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ORIGINAL PAPER

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Salivary gland extract from *Ixodes ricinus* tick modulates the host immune response towards the Th2 cytokine profile

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Abstract In our previous work, the salivary gland extract (SGE) from *Ixodes ricinus* ticks impaired T-lymphocyte proliferation and clearly modulated the immune response towards the Th2 pattern in human peripheral blood mononuclear cell culture. In the present work, the results obtained on mouse splenocytes are compared with those on human leukocytes. ELISA (protein level) and RNAse protection assay (mRNA level) showed that SGE enhanced interleukin (IL)-1α, IL-1β, IL-1Ra, IL-6, and IL-12p40 cytokines, whereas production of IL-2, IL-5, IL-10, and IL-13 was decreased. The minute levels of IL-9, IL-15 and IL-12p70 were not changed after the addition of tick saliva. IL-4 was upregulated, whereas the production of gamma interferon and migratory inhibition factor was downregulated after the addition of SGE. Tick saliva decreased concanavalin A-stimulated spleen cell proliferation and the percentage of activated T-cells. We conclude that the Th2 polarization did not involve all of the cytokines tested. However, the Th2 subset-augmenting effect of tick saliva was confirmed.

Introduction

Compared to other blood-feeding ectoparasites, ticks need an unusually long feeding period (days or weeks) until they are fully engorging and detach from their host (Bowman et al. 1996). While obtaining a bloodmeal, ixodid ticks returns excess fluid back into the host via the saliva. During the feeding period, blood flowing into the feeding lesion can clot, and immune responses of the host can counteract tick feeding. Thus it is not surprising that tick saliva was found to exert immunomodulatory effects (reviewed in Gillespie et al. 2000). The Th2 response-promoting effect of tick saliva (Ferreira and Silva 1999; Schoeler et al. 2000; Mejri et al. 2001), the reduction of the T-cell proliferative response (Bergman et al. 1995; Ferreira and Silva 1999) and impaired phagocyte function (Kopecký and Kuthejlová 1998; Gwakisa et al. 2001) have all been well defined.

Tick-induced host immunomodulation facilitates bloodmeal acquisition and is an important factor in the transmission and establishment of the tick-borne disease-causing agents *Borrelia burgdorferi* and tick-borne encephalitis (TBE) virus (Labuda et al. 1993; Wikel 1999). Recently, several immunomodulatory factors have been discovered in tick saliva (Mulenga et al. 1999; Wang and Nuttal 1999; Bergman et al. 2000; Gillespie et al. 2001; Jaworski et al. 2001; Tsuda et al. 2001). Some of these were found as potential vaccine candidates for the control of tick infestation (Mulenga et al. 1999; Tsuda et al. 2001).

Many examples of the immunomodulatory effects of tick saliva have been described for various tick species (Brossard and Wikel 1997; Wikel and Bergman 1997). Differences between the immunomodulatory abilities of larvae, nymphs or adults have recently been reported (Mejri et al. 2001). Many tick species, as with other haematophagous arthropods, do not show strict host specificity. During their life cycle, such species feed on several hosts, often of different vertebrate species.

The present study, together with our previous work (Kovář et al. 2002), was undertaken to compare the effect of tick saliva on two mammalian hosts: the house mouse (*Mus musculus*), a common experimental animal, and humans, potential victims of tick-borne diseases. We determined the impact of *Ixodes ricinus* salivary gland extract (SGE) on mouse lymphocytes and compared the results with those obtained on human peripheral blood

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leukocytes (Kovář et al. 2002). The same SGE preparation, mitogens and experimental protocols were used in both the mouse and the human models. In addition, we used a ribonuclease protection assay (RPA) to test the influence of tick saliva, not only on the release, but also on mRNA expression of various cytokines.

Materials and methods

Salivary gland extract

Adult *I. ricinus* ticks were collected by flagging in localities in the southern Czech Republic known to be free of TBE virus. The ticks were screened for *Borrelia burgdorferi* by PCR with negative results. They were then fed in groups of ten mating pairs within retaining cells attached to the backs of guinea pigs. After 5 days, the engorged female ticks were removed, and their salivary glands dissected out under sterile conditions and pooled. The 5-day period during which the ticks had fed on mice was chosen because of the optimal effect of the SGE prepared on the saliva activated transmission of TBE virus (Labuda et al. 1993). After washing three times in phosphate-buffered saline, the salivary glands were homogenized by sonication and clarified by centrifugation at 10,000 *g* for 10 min. The protein concentration of clarified SGE was determined using the BioRad protein estimation kit (BioRad, Hercules, Calif.). Aliquots of the SGE preparations were stored at –70°C. SGE at a concentration of 20 μg/ml had no effect on spleen cell viability as determined by the trypan blue exclusion test.

