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ANTIBODY-STIMULATING FACTOR SECRETED BY SPLEEN CELLS in vitro

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The intensity of antibody formation in irradiated syngeneic recipients was studied in relation to its restoration by various cell suspensions. A lymphocyte suspension free of macrophages restored the ability of the irradiated recipients to respond to sheep's red blood cells only weakly. Addition of peritoneal or splenic macrophages to the lymphocytes led to a sharp increase in plaque formation. The same response was produced by injecting a suspension of spleen cells incubated beforehand in vitro for 3 h at 37°C into the recipients. After incubation of the spleen cell suspension in this way in the absence of antigen, a factor capable of sharply increasing antibody formation in irradiated recipients on restoration of their immune response by injection of donors' lymphocytes was secreted into the medium. The antibody-stimulating factor was not produced after incubation of lymphocytes for the same times. It is suggested that the antibody-stimulating factor appearing in the medium during culture of spleen cells in vitro in the absence of antigen is formed by cells of the mononuclear phagocytic system.

KEY WORDS: macrophages; antibody-stimulating factor; lymphocytes.

The role of macrophages in the realization and regulation of the immune response is undisputed. Numerous investigations have shown that macrophages and their products can both stimulate and depress the immune response. Stimulation of lymphocytes in vitro by antigens or mitogens does not take place in the absence of macrophages or of the factor secreted by them which activates lymphocytes [3]. However, under certain conditions macrophages may depress the response of lymphocytes to antigens and mitogens [2]. Most of these investigations were undertaken in vitro. It has not yet been established what role in the effects exhibited by macrophages is played by their number and functional activity or whether this depends on the presence or absence of a particular subclass of cells.

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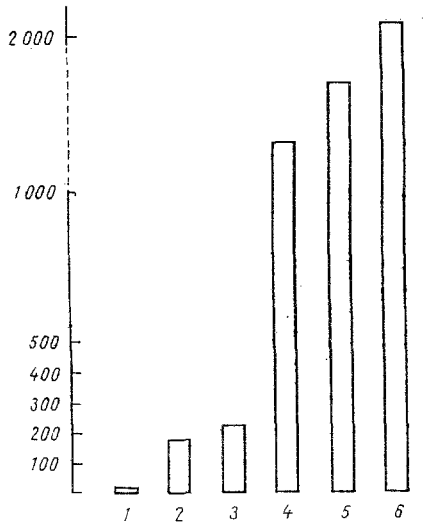

Fig. 1. Restoration of response to irradiated recipients by means of cell suspensions. Ordinate, here and in Figs. 2 and 3: number of PFC per spleen. 1) Injection of SRBC ($17.5 \cdot 10^7$) only; 2) lymphocytes ($1 \cdot 10^7$) + SRBC; 3) freshly prepared suspension of spleen cells ($1 \cdot 10^7$) + SRBC; 4) peritoneal exudate macrophages ($1 \cdot 10^6$) + lymphocytes + SRBC; 5) splenic macrophages ($1 \cdot 10^6$) + lymphocytes + SRBC; 6) spleen cells incubated for 3 h in vitro + SRBC.

The object of this investigation was to compare splenic and peritoneal macrophages for their ability to restore antibody formation in irradiated recipients.

EXPERIMENTAL METHODS

Experiments were carried out in a syngeneic system. Male CBA mice weighing 18-20 g were used as donors of the cells and as recipients. Sheep's red blood cells (SRBC) were used as the antigen. Before injection into the recipients, the antigen was added to cell suspensions, which were incubated together for 30 min at 37°C and injected intravenously in a dose of $17.5 \cdot 10^7$ SRBC per mouse. All cell suspensions were injected in 0.1 ml physiological saline.

On the fifth day the recipients' spleens were removed and the number of plaque-forming cells (PFC) determined by Jerne's method.

Macrophages were obtained from the peritoneal cavity of the mice on the fourth day after intraperitoneal injection of 2 ml nutrient broth. The peritoneal cavity was washed out with 2 ml of medium No. 199 with heparin. Peritoneal exudate cells was incubated for 2 h at 37°C in medium No. 199 ($1 \cdot 10^6$ cells/ml). After removal of nonadherent cells and careful rinsing of the monolayer the medium was replaced by fresh and incubation continued for another hour. The monolayer was repeatedly rinsed, the adherent cells were removed with a soft spatula, and their concentration was adjusted to $1 \cdot 10^7$ with physiological saline.

Macrophages and lymphocytes were obtained from the spleens by the same method. Homogenates of the spleens in medium No. 199 with heparin were poured into flasks in a concentration of $1 \cdot 10^7$ cells/ml medium.

To obtain lymphocytes the nonadherent cells were collected after incubation of the splenic suspension for 90 min, transferred to fresh flasks, and incubated for a further 1.5 h to separate them better from the adherent cells. The suspension of nonadherent spleen cells was then centrifuged at 800 rpm for 15 min. The residue was diluted in physiological saline to a concentration of $1 \cdot 10^8$ cells/ml.

To obtain splenic macrophages, after removal of the nonadherent cells the monolayer was washed with medium No. 199 and then incubated further in a fresh portion of medium (the total duration of incubation of the cells in the flasks was 3 h). The adherent cells were then removed and resuspended in physiological saline to a concentration of $1 \cdot 10^7$ cells/ml.

