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EFFECT OF SPECIFIC ALLOANTISERUM AGAINST T SUPPRESSORS ON RESISTANCE OF MICE TO TUBERCULOSIS

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The writers showed previously that mice of the inbred line I/St are highly susceptible to infection with tuberculosis, whereas those of inbred line A2G are resistant [2]. Parameters of immunity to tuberculosis, such as the level of the delayed-type hypersensitivity (DTH) reaction and of antibody synthesis to tuberculin, were opposite in mice of these lines at the same times after infection: a high level of DTH and a low level of antibodies in A2G mice and the opposite picture in I/St mice [1]. These data confirm the earlier concept [3] of immunological causes of sensitivity and resistance to tuberculosis and the protective role of cellular immunity in this disease.

Immunologic reactions to any antigens, including intracellular pathogenic agents, are under the control of interacting immunocompetent cells [9], among which a special place is occupied by the various subpopulations of T suppressors. After the surface marker of T suppressors had been established — a product of the I-J subregion of the H-2 complex [11], the use of alloantisera against allelic products of the I-J locus (loci) yielded interesting data on the part played by suppressor I-J-positive cells in antitumor immunity [6, 7]. However, this approach has not yet been used to study immunity against infection.

This paper describes a method of obtaining alloantisera against the product of the I-J locus and gives the results of experiments to study the effect of these antisera *in vivo* on survival of mice after infection with tuberculosis.

EXPERIMENTAL METHOD

Inbred and congeneic recombitant mouse lines A2G, A/Sn, A.TL, B10.A(5R), and B10.HTT were reared in the nursery of the authors' Institute. DBA/2 mice used to obtain hybrid recipients were obtained from Z. K. Blandova (Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, Moscow Province), and the B10.A(3R) mice were obtained from B. D. Brondz (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow), to whom the writers are grateful.

Preparation of the alloantisera took place in two stages. First, blast cells were obtained from donors' thymus cells by stimulation with concanavalin A (con A) *in vitro*. By this procedure it is possible for I-J antigen to appear on the cell surface and, in addition, con A induces preferential proliferation of T suppressors [5]. Second, the recipients were immunized with the blast cells thus obtained. The stages are so described below.

Con A-blasts were obtained as follows. In B10.A(5R) and B10.A(3R) mice aged 8-10 weeks cells were isolated from the thymus by pressing the gland through a stainless steel sieve with pore diameter 100 mesh, by means of a Teflon pestle. This stage, and also subsequent rinsing of the cells twice, were carried out in medium 199 (Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR, Moscow), containing 10% heat-inactivated (56°C, 45 min) embryonic calf serum (Flow Laboratories, England) and 15 mM HEPES (from the same firm). After rinsing the cells were transferred to culture medium of the fol-

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lowing composition: tissue culture medium L-15 (Flow Laboratories) containing 10% inactivated embryonic calf serum, 15 mM HEPES, 2 mM L-glutamine (Flow Laboratories), 100 μg/ml gentamycin (Flow Laboratories), and 10^{-5} mole/ml 2-mercaptoethanol (from Serva, West Germany). The cells were cultured in glass flasks treated with silicone (Serva), in a total volume of 5 ml of cell suspension per flask. The cell concentration was $4 \cdot 10^6$/ml. To simulate blast transformation, con A (from Serva) was added to the thymocyte culture in a concentration of 3 μg/ml. The flasks were then incubated for 48 h at 37.5°C.

To estimate stimulation of DNA synthesis in the cultures, [^{3}H]thymidine (1 μCi/ml, specific activity $1 \cdot 10^3$ to $2 \cdot 10^3$ μCi/mmole) was added to some flasks 5 h before the end of culture, after which incorporation of the radioactive label was measured in stimulated and unstimulated (control) cultures on an SBS-1 liquid scintillation counter (USSR). The stimulation index (SI) was calculated by the following equation

$$SI = \frac{\text{Number of counts in experiment} - \text{number of counts in control} - \text{nonspecific background.}}{\text{number of counts in control} - \text{nonspecific background}}$$

In all cases SI varied from 22 to 30.

(DBA/2 × 3R)F_1 and (DBA/2 × 5R)F_1 hybrids of both sexes, aged 2-4 months, were immunized with 5R and 3R cells, respectively. (DBA/2 × 3R) anti-5R antiserum was called AS-T10, and DBA/2 × 5R anti-3R serum was called AS-T11. The con A-blasts obtained beforehand were washed three times with medium 199, resuspended in the same medium, and injected intraperitoneally in a dose of 10^7 cells per mouse six or seven times at weekly intervals. The first immunization was carried out together with Freund's complete adjuvant (from Difco, USA), for which purpose 0.25 ml of cell suspension was emulsified in 0.25 ml of adjuvant; the rest of the immunizations were given without adjuvant. Blood was taken from the retroorbital sinus of the mice ten days after the 6th and 7th immunization, and the resulting antiserum was kept at −40°C until use. The results obtained with both pools were identical.

For the cytotoxic test the antisera were absorbed beforehand (45 min at 4°C) with an excess of a mixture of spleen and lymph node cells of the corresponding recipient: AS-T10 to (DBA/2 × 3R)F_1 cells, AS-T11 to (DBA/2 × 5R)F_1 cells, in order to remove autoantibodies from them. After absorption the microlymphocytotoxic test was set up by the method described previously [8], with slight modifications. Rabbit complement of low toxicity (from Cederlane Laboratories, Canada), diluted according to the manufacturer's recommendations, was used in the reaction. Nonspecific cytotoxicity in the negative controls did not exceed 5% of dead cells.