Preparation and proliferation of spleen cells

Spleens from BALB/c mice were harvested and splenocytes, in RPMI 1640 medium supplemented with 5% fetal bovine serum, were added to the wells of a 96-well plate (Nunc, Roskilde, Denmark). A total of 2×10^5 spleen cells per well were stimulated with 5 μg/ml concanavalin A (ConA) or 2 μg/ml lipopolysaccharide (LPS) from *Salmonella typhimurium* or control medium ± SGE (0.05, 0.5, 5 or 20 μg/ml). After 24, 48 or 72 h-incubation at 37°C in 5% CO_2, 50 μl of 18.5 kBq of [^{3}H]thymidine (Nicom, Prague, Czech Republic) was added per well, followed by 6 h incubation at 37°C in 5% CO_2. Splenocytes were collected using a cell harvestor (Tomtec, Hamden, Conn.) onto glass filter mats. Dried filters were placed into polyethylene minivials and plastic scintillator MeltiLex A (Wallac, Turku, Finland) was applied by heating. Counting was performed in a 1450 MicroBeta TriLux scintillation counter (Wallac). Aliquots of the supernatants from these cultures were stored at –70°C for cytokine detection. The results show the mean of five wells for each experiment.

Cytokine detection

ELISA kits were used for the interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-10 and gamma interferon, IFN-γ (Genzyme, Cambridge, Mass.) and IL-12p70, IL-12p40 (R and D Systems, McKinley, Neb.) detection in culture supernatants. Assays were performed and data were analyzed following the manufacturer's instructions. To determine the concentration of cytokines in the culture supernatants, standard curves were constructed using recombinant cytokines, and cytokine concentrations in test samples were determined from the corresponding concentration values on the standard curve. The results show the mean of triplicate wells for each experiment.

Flow cytometry

Control or SGE-treated splenocytes after 24, 48, or 72 h proliferation were collected and red blood cells were lysed over 5 min in lysing solution (0.83% NH_4Cl, 0.1% $KHCO_3$, 0.0037% $Na_2EDTA.2H_2O$, pH 7.2–7.4). Splenocytes were washed in washing buffer (PBS buffer with 1% BSA and 0.1% NaN_3) and, to avoid non-specific Fcγ-Fcγreceptor interactions, 2×10^5 cells (in 10 μl washing buffer) were incubated with 0.5 μg of anti Fc-γ antibodies (in 10 μl) at 4°C. After 5 min incubation, 0.2 μg (in 10 μl) fluorescein isothiocyanate (FITC) and phycoerythrin (PE) labelled antibodies, purchased from PharMingen (San Diego, Calif.), were added followed by 40-min incubation at 4°C. After removing unbound antibodies, flow cytometry analysis was performed using a fluorescence-activated cell sorter (FACSort, Becton Dickinson Immunocytometry Systems, San Jose, Calif.). Before analysis, 10 μl propidium iodide (final concentration 1 μg/ml) was added to permit the discrimination of dead cells and their exclusion from the analysis. Antibody-conjugate combinations used for two colour cytometry were CD3-FITC/CD19-PE, CD4-FITC/CD8-PE, CD3-FITC/CD71-PE, CD3-FITC/Ly-49c-PE. The combination CD45-FITC/CD11b-PE was used to define the light-scatter gate that distinguishes lymphocytes from monocytes, unlysed red blood cells and debris. The results show the data from a single experiment, which is representative of the three similar experiments performed.

