The Θ Antigenicity of Lymphoid Organs of Mice Bearing the Ehrlich Ascites Tumor

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SUMMARY
Murine lymph node and thymus cells were tested for their sensitivity to the cytotoxic effect of Θ antibodies at various intervals after i.p. implantation of the Ehrlich ascites tumor. Within 16 days after tumor inoculation, both thymus and lymph node cells of RIII mice became almost completely refractory to Θ-AKR antibodies. In C57BL mice, thymus cells lost their sensitivity to Θ-C3H antibodies within 15 days after tumor implantation, while lymph node cells retained their sensitivity to Θ antibodies even after prolonged periods of tumor growth. Thymus cells of Ehrlich ascites tumor-bearing (RIII X C57BL) F1 hybrid mice lost their sensitivity simultaneously to Θ-AKR and Θ-C3H antibodies, while lymph node cells of these hybrids showed no change in their sensitivity to Θ antibodies.

The disappearance of cells sensitive to the cytotoxic effect of Θ antibodies from lymph nodes of tumor-bearing RIII mice seems to indicate that tumor growth may lead to depletion of thymus-dependent lymphocytes.

INTRODUCTION
Interest in the immunological capability of tumor-bearing hosts has been stimulated by the discovery that tumors may possess specific antigens, which may elicit an immune response by the host (6, 9, 10, 13). The progressive growth of tumors in spite of their potential immunogenicity may be due to various mechanisms (1, 10, 13). Tumor growth may "override" the immune response of the host or the production of humoral antibodies may cause "immunological enhancement" of tumor growth. An additional factor may be the general impairment of immune capacity of tumor-bearing hosts, but this appears to be more characteristic of advanced stages of the disease (11, 12). Southam (25) has recently summarized the evidence for deficiencies in specific and nonspecific serum factors and in the cellular reactions of cancer patients.

Some insights into the nature of the impairment of immune apparatus of tumor-bearing hosts may be gained by antigenic analysis of their lymphoid organs (19). It was previously shown (21) that the thymus cells of mice bearing the EAT3 lost their TL antigenicity (3) and at the same time their sensitivity to the cytotoxic effect of guinea pig serum (20) was also lost. No concomitant decrease of their H-2 antigenicity could be detected (16, 21). In the present study, the effect of EAT growth on the Θ antigenicity (15) of the thymus and lymph nodes was analyzed.

MATERIALS AND METHODS
Mice. Mice of the C57BL/6 and RIII/Jem inbred strains and (RIII X C57BL) F1 hybrids were used as donors of lymphoid cells for cytotoxic studies. AKR/J and C3H/An mice were used for the production of Θ antibodies. The origin of these strains was reported previously (22).

Tumor. Ten million EAT cells were injected i.p. into C57BL/6, RIII, or (RIII X C57BL) F1 hybrid mice.

Sero logical Techniques. Pools of isoantisera against the Θ-AKR antigen were prepared by repeated i.p. injections of AKR/J spleen cells into C3H/An mice, while isoantisera against the Θ-C3H antigen were prepared by repeated i.p. injections of C3H/An spleen cells into AKR/J recipients. One week after the last injection, mice were bled from the retroorbital sinus through a glass capillary. Sera were stored at −20°.

The cytotoxic test used was a modification by Boyse et al. (4) of the method of Gorer and O’Gorman (7). The complement used for the cytotoxic test with thymus cells was absorbed with agar to remove its natural cytotoxicity for murine thymus cells (20), according to a method developed by Cohen and Schlesinger (5). Agar, 80 mg, was added to 3 ml of guinea pig serum diluted 1:3 in 0.9% NaCl solution. The mixture was kept on ice for 1 hr and shaken frequently. Following absorption for 1 hr, the mixture was centrifuged at 3000 rpm for 10 min. The absorbed guinea pig serum was used in cytotoxic tests without further dilution. It was found to be devoid of toxicity for mouse thymus cells while retaining its complement activity.
RESULTS

The Effect of EAT on Lymphoid Cells of RIII Mice. In repeated experiments, tumor growth was accomplished by a reduced sensitivity of both the lymph nodes and thymus cells to the cytotoxic effect of Θ antibodies and complement. The time of onset of these changes and their severity varied with various pools of Θ-AKR isoantisera and among individual tumor-bearing mice. A slight reduction in the sensitivity of lymph node cells to the cytotoxic effect of Θ isoantibodies could be detected in some RIII mice as early as 8 days after tumor inoculation. Chart 1 illustrates the reduction in the sensitivity of lymph node cells to Θ isoantibodies observed 10 and 14 days after tumor inoculation. The lymph node cells of RIII mice tested 16 days after tumor inoculation were almost completely refractory to the cytotoxic effect of Θ antibodies. Only a partial decrease in the Θ isoantigenicity of the thymus of tumor-bearing RIII mice was detected within 2 weeks after tumor implantation (Chart 2). However, the thymus cells of mice examined 16 days after tumor inoculation lost almost completely their sensitivity to the cytotoxic effect of Θ isoantibodies (Chart 2).

The Effect of EAT on Lymphoid Cells of C57BL Mice. Lymph node cells of C57BL mice did not show any decrease in their sensitivity to the cytotoxic effect of Θ-C3H antibodies, even after prolonged periods of tumor growth (Chart 3). If anything, lymph nodes of tumor-bearing C57BL mice showed a slightly elevated sensitivity to the cytotoxic effect of Θ antibodies.

Whereas 16 days of tumor growth were required to reduce substantially the sensitivity of RIII thymus cells to Θ-AKR antibodies, a similar drop in the sensitivity of C57BL thymus cells to Θ-C3H antibodies was already noted 16 days after tumor inoculation (Chart 4). Fifteen days after tumor implantation, the thymus cells lost their sensitivity almost completely.

