

shaped bacteria grow in the form of microcolonies in this spring, data are presented as number of cells per microcolony. Generation times calculated from the exponential phase of growth of these two experiments are 2.5 and 3.0 hours. In other springs, where growth does not occur in discrete microcolonies, results are expressed as numbers of cells per microscope field. Calculated generation times are summarized in Table 2. Growth rates of filamentous bacteria that also occur in some of these springs have been determined, and their generation times are similar to those of unicellular bacteria. Qualitative observations of bacterial growth have also been made in many other alkaline springs in Yellowstone Park, and in every spring bacteria accumulated rapidly upon microscope slides, although rate of accumulation varied from spring to spring. From these results we conclude that not only

is bacterial growth occurring at temperatures greater than 90°C, but that the rates are surprisingly rapid.

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References and Notes

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2. Boulder Spring is west of Fountain Freight Road in the Fairy Creek area. The other springs are apparently unnamed, and we have given them trivial names for the purposes of this report. Geyserino is a superheated semi-eruptive spring in the White Creek basin east of Firehole Lake Loop Road. Pool A is in front of a cold-water marsh northeast of Geyserino. Steppbrother is a superheated semi-eruptive spring in the White Creek valley east of Five Sisters springs. Porcupine Spring is in the Porcupine Hills group and is a superheated spring near drill hole Y-13 of the U.S. Geological Survey.
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Antigenic Changes in Lymph-Node Cells after Administration of Antiserum to Thymus Cells

Abstract. Mice of the RIII and C57BL strains were treated with rabbit antiserum to thymus (ATS), and cells of their lymph nodes were analyzed serologically at intervals after treatment. While lymph-node cells of untreated mice were sensitive to the cytotoxic effect of isoantibodies against the theta antigen, lymph-node cells of ATS-treated mice showed a significantly reduced sensitivity. Three days after ATS treatment lymph-node cells of most mice were completely refractory to the cytotoxic effect of theta antibodies. Administration of normal rabbit serum elicited only a slight reduction of the sensitivity of lymph-node cells to the cytotoxic effect of theta antibodies. The results support the hypothesis that ATS treatment selectively affects a population of thymus-dependent circulating lymphocytes.

The administration of heterologous antiserum to lymphocyte (antilymphocyte serum, ALS) results in a depression of the immunological competence of the adult organism (1). The mechanism of action of ALS is still unclear, and several hypotheses have been put forward (2). Several lines of evidence indicate that a prerequisite for the activity of ALS is its capacity to bind complement. Pepsin digestion (3) or exposure to low pH (4) not only abrogates the capacity of ALS to bind complement but also prevents its immunosuppressive activity. It therefore seems that at least part of the immunosuppressive effect of ALS may be due to its cytotoxic effect on cells involved in the immune response (5). Evidence has been presented that some populations

of lymphocytes are eliminated in ALS-treated animals. After ALS treatment, lymph nodes may be depleted of lymphocytes (6, 7), and the population of circulating long-lived lymphocytes decreases significantly (8).

In our present study, the effect of rabbit antiserum to thymus cells (ATS) on the theta isoantigenicity of lymph-node cells was analyzed. The theta isoantigen system of the mouse is a non-H-2 antigen system characterized by its high concentration in the thymus and brain (9). In the presence of guinea-pig complement antibodies against the theta isoantigens show a significant cytotoxic effect not only on thymus cells but also on lymph-node cells, while there is no such effect on cells resident in the spleen or in Peyer's

patches (9, 10). We now report that lymph-node cells of ATS-treated mice transiently lose their sensitivity to the cytotoxic effect of antibodies against theta isoantigens.

ATS was prepared by repeated intraperitoneal injections of RIII thymus cells. The effect of ATS was studied in mice of the inbred RIII/Jem and C57BL/6 strains. These strains contain respectively the theta AKR and theta C3H isoantigens (11). Experimental mice received four intraperitoneal injections of 0.25 ml of ATS on alternate days and were killed at intervals after the last injection. Some control mice received four intraperitoneal injections of 0.25 ml of normal rabbit serum (NRS).

Cytotoxic tests were performed with suspensions of cells obtained from axillary and inguinal lymph nodes. A modification (12) of the cytotoxic test of Gorer and O'Gorman (13) was used. The isoantisera used were prepared by repeated administration of allogeneic spleen cells. The AKR/J antiserum to C3H was used for detection of the theta C3H antigen, while the C3H antiserum to AKR/J was used for detection of theta AKR antigen. The ATS was unabsorbed, polyvalent, rabbit antiserum to mouse thymus cells and is assumed to contain heterologous antibodies against a variety of mouse antigens. On the other hand, the reagents used employed for the detection of theta isoantigens (that is, the C3H antiserum to AKR/J and the AKR/J antiserum to C3H) were strictly specific for theta isoantigens.

