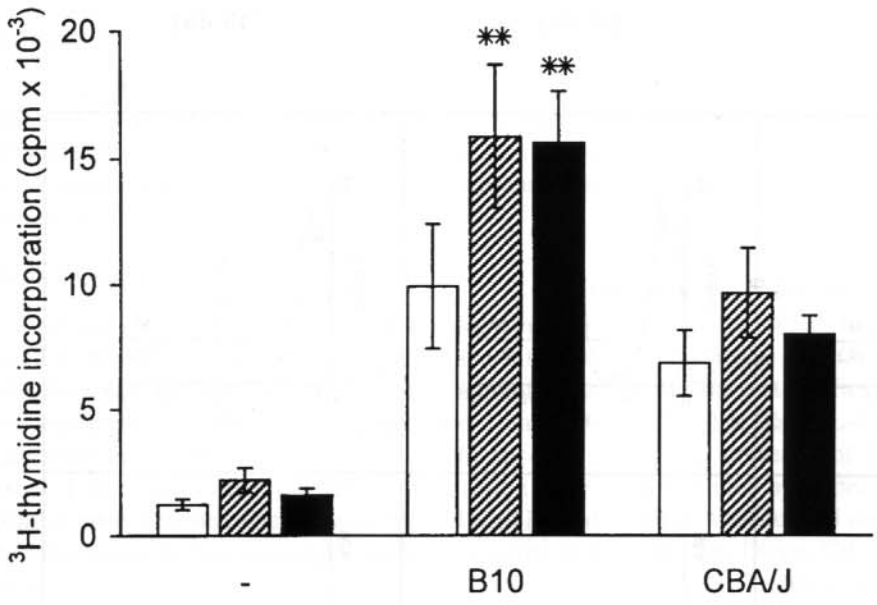

Fig. 3. MLC reactivity of PP cells from control and orally immunized mice. PP cells from control BALB/c mice (white bars) or BALB/c mice 1 day (shaded bars) or 7 days (black bars) after the last immunization dose of 30×10^6 spleen cells from B10 mice were cultured unstimulated (-) or were stimulated with irradiated (25 Gy) B10 or CBA/J spleen cells. Each bar represents average (± SE) from 9 individual mice. Values with asterisks are significantly ($^{**}P < 0.01$) different from the control.

containing 5' and 3' primers (Strategene, La Jolla, CA). After RT-PCR, the products were electrophoresed on an ethidium bromide-stained agarose gel.

Flow cytometry

The proportion of $CD4^+$, $CD8^+$, $CD11^+$ and $CD25^+/CD4+$ cells in PP from control and orally immunized mice was determined by flow cytometry (FACSTAR, Becton Dickinson, Mountain View, CA) using labelled monoclonal antibodies purchased from PharMingen (San Diego, CA).

Statistics

The statistical significance of diferences between the means of individual groups was calculated using Student's t-test.

Results

Effect of oral immunization with allogeneic cells on the growth of allogeneic tumours

Untreated BALB/c mice and BALB/c mice immunized orally for 10 consecutive days with B10 or CBA/J spleen cells were inoculated subcutaneously with 12×10^6 cells of MC 11 sarcoma of the B10 origin. As demonstrated in Fig. 1, allogeneic tumours grew progressively in untreated recipients or in mice immunized orally with CBA/J cells, but the tumour growth was completely inhibited in mice immunized orally with B10 cells.

Phenotype of Peyer's patch cells from orally immunized mice

Flow cytometry analysis of PP cells from control and orally immunized BALB/c mice showed that the proportion of $CD4^+$, $CD8^+$, $CD11^+$ and $CD25^+/CD4^+$ cells was not significantly changed after oral immunization with B10 cells (Fig. 2).

Proliferative response of PP cells after oral immunization

Peyer's patch cells from mice orally immunized with allogeneic cells and tested 1 and 7 days after the last immunization dose displayed significantly increased proliferative response in MLC when stimulated with antigens used for immunization (Fig. 3). The response was antigen specific, since no significant differences in the proliferative response between cells from control and orally immunized mice were observed when the cells were stimulated with third-party CBA/J antigens (Fig. 3).

Cytokine production by Peyer's patch cells from control and orally immunized mice

Peyer's patch cells from control and orally immunized BALB/c mice were stimulated with allogeneic B10 cells *in vitro* and production of IL-2, IL-4, IL-10 and IFN-γ was measured by ELISA. Preliminary experiments showed that the optimal level of IL-2 was achieved after 48 h, IFN-γ after 72 h, and IL-4 and IL-10 after 96 h of incubation of the reactive cells with the antigen. As demonstrated in Fig. 4, the production of IFN-γ was enhanced in immunized mice, while the production of IL-4 was significantly decreased after oral immunization. The production of IL-2 and IL-10 was only slightly increased in orally immunized mice.

To exclude the possibility that the increased levels of IFN-γ found in PP cells after oral immunization were not due to different consumption of the produced cytokine, the expression of the gene for IFN-γ was determined. As shown in Fig. 5, the level of IFN-γ mRNA was increased in orally immunized mice. This result corresponds to the finding at the protein level.

