Phenotype, Immunological Reactivity and Cytokine Production Profile of Peyer's Patch Cells from Mice Immunized Orally with Allogeneic Cells

(oral immunization / Peyer's patches / phenotype / proliferation / cytokine production)

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Abstract. Mice of inbred strain BALB/c were immunized orally for 10 consecutive days with fresh spleen cells from allogeneic C57BL/10 (B10) donors. The immunized mice displayed significant allotransplantation immunity *in vivo* as demonstrated by resistance to the growth of allogeneic tumours induced by high doses of tumour cells. No significant changes in the proportion of the major T cell subsets in PP of immunized mice were found 1 or 7 days after the last immunization dose. However, PP cells from orally immunized mice displayed stronger proliferative response after stimulation with cells used for oral immunization than the cells from control animals. Similarly, after stimulation *in vitro* with specific alloantigens, PP cells from orally immunized mice produced more IFN-γ than the cells from control recipients. On the contrary, the production of IL-4 was significantly decreased in the immunized mice. The production of IL-2 by PP cells after oral immunization was not significantly changed and IL-10 was only slightly increased. The results thus show that oral immunization with allogeneic cells induces systemic transplantation immunity which can be demonstrated already in Peyer's patches by increased cell proliferation after immunization with specific alloantigens and by changes in cytokine production.

Administration of antigen by oral route modulates the immune response of the recipient, but the results are far from being uniform. In some models inhibition of immune response after oral immunization was observed and the phenomenon has been called mucosal tolerance (Weiner et al., 1994; Garside and Mowat, 1997). Other studies, however, described rather stimulation of immunity, including enhancement of antibody production and activation of cell-mediated immunity (Yang et al. 1990; Blanas et al., 1996). Among the factors which decide about the outcome of the oral immunization, the type, dose and form of the antigen are the most important. In general, soluble antigens preferentially induce tolerance, while oral administration of particulate antigens leads to immunity. Also, at the cytokine production level, oral immunization yields contradictory results. In some cases, mucosal tolerance is associated with increased production of Th2 cytokines such as interleukin (IL)-10 and IL-4 and with high production of transforming growth factor (TGF)-ß (Friedman and Weiner, 1994; Ma et al., 1998), but in other models enhanced production of interferon (IFN)-γ and decreased secretion of IL-4 were observed (McMenamin et al., 1994; Marth et al., 1996; Chen et al., 1997). It still remains difficult to predict the final outcome of oral immunization because more factors, both endogenous and exogenous, including genotype, immune status and age of the recipient, frequency and timing of immunization, decide whether immunity or tolerance will prevail (Peng et al., 1989; Faria et al., 1993). Distinct cytokine production patterns can also be found in mucosal and peripheral lymphoid organs (Tonkonogy and Swain, 1993).

Peyer's patches (PP) represent the primary site for uptake and presentation of ingested antigens in the intestine. Since this lymphoid tissue possesses immunological particularities distinct from other lymphoid organs (Iwasaki and Kelsall, 1999), we determined changes in the immunological reactivity and in cytokine production of PP cells after oral immunization with fresh allogeneic cells. It has already been shown that oral immunization with allogeneic cells can induce hyporeactivity to alloantigens or even tolerance of allografts (Sayegh et al., 1992; He et al., 1996). We found, however, that feeding of mice with allogeneic cells for 10 consecutive days induces systemic transplantation immunity which can be demonstrated already in PP cells by increased alloantigen-induced cell proliferation, enhanced production of IFN-γ and decreased secretion of IL-4.

Received February 11, 2000. Accepted March 23, 2000.

This work was supported by grants No. 310/99/0360, 310/97/1261 and 310/99/D044 from the Grant Agency of the Czech Republic, grant No. 3964-3 from the Ministry of Health of the Czech Republic, Project VS 97 099 from the Ministry of Education of the Czech Republic, and NATO Linkage grant CRG.LG 972853.

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Abbreviations: IFN – interferon, IL – interleukin, MLC – mixed lymphocyte culture, PBS – phosphate-bufferred saline, PP – Peyer's patches, RT-PCR – reverse transcription-polymerase chain reaction, TGF – transforming growth factor.

Material and Methods

Animals

Mice of inbred strains BALB/c ($H2^d$), C57BL/10Sn (B10) ($H2^b$) and CBA/J ($H2^k$) from the breeding colony of the Institute of Molecular Genetics, Prague, were used in the experiments. Animals of both sexes were used at the age of 7–10 weeks.

