role in protection of the antigenic determinants of OVA may also be played by its greater resistance to acid denaturation in the stomach compared with BSA [2].

The greater quantity of OVA than of BSA entering the internal medium of the body may lead to differences in the immune response to these proteins. It was shown, in particular, in [12] that, depending on the dose of food protein assimilated, it may behave both as an immunogen and as a tolerogen.

During separate administration of different proteins to animals in experiments to study digestion and absorption, interpretation of the results may be made difficult by the considerable dispersion of the experimental data [8, 11]. It can be concluded from the present investigation that the simultaneous administration of two labeled food antigens to an animal, followed by selective immunosorbent antigen determination, enables the permeability of the protective barriers of the body for different antigenic structures to be effectively compared.

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THYMUS TARGET CELLS UNDER MACROPHAGE CONTROL DURING THE FORMATION OF GRAFT VERSUS HOST REACTION EFFECTORS

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KEY WORDS: macrophages; thymocytes; graft versus host reaction.

Thymocytes (TC) are weak inducers of the graft versus host reaction (GVHR) [2]. Meanwhile the writers have shown that maturation of thymus cells to functionally active effectors can take place under the influence of short-term incubation with peritoneal exudate macrophages [1]. The thymus is known to contain different populations of lymphoid cells, differing not only in their location in the organ (cortex, medulla), but also in the antigenic properties of their surface membranes, immunocompetence, sensitivity to corticosteroids and irradiation, and so on.

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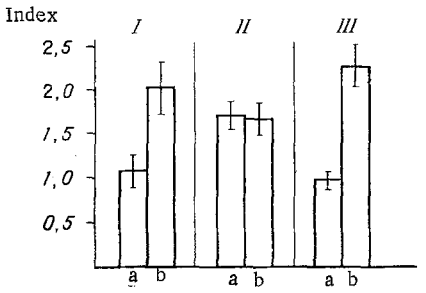

Fig. 1

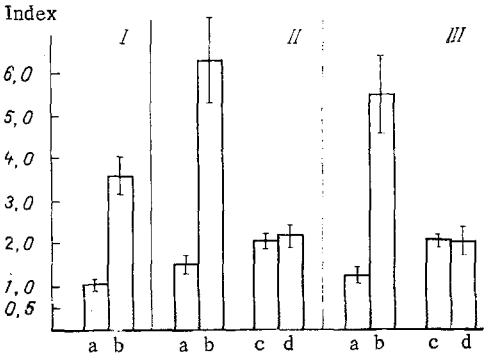

Fig. 2

Fig. 1. Intensity of GVHR induced by cortisone-resistant and radioresistant TC after contact with macrophages. I: a) Intact TC, b) TC + macrophages; II: a) cortisone-resistant TC, b) cortisone-resistant TC + macrophages; III: a) TC irradiated with a dose of 300 R, b) irradiated TC + macrophages.

Fig. 2. GVHR induced by TC migrating into different lymphoid organs. I: a) Intact TC, b) TC + macrophages; II: a) spleen cells of lethally irradiated mice injected with syngeneic spleen cells, b) the same cells + macrophages, c) lymph node cells of lethally irradiated mice into which syngeneic spleen cells were transplanted, d) the same cells + macrophages; III: a) spleen cells of lethally irradiated mice into which syngeneic thymus cells were injected, b) the same cells + macrophages, c) lymph node cells of lethally irradiated mice into which syngeneic thymus cells were transplanted, d) the same cells + macrophages.

It was accordingly decided to investigate with which thymus cell population macrophages interact or, in other words, to determine which class of TC are the targets for regulatory action of phagocytic monocytes.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice and (CBA × C57BL)6/F_1 hybrids. Phagocytic monocytes were obtained from peritoneal exudate. These cells were obtained and a monolayer of macrophages prepared as described previously [1]. Thymus cells from CBA mice were added to the monolayer of macrophages. Contact between TC and macrophages lasted 4-18 h. At the end of that time the TC were harvested, washed, and injected subcutaneously in a dose of 2×10^6 into the footpad of the animal's hind limb. TC from F_1 mice were injected into the opposite footpad. The intensity of the GVHR was assessed by an index of enlargement of lymph nodes, determined 7 days after injection of the cells as the ratio of the number of cells in the popliteal nodes of the right and left limbs [4]. Mice of the F_1 group, into which intact CBA TC were injected, served as the control. The mice were irradiated on a "Stebel'-3A" apparatus. The dose rate was 900 R/min. Hydrocortisone acetate (from Gedeon Richter, Hungary) was injected intraperitoneally in a dose of 2.5 mg/mouse 48 h before removal of the thymus [6]. Separation of TC into cortical and medullary with the aid of peanut lectin was carried out as in [9]. Each experimental group contained from seven to 20 animals. The results were subjected to statistical analysis with calculation of the arithmetic mean and standard error and the level of significance (P) by Student's test.

EXPERIMENTAL RESULTS

In the modern view at least two subpopulations of T cells take part in the GVHR: T_1 and T_2 [2, 7, 8]. Subpopulation T_2 has an effector function and T_1 a regulatory function, acting as amplifier of the basic process. Various experimental approaches were used in an attempt to discover which subpopulation of thymus T cells is acted upon by macrophages during maturation of GVHR effectors.

