marker. According to our preliminary data, these suppressors are resistant to the action of CP: injection of 100 mg/kg CP 2 days or 1 day before adoptive transfer did not abolish the suppressor activity of splenocytes from tolerant mice.

Helper T cells are known to cross-react to mammalian erythrocytes [1]. The weakened immune response not only to RRBC, but also to SRBC, which we observed on the 10th day after induction of tolerance, may therefore be explained as the result of inactivation or deletion of helper T cells. Since suppression of the immune response by splenocytes of the tolerant mice was strictly specific at both times of testing, it can be concluded that the specificity of suppressor T cells is narrower than that of helper T cells involved in the response to xenogeneic antigens. These data showing more specificity of suppressor T cells are in agreement with results obtained by other workers [1, 2].

It can be concluded from the results of these investigations of tolerance to xenogeneic antigens that immunologic tolerance in adult animals in the early stages after tolerogenic treatment is maintained by two mechanisms: by highly specific suppressor T cells and by a clonal deficit of helper T cells, which exhibit weaker specificity. Later, $I\text{-}J^+$ suppressor T cells evidently become the factor determining maintenance of areactivity.

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REGULATION OF IMMUNITY OF MICE TO TUBERCULOSIS BY GENES OF THE H-2 COMPLEX

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Genetic control of susceptibility and resistance to virulent pathogens, including to *Mycobacterium tuberculosis* and *M. bovis*, in inbred mice has been shown to be polygenic in character [2, 7, 8]. Besides the Bog-Ity-Lsh gene (genes), mapped in mouse chromosome 1 [5, 11, 12], and other genetic loci determining the level of natural resistance of mice to these pathogens, an important role in the regulation of immunity to tuberculosis is played by genes of the H-2 complex, which evidently determine the acquired immune response to infectious antigens [4]. Data obtained in recent years by T-lymphocyte cloning show that the immune response to antigens of intracellular agents, including mycobacteria, is restricted by products of genes in the I region of the H-2 complex [6, 9]. Meanwhile the involvement of H-2 genes in the formation and regulation of immunity to tuberculosis in vivo has virtually not been investigated and there are no reliable estimates of the function of the IR-genes of the H-2 complex in determination of the level of the immune response to tuberculosis and of resistance to the disease.

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TABLE 1. Parameters of Resistance to Tuberculosis of Mice Congeneic for H-2, on Infection with *M. tuberculosis*

Line of mice	H-2 genotype							Intact mice		Vaccinated mice	
	K	A_β	A_α	E_β	J	E_α	D	day of death	DTH**, mm	day of death	DTH, mm
C57BL/10SnY	b	b	b	(b)*	b	(b)*	b	24,7±1,7	0,21±0,07	74,2±3,3	0,45±0,17
B10.D2/SnY	d	d	d	d	d	d	d	35,4±2,1	0,27±0,09	n.t.	0,40±0,04
B10.M/SnY	f	f	f	(f)*	f	(f)*	f	34,3±2,0	n.t.	26,4±1,6	0,24±0,09
B10.RIII/SnY	r	r	r	r	r	r	r	30,1±1,9	0,23±0,08	n.t.	n.t.
B10.A/SnY	k	k	k	k	k	k	d	37,7±2,7	0,28±0,06	75,0±9,6	0,48±0,17
B10.D2(R107)	b	b	b	(b)*	b	(b)*	d	36,3±3,0	n.t.	67,8±4,7	0,42±0,18
B10.A(4R)	k	k	k	(k/b)*	b	(b)*	b	33,6±1,1	0,23±0,06	64,2±7,1	0,37±0,08

Legend. n.t.) Not tested. *$E_\beta E_\alpha^b$ and $E_\beta E_\alpha^f$ not expressed; $E_\beta^{k/b}$ not expressed in the presence of the E_α^b allele. **DTH determined 3 weeks after infection.

