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Mise en place et dynamique de la réponse immunitaire chez des souris BALB/c infestées par des nymphes de la tique *Ixodes ricinus*

par

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Publication n° 1

SPLEEN ACCESSORY CELL ANTIGEN PROCESSING AND *IN VITRO* INDUCTION OF SPECIFIC LYMPHOCYTE PROLIFERATION IN BALB/c MICE INFESTED WITH NYMPHAL *Ixodes ricinus* TICKS

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INTRODUCTION

Ticks are hematophagous organisms that transmit numerous human and veterinary diseases. They also induce an anti tick immune response during their blood meal. Thus, rabbits repeatedly infested with *I. ricinus* nymphs and adults produce IgG antibodies reacting against some salivary gland and integumental proteins from female ticks[1]. In contrast, experimental infestations of BALB/c mice with nymphal *I. ricinus* do not generate anti tick antibodies (IgG) or only occasionally. However mice immunized with salivary gland extract (SGE) develop antibodies that recognize tick integumental and salivary gland proteins (Fig 1).

In this work we attempted to establish the ability of T lymphocytes from tick infested mice to respond *in vitro* to tick SGE and integumental extracts (IE), after spleen accessory cells (SAC) antigen processing.

MATERIALS AND METHODS

Animals and infestations

Eight to 12 weeks old BALB/c mice purchased from IFFA CREDO (Arbresle, FRANCE) were used for these experiments. Mice were infested with 15 nymphal *I. ricinus* ticks each. Ticks were reared in our laboratory.

Tick protein extracts

Integuments and salivary glands were dissected from partially fed female ticks. Antigenic extracts were prepared following the procedures previously described[1]. Protein concentration was determinated by the Coomassie blue method. SGE and IE were finally sterilized through a 0.2 µm millipore filter and stored at -20°C until use.

Antigen processing test

A pool of SAC were obtained from 5 BALB/c mice killed 9 days after infestation. $5x10^5$ spleen cells (red blood cells free) per well were incubated in a 96-well flat bottomed plate at 37°C in a 5% CO_2 saturated atmosphere. Two hours later non adherent cells were removed and the wells washed three times with 10% FCS in PBS (pH 7.4). Adherent cells were incubated 20 min with or without chloroquine (0.25 mM/well). Tick extracts (20 µg/well) were added and adherent cells were incubated for an additional three and half hours. In control wells, only culture medium was added. Each well was washed four times. $4x10^5$ lymph node cells of these same infested mice were added. After 96 hours incubation, 0.1 µCi/well of [^{3}H] thymidine was added. The uptake of tritiated thymidine was determinated 24 hours later by liquid scintillation counting.

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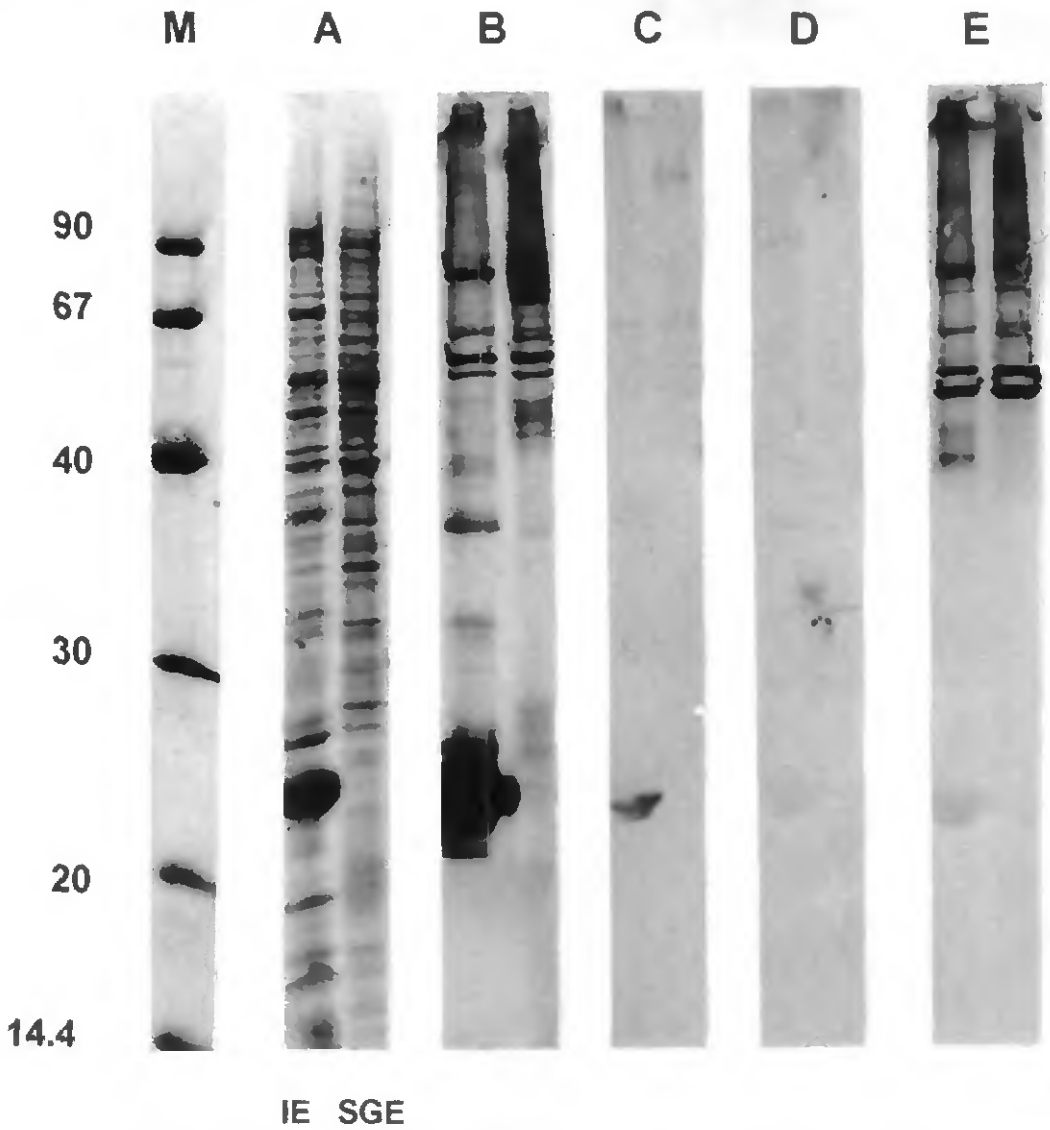