In the experiments of series I the effect of macrophages was studied on radioresistant and cortisone-resistant thymus target cells compared with the intact population. T_2 are known to be cortisone-resistant and T_1 to be radioresistant when a sublethal dose of irradiation is used [2, 3]. The effectiveness of interaction of macrophages with TC of donors treated with hydrocortisone and with TC of mice irradiated in a dose of 300 R was compared. Macrophages were incubated with TC for 4 h. As Fig. 1 shows interaction between TC and macrophages was not accompanied by any change in the properties of this cell subpopulation. They were initially

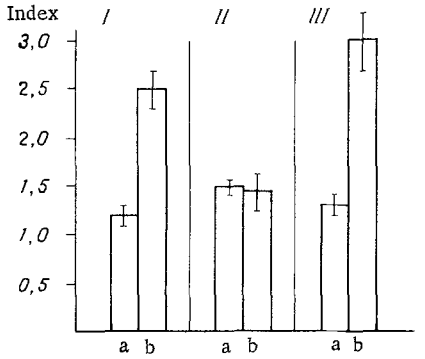

Fig. 3. GVHR induced by cortical and medullary TC separated by means of peanut lectin. I: a) Intact TC, b) TC + macrophages; II: a) medullary TC, b) medullary TC + macrophages; III: a) cortical TC, b) cortical TC + macrophages.

highly reactive in the GVHR. It was a different matter with TC irradiated in a sublethal dose. Contact of radioresistant TC with macrophages led to a sharp increase in their inducing properties.

T_1 and T_2 lymphocytes are known to migrate into different lymphoid organs. The T_2 subpopulation concentrates mainly in lymph nodes, T_1 in the spleen [2, 8]. In the present investigation, in order to separate T_1 and T_2 lymphocytes syngeneic spleen cells in a dose of 100×10^6 or thymus cells in the same dose were injected intravenously into irradiated (1000 R) CBA mice. The mice were killed 24 h later and the lymph node and spleen cells were removed and cultured with macrophages for 18 h. Intact TC and also lymph node and spleen cells isolated from irradiated recipients and not cultured with macrophages served as the controls. After the end of incubation the cells were harvested and injected into F_1 recipients. It will be clear from Fig. 2 that cells migrating into the spleen were the targets for the controlling influence of macrophages. Contact between macrophages and cells isolated from lymph nodes caused no change in their properties. Like cortisone-resistant TC, cells migrating into lymph nodes independently induce a GVHR of a level comparable with intact TC.

Peanut (Arachis hypogaea) lectin has the property of agglutinating cortical TC but does not interact with medullary cells [5, 9]. By using the reagent the original TC population was separated into two subpopulations and the effect of interaction of these subpopulations with macrophages for 4 h was studied. It will be clear from Fig. 3 that incubation of macrophages with cortical TC led cells which were unable by themselves to induce the GVHR to induce an intensive GVHR. The inducing properties of the medullary TC were unchanged after contact with macrophages. Medullary TC, it will be noted, possess lower reactivity than cortisone-resistant cells, although these cells were located in the medulla of the thymus.

The results thus show that the targets for the regulatory effect of macrophages are cortisone-sensitive, relatively radioresistant cells, located in the cortex of the thymus and migrating into the spleen, i.e., in their characteristics as a whole they correspond to T_1 cells. Interaction of the subpopulation of immature precursors of T effectors of GVHR located in the cortex of the thymus with macrophages for several hours probably facilitates their functional maturation. As a result, cells capable of developing a more intensive GVHR than intact TC accumulate. Maturation of GVHR precursors probably takes place under the influence of one (or several) macrophagal monokines. This hypothesis is based on the results of a series of investigations which showed that the humoral factors of macrophages can produce differentiation of immature TC to T cells [10].

The aim of future research will be to determine the actual role of macrophagal monokines in the process described above and to determine the molecular nature of these monokines.

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PRODUCTION OF ANTIBODIES TO INFLUENZA A VIRUS BY HUMAN LYMPHOID CELLS IN VITRO

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KEY WORDS: antibodies; influenza virus; lymphoid cells.

Reports have recently been published on the possibility of reproducing the production of antibodies against different antigens by human lymphoid cells in culture in vitro. These antigens include xenogeneic erythrocytes and poliomyelitis and influenza viruses. Peripheral blood cells have been used to study antibody production against influenza virus [9, 10, 12].

The method of studying antibody production against influenza virus in vitro can be used to obtain data on the mechanisms of antibody formation and investigations of immunologic memory. A more profound study of the latter problem is necessary with the obtaining of data showing that strain-specific immunity to influenza lasts for a very long time [3, 5]. Memory cells remain in the peripheral blood for several weeks [8]. With these facts and the characteristics of the pathogenesis of influenza in mind, it was decided to study antibody production against influenza A (H3N2) virus by cells of the tonsils and mediastinal lymph nodes – organs draining the region of the "portals of entry" of influenzal infection, where memory cells persist for a long time [2].

EXPERIMENTAL METHOD

Influenza virus A/Leningrad/385/80 (H3N2), grown in a culture of MDCK cells (transplantable canine kidney cells) was used in the experiments. The tonsils and lymph nodes were obtained from adults of both sexes undergoing tonsillectomy for chronic tonsillitis and respiratory diseases. After removal the lymph nodes and tonsils were placed in flasks with Eagle's medium or medium 199 containing 10% bovine serum, 100 units/ml penicillin and streptomycin, and 200 μg/ml kanamycin, and received in the laboratory not later than after 1 h. The same medium was used to wash the cells. Pieces of tissue with no visible signs of inflammation were excised from the tonsils with scissors, transferred to sterile petri dishes with medium, washed 3 times, and again transferred to sterile dishes. Lymph nodes were freed from extraneous tissue and treated in the same way as the tonsils. Cells were isolated from the lymph nodes and tonsils by means of dissection needles, collected in test tubes, and washed twice on the TsLR-1 centrifuge at 800 rpm for 5 min at 4°C. The supernatant after the last washing was collected for determination of antibodies. The residue was resuspended in culture medium.

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