Oral immunization

Suspensions of spleen cells from B10 mice were prepared in phosphate-buffered saline (PBS) and 30×10^6 cells/dose/day in a volume of 0.2 ml were administered directly into the stomach using a gavage tube. Totally, 10 daily immunization doses were applied and the mice were tested 1 or 7 days after the last immunization dose. Control mice were untreated or were treated with PBS at the same time schedule as were immunized experimental mice.

Allogeneic tumour growth

Cells of fibrosarcoma MC11, originally induced by methylcholanthrene in a B10 male mouse (Bubeník et al., 1978), were grown *in vitro* in tissue culture. To induce tumours, control and orally immunized BALB/c mice were injected subcutaneously in a dorsal part of the body with 12×10^6 of MC11 sarcoma cells in a volume of 0.2 ml of PBS. The size of the tumours was measured every other day.

Our prelimiary experiments showed that less than 10^3 MC11 cells induce progressively growing tumours in syngeneic B10 mice. However, due to transplantation immunity, at least 10×10^6 MC11 cells are required to induce tumours in allogeneic BALB/c mice. After injection of 12×10^6 MC11 cells, the tumours grew in all untreated recipients.

Preparation of Peyer's patch cells

Peyer's patches were isolated from washed small intestine of individual normal or orally immunized mice and single cell suspensions were prepared in RPMI 1640 medium (Sigma, Chemical Co., St. Louis, MO) contaning 10% heat-inactivated foetal calf serum (Sigma), antibiotics (100 units/ml of penicillin, 100 μg/ml of streptomycin), 10 mM HEPES buffer and 5×10^{-5} M 2-mercaptoethanol (hereafter referred to as complete RPMI 1640 medium). The cells were centrifuged and diluted to appropriate concentrations.

MLC reaction

Peyer's patch cells (1.5×10^5/well) from control or orally immunized mice were incubated with irradiated (25 Gy) stimulator spleen cells (2×10^5/well) in 0.2 ml of complete RPMI 1640 medium in 96-well tissue culture plates (Nunclon, Roskilde, Denmark). Cell proliferation was determined by adding 0.5 μCi of ^{3}H-thymidine/well for the last 6 h of the 96-h incubation period (Institute for Research and Application of Radioisotopes, Řež, Czech Republic).

Cytokine production and determination

Peyer's patch cells from control or immunized mice were incubated in a volume of 1 ml of complete RPMI 1640 medium in 24-well tissue culture plates (Flow Laboratories, McLean, MI) alone or with irradiated (25 Gy) stimulator spleen cells. The final concentration of each cell type was 1.5×10^6 cells/ml. Cell culture supernatants were harvested after 48 h (IL-2 determination), 72 h (IFN-γ) or 96 h (IL-4 and IL-10).

The presence of IL-2, IL-4, IL-10 and IFN-γ was determined by a specific ELISA using a pair of anti-cytokine monoclonal antibodies purchased from PharMingen (San Diego, CA) as we have described (Hašková et al. 1999). For the quantification of the reaction, cytokine standards (all purchased from Genzyme, Cambridge, MA) were included in all ELISA determinations.

Reverse transcription-polymerase chain reaction (RT-PCR)

Peyer's patch cells from control and orally immunized BALB/c mice were stimulated for 24 h with irradiated (25 Gy) B10 spleen cells. Total RNA was isolated using TRIsol Reagent (Life Technologies, Grand Island, NY) and two micrograms of total RNA were reverse-transcribed into cDNA in 20 μl reaction mixture as we described in detail elsewhere (Holáň et al., 1998). Two μl of cDNA preparation was amplified in a PCR cycler (MJ Research, Watertown, MA) in the reaction mixture

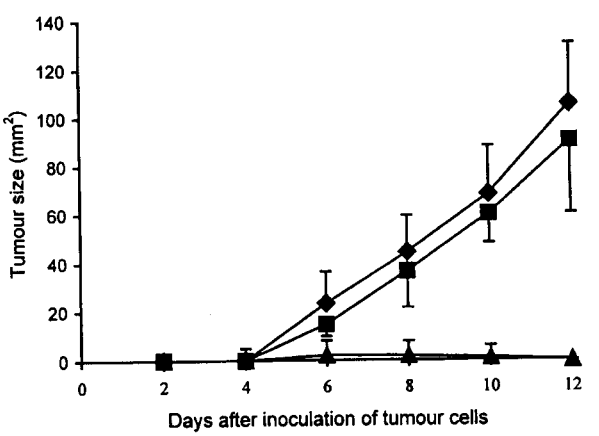

Fig. 1. The growth of allogeneic tumours in control and orally immunized mice. Control BALB/c mice (rhombuses) and BALB/c mice tested 7 days after the last oral immunization dose with 30×10^6 spleen cells from B10 (triangles) or CBA/J (squares) donors were inoculated subcutaneously with 12×10^6 cells of sarcoma MC11 originally induced in a B10 mouse. The size of growing tumours was measured every other day. Each group consists of 6 mice.