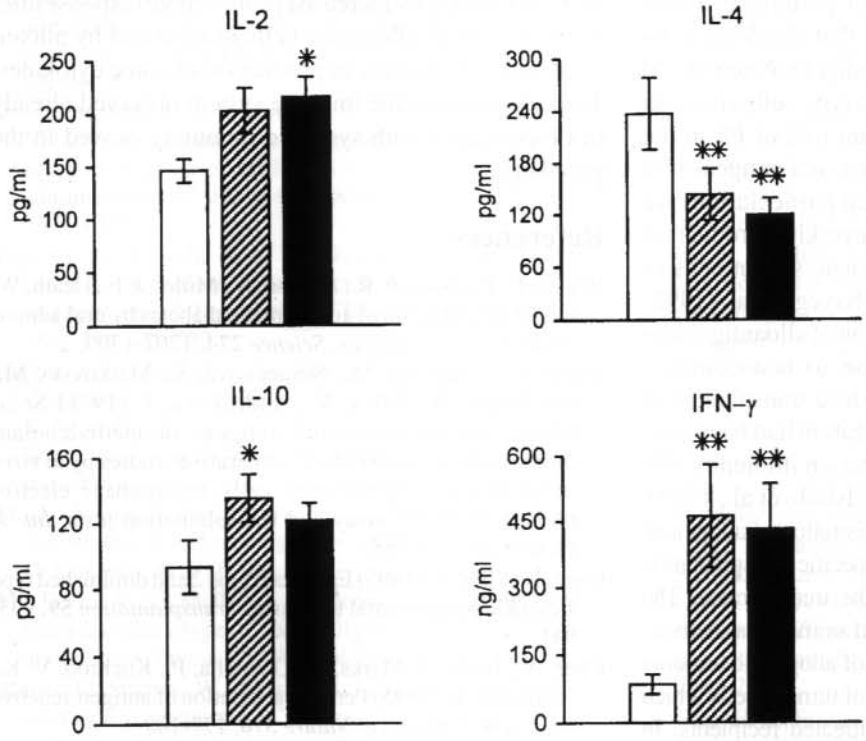

Fig. 4. Cytokine production by PP cells from control and orally immunized mice. PP cells from control BALB/c mice (white bars) or BALB/c mice 1 (shaded bars) or 7 (black bars) days after the last oral immunization dose of 30×10^6 B10 spleen cells were stimulated *in vitro* and the production of IL-2, IL-4, IL-10 and IFN-γ was determined by ELISA. Each bar represents average (± SE) from 6 individual mice. Values with asterisks differ significantly ($^*P < 0.05$, $^{**}P < 0.01$) from values of control mice.

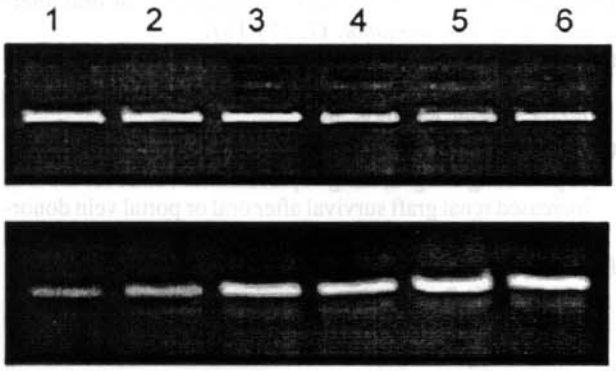

Fig. 5. Expression of mRNA for IFN-γ in PP cells from control and orally immunized mice. PP cells from control BALB/c mice (lanes 1 and 2) or BALB/c mice 1 (lanes 3 and 4) or 7 (lanes 5 and 6) days after the last oral immunization dose with 30×10^6 B10 spleen cells were stimulated for 24 h *in vitro* with irradiated (25 Gy) B10 spleen cells. Total RNA was isolated, reverse transcribed, and amplified with primers for ß-actin (top) and IFN-γ (bottom). PCR products were electrophoresed and the ethidium bromide-stained gel is shown.

Discussion

The intestine contains more T cells than the rest of the body combined. The reason is to ensure the protection of the individual since the mucosal surfaces, such as the intestinal and the respiratory tracts, represent the major route by which foreign antigens gain access to the body. The general characteristic feature of the immune response in the gut is that protective cell-mediated and humoral immune responses against invading pathogens are allowed to proceed whilst pathogenic responses against innocuous inhaled antigens, dietary proteins or resident bacteria are prevented. However, in experimental models of oral immunization it is still difficult to predict the final result.

Administration of antigen by oral route modulates systemic immune response of the recipient and induces changes in cytokine production. Although some authors described, after oral immunization, a shift of cytokine production to Th2 type, characterized by enhanced production of IL-4 and IL-10 and decreased secretion of IL-2 (Neurath et al., 1996; Ma et al., 1998), or even to Th3 type of cytokine response characterized, in addition to the above cytokines, by secretion of TGF-ß (Chen et al., 1995; Hancock et al., 1995), in other models of mucosal immunization aberrant patterns of cytokine production were observed. The latter were characterized mainly by increased production of IFN-γ and decreased secretion of IL-4 (Hoyne and Thomas, 1995; Marth et al., 1996). The Th2/Th3 type of response was observed preferentially in the models of oral tolerance, where inhibition of systemic immunity after oral immunization was observed. A higher secretion of IFN-γ was found when oral immunization led to immunity or when cytokine production was tested in PP. It was also shown that local differences in cytokine production may occur (Tonkonogy and Swain, 1993).

Peyer's patches are the most important part of the immune system of the gastrointestinal tract and they represent the primary site for the uptake of antigen which was ingested. Lagoo et al. (1994) demonstrated high spontaneous levels of transcripts for multiple cytokines