Hence, depending on the type of interacting immunoglobulins, the process of specific complex formation takes place at different speeds and intensities, and it is most evident for IgM. Consequently, depending on the character of the curves obtained, the class of immunoglobulins being synthesized at a given moment can be determined. Disturbance of the internal structure of the immunoglobulin macromolecules (in particular by heating) leads to a change in the mechanism of complex formation; the important question here is which of the interacting components undergoes denaturation. The sharpest decrease in complex formation is observed in the case of thermal denaturation of the antiserum, because of partial loss of specificity of the antibody macromolecules on heating.

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EVALUATION OF THE IMMUNOHISTOLOGICAL STATE OF LYMPH NODES REGIONAL WITH RESPECT TO TUMORS

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The immunoreactivity of lymph nodes regional with respect to the tumor was studied in 22 patients with melanoma. Blast-transformation, rosette-formation, and immunofluorescence tests were used to detect T- and B-lymphocytes. Lymphocytes were isolated from one half of the lymph node, the other half was used for histology. The results showed that function of the regional lymph nodes correlates well with morphology.

KEY WORDS: immunreactivity; lymphocytes; lymph node.

The study of the efficacy of the antitumor barrier of the various functional systems in man is a very urgent problem to which many investigations have been devoted [1, 2, 4, 6, 9, 11, 12].

The present writer [8] has shown that the immunologic activity of T- and B-lymphocytes from lymph nodes with tumor metastases is lower than that of lymphocytes from lymph nodes without metastases. These results naturally have posed fresh problems: What morphological changes are observed in the lymph nodes besides signs of metastasization, to correspond to these different immunologic backgrounds? The work of Tsakraklides et al., [11, 12] confirmed the value of studying immunomorphological changes in lymph nodes from cancer patients. The investigation described below was devoted to this purpose. The results of immunologic tests to study T- and B-lymphocytes in vitro were used as criteria of immunoreactivity.

The state and the cell composition of the cortical and paracortical zones, the medullary layer, and the sinuses of the lymph nodes served as steps in the histological analysis.

The results were accordingly combined into three groups: severe, moderately severe, and mild reactive changes, which can also be represented as marked hyperplasia, moderate hyperplasia, and hypoplasia of the lymphoid tissue of the lymph nodes.

The investigations were based on the recommendations of Cottier et al., [3] on standardization of morphological signs of immunologic activity of lymph nodes.

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Altogether 22 patients with malignant melanoma of the skin were studied.

EXPERIMENTAL METHOD

The lymph nodes taken for investigation were cut transversely along the minor axis. One half of the lymph node was used for histological investigation, the other for immunologic tests. To prepare histological sections the material was fixed in Zenker-formol and Carnoy's solution and embedded in paraffix wax. Sections were stained by Giemsa and Brachet's methods. The morphology of the lymph nodes was assessed by a scheme similar to the recommendations of Cottier et al., [3]. Lymphocytes were isolated in the cold under sterile conditions by gentle homogenization of the lymphoid tissue.

Of the various immunologic tests for evaluation of T- and B-lymphocytes, the spontaneous rosette-formation (RFT), the blast-transformation test with PHA (BTT), and the direct immunofluorescence test (IFT) were used.

In the RFT, determining the ability of T-lymphocytes to form rosettes with heterogeneic sheep's red blood cells in vitro, techniques developed in the Laboratory of Clinical Immunology, Oncologic Scientific Center, Academy of Medical Sciences of the USSR [4] and the investigations of Pang et al., [10] were taken into account. The percentage of rosette-forming cells (RFC), namely lymphocytes with at least three red blood cells attached to them, was determined by counting at least 100 lymphocytes in a section.

The blast-transformation test (BTT), determining the ability of T-lymphocytes to react to antigen, was carried out by a radiometric method with thymidine-^{3}H. Lymphocytes were stimulated by phytohemagglutinin (PHA) from Difco. Incorporated activity was counted with the Nuclear Chicago Mark II liquid scintillation counter. The results were assessed through the stimulation index – the ratio of the stimulated response to the unstimulated, expressed as the number of counts per minute, allowing for background activity.