It was accordingly decided to make a comparative study of the course of the disease in intact mice congeneic with respect to H-2, and mice vaccinated beforehand with BCG, infected with tuberculosis and to study the direct participation of products of individual H-2 loci in the formation of the immune response to PPD in vitro.

EXPERIMENTAL METHOD

Mice of lines B10.D2/SnY, B10.M/SnY, B10.A/SnY, B10.RIII/SnY, and B10.D2(R107)/EgY were generously provided by Z. K. Blandova (Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR), CBA/Sto mice were obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, and mice of lines C5BL/10YCit and B10.A(4R)/DvCit were maintained in the nursery of our own Institute. Mice were used in the experiments at the age of 3-5 months.

The methods of infecting the mice with a virulent strain of *M. tuberculosis*, of BCG vaccination, of conducting the tuberculin tests, and of the blast transformation of lymphocyte reaction to PPD were described previously [1, 3].

Cells in culture were treated with the following monoclonal antibodies: 14V18, anti-$I\text{-}A^k$ (Cederlane Laboratories, Canada), final dilution in the culture 1:200; 10-2-16, anti-$I\text{-}A_\beta^k$, final dilution 2 μg/ml; 3/14, anti-$I\text{-}E^k$, final dilution of hybridoma supernatant 1:50. Antibodies 10-2-16 and 3/14 were generously provided by A. Rudenskii (All-Union Genetics Research Institute, Moscow).

EXPERIMENTAL RESULTS

Table 1 gives data on tuberculin sensitivity and survival of intact and vaccinated mice of lines congeneic with respect to H-2, infected with virulent strain H37Rv of *M. tuberculosis*.

The mice infected with tuberculosis survived for 30 to 37 days, except those of the B10 line with $H\text{-}2^b$ haplotype (24.7 days). The intensity of the tuberculin tests varied from 0.21 to 0.28 mm, with no statistically significant differences. The survival rate of mice of the same lines, vaccinated 5 weeks before infection (dose of BCG 1 mg/mouse) was increased by 2-2.5 times (64-75 days) and the intensity of the tuberculin tests was 0.37-0.38 mm. Vaccinated B10.M ($H\text{-}2^f$) mice were the exception, for their survival rate was actually lower than that of mice of the same line infected primarily (26.4 ± 1.6 and 34.3 ± 2.0 days respectively; $p < 0.05$). The delayed-type hypersensitivity (DTH) reaction (tuberculin tests) was also lowest in these mice (0.24 ± 0.09 mm). It was shown previously [2] that subcutaneous injection of low doses of BCG protects mice of virtually all lines to some degree or other against infection with tuberculosis. The use of a high dose of BCG showed that immunity and resistance are abolished in mice with the $H\text{-}2^f$ haplotype, whereas in mice with other H-2 haplotypes, marked immunity to tuberculosis is induced. This confirms our previous hypothesis [2] that, for a protective effect to be obtained, different doses of BCG must be used, and it shows that the development of protective immunity depends on genes of the H-2 complex. This is a conclusion of practical importance, since, first, typing for MHC genes presents no difficulties and, second, it follows from the data we obtained that, when the experimental results on association of the HLA genotype with morbidity from tuberculosis are interpreted, it must be recalled that most patients are vaccinated with BCG and not infected primarily, and doses of the vaccines naturally were not chosen individually.

TABLE 2. Action of Anti-Ia Antibodies on Proliferative Response to PPD in Vitro of Lymph Node Cells of Mice Infected with Tuberculosis

Line of mice	Treatment of culture	Proliferation, cpm		Index of stimulation
		to PPD	control	
B10	—	3137±491	1370±210	2,29
	Anti-I-A^{k*}	2901±374	1148±235	2,53
B10.A(4R)	—	4747±602	1530±224	3,10
	Anti-I-A^{k*}	1338±291	1004±107	1,33
CBA/Sto	—	4936±707	1493±216	3,31
	Anti-I-A^{k**}	1993±343	1045±119	1,91
	Anti-I-E^{k***}	6030±942	2035±506	2,96
	Anti-I-A^{k}+ Anti-I-E^{k***}	1091±124	855±91	1,28

Legend. *) 14V18 antibodies (see: Experimental Method), **) 10-2-16 antibodies, ***) 3/14 antibodies.