RNase protection assay

Total RNA was extracted from control and SGE-treated splenocytes after 24, 48 and 72 h of proliferation using the RNA Blue reagent according to the manufacturer's instructions (Top-Bio, Czech Republic). Total RNA was quantified by absorbance at 260 nm and 10–20 μg was dried by vacuum centrifugation for 1 h. A RiboQuant Multi-probe RNase Protection Assay System (Pharmingen) was used to determine the levels of cytokine mRNA transcripts following the manufacturer's instruction. Two multi-probe template sets mCK-1 and mCK-2, consisting of IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-15, IFN-γ and IL-1α, IL-1β, IL-1RA, IL-6, IL-10, IL-12p35, IL-12p40, IFN-γ, migratory inhibition factor (MIF), respectively, were used. L32 and GAPDH housekeeping genes were used in both sets. α[^{32}P] UTP (Izotop, Budapest, Hungary) was used for the synthesis of anti-sense RNA probe sets. ^{32}P-labeled protected fragments were resolved by electrophoresis on 5% Long Ranger gel (BMA, Rockland, Me.). Gels were dried and radioactivity visualized by phosphoimaging or by exposure to an X-ray film at –80°C.

Statistical analysis

The significance of any differences obtained between experimental groups in the proliferation and cytokine detection experiments was evaluated by the Student's *t*-test. In all statistical analyses, significance was assessed at the $P < 0.05$ level.

Results

Proliferative responsiveness of mouse splenocytes

On the 1st and 2nd days of the experiment, the ConA-stimulated proliferative responsiveness of mouse splenocytes was not significantly affected by 20 μg/ml SGE. SGE-induced inhibition (up to 85%) of ConA-stimulated splenocytes was observed only on the 3rd day of the experiment. Experiments with doses of 0.05, 0.5, 5, and 20 μg/ml of SGE showed a dose-dependent effect. Whereas 0.05 μg/ml did not have any significant effect on ConA-stimulated mouse splenocytes, 0.5 and 5 μg/ml significantly inhibited the proliferation of splenocytes by upto 13% and 73% respectively on the 3rd day of the experiment. Incubation of non-stimulated spleen cells

with SGE did not exert any effect on cell proliferation. *I. ricinus* SGE did not affect the LPS-stimulated proliferation at any time during the experiment.

Cytokine production

Mouse spleen cells were stimulated with ConA or LPS with or without SGE, and culture supernatants were harvested after 24, 48, and 72 h. These were then assayed for various cytokines at a protein level. Total RNA was extracted from cultured splenocytes and cytokine mRNA was detected at the same time points. An SGE concentration of 20 μg/ml was used in all cytokine detection experiments.

IL-1 family

Tick SGE significantly stimulated the production of mouse IL-1α, IL-1β and IL-1Ra (Table 1). In the case of IL-1α protein, significant stimulation was obtained

Table 1. Effect of SGE from partially fed *Ixodes ricinus* females on cytokine protein and mRNA levels in cultures of mouse splenocytes. Cells were stimulated with ConA or LPS and co-stimulated with SGE. The data show the percentage of change vs control group without SGE

Cytokine	Hours of proliferation	Control + SGE		ConA + SGE		LPS + SGE	
		Protein	mRNA	Protein	mRNA	Protein	mRNA
IL-1α	24	+113[a]	+50	+25[a]	c	+80	+150
	48	+114[a]	+86	+18[a]	0	+16[a]	+30
	72	+148[a]	+290	+109	c	+16[a]	+100
IL-1β	24	+100	+80	+73	c	+10[a]	+52
	48	+233	+230	+81	c	+21[a]	+49
	72	+275	+440	+60	c	+21[a]	+66
IL-R[a]	24	b	+202	b	+50	b	+25
	48	b	+196	b	+9	b	+71
	72	b	+146	b	+40	b	+41
IL-2	24	–18[a]	c	–12[a]	0	b	c
	48	+11[a]	c	–70	–15	b	c
	72	+15[a]	c	–100	–60	b	c
IL-4	24	c	c	+135	+231	c	c
	48	c	c	+25[a]	+100	+100[a]	c
	72	c	c	+4[a]	–75	c	c
IL-5	24	b	c	b	–20	b	0
	48	b	0	b	–106	b	c
	72	b	c	b	–86	b	c
IL-6	24	+100[a]	+98	+14[a]	+34	–19[a]	+5
	48	+100[a]	+45	+18[a]	+14	+14[a]	+33
	72	+100[a]	+100	+21[a]	–5	+21[a]	+16
IL-9	24	b	c	b	c	b	c
	48	b	c	b	c	b	c
	72	b	c	b	c	b	c
IL-10	24	–15[a]	–33	–8[a]	–20	–6[a]	–78
	48	–8[a]	–54	–60	–89	–56	–36
	72	–58[a]	–46	–53	–57	–33	+6
IL-12p35	24	b	+12	b	+4	b	0
	48	b	0	b	c	b	c
	72	b	+10	b	+8	b	+9
IL-12p40	24	+300	c	+129	+5	+61[a]	0
	48	+156	+10	+70	+56	+81[a]	–4
	72	+129	+40	+207	+38	+39[a]	c
IL-12p70	24	c	b	+140[a]	b	–100[a]	b
	48	c	b	+200[a]	b	–100[a]	b
	72	–100[a]	b	+100[a]	b	–50[a]	b
IL-13	24	b	c	b	–26	b	c
	48	b	c	b	–68	b	0
	72	b	c	b	–43	b	c
IL-15	24	b	c	b	c	b	c
	48	b	c	b	c	b	0
	72	b	c	b	c	b	c
IFN-γ	24	+1,403	+689	–22	–20	–40	–56
	48	+1,931	+412	–23	–94	–47	–54
	72	+100	+725	–20	–82	–45	–22
MIF	24	b	+6	b	0	b	–45
	48	b	–8	b	c	b	–12
	72	b	0	b	0	b	–50

