Peculiarities of *In Vitro* Immune Response to Mycobacterial Antigens in Inbred I/St Mice

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Comparative study on inbred mouse stains showed that *in vitro* response of T lymphocytes from tuberculosis-susceptible I/St mice to mycobacterial antigens did not differ from that of T lymphocytes from resistant mouse strains. The defect appeared only in the presence of virulent mycobacteria and was not related to T lymphocytes.

Key words: *tuberculosis; genetic control; immune response*

Among more than 30 inbred mouse strains, I/St mice are the most sensitive to tuberculosis [1,2,8]. Their susceptibility to infection is related to alleles of some unlinked loci [5]. Some of these alleles in the homozygous state cause deficiency of the antitubercular defense system and probably suppress the expression of nonallelic genes responsible for resistance against tuberculosis [2,7,8]. Despite intensive research [6,8], it is not known whether this extreme susceptibility of I/St mice is due to their inability to resist virulent *Mycobacterium tuberculosis* (MBT) at an early stage of infection (defect of natural resistance) or to ineffective immune response to mycobacterial antigens (defect of acquired immunity).

This work was designed to evaluate the response of cultured immune lymphocytes from I/St mice to mycobacterial antigens and the roles of T cells and antigen-presenting cells (APC) in this response.

MATERIALS AND METHODS

Inbred I/St (H-2^j), C3H.JK (H-2^j), and A/Sn (H-2^a) mice, and their F1 hybrids were studied. The mice were immunized with ultrasound-destroyed (USD) or live *Mycobacterium tuberculosis* (virulent strain H37Rv) in complete Freund's adjuvant. The antigens were injected into the pads of both hind paws in a dose of 50 μg per paw [8]. Popliteal lymph nodes were removed 14 days postinjection, and cell suspension was prepared for *in vitro* culturing. T cell-enriched suspensions were isolated by double fractionation of lymphocytes in columns packed with nylon wool [4,6]. Unfractionated splenocytes, lymph node cells, and T cells were studied in a 72-h proliferation test *in vitro* [3,8]. APC (spleen cells from intact mice treated with 50 μg/ml mitomycin C) were added to cultured T cells (more than 90% $CD3^+$). Lethal acute tuberculosis was modeled by intravenous administration of high doses of MBT H37Rv [1,7].

RESULTS

After intravenous administration of MBT in lethal doses, the mean survival times were 21.4±2.2, 28.3±3.2, 42.4±4.2, 51.3±6.6, and 58.4±5.8 days for strains I/St, C3H.JK, A/Sn, (C3H.JK×I/St)F1, and (A/Sn×I/St)F1, respectively. Thus, F1 hybrids displayed higher resistance to tuberculosis in comparison with parent strains, which is characteristic of virtually all previously examined hybrids [1,7].

The next goal was to compare *in vitro* responses of cultured lymph node cells from mice immunized with H37Rv USD and injected with live MBT H37Rv, and to find out whether highly sensitive I/St mice differ by these parameters from other mouse stains.

Experiments with nonfractionated lymph node cells from mice immunized with USD H37Rv showed that resistant A/Sn mice and both highly resistant F1 hybrids and I/St mice responded to antigenic stimulation

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TABLE 1. *In Vitro* Proliferation (cpm) of Immune Cells from Lymph Nodes of Mice with Different Resistance to Tuberculosis (*M*±*m*, *n*=5-7)

Mouse strain	Immunization	Control	H37Rv USD
I/St	H37Rv USD	12,467±2967	80,779±5976
	Live MBT	71,256±21,200	48,842±28,709
A/Sn	H37Rv USD	12,625±1347	65,747±7639
	Live MBT	20,187±7549	22,391±8439
(A/Sn×I/St)F1	H37Rv USD	42,377±13,047	91,832±1220
	Live MBT	6334±434	10,556±4803
(I/St×C3H.JK)F1	H37Rv USD	26,591±1764	104,571±12,376

(Table 1). In contrast, the antigen significantly suppressed *in vitro* proliferation of cells from I/St mice infected with live MBT, but had practically no effect on proliferation of cells from A/Sn mice receiving live bacteria (Table 1).

It is interesting that in the absence of the antigenic stimulus, cultured cells from I/St mice infected with live MBT displayed significantly higher proliferation rates than cells from mice immunized with H37Rv USD. This can be explained by inability of I/St mice to suppress the reproduction of MBT H37Rv, which results in hyperactivation of the immune responses.

In further experiments we compared the response of immune T cells from I/St mice to mycobacterial antigens and antigen presentation by their APC. T cells were isolated from lymph nodes of I/St and (C3H.JK×I/St)F1 mice injected with MBT into paw pads. C3H.JK mice have a haplotype H-2^j identical to that of I/St. Thus, their F1 hybrids are fully H-2-compatible with I/St mice, and our previous experiments showed that they are highly resistant to tuberculosis. This determined the choice of these mice and I/St as the source of T lymphocytes and APC. Experiments showed that T cells from I/St mice respond to H37Rv USD, and APC from intact I/St mice are functionally active (Fig. 1).

Thus, there is no difference between I/St and other mouse strains in the ability to respond to mycobacterial antigens *per se*. In I/St mice, T cells and APC are capable of developing immune responses; however, a unique genetically determined deficiency of the antitubercular defense system becomes apparent in the presence of live MBT, which is probably due to a defect in the macrophage system.

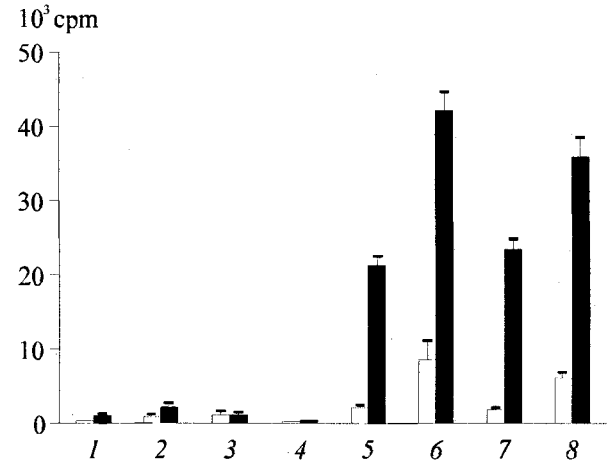

Fig. 1. Proliferative responses of immune T lymphocytes to USD of H37Rv micobacteria in the presence of various antigen-presenting cells (APC): *1*) T cells from I/St without APC; *2*) T cells from F1 without APC; *3*) APC from I/St without T cells; *4*) APC from F1 without T cells; *5*) APC and T cells from F1; *6*) APC and T cells from I/St; *7*) T cells from F1 and APC from I/St; *8*) T cells from I/St and APC from F1. Light columns: control; dark columns: in the presence of H37Rv USD.

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