The Effect of EAT on Lymphoid Cells of (RIII × C57BL) F1 Hybrid Mice. The growth of EAT has a different effect on the sensitivity of lymphoid cells of RIII and C57BL mice.
to Θ isoantibodies. This difference could be attributed to 2 different underlying mechanisms: (a) since RIII and C57BL carry 2 different Θ alleles (Θ-AKR and Θ-C3H, respectively), it could be assumed that tumor growth had different effects on the expression of these 2 antigens in lymphoid cells; (b) alternatively, the 2 strains of mice could differ in other biological aspects in their response to tumor growth. It was therefore of interest to analyze the effect of tumor growth on the 2 allelic antigens in the same host, i.e., in the (RIII × C57BL) F1 hybrid.

The growth of EAT had no significant effect on the sensitivity of lymph node cells of F1 hybrids to either Θ-AKR or Θ-C3H antibodies. In contrast, the thymus of F1 hybrid mice showed a significant reduction as to both Θ-AKR and Θ-C3H antibodies within 12 days after tumor inoculation (Charts 5 and 6). It is clear, therefore, that unlike those in the parental strains the lymphoid cells of (RIII × C57BL) F1 hybrids displayed parallel changes in their sensitivity both to Θ-AKR and Θ-C3H antibodies.

DISCUSSION

Previous studies have shown that the thymus of tumor-bearing animals loses its TL antigenicity and its distinctive sensitivity to guinea pig serum (17, 21). These serological changes occurred within 4 days after i.p. inoculation of EAT into mice of the A and SJL/J strains. In the present study, it was found that the thymus cells of tumor-bearing RIII and C57BL mice lose their sensitivity to the cytotoxic effect of Θ isoantibodies. There was a difference in the rate at which these changes occurred in the thymus of the 2 strains of mice. The disappearance of the sensitivity of thymus cells to Θ isoantibodies in both strains occurred much later than the disappearance to TL antigenicity and sensitivity to guinea pig serum. While it took 12 to 16 days of tumor growth before thymus cells lost their sensitivity to Θ antibodies, 4 days of tumor growth sufficed for the complete disappearance of TL antigenicity and sensitivity to guinea pig serum. The reason for these differences is not clear at present. It may be that there is a difference in the metabolic processes involved in the expression of these various antigens in the thymus. Related to this may be the observation that, while the treatment of mice of the A strain with gonadal hormones produces a significant drop in the TL antigenicity, this treatment had no effect on the Θ antigenicity of the thymus cells (18). Alternatively, the Θ antigen may be present on a population of thymus cells which is more resistant to the effects of tumor growth and of hormone administration than the population of TL-positive cells.

It has recently been shown that the population of lymph node cells that are sensitive to the cytotoxic effect of Θ antibodies is thymus dependent (2, 14, 23, 24). Both neonatal and adult thymectomy result in loss of sensitivity of lymph node cells to Θ antibodies. Reconstitution experiments have indicated that at least some of these thymus-dependent cells are actually derived from the thymus (24). This population of cells was found to disappear temporarily after the administration of heterologous antilymphocyte serum in both RIII and C57BL mice (14, 23). In the present study, it was found that prolonged growth of EAT in RIII mice was accompanied by the disappearance of sensitivity of lymph node cells to the cytotoxic effect of Θ antibodies. No such effect could be observed in the lymph nodes of tumor-bearing C57BL or (RIII × C57BL) F1 hybrid mice. If anything, sometimes there was an increased sensitivity. Further studies should clarify whether other host-tumor combinations may lead to similar antigenic changes.

Although the population of Θ-sensitive cells in the normal lymph node is thymus dependent, the drop in the sensitivity of lymph node cells of RIII mice to the cytotoxic effect of Θ antibodies probably is not a direct consequence of the antigenic changes occurring in the thymus. The antigenic changes in the thymus did not precede those found in the lymph nodes but occurred in parallel. Following thymectomy of nontumor-bearing animals, 4 weeks had to elapse before antigenic changes became apparent in the lymph nodes (24). Thymectomy of tumor-bearing animals did not accelerate the antigenic changes in the lymph nodes (M. Loring and M. Schlesinger, unpublished data). Finally, in
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C57BL mice, in spite of early antigenic changes in the thymus of tumor-bearing animals, no change was demonstrable in their lymph nodes, even after prolonged tumor growth. It seems, therefore, that tumor growth exerts its biological effect independently on both lymphoid organs.

The antigenic changes occurring in lymphoid cells of EAT-bearing RIII mice could be due either to the selective elimination of cells characterized by their sensitivity to Θ antibodies or due to the disappearance of the Θ antigen in individual lymphoid cells. It was hoped that antigenic analysis of F1 hybrids would throw some light on this problem. Tumor growth had different effects on the serological properties of lymphoid cells of C57BL and RIII mice. If, in the F1 hybrid, tumor growth had a different effect on the sensitivity to antibodies against the Θ antigens determined by the 2 parental strains, this would unequivocally rule out the possibility that tumor growth results in the selective elimination of a certain cell population. However, since the behavior of the Θ-C3H and Θ-AKR antigenicity in tumor-bearing F1 hybrids was parallel, both hypotheses could account for the antigenic changes observed.

Tumor-bearing hosts may show an impaired immunological capacity in advanced stages of the disease. Cell-mediated immune reactions are particularly impaired (11, 12, 25). The changes found in the lymph nodes of RIII mice suggest that depletion of the population of thymus-dependent lymphocytes may contribute to the immunological deficiency in advanced cancer. If this assumption is valid, it is to be expected that the administration of thymus cells may restore the immunological capacity of tumor-bearing hosts.

REFERENCES

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