Figure 1. Integument or salivary gland extracts from partially fed females were separated on SDS-PAGE 12%, transferred on nitrocellulose paper and stained with colloïdal gold (panel A). Immunoblot probed with sera of pluriinfested rabbits with adults (panel B) or by sera of mice pluriinfested with nymphs (panels C and D) show that mice do not generate anti-tick antibodies or only occasionally. In contrast, mice immunized with salivary gland extract develop antibodies which recognize tick integumental as well as salivary gland proteins (panel E). M: molecular weight markers of 90, 67, 40, 30, 20, and14.4 kDa. IE: integumental extract. SGE: salivary gland extract.

RESULTS AND DISCUSSION

Table 1: BALB/c mice infested with nymphal *I. ricinus* ticks: Chloroquine effect on *in vitro* stimulation of lymph node cells by mice spleen accessory cells pulsed with tick extracts.

	Uptake of tritiated thymidine (mean cpm of quadruplicate ± SD)	
	Chloroquine 0.25 mM	Without Chloroquine
Controls (culture medium)	5417 ± 1241	4648 ± 1602
SGE (20µg/well)	4794 ± 1547	14443 ± 2675 *
IE (20µg/well)	4681 ± 2745	4066 ± 2913

(*) Signigicant difference using Student's t-test ($p<0.01$)

SGE but not IE is able to stimulate T lymphocytes from mice infested with ticks. In this assay, spleen adherent cells from infested mice process SGE immunogens. Pretreatment of accessory cells with chloroquine abolishes the *in vitro* stimulation of lymph node cells.

Nine days after tick fixation, SAC process and present salivary gland antigens with efficiency to lymphocytes from draining lymph nodes near tick fixation sites. Treatment of SAC with chloroquine fully inhibits this phenomenon. Under the same conditions but pulsed with IE, lymphocytes are not stimulated. Our observations suggest that, during tick feeding, mice antigen presenting cells (APC) selectively trap immunogens contained in tick saliva and process them before triggering anti tick specific immune response. Nine days after tick fixation, spleen cells do not respond when stimulated with SGE (results not shown). This unresponsiveness is apparently due to the regionalisation of T specific lymphocytes and not to APC inefficiency. APC treatment with chloroquine during antigen pulsing period abolishes their subsequent capacity to present foreign antigen to T cells but left the MLR largely intact [2]. Our work corroborates this observation. Chloroquine raises intracellular pH interfering with processing and presentation of antigen by MHC class II molecules [3].

In BALB/c mice infested with *I. ricinus* nymphs some antibodies reacting against one integumental antigen of 25 kDa was detected using western blot (Fig 1). We have shown that IE probably does not contain immunogen proteins which can be processed and presented by mice APC to T cells. IgG antibodies developed against tick saliva probably cross react with IE proteins.