The mice were infected with tuberculosis by the scheme described previously [1, 2]. To study the action of AS-T10 and AS-T11 on the survival rate of the infected mice, the experimental animals were given two intravenous injections, simultaneously with the suspension of mycobacteria and 24 h after infection, each consisting of 17 μl of antiserum diluted in 0.2 ml physiological saline. Control animals received injections of normal mouse serum by the same scheme.

EXPERIMENTAL RESULTS

Data on the cytotoxic activity of the AS-T10 and AS-T11 antisera against target cells of different lines are given in Table 1. AS-T10 antiserum contained antibodies which reacted with target cells carrying the I-J^k allele, against which immunization had been carried out. As might be expected, the antiserum reacted with only 25-30% of the target cells, for the I-J antigen is represented in only one subpopulation of T cells and is not expressed on the surface of other lymphocytes. The reaction was highly specific, for the antiserum did not react with cells of B10.HTT mice, which carry the I-E^k allele but not the I-J^k allele.

Antiserum AS-T11 is reciprocal relative to AS-T10. Nevertheless, antibodies giving a cytotoxic reaction with target cells carrying I-J^b antigen, against which the animals were immunized, could not be found in it. In the writers' opinion, the reason for this inequality of reciprocal immunizations may be a difference in the requirement of alleles of certain genes of the immune response for antibody synthesis against allelic antigenic products. In that case, those alleles of immune response genes which are "introduced" by the DBA/2 line into the hybrid recipient are suitable for antibody generation against the I-J^k product but are not suitable for the production of anti-I-J^b antibodies. This interpretation was first suggested to explain the negative results of some tests of cellular immunity [10]. Our own data indicate once again the need for special selection of hybrid recipients in order to obtain antisera against weak antigens.

TABLE 1. Cytotoxic Action of AS-T10 and AS-T11 Antisera on Lymph Node Cells from Different Lines of Mice

Antiserum	Target cells	H-2 genotype	Percent of reacting cells	Denominator of titer
AS-T10	A/Sn	$K^kA^kJ^kE^kC^dS^dD^d$	27,4±3,7	16
AS-T10	A2G	$K^kA^kJ^kF^kC^dS^dD^d$	27,3±2,8	16
AS-T10	B10·AKM	$K^kA^kJ^kE^kC^kS^kD^d$	33,7±5,1	16
AS-T10	A·TL	$K^sA^kJ^kF^kC^kS^kD^d$	26,2±3,1	8
AS-T10	B10·HTT	$K^sA^sJ^sE^kC^kS^kD^d$	<7	—
AS-T11	C57BL/10	$K^bA^bJ^bE^bC^bS^bD^b$	<6	—
AS-T11	B10·D2(R107)	$K^bA^bJ^bE^bC^bS^bD^d$	<7	—
AS-T11	B6-H-2^{bm3}	$K^{bm3}A^bJ^bE^bC^bS^bD^b$	<7	—

Legend. In all variants of the negative control (normal serum with complement, antiserum without complement, target cells of another genotype) the fraction of dying cells did not exceed 5-6%.

TABLE 2. Effect of Dilution of Anti-I-J Antisera on Survival of Different Lines of Mice after Infection with Tuberculosis

Line	I-J allele	Antiserum injected	Specificity of antiserum	Time of death, days (M ± m)
A2G	k	AS-T10	Anti-$I\text{-}J^k$	74,3±6,7 (7)
A2G	k	NS	—	56,3±4,1 (7)
I/St	j	AS-T10	Anti-$I\text{-}J^k$	19,6±1,8 (5)
I/St	j	NS	—	20,5±2,0 (6)
B6	b	AS-T11	Anti-$I\text{-}J^b$	38,8±6,2 (7)
B6	b	NS	—	42,1±6,9 (9)

Legend. Number of mice in group shown in parentheses; NS) normal mouse serum.

To obtain results reflecting the cytotoxic action of AS-T10 and AS-T11 antisera on target cells, their activity was investigated in a function test. Mice of different lines, infected with tuberculosis, were given an injection of microdoses of antisera and their activity was assessed according to their effect on survival of the recipients. The results of this experiment are given in Table 2.

AS-T10 antiserum, with cytotoxic activity and specific for antigen $I\text{-}J^k$, significantly ($P < 0.01$) increased the time of survival of mice of the A2G line, resistant to infection. These mice carry the $I\text{-}J^k$ allele, against which this antiserum was active in the cytotoxic test. Meanwhile injection of AS-T10 into mice of line I/St, susceptible to tuberculosis, did not affect the survival of these animals. This fact, first, confirms the specificity of the test antiserum, for line I/St does not carry the allele of the I-J locus against which the animal was immunized (the J allele, but not the k), and second, it indicates indirectly that the cytotoxic and therapeutic activity of the antisuppressor antiserum is connected with the presence of active anti-I-J antibodies in it. Further evidence is given by the fact that AS-T11 antiserum, which has no cytotoxic action, did not affect survival of C57BL/6 mice after injection, despite the presence of $I\text{-}J^b$ antigen, against which immunization had been carried out, on their T suppressors.

We thus obtained an alloantiserum against antigen I-J, the specific marker of T-suppressors in mice, which had a marked therapeutic effect *in vivo* on the course of experimental tuberculosis. Similar results have been obtained with respect to antitumor immunity [6], for which the principal protective role of T-cell immunologic reactions is not in dispute [4]. According to the concept expressed previously [1], T-cells produce resistance in tuberculosis also. It can be tentatively suggested that specific blocking or (and) "knocking out" of T-suppressors by means of anti-I-J antiserum leads to derepression of certain T-dependent immunologic reactions which favor prolongation of survival of the corresponding recipients.

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