that, despite the presence of antigen in the macrophages taken both from the vaccinated and from the infected animals, macrophages from the latter were destroyed to a greater degree.

The lymphocytes of the immune organism thus have a more marked cytotoxic action on antigen-containing target cells than the corresponding cells taken from animals with widespread tuberculosis, i.e., interaction between lymphocytes and target cells is not only specific, but also depends on the state of the sensitized cells. It can be postulated that the more active destruction of macrophages taken from infected animals is evidently due not to the quantity of antigen which they contain, but to their greater vulnerability. It is interesting to note that there is a definite parallel between the dynamics of the reaction described above and the blast-transformation reaction, whereas the inhibition of migration reaction (which is also a test of HDT) had a completely opposite dynamics: the two first tests give the strongest positive results if the intensity of immunity is high, while the last test is more strongly positive in the presence of widespread tuberculosis.

It is noteworthy that according to recent findings sensitized lymphocytes [6] or cytokinins [7] can potentiate the effectiveness of phagocytosis.

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