from beneath it by the flow of liquid. Protein cannot enter the trap because of the high (30%) concentration of gel. After absorption of the liquid from K_1 the boundary passes across the first reservoir and a second SB is established relative to K_2. Under these circumstances proteins in front of K_2 are "absorbed" at the boundary for the reasons examined in connection with the first stationary position of the boundary.

Protein fractions whose value of m is less than that of the closing ion, i.e., those with $U_R < 0$, under SB conditions will be carried toward the cathode and lost. During work with glycine and the closing ions, some of the "slow" proteins, namely WM and γ-G, are lost in this way (Fig. 2). To keep all these proteins in ITP, β-alanine or GABA, which have much lower values of m, must be used instead of glycine. In that case equilibrium develops at lower values of L_1/L and the distance from K_1 to the cathodic border must be increased.

The technique described above is very simple, because no special equipment is required to create the counterflow [4] or to detect the protein zones. It can be used for work with very dilute solutions of proteins and can readily be combined with immunodiffusion [1] and immunoelectrophoresis [2].

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ROLE OF THE ADHESIVE LYMPHOCYTE POPULATION IN AREACTIVITY TO HEPATOMA 22a

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The role of the adhesive fraction of T lymphocytes in areactivity of mice to hepatoma 22a was studied. Experiments showed that removal of the adhesive fraction from a suspension of spleen cells enriched with T lymphocytes potentiates cellular immunity in mice tolerant to hepatoma.

KEY WORDS: antitumor immunity; tolerance; hepatoma 22a; migration of macrophages; T lymphocytes.

Among the many factors influencing manifestation of immune reactions to tumors an important role is ascribed to immunologic tolerance, development of which is due to the existence of antigens common to embryos and tumors, and also to the appearance of soluble tumor antigens in the circulation. In previous experiments with mouse hepatoma 22a the writers showed that contact with tumor antigens in the neonatal period not only leads to subsequent stimulation of growth of an inoculated tumor, but is also accompanied by depression of antitumor immunity in mice thus rendered tolerant [1].

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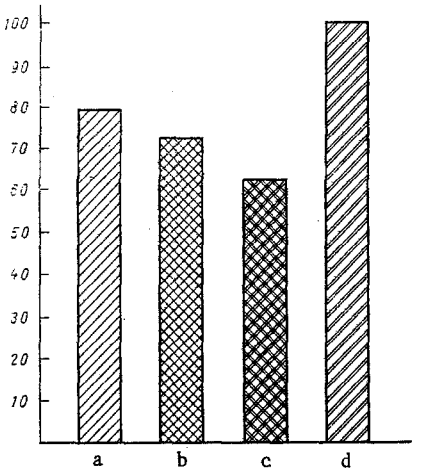

Fig. 1. Migration of macrophages in the presence of lymphocytes and their subpopulations isolated from the spleen of mice tolerant to hepatoma. Abscissa: a) total population of lymphocytes; b) T lymphocytes; c) T lymphocytes after removal of adhesive cells; d) control; ordinate, migration of macrophages, %.

The immune response to tumors is effected by different subpopulations of lymphocytes, among which suppressor cells with immunoregulatory properties have attracted particular attention. According to information in the literature, cells of this type take part in tolerance formation and they probably belong to the adhesive fraction of T lymphocytes [2, 3, 5].

The object of the present investigation was to study the role of suppressor cells in areactivity to hepatoma. For this purpose the effect of the adhesive population of T lymphocytes on reactions of cellular immunity was studied in mice tolerant to hepatoma.

EXPERIMENTAL METHOD

Mouse hepatoma 22a was chosen as the experimental tumor. During the first 3 days after birth, C3HA mice were given injections of a saline extract of hepatoma cells in a total dose of 0.75 mg protein per animal. At the stage of sexual maturity experimental and control mice were given an intraperitoneal injection of $2 \cdot 10^4$ hepatoma cells. There were 30 animals in the experimental and 20 in the control group. The effect of different subpopulations of lymphocytes of the tolerant mice on the manifestations of cellular immunity was studied by the macrophage migration inhibition test using capillary tubes in Poupon's modification [4]. Macrophages from normal mice, migration of which is modified in the presence of sensitized lymphocytes, were used in this modification. Peritoneal macrophages were isolated from the peritoneal cavity of intact mice 72 h after intraperitoneal injection of 2 ml of 5% Difco peptone.

Lymphocytes were isolated from the spleens of the experimental and control mice and separated from erythrocytes and macrophages. To remove erythrocytes, the washed spleen cells were resuspended in 1 ml physiological buffered saline (PBS), 8 ml of 0.83% NH_4Cl was added, the mixture was incubated for 5 min at 4°C, and the cells washed and resuspended in 2 ml of the same buffer. Macrophages were separated with the aid of iron carbonyl. To do this, 50μg iron carbonyl powder was added to 2 ml of a suspension of spleen cells and the sample incubated for 30 min at 37°C with periodic mixing. The macrophages, loaded with iron, were sedimented by means of a magnet. The cell fraction enriched with T lymphocytes was obtained by centrifuging the cell suspension in a ficoll-verografin (density 1.09) gradient, enabling sedimentation of the B cells by the use of rosette formation. After separation of B lymphocytes, further fractionation of the T cells was carried out by adsorbing the adhesive cells on a plastic surface during incubation at 37°C for 15 min.

To carry out the macrophage migration inhibition test, the lymphocytes or their subpopulations obtained as described above were mixed in equal volumes, 0.2 ml of each, with macrophages and hepatoma cells ($20 \cdot 10^6$ macrophages, $10 \cdot 10^6$ lymphocytes, and $5 \cdot 10^6$ tumor cells in 1 ml) and capillary tubes (diameter 0.7 mm, length 70 mm) were filled with the resulting suspension and incubated at 37°C in special chambers with medium No. 199 in the presence of 15% normal mouse serum. The zones of migration of the cells were drawn on paper by means of a drawing apparatus, cut out, and weighed, and the percentage migration was calculated by the equation:

$$\% \text{ migration} = 100 \times \frac{\text{mean weight of migration zones in experiment}}{\text{mean weight of migration zones in control}}.$$

EXPERIMENTAL RESULTS

By carrying out the experiments in this way it was possible to assess cellular immunity of the tolerant mice by using a mixture of lymphocytes and a fraction enriched with T lymphocytes; the effect of the adhesive fraction of T lymphocytes on the immune reactions could also be studied.

Experiments showed that reactions of cellular immunity detected during growth of the hepatoma in the tolerant mice were weak and were manifested in only 50% of the mice. The percentage migration of macrophages in mice of the experimental group (Fig. 1) was 78, compared with 100% migration in the control, i.e., inhibition of macrophage migration was on a very small scale.

Similar results were obtained also when the fraction enriched with T cells was used in the macrophage migration inhibition test: The percentage migration of macrophages fell to 72. The results were somewhat different after further fractionation of the T cells into subpopulations. For instance, removal of the adhesive fraction of lymphocytes from the splenic suspension enriched with T lymphocytes revealed clearer inhibition of macrophage migration in 50-60% of animals. In the experiments of this series migration of the macrophages was reduced to 62%, evidence that the reactions of cellular immunity are stronger in mice tolerant to tumors.

The results suggest that the adhesive fraction of T lymphocytes plays a definite role in the regulation of the immune response of the animal to tumors. However, because this effect was not found in all tolerant mice, a further study of the role of complex cooperative interaction between cells of the lymphoid systems in areactivity to tumors is necessary.

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