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Immunological Memory in CBA and CBA/N Mice after Vaccination with *Mycobacterium bovis* (BCG)

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In inbred CBA and CBA/N mice immunological memory was induced by subcutaneous injection of *Mycobacterium bovis* (BCG). Experiments with adoptive transfer of spleen T cells and ionomycin-resistant T cells (memory cells) between CBA and CBA/N mice in various combinations showed that immunological memory was not formed in CBA/N mice, but can be induced by adoptive transfer of cells from CBA mice.

Key Words: *immune memory; tuberculosis; adoptive transfer*

Our previous studies showed that vaccination with *Mycobacterium bovis* (BCG) does not protect inbred CBA/N mice against infection with virulent *M. tuberculosis* H37Rv strain [1,6]. CBA/N mice differ from the parental CBA strain (BCG effectively protects CBA mice from tuberculosis) by *xid* mutation (X-linked immunodeficiency) determining some defects in B cell maturation [4]. We showed that lymphoid cells of BCG-vaccinated CBA/N mice are characterized by reduced spontaneous and antigen-specific proliferative activity. Defective response to BCG vaccination is linked with X chromosome and, probably, with *xid* mutation [6], though the mechanisms underlying the absence of protective effect of BCG vaccination remain unknown.

Immunological memory (IM) mediated by long-living resting memory T cells is an important component of immunity formed after BCG vaccination [2]. Memory cells after repeated contact with mycobacterium antigens synthesize effector cytokines and protect the host organism against tuberculous infection [3].

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In the present study we examined the effect of *xid* mutation on the formation and effector phase of IM in mice after BCG vaccination.

MATERIALS AND METHODS

Inbred CBA and CBA/N mice were vaccinated subcutaneously with live *M. bovis* (BCG) culture [1,2]. Vaccinated mice receiving chemotherapy 5 weeks after BCG vaccination served as IM-carrying donors [2,3]. Experiments were started at least 4 months after termination of treatment, *i.e.* after termination of the active phase of the immune response [3]. Sublethally irradiated recipient mice were intravenously injected with spleen T cells enriched by double passage through a nylon filter or ionomycin-resistant cells (IMC) containing mainly resting memory T cells [5]. One day after adoptive transfer the mice were intravenously infected with virulent *M. bovis* H37Rv strain. The efficacy of transfered immunity was evaluated by the number of mycobacteria in mouse spleen 14 days postinfection. Phenotypic study of CBA and CBA/N mouse cells was performed by flow cytometry on a laser EPICS ELITE fluorimeter (Coulter) using anti-CD4, anti-CD8, and anti-$TCR_{\alpha\beta}$ antibodies (FarMingen).

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RESULTS

In our previous experiments we reproduced IM of BCG vaccination in CBA mice and showed that adoptive immune transfer from mice with IM effectively protected intact mice from infection with virulent mycobacteria. In the present study we studied adoptive transfer of antituberculous resistance from mice with IM in the heterogenous system (between CBA and CBA/N strains). Cell transfer between these strains was not accompanied by rejection of donor cells because the strains differ only in *xid* mutation. A pronounced protective effect was observed only for CBA→CBA transfer (Table 1). It should be noted that the effect of adoptive transfer was tested 2 weeks postinfection, because protection transferred by memory cells was most effective at this term [3].

We previously showed that the most effective antituberculous protection was achieved by adoptive transfer of resting IRC in syngeneic donor-recipient combination [2]. To compare the protective properties of cells from CBA and CBA/N mice we used adoptive transfer of IRC isolated from CBA and CBA/N mouse spleen to intact CBA and CBA/N mice. IRC from both mouse strains possessed protective properties, however, the efficacy of transfer of antituberculous resistance was different. The number of mycobacteria isolated from the spleen after CBA→CBA/N transfer was 3-fold lower than after CBA/N→CBA/N transfer (Table 2). This finding suggests that the protective effect of memory T cells transferred from CBA to CBA/N mice is realized at the effector defense stage, while own IM against the pathogen is not formed in CBA/N mice, probably due to impaired transformation of mycobacterium-activated T cells into resting memory T cells. Another explanation can be incorrect presentation of antigens to T cells by antigen-presenting cells. However, even after normal antigen presentation by T cells of CBA mice, T cells of CBA/N mice cannot effectively transfer antituberculous immunity. Thus, the inability of T cells from CBA/N mice to transfer the antituberculous immunity can be explained by the absence of antigen-specific memory T cells in this population.

The study of cell subpopulations of the spleen and lymph nodes showed that the relative content of T cells and the content of $CD4^+$ and $CD8^+$ in intact CBA/N mice is higher than in CBA mice due to markedly reduced content of B cells. Repeated infection of mice with IM insignificantly changed the content of $CD4^+$ and $TcR^+_{\alpha\beta}$ cells in CBA mice and significantly increased it in CBA/N mice.

TABLE 1. Adoptive Transfer of IM by Spleen T Cells between CBA and CBA/N Mice ($M \pm m$)

Donor	Recipient	CPU/spleen
CBA	CBA	$(2.2 \pm 0.5) \times 10^4$
CBA	CBA/N	$(2.7 \pm 0.7) \times 10^5$
CBA/N	CBA	$(2.8 \pm 0.8) \times 10^5$
CBA/N	CBA/N	$(4.6 \pm 1.3) \times 10^5$
CBA intact	CBA	$(2.7 \pm 0.2) \times 10^5$
CBA/N intact	CBA/N	$(5.2 \pm 0.4) \times 10^5$

Note. Here and in Table 2 all differences from the combination CBA→CBA are significant ($p<0.001$).

TABLE 2. Adoptive Transfer of Immunological Memory by Ionomycin-Resistant Cells ($M \pm m$)

Donor	Recipient	CPU/spleen
CBA	CBA	$(1.4 \pm 0.1) \times 10^4$
CBA	CBA/N	$(2.2 \pm 0.3) \times 10^5$
CBA/N	CBA	$(5.5 \pm 0.3) \times 10^5$
CBA/N	CBA/N	$(7.5 \pm 0.4) \times 10^{5*}$
—	CBA	$(6.5 \pm 1.1) \times 10^5$
—	CBA/N	$(9.1 \pm 0.7) \times 10^5$

Note. *$p<0.001$ compared to combination CBA→CBA/N.

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