sections of the reproductive tract. Spleens were homogenized and the cell suspension was passed through a 110 μm mesh, washed three times by centrifugation at 400 *g* for 5 min each time and resuspended in PBS. Blood of the treated mice collected by heart puncture into heparinized tubes was diluted 1 : 1 (v/v) with PBS, and WBC were separated on columns of Ficoll-Paque (Pharmacia, Uppsala, Sweden). Separated cells (6×10^6 cells/ml) were incubated with an equal amount of LIS-4 antibody diluted 1 : 10 (v/v) in PBS at 22°C for 20 min, thoroughly washed with PBS-Tween, further incubated for the next 20 min with porcine anti-mouse immunoglobulin (SEVAC, Prague, Czech Republic) conjugated with fluorescein isothiocyanate diluted 1 : 60 in PBS, and preabsorbed with tissue homogenate from mouse cervix, vagina, uterus and spleen. A droplet of cell suspension was placed on a glass slide and examined with an Orthoplan Leitz microscope (Leitz, Wetzlar, Germany) equipped with a halogen-quartz lamp using a FITC interference filter. WBC suspension from mice subjected to intrauterine infusion with PBS instead of ISF served as a negative control. Both normal mouse serum and LIS-4 supernatant preabsorbed with ISF (4 mg/ml) were also used as controls.

Statistical analysis

The significance of the differences between experimental and control groups was analyzed using Student's t-test.

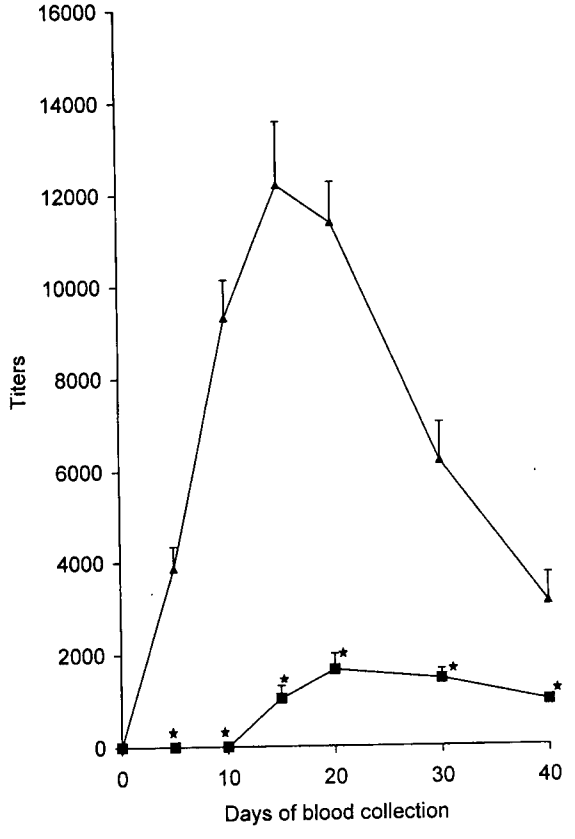


Fig. 1. Effect of 6-fold administration of 100 μg ISF on primary antibody response to KLH in mice without booster. Mice were immunized with KLH only (triangles) or treated with KLH plus intrauterine infusion of 100 μg ISF (squares). ISF treatment lasted for 6 days. The mice were bled on indicated days and serum KLH antibody titers were measured by ELISA. The mice did not receive secondary immunization. Values are expressed as the mean ± SD of antibody titer from 3 different experiments with 5 animals at each time point. Antibody titers of mice immunized with SRBC were similar to those of mice immunized with KLH, and have therefore been omitted. *P < 0.01 versus control mice

