synthetic β-N-acetylglucosamine [14]. The data described above are evidence that antibodies differing in their specificity can be isolated on such immunosorbents.

The isolation of antibodies against A polysaccharide which differ in their affinity and specificity, and their subsequent investigation by the direct immunofluorescence method, opens up new prospects for the study of cross reactions between antigenic determinants of A polysaccharide and the tissue antigen of man and animals.

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EFFECT OF METHOTREXATE ON ALLOANTIGEN EXPRESSION IN A POPULATION OF MOUSE LYMPH NODE CELLS

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The effect of intraperitoneal injection of methotrexate on alloantigen expression was studied in a population of lymph node cells from (CBA × C57BL/6j)F_1 mice. For this purpose, cells of F_1 mice, intact or receiving methotrexate, were injected into the foot of CBA mice and the increase in size of the regional popliteal lymph node compared with the contralateral (intact) side was determined. Methotrexate in doses of 50 and 75 mg/kg significantly increased the alloantigenicity of the lymph node cell population of the F_1 mice.

KEY WORDS: lymphocytes; alloantigen expression; methotrexate.

The effects of certain chemical substances and, in particular, of antitumor agents on the expression of transplantation antigens in populations of lymphocytes have been published. For instance, Haspel et al. [4], who studied human lymphoblastoid cell lines *in vitro*, observed a decrease in the number of HLA-antigens on the surface of the cells under the influence of puromycin. Neither actinomycin D nor cycloheximide had any such action. Guttman [3] showed that the lymphocytes of rats receiving cyclophosphamide largely lose their immunogenicity for animals of a different line. In his opinion, cyclophosphamide can depress more or less selectively the synthesis of histocompatibility antigens. Lindahl et al. [5] investigated the effect of interferon on expression of H-2 antigens in mouse lymphoid tissue. Both in experiments *in vitro* and after administration of interferon *in vivo*, expression of H-2 antigens on

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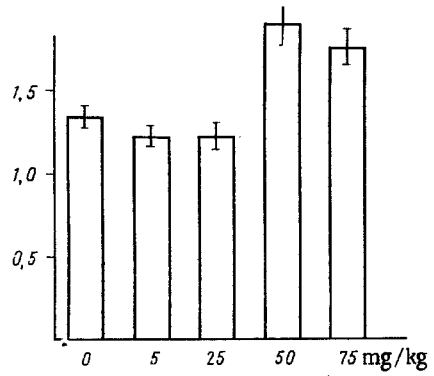

Fig. 1

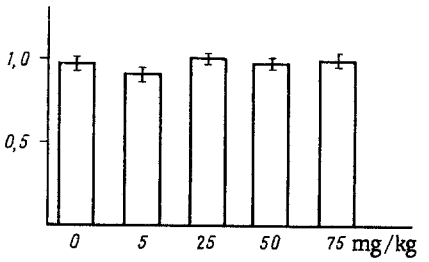

Fig. 2

Fig. 1. Dependence of antigenicity of lymph node cells of (CBA × C57BL/6j)-F_1 mice on dose of methotrexate. Abscissa, dose of methotrexate (in mg/kg) injected into F_1 mice (cell donors); ordinate, ratio of weight of experimental to weight of intact lymph node of CBA mice (recipients of F_1 cells).

Fig. 2. Results of control $F_1 \rightarrow F_1$ transplantations (pooled results of five analogous experiments). Abscissa, dose of methotrexate (in mg/kg) injected into F_1 mice (cell donors); ordinate, ratio of weight of experimental to weight of intact lymph node in F_1 mice (cell recipients).

thymus and spleen lymphocytes was increased. Cortisone had a similar action but only on thymus cells; unlike interferon, moreover, it caused hypoplasia of both organs. Majsky et al. [6], in the course of repeated leukotyping of cancer patients (including patients with tumors of nonhematogenous nature), observed significant changes in expression of HLA-antigens on peripheral blood leukocytes under the influence of courses of chemotherapy. For the overwhelming majority of antitumor agents, their effect on expression of transplantation antigens has not yet been studied, although the investigation of this aspect of the action of such preparations could lead to an understanding of their systemic antitumor effect at the level of the whole organism.

The object of the investigation described below was to study the effect of methotrexate on alloantigen expression in a lymph node cell population of intact mice.

EXPERIMENTAL METHOD

Male CBA or (CBA × C57BL/6j)F_1 female mice aged 3-4 months were used. The F_1 mice were given an intraperitoneal injection of 0.2 ml of a solution of methotrexate (Lederle) in doses of 5, 25, 50, and 75 mg/kg. Each dose of the drug was injected into four to six mice. On the 4th day after the injection the inguinal, axillary, brachial, and mesenteric lymph nodes were removed from the animals. Material from mice receiving the same dose of the drug was pooled. Medium No. 199 was used as the working solution. To obtain a cell suspension, tissue was homogenized in a glass Potter's homogenizer and filtered through three layers of gauze. The cells were washed three times by centrifugation in volumes of 10-15 ml (500 rpm) and the concentration of nucleated cells was adjusted to 100 million cells/ml. The CBA mice were given an injection of 5 million cells in a volume of 0.05 ml into the foot of the left hind limb. In control groups, cells from intact F_1 mice were injected into CBA mice, and syngeneic $F_1 \rightarrow F_1$ transplantations were carried out from donors receiving methotrexate and from intact F_1 mice. Each group contained 8 to 10 recipients. On the 6th day the animals were killed, their popliteal lymph nodes were weighed on VT-20 torsion scales, and the weight index (weight of the left lymph node/weight of the right lymph node) was calculated. The reaction was determined from the difference between the arithmetic mean indices in the control (intact $F_1 \rightarrow$ CBA) and experimental groups. Statistical analysis of the results was carried out by Student's t-test.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1, which summarizes the results of five analogous experiments, that injection of methotrexate into F_1 mice in doses of 5 and 25 mg/kg did not affect the antigenicity of their lymph node cells in the hybrid—parent system.

With an increase in the dose of methotrexate to 50 and 75 mg/kg a significant increase in antigenicity was observed (the index of the lymph nodes of the recipients was 1.91 and 1.77 respectively, compared with 1.35 in the control; $P < 0.001$).

At least two conclusions can be drawn from these results. First, after injection of a definite dose of methotrexate into F_1 mice their lymph node cells became more antigenic for the parents than cells of intact F_1 mice. Second, if this optimal dose of the drug was exceeded, the phenomenon was not enhanced.

Control $F_1 \rightarrow F_1$ transfers (Fig. 2) were carried out to rule out any possible effect of nonspecific toxicity of the lymphocytes under the influence of methotrexate. In this case the response to cells from donors receiving methotrexate did not differ from the response to cells of intact donors ($P > 0.05$).

The results of these experiments can be explained on the grounds that methotrexate causes changes in the lymph node cell population and increases the percentage of cells carrying large numbers of alloantigens, or alternatively methotrexate affects alloantigen expression directly in the cells. The problem of alloantigen expression under the influence of various factors (chemotherapy, pathological processes including growth of tumors), despite research in this field, is still relatively unstudied. For tumors a characteristic feature is known to be the loss of several antigens, including transplantation antigens. Information has recently been published, however, on expression of "normal" transplantation antigens of a haplotype foreign for the particular individual, on tumor cells [1, 2, 7, 8]. The physiological importance of changes in expression of alloantigens on normal tissues, especially during growth of tumors, is in general still unknown. The study of alloantigen expression on cells of normal and tumor tissues during chemotherapy and immunotherapy appears promising.

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