B-lymphocytes were determined by immunofluorescence analysis of surface immunoglobulin receptors by the direct Coombs' method, after subsequent modification, notably by Lezhneva [5]. At least 100 cells were counted with the aid of specific fluorescence on the lymphocytes in UV light in a luminescence microscope. Diffusely fluorescent cells, and also cells with fluorescent granules in their cytoplasm, were disregarded during counting. The results were subjected to mathematical analysis [7].

EXPERIMENTAL RESULTS

Immunohistological analysis of lymph nodes regional with respect to the tumor from patients with melanoma of the skin showed that, besides morphological changes, the functional activity of the lymphocytes, as revealed by the RFT and BTT, also changed. For instance, in the presence of severe morphological changes (marked hyperplasia of lymphoid tissue) the greatest number of RFC was observed in the lymph nodes ($21 \pm 4.8\%$), in the presence of moderate reactive changes (moderate hyperplasia of lymphoid tissue) there were few RFC ($11.6 \pm 1.5\%$), and in the presence of mild reactive changes (hypoplasia of the lymph nodes) the smallest number of RFC was found ($5.2 \pm 1.2\%$). The differences are statistically significant, $P < 0.05$.

Analysis of T-lymphocyte function by the BTT with PHA showed that whereas in the presence of severe reactive changes the BTT index was 4.8 ± 1.5, when mild reactive changes were present it fell to 1.7 ± 0.16 ($P < 0.02$). In the presence of moderate reactive changes the BTT index was 3.7 ± 0.9 ($P > 0.05$ compared with previous groups).

However, although T-cell function was depressed in the presence of reactive changes ranging from severe to mild, no statistically significant differences were revealed by the direct immunofluorescence test in the B-lymphocytes. Severe, moderately severe, and mild reactive changes in the lymphoid tissue of the lymph nodes corresponded to BTT indices of $15.2 \pm 2.4\%$, $15.1 \pm 2.3\%$, and $12.6 \pm 2.3\%$ for B-lymphocytes.

The results of this investigation suggest considerable variability, both qualitative and quantitative, in the T-lymphocyte population depending on reactive changes in the lymphoid tissue of patients with malignant melanoma of the skin.

The intensity of functional activity of lymphocytes from lymph nodes regional with respect to the tumor thus corresponds to definite reactive changes in the lymph nodes (depending on the severity of those changes). Changes in lymphoid tissue also were found to correlate with the clincial course of the disease: In patients with high immunomorphological indices the course of the disease was favorable, whereas in patients with average and low immunomorphological indices the course of the disease was complicated in about 30% of cases by recurrence or metastasization.

Assessment of the immunologic activity of lymph nodes regional with respect to the tumor is thus an important stage in the analysis of the patient's immunologic status.

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IMMUNOLOGIC TOLERANCE AND IMMUNITY TO TUBERCULOSIS

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The intensity of immunity to tuberculosis and resistance to the disease in the case of infection with the virulent strain Mycobacterium tuberculosis $H_{37}Rv$ were studied in a model of immunologic tolerance induced in CBA mice by injection of a large dose of BCG polyantigen and the immunodepressant cyclophosphamide. Cellular immunity to tuberculosis (tested by the blast-transformation and tuberculin skin tests) was shown not to develop in tolerant animals after BCG vaccination, and vaccination had no protective effect under these circumstances in the group of tolerant mice.

KEY WORDS: immunologic tolerance; immunity to tuberculosis.

The study of the mechanisms of immunologic tolerance (areactivity) as one form of immune response has been the subject of much research [1-3, 7, 8]. This phenomenon is known to have both a positive and a negative effect on the course of various experimental infections [4-6]. However, it is not clear what is the role of tolerance in tuberculosis.

In this investigation the effect of induced tolerance was studied on the character of formation of immunity to tuberculosis after BCG vaccination and its role in resistance to infection with tuberculosis.

EXPERIMENTAL METHOD

Experiments were carried out on 615 male CBA mice weighing 16-18 g. Tolerance was induced by intraperitoneal injection of a combined antigen obtained by disintegrating BCG vaccine in a ball mill (in a dose of 20 mg), followed after 48 h by intraperitoneal injection of cyclophosphamide (CP) in a dose of 100 mg/kg.

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