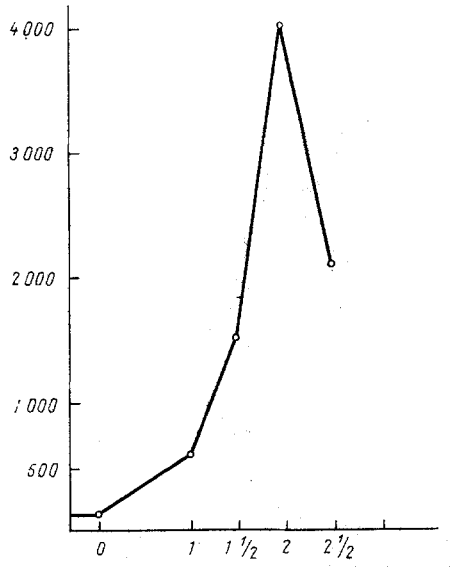

Fig. 2. Dynamics of formation of stimulating factor. Abscissa, duration of incubation (in h).

EXPERIMENTAL RESULTS

As Fig. 1 shows, the irradiated recipients did not respond to injection of SRBC, and a lymphocyte suspension freed from macrophages restored their ability to respond only weakly (186 PFC per spleen). Virtually the same response was given by recipients which received a fresh suspension of spleen cells: 228 PFC per spleen. Addition of macrophages of any origin (peritoneal or splenic) to the lymphocytes led to a sharp increase (tenfold) in plaque formation (up to 1120-1730 PFC per spleen). The same response was produced by injecting a suspension of spleen cells incubated beforehand in vitro into the recipients.

Since preliminary incubation did not affect the ability of the lymphocytes to restore the response and since a fresh suspension of spleen cells, in which the majority naturally were macrophages, also gave a weak response, it was suggested that during incubation of the macrophages in vitro a certain factor stimulating antibody formation is produced in the absence of antigen.

In the next series of experiments attempts were made to determine the dynamics of formation of the stimulating factor. For this purpose, spleen cells in a dose of $1 \cdot 10^7$/ml were incubated in medium No. 199 at 37°C and injected into recipients 1, 1.5, 2, 2.5, and 3 h after the beginning of incubation. As Fig. 2 shows, the suspension of spleen cells began to be activated 1 h after the beginning of incubation, and gave the highest response (4000 PFC per spleen) after 2 h, after which there was a tendency for its activity to diminish. To determine whether the activating factor is liberated into the medium, the spleen cells were sedimented by centrifugation after incubation for 3 h and the resulting supernatant was added to lymphocytes or to a fresh suspension of spleen cells in the same proportions as those in which it was obtained. The mixtures were incubated for 30 min at 37°C. The sedimented cells were then diluted with physiological saline and injected into recipients in a dose of $1 \cdot 10^7$ per mouse. As Fig. 3 shows, the supernatant activated lymphocytes and fresh spleen cells by a no lesser degree than addition of macrophages or prolonged incubation of spleen cells (by 15 times).

These observations suggest that during incubation of the suspension of spleen cells in vitro at 37°C a factor capable of sharply increasing antibody formation in irradiated recipients during restoration of their capacity for immunogenesis by injection of donors' lymphocytes or of a fresh suspension of spleen cells is secreted into the medium. The production of this factor reached a maximum between 2.5 and 3 h of incubation. The antibody-stimulating factor was not produced during incubation of lymphocytes for the same times, despite the fact that the cell suspension contained many macrophages during the first 1.5 h of incubation. Contact between lymphocytes and macrophages in vitro for only 90 min is probably not sufficient for the necessary quantity of the factor to be produced, and absence of its production during continued incubation of the lymphocyte suspension, now deprived of its adherent cells, is clear evidence that the lymphocytes themselves are not its source.

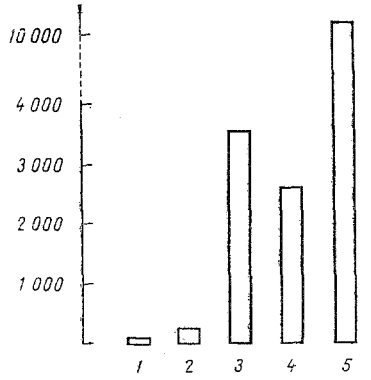

Fig. 3. Restoration of response to irradiated recipients by means of supernatant. 1) Lymphocytes ($1 \cdot 10^7$) + SRBC; 2) freshly prepared suspension of spleen cells ($1 \cdot 10^7$) + SRBC; 3) lymphocytes treated with supernatant ($1 \cdot 10^7$) + SRBC; 4) freshly prepared suspension of spleen cells ($1 \cdot 10^7$) treated with supernatant + SRBC; 5) incubated suspension of spleen cells ($1 \cdot 10^7$) + SRBC.

It can tentatively be suggested that the antibody-stimulating factor secreted into the medium during culture of spleen cells in vitro in the absence of antigen is produced by cells of the mononuclear phagocytic system, although the possible role of contact between these cells and lymphocytes in the initial period of incubation cannot be completely ruled out.

A factor activating lymphocytes is known to be produced by macrophages and to act mainly on T lymphocytes, increasing their sensitivity to mitogenic agents [1]; the dynamics of its production also differs from the dynamics of production of the antibody-stimulating factor [3]. Nevertheless, it will be necessary to investigate the relations between these products secreted by the cells and to undertake a detailed analysis of the antibody-stimulating factor. The inability of macrophages from irradiated donors to transmit antigenic information may perhaps depend on disturbance, as a result of irradiation, of the ability of the macrophages to produce the factor stimulating antibody formation.

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