[a]The difference versus the control group was not significant
[b] The respective experiment has not been done
[c] No detectable levels

on the 3rd day in ConA-stimulated, and on the 1st day in LPS-stimulated wells. SGE-treated wells without a mitogen did not show any significant IL-1α protein change. mRNA production showed the strongest stimulation on the 3rd day in SGE-treated wells without a mitogen. Whereas *I. ricinus* SGE had no significant effect on the production of IL-1β in LPS-stimulated wells, ConA-stimulated or control wells incubated with SGE produced significantly higher levels of this cytokine during the whole 3-day period when compared to wells without SGE. Whereas ConA did not stimulate detectable IL-1β mRNA production, both LPS and control nonstimulated wells showed enhanced levels of IL-1β mRNA after the addition of SGE. In addition, the mRNA production of IL-1Ra was enhanced by SGE. The strongest stimulation was shown in wells without a mitogen.

Th1 cytokines

Mouse splenocytes incubated with tick SGE showed a decrease in Th1 cytokine release (Table 1). Whereas the IL-2 protein levels were not significantly affected in the control wells, the production of IL-2 was significantly decreased on the 2nd and 3rd days of proliferation after the addition of SGE to ConA-stimulated wells. A 60% decrease of IL-2 mRNA in ConA + SGE-treated wells was observed on day 3 of the experiment. LPS-stimulated splenocytes did not produce measurable IL-2. The production of the IL-12p70 molecule was not significantly affected by SGE, whereas levels of IL-12p35 mRNA showed a minute enhancement after SGE treatment. Protein and mRNA levels of the IL-12p40 subunit were significantly higher after the addition of SGE to control or ConA-stimulated wells, but were not affected in LPS + SGE-treated wells. ConA or LPS did not stimulate mouse splenocytes to produce detectable levels of IL-15 mRNA, and this was not changed after SGE treatment.

Th2 cytokines

Splenocytes treated with SGE produced increased levels of most of the Th2 cytokines (Table 1). The addition of SGE to wells containing ConA-stimulated spleen cells raised IL-4 protein elaboration for all 3 days of the experiment, but a significant increase occurred only on the 1st day of incubation. IL-4 mRNA was markedly enhanced on days 1 and 2, then it decreased below the control, SGE-untreated level. Control and LPS-stimulated wells showed negligible concentrations of both IL-4 protein and mRNA. ConA + SGE-treated splenocytes produced lower levels of IL-5 mRNA, compared with ConA-stimulated cells. SGE slightly upregulated the production of both mRNA and protein of IL-6 in all control and ConA-stimulated and in most of LPS-stimulated wells. The upregulation was not significant at the protein level. BALB/c splenocytes produced undetectable levels of IL-9 mRNA which were not changed after the addition of SGE. SGE significantly decreased the elaboration of IL-10 in ConA or LPS-stimulated splenocyte cultures on days 2 and 3 of the experiment. This inhibition was confirmed at the mRNA level. SGE also lowered the amounts of IL-13 mRNA produced by ConA-stimulated splenocytes. SGE enhanced the production of both IFN-γ protein and mRNA in control wells, whereas in ConA and LPS-stimulated wells this production was inhibited.