ACKNOWLEDGMENTS

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REFERENCES

1) B. Rutti & M. Brossard (1989) Repetitive detection by immunoblotting of an integumental 25-kDa antigen in *Ixodes ricinus* and a corresponding 20-kDa antigen in *Rhipicephalus appendiculatus* with sera of pluri infested mice and rabbits. *Parasitol. Res.* 75, 325

2) K.C. Lee, M. Wong & D. Spitzer (1982) Chloroquine as a probe for antigen processing by accessory cells. *Transplantation* 34, 150

3) R.N. Germain & D.H. Margulies (1993) The biochemistry and cell biology of antigen processing and presentation. *Ann. Rev. Immunol.* 11, 402

Publication n° 2

Immunology 1995 **85** 120–124

In vitro production of interleukin-4 and interferon-γ by lymph node cells from BALB/c mice infested with nymphal *Ixodes ricinus* ticks

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SUMMARY

In this study we compared the ability of lymphocytes taken from axillary and brachial lymph nodes of BALB/c mice that had been infested once three times with 15 nymphal *Ixodes ricinus* ticks, to produce interleukin-4 (IL-4) and interferon-γ (IFN-γ) after *in vitro* stimulation with concanavalin A (Con A). They released high levels of IL-4 and low levels of IFN-γ. An increase of IFN-γ between the first and the third tick infestation was observed. Salivary gland extracts from female *I. ricinus* ticks induced specific *in vitro* proliferation of lymphocytes from infested mice. IL-4 production was correlated with the salivary gland extracts' ability to stimulate tick-specific lymphocyte proliferation. Its levels remained high from the first to the third infestation. IFN-γ production was not necessarily associated with tick salivary gland antigen stimulation. In BALB/c mice, anti-tick immune response induction is regional and the contribution of other similar secondary lymphoid organs is negligible. Only cells from the lymph nodes which drained the tick-fixation site proliferated *in vitro* in the presence of tick antigens, and when stimulated with Con A produced IL-4 and IFN-γ.

INTRODUCTION

The mice immune response against parasite infection is modulated by the cytokine pattern.[1,2] Humoral and cellular responses are regulated by two subclasses of T-helper cells. Th1 cells are responsible for cellular responses such as delayed-type hypersensitivity (DTH), as well as some humoral responses which generate IgG1 production, and Th2 cells play an important role in humoral responses. The main cytokine pattern produced by Th1 or Th2 cells are, respectively, interferon-γ (IFN-γ) and interleukin-2 (IL-2) or IL-4 and IL-5. Other subsets with different patterns exist either as precursors, such as Th0, or as effector cells.[3,4] In the immune response mechanism, the role of IL-4 has been studied extensively. The concentration of this cytokine regulates the immunoglobulin isotype switching.[5]

A few observations have been made on the immune relationship between mice and tick immature stages. A high production of IgE is necessary for WBB6F1 +/+ mice infested with *Haemaphysalis longicornis* larvae to acquire resistance.[6] BALB/c mice infested with *Ixodes ricinus* nymphs regularly produce IgE (unpublished results) and sometimes IgG anti-tick antibodies. Detection of antibody production was recently reported in this host–parasite system.[7]

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BALB/c mice infested with *I. ricinus* nymphs develop immediate-type hypersensitivity and DTH but fail to acquire resistance after repeated infestations.[8] DTH consists of a cascade of events, and T cells mediate the early aspect of DTH, releasing antigen-specific factors that, upon encountering antigen, activate local serotonin-containing cells such as mast cells.[9] However, the ability of the T-cell population to mediate the late component of DTH is independent of further release of serotonin.[10] ITH is a cutaneous reaction generated by IgE antigen linkage that induces mast cell degranulation at the tick's feeding site. Using *in situ* hybridization, previous experiments have shown that at the beginning of tick infestation IL-4 and IFN-γ mRNA can be observed regularly in the draining lymph node cells.[11] At the tick-binding site, however, only IFN-γ mRNA-positive cells increased in number during repeated infestations. The authors' suggestions were focused on the tick's ability to modulate mice immune reactions, a possibility that was investigated previously using *in vitro* bioassays.[12] In fact, prostaglandin E_2 (PGE_2), which has been detected in *Amblyomma americanum* and some other tick species' saliva, inhibits Th1 but not Th2 lymphokine production by *in vitro* direct contact.[13–15]

The aim of this work was to attempt to establish whether BALB/c mice infested by *I. ricinus* ticks develop a T-specific immune response. The ability of tick-specific effector lymphocytes to produce IL-4 and IFN-γ, cytokines *in vitro* was investigated and the prominent role of IL-4 and IFN-γ in this parasitic relation is discussed, without considering their pleiotropic action regularly reported in the literature.[16,17]

MATERIALS AND METHODS

Animals and infestations

Eight- to 12-week-old female BALB/c mice (IFFA-CREDO, Arbresle, France) were used for these experiments. The mice were infested with 15 *I. ricinus* nymphs placed in plastic capsule, as reported elsewhere.[8] The ticks were reared in our laboratory according to the method described previously.[18] Successive infestations were inter-spaced by 10 days and each experiment was done on a group of five mice. Mice flanks were alternated during these