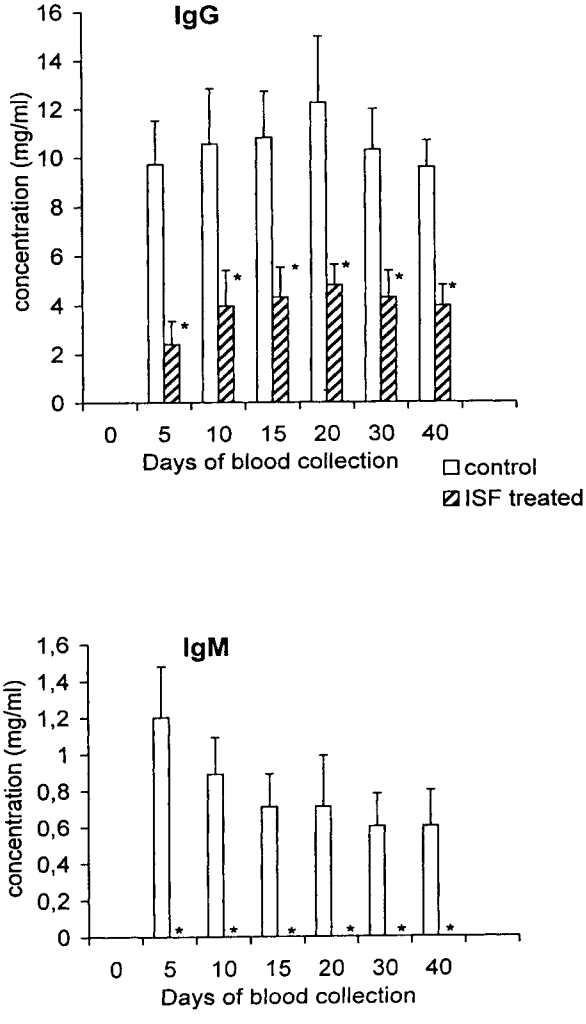


Fig. 2. Effect of 6-fold administration of 100 μg ISF infused via uterus on absolute concentrations of IgG and IgM in mice. Concentrations of IgG and IgM in the antisera of mice immunized with KLH only are designated as control group. Concentrations of IgG and IgM in the antisera of ISF-treated and KLH-immunized mice are designated as experimental groups. The results are expressed in mg of immunoglobulins per ml evaluated by sandwich ELISA in the sera collected on indicated days from mice with no secondary immunization. Data represent the mean ± SD of immunoglobulin concentration from 3 different experiments with 5 mice at each time point. *P < 0.01 versus control mice

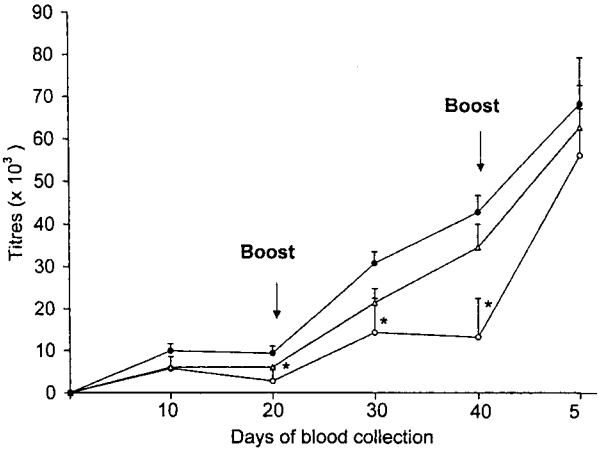


Fig. 3. Effect of 6-fold administration of ISF on duration of the immune suppression of antibody response to KLH in mice. Mice were immunized with KLH only (black circles) and with KLH plus intrauterine infusion of 100 μg ISF (white circles) or 50 μg of ISF (triangles). The mice were bled on indicated days and serum titers of antibodies were measured by ELISA. All mice received additional immunizations with KLH on days 20 and 40. Values are expressed as the mean ± SD of antibody titer for 5 animals at each time point. Values of antibody titer in mice immunized with SRBC were similar to those for mice immunized with KLH, and have therefore been omitted. *P < 0.01 versus control mice