Migration inhibitory factor

MIF, tested only at the mRNA level, was produced by both control and LPS- as well as ConA-stimulated splenocytes. SGE decreased MIF in the LPS-stimulated, but not in the ConA-stimulated or control wells (Table 1).

Flow cytometry

Tick SGE significantly lowered the percentage of CD3 + CD71 + cells (activated T-lymphocytes) in mouse splenocyte cultures stimulated with ConA. The highest suppression (up to 88%) of CD3 + CD71 + cells was shown after 48 h of proliferation (Fig. 1), whereas the 3rd day showed a suppression of up to 35%. ConA + SGE-treated wells showed no changes after 24 h of incubation, compared to ConA-stimulated wells only. *I. ricinus* SGE had no effect on non-activated T-cells, Th- and Tc-lymphocytes, B-cells and NK cells.

Discussion

Only a few previous publications have compared the influence of tick saliva on the immune systems of different hosts. These experiments showed slight differences in the case of individuals from related species (Ramachandra and Wikel 1995), whereas genetically more distant animals reacted differently (Bechara et al. 1994; Szabó et al. 1995). In the present discussion, the results obtained with murine splenocytes are compared with those of human peripheral blood leukocytes (Kovář et al. 2002).

The impact of tick saliva on the ConA- or LPS-stimulated proliferation of mouse lymphocytes was described earlier and the inhibitory effect of tick saliva was used for the identification and characterization of immunomodulatory molecules in tick saliva (Bergman et al. 1995, 2000). In our experiments, an inhibitory effect of *I. ricinus* SGE on ConA-stimulated splenocytes was shown on the 3rd day of proliferation, while human PBMC proliferation was significantly inhibited on days 2 and 3 of the experiment (Kovář et al. 2002). The