Cytotoxic indices were calculated from the results of the cytotoxic tests as follows (14): the difference between the percentages of nonviable experimental and nonviable control cells divided by the percentage of viable control cells, the result then being multiplied by 100. (Figures 1 and 2 show the mean of cytotoxic indices obtained in tests with six to ten individual mice per experimental group. Two days after the last injection of ATS to RIII mice, the lymph nodes of these mice showed a significant reduction in sensitivity to antibodies against the theta AKR antigen as compared to untreated controls (Fig. 1). Three days after the last injection of ATS, the lymph-node cells of all RIII mice tested were highly refractory to the cytotoxic effect of theta AKR antibodies. Lymph-node cells of most mice lost completely their sensi-

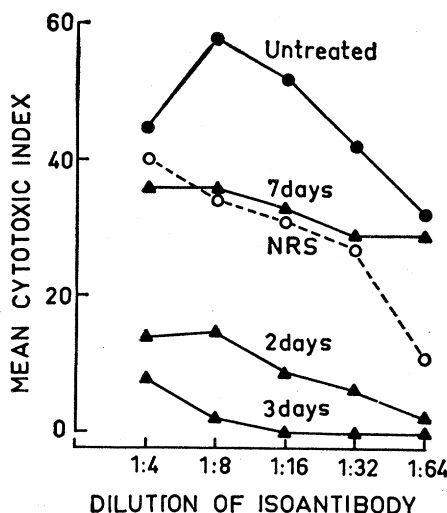


Fig. 1. Mean cytotoxic index obtained with RIII lymph-node cells at various dilutions of C3H antiserum to AKR/J, in the presence of guinea pig complement. ●—●, Lymph nodes from untreated control mice. ▲—▲, Lymph nodes, obtained 2, 3, and 7 days after ATS treatment. ○—○, Lymph nodes obtained 3 days after NRS treatment.

tivity to these cytotoxic antibodies. In the remaining mice not more than 10 percent of the lymph-node cells were killed at any antibody dilution. The sensitivity of lymph-node cells of RIII mice to theta AKR antibodies returned to normal or slightly subnormal levels

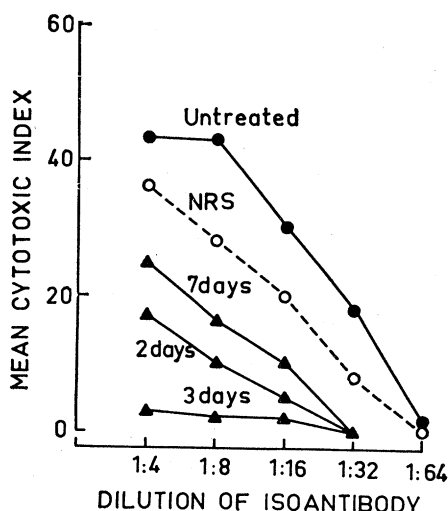


Fig. 2. Mean cytotoxic index obtained with C57BL/6 lymph-node cells at various dilutions of AKR/J antiserum to C3H, in the presence of guinea pig complement. ●—●, Lymph nodes from untreated control mice. ▲—▲, Lymph nodes obtained 2, 3, and 7 days after ATS treatment. ○—○, Lymph nodes obtained 3 days after NRS treatment.

within 7 days after the last injection of ATS.

The effect of ATS prepared by immunization of rabbits with thymus cells of RIII mice was also studied in C57BL mice. The lymph-node cells of C57BL mice showed partial loss of sensitivity to theta C3H antibodies 2 days after the last injection of ATS and were completely refractory 3 days after the last injection (Fig. 2). Within 7 days after the last injection of ATS the lymph-node cells of C57BL mice regained only partially their sensitivity to theta C3H antibodies.

Treatment of either RIII or C57BL mice with NRS resulted in only a slight reduction of the sensitivity of lymph-node cells to theta antibodies. The results obtained 3 days after the last injection of NRS are shown in Figs. 1 and 2.

After treatment with ATS, lymph-node cells showed a significant reduction in their sensitivity of theta isoantibodies, but no change in their sensitivity to the cytotoxic effect of H-2 isoantibodies could be detected. In contrast to lymph-node cells, thymus cells of ATS-treated mice did not show any reduction in their sensitivity to the cytotoxic effect of theta isoantibodies.

Our study shows that ATS treatment results in a temporary disappearance from lymph nodes of lymphocytes characterized by their sensitivity to theta isoantibodies and complement. This effect could be due to blocking of antigen sites by adsorbed antibody (15), or it could reflect antigenic modulation of the cells similar to that observed with thymus and leukemia cells as a result of antibody against the TL antigen (16). The latter possibility is unlikely, because ATS produced by immunization with RIII thymus cells was equally effective in eliminating the theta AKR antigenicity of RIII mice and the theta C3H antigenicity of C57BL mice.

An alternate interpretation of our findings is that treatment with ATS eliminates a cell population characterized by a high concentration of the theta antigen. Several studies indicate that ALS and ATS selectivity destroy thymus-dependent lymphocytes; ALS reduces the population of long-lived lymphocytes (8) which are thought to be derived from the thymus (17). Leuchars *et al.* (18) using chromosome markers showed that ALS treatment transiently inhibits the mitosis of thy-

mus-derived cells in the spleen. Turk and Willoughby (6) found that ATS treatment results in the elimination of lymphocytes from the paracortical area of lymph nodes. This area has been shown to be thymus-dependent in that it is depleted upon thymectomy (19). The possibility that the disappearance of theta positive cells from the lymph nodes of ATS-treated mice reflects the disappearance of a population of thymus-dependent cells is strengthened by the recent finding (20) that thymectomy results in a permanent loss of theta-positive cells from the lymph nodes.

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