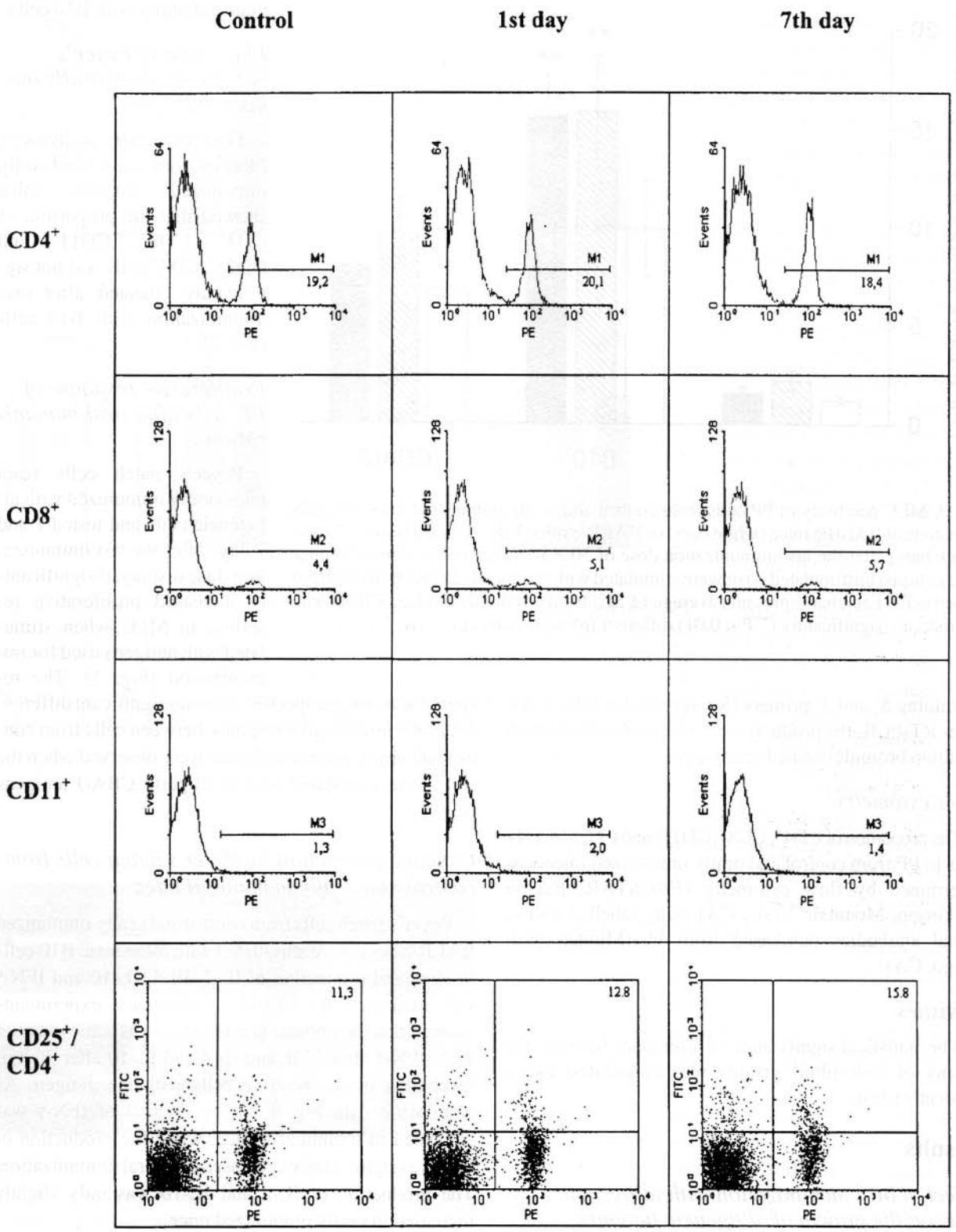

Fig. 2. Proportion of CD4+, CD8+, CD11+ and CD25+/CD4+ cells in Peyer's patches from control BALB/c mice (Control) and BALB/c mice tested 1 (1st day) or 7 (7th day) days after the last oral immunization dose with 30×10^6 B10 cells. The percentages of positive cells were determined by flow cytometry.