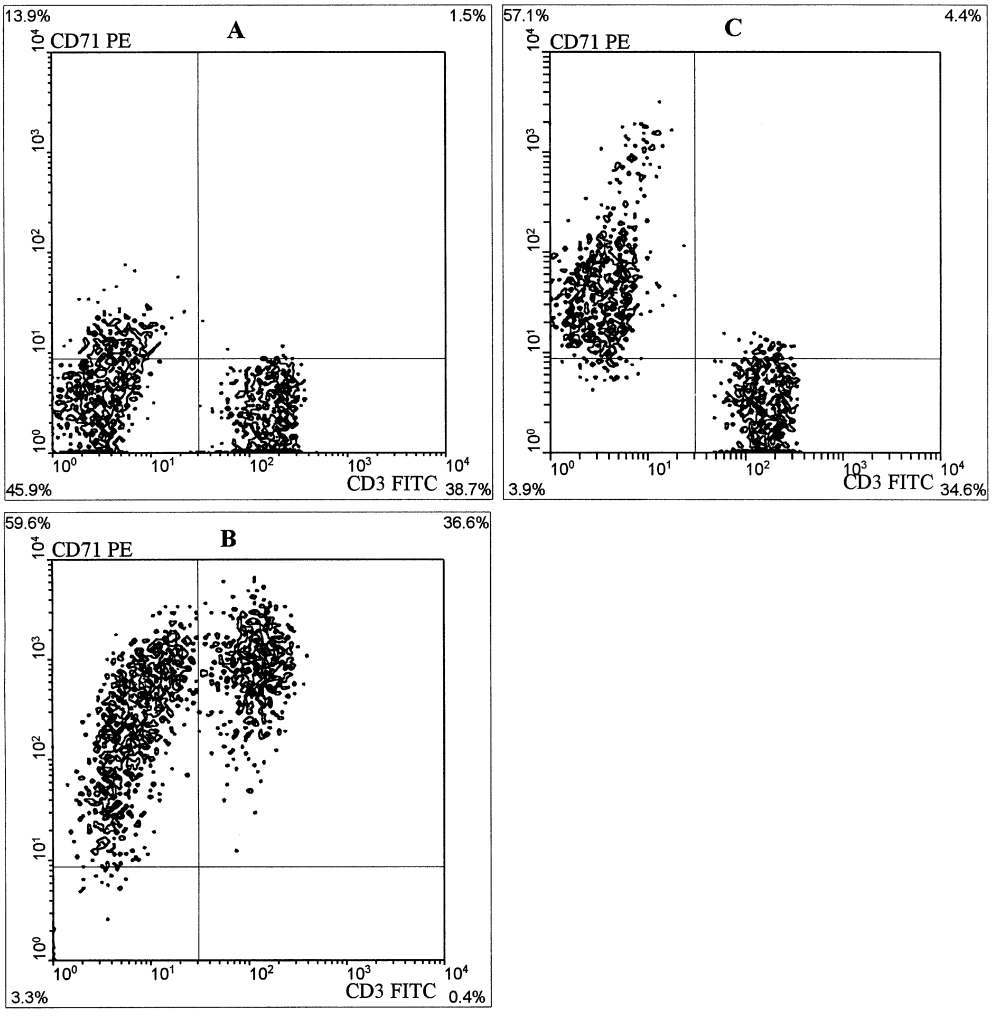

Fig. 1A–C. Effect of salivary gland extract (SGE) from partially fed *Ixodes ricinus* females on the appearance of $CD3^+CD71^+$ double positive T-cells. Mice splenocytes were stimulated 2 days in vitro with ConA in **B** the absence of or **C** the presence of SGE. **A** Non-stimulated spleen cells were used as a control

proliferation of both human and murine lymphocytes was inhibited in a dose-dependent manner, and the dose required for the significant suppression of proliferation was ten times lower for mouse lymphocytes (0.5 μg vs 5 μg/ml). SGE decreased the number of activated T-lymphocytes in both human and murine cultures (Kovář et al. 2002).

Our experiments showed that SGE enhances the production of IL-1α and IL-1β at the protein level in both human (Kovář et al. 2002) and mouse lymphocyte cultures, and IL-1α, IL-1β and surprisingly also the IL-1 receptor antagonist (IL-1Ra) at the mRNA level in mouse experiments. IL-1Ra is structurally similar to IL-1β, but it is a receptor antagonist with no agonist activity. IL-1 is a strong pro-inflammatory cytokine but secreted IL-1, i.e. mostly IL-1β (Dinarello 1997), could also act as a co-stimulator for the proliferation of Th2 lymphocytes (Lichtman et al. 1988; Dinarello 1997). Considering the fact that low levels of receptors (less than ten on some cells) and low concentrations of IL-1 can induce a biological response (Dinarello 1997), it is difficult to deduce from our data whether IL-1Ra antagonized the biological activities of IL-1 in our experiments.

In the early stages of infection, levels of IL-4 and IL-12 determine whether the cellular (Th1) or humoral (Th2) immune response activates. The bioactive IL-12 (p70) molecule is heterodimeric, composed of two disulfide-bound subunits, p35 and p40. The p35 subunit is secreted only as part of the p70 heterodimer, whereas a homodimer of p40 subunits $(p40)_2$ has been shown to be a potent IL-12 antagonist (Wang et al. 1999). After SGE treatment, the levels of p35 subunit mRNA were slightly enhanced, whereas the protein levels of the whole IL-12 molecule were not significantly affected. Subunit p40 was significantly stimulated at both the protein and mRNA levels. This indicates that *I. ricinus* SGE can inhibit the activity of IL-1 via the $(p40)_2$ and, together with enhanced production of IL-4, polarizes the cytokine profile toward Th2 (Ferreira and Silva 1999; Schoeler et al. 2000; Mejri et al. 2001; Kovář et al. 2002). Significant stimulation of IL-4 protein production after SGE treatment was observed only on the first day of proliferation and subsequently it became lower day by day. Similar results were obtained when IL-4 mRNA was detected. IL-4 mRNA detected on day 3 of proliferation was even inhibited in SGE-treated cultures. This could mean that the high levels of IL-4 needed for Th2

polarization in the early stages of tick-induced immunomodulation are lowered later, once the Th2 response is established. The question is whether only 24 h of the IL-4 environment is sufficient for the differentiation of the Th2 subset (Rogers and Croft 1999). On the other hand, in the initial stages of infection, the cytokine environment is only one of the factors influencing the Th1/Th2 balance (Constant and Bottomly 1997; Rogers and Croft 1999). IL-4 independent (Noble et al. 2001) and IL-1-stimulated Th2 lymphocyte proliferation has been described (Lichtman et al. 1988; Huber et al. 1996; Dinarello 1997).