On the basis of data on differences in survival rate and immune response between B10 and 4R lines, which are I-E-negative and differ only with respect to H-2K and $A_\beta A_\alpha$ genes, it now became possible to study the involvement of the I-A and I-E regions, which are known to carry genes of the immune response ($A_\beta A_\alpha$, $E_\beta E_\alpha$), in immunity to tuberculosis.

Table 2 gives the results of analysis of the role of individual class II H-2 loci in immunity to tuberculosis in vitro. The proliferative activity of lymph node cells of infected mice was investigated in a 4-day culture with PPD. It will be clear from Table 2 that addition of anti-I-A^k antibodies (4R mice do in fact possess this allele, see Table 1) to a culture of B10.H(4R) cells lowered the index of stimulation of the lymphocytes, but these same antibodies did not affect proliferation of lymphocytes of B10 mice carrying the I-A^b allele.

Anti-I-A^k antibodies depressed the proliferative response of lymphocytes of CBA mice from 3.31 to 1.91, and anti-I-E^k antibodies depressed it to 2.96; a mixture of anti-I-A^k and anti-I-E^k antibodies lowered it to 1.28. Blocking of I-A^k molecules on 4R cells and of I-A^k + I-E^k molecules on CBA cells thus led to almost total inhibition of the proliferation of these cells in response to PPD (incidentally, I-E molecules in general are not expressed in 4R mice). The results given above suggest that both products of the I region (I-A and I-E) participate in the presentation of antigenic determinants of PPD to lymphocytes, acting as restricting elements of antigen — presenting cell — T lymphocyte interaction. The principal determinant restricting the response in this case is the product of the I-A locus, in agreement with data in the literature [6, 10]. The fact that in the publications cited all specific T clones to PPD were restricted only with respect to I-A can be explained by the use of the I-E-negative B10 line as the source of T cells.

The results thus indicate the important role of Ir-genes of the H-2 complex in the regulation of the secondary specific immune response and resistance of mice and the decisive role of products of the I-A locus in control of the proliferative response of lymph node cells to PPD.

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T-CELL IMMUNODEFICIENCY IN MICE RECEIVING LECTIN AND CYCLOPHOSPHAMIDE

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Successive injections of an antigen and cyclophosphamide (CP) can induce immunologic tolerance to that particular antigen [3]. It has been suggested [2, 6, 16] that one cause of this effect is selective elimination by the cytostatic of lymphocytes stimulated by the antigen. It can accordingly be postulated that the use of the T-cell mitogen, lectin, instead of the specific antigen in the analogous situation would induce general T-cell immunodeficiency, but without at the same time disturbing B-cell functions. The aim of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL/6)F_1 hybrid mice weighing 18-20 g. Lectin from *Lens culinaris* (LcA), obtained and generously provided by Dr. I. Hilgert of the Czechoslovak Institute of Molecular Genetics, and concanavalin A (ConA), from Difco (USA), were used in the experiments. The LcA (1 mg) and conA (100 μg) were injected intravenously in a volume of 0.5 and 0.2 ml respectively in sterile physiological saline.

Cyclophosphamide (Cyclophosphan, from Saransk Medical Preparations Factory) was dissolved in sterile distilled water and injected intraperitoneally in a dose of 200 mg/kg, 2 days after the lectin.

TABLE 1. Suppression of Immune Response to SRBC Induced by Consecutive Injections of T-Mitogens and CP (immunization 7 days after CP)

Series of experiments	Pretreatment of animals	Number of mice	Number of AFC to SRBC in spleen 4 days after immunization
I	LcA+CP	17	4 894
	LcA	17	75 242
	CP	16	56 612
	—	10	221 109
II	ConA+CP	11	12 236
	ConA	12	142 144
	CP	11	73 620
	—	11	248 593

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