Results

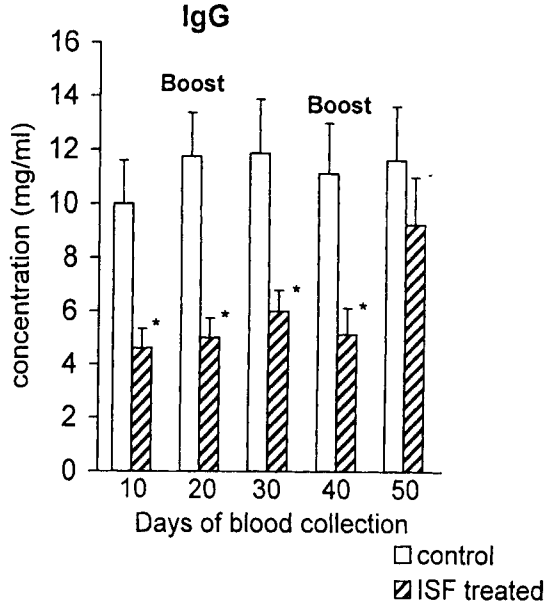
Effect of ISF on primary antibody response to challenging antigens (KLH, SRBC) and concentration of IgG and IgM

The intrauterine infusion of 100 μg ISF completely suppressed the primary antibody response to KLH or SRBC from day 0 to 10 after primary immunization (Fig. 1). A low titer of antibodies was detected in the sera collected 15 to 40 days after immunization. In the sera of control mice immunized but not treated with ISF, an increased antibody titer was detected from day 5 with maximum on days 15 to 20 after immunization. After day 30 the antibody titer decreased rapidly.

The intrauterine treatment with 100 μg ISF decreased the concentration of IgG and IgM in the blood sera collected from the experimental ISF-treated mice after the primary immunization with KLH. The concentrations of IgG and IgM did not differ significantly in the sera of control mice collected 5 to 40 days after immunization (Fig. 2). However, the concentration of IgG was substantially decreased (approximately to 60%) in the sera of ISF-treated mice. IgM was not detected in the sera of ISF-treated mice collected from day 5 to 40 after the primary immunization.

Duration of the immune suppression

We determined the duration of the immune suppression induced by ISF intrauterine treatment after the primary and secondary immunizations with KLH or SRBC (Fig. 3). The treatment with 100 μg of ISF suppressed the primary KLH antibody response by 93% in comparison to antibody titers in the antisera of control mice ($P<0.01$). Suppression of the secondary antibody response with 100 μg of ISF was also significant (suppression by 67%, $P< 0.01$). The suppression of the antibody response to KLH after the third immunization (on day 40) was not significant.



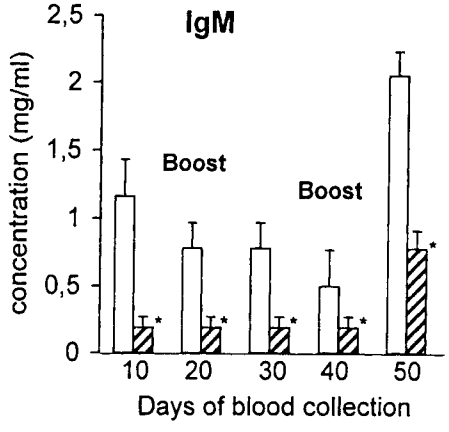


Fig. 4. Effect of 6-fold administration of 100 μg ISF infused via uterus on immunoglobulin IgG and IgM concentrations. The mice received additional immunizations on days 20 and 40. The results are expressed in mg of immunoglobulin per ml of serum evaluated by sandwich ELISA in the antisera of ISF-treated and KLH-immunized mice (squares – experimental group) and ISF-untreated but KLH-immunized mice (squares – control group). Data represent the mean ± SD from 3 different experiments with 5 mice versus control mice in each group. *P < 0.01.