A Th2 cytokine, IL-13, has similar biological activities as IL-4 and its mRNA levels were lowered for the whole 3 days of the experiment in SGE-treated cultures. SGE enhanced mRNA levels in mouse and protein levels in human lymphocyte cultures of another Th2 cytokine, IL-6.

The SGE-induced decrease in IL-10 at both the protein and mRNA levels reported in this paper has recently been described in a murine macrophage cell line (Gwakisa et al. 2001). Increased levels of IL-1α, IL-1β, IL-6 and unaffected levels of IL-12p70 correlate with lower levels of IL-10, a potent inhibitor of these cytokines (Moore et al. 2001). The SGE-induced decrease in IL-10 could result from the inhibition of macrophages, as demonstrated by Gwakisa et al. (2001), together with the inhibition of the still controversial regulatory CD4+ T-cell subset (Moore et al. 2001). Studies in vivo revealed that IL-10 could exert different effects depending upon the timing, dose, and location of expression. In some scenarios, the expected immunosuppressive activities were observed, while in others IL-10 enhanced the immune and inflammatory responses (Moore et al. 2001). The inhibited production of IL-10 after SGE treatment is in contrast not only with our previous experiments with human PBMC (Kovář et al. 2002), but also with other findings (Ferreira and Silva 1999; Schoeler et al. 1999, 2000). Another contrast with a recently published paper by Schoeler et al. (2000) is the SGE-induced lowered production of IL-5 mRNA shown in the present paper. Eosinophils, as the main anti-parasitic effector cells, can cause tick damage while infiltrating the bite site (Wikel et al. 1994), and therefore the inhibition of the eosinophil growth factor, IL-5, could be advantageous for tick infestation.

SGE reduced the levels of IL-2, a principal autocrine growth factor of Th1 lymphocytes, at both the protein and mRNA levels, in a similar way to that observed in human cell cultures (Kovář et al. 2002) and to other data obtained in experiments with mouse cells (Ferreira and Silva 1999; Schoeler et al. 1999, 2000). Recently, IL-2-binding protein was described in tick saliva. This could cause a decrease in the biological activity of this cytokine at the site of the tick bite (Gillespie et al. 2001).

The levels of another important Th1 cytokine, IFN-γ, were enhanced at both the protein and mRNA levels in control and suppressed in mitogen-stimulated wells treated with SGE. Similar results, up- and downregulation of IFN-γ, were described by Schoeler et al. (1999) who used ex vivo cultures from repeatedly infested BALB/c mice. In human PBMC, SGE significantly reduced the levels of IFN-γ during the whole 3-day proliferation period (Kovář et al. 2002).

MIF has various roles; from inducing inflammation to activating macrophages and T-cells. A homologue of this cytokine was found in tick saliva (Jaworski et al. 2001). In our experiments, we observed the inhibition of the production of MIF mRNA after the SGE treatment of LPS-stimulated cells. There could be some minor differences in biological activities between the tick and human MIF, and the ticks could take advantage of the inhibition of host MIF and secretion of its own homologue. The advantage of this could be in facilitating tick feeding by increasing blood flow and the inhibition of macrophage migration with minute stimulation of inflammation (Jaworski et al. 2001).

The primary aim of this work was to compare the effect of SGE obtained from *I. ricinus* on mouse splenocytes and human leukocytes from peripheral blood (Kovář et al. 2002). Both cell cultures showed an enhanced production of IL-1α, IL-1β, IL-6 and partially of IL-4, as well as the downregulation of IL-2 and partially of IFN-γ. The main difference between human and mouse cells was in the downregulation of IL-10, shown in the present work, which was observed at both the mRNA and protein levels. The inhibitory effect of tick SGE on T-cell proliferation and the lowered percentage of activated T-cells were similar in both human and mouse experiments.

Tick saliva induces the augmentation of Th2 and the inhibition of Th1 cytokines, as described previously (Ganapamo et al. 1996; Ferreira and Silva 1999; Schoeler et al. 1999, 2000; Mejri et al. 2001; Kováø et al. 2002). The results obtained in the present work mostly indicate a enhancement of the Th2 pattern. The inhibition of IL-10 described in this work and in that by Gwakisa et al. (2001) could mean that other cells, e.g. macrophages, are included in the tick saliva-affected production of cytokines in addition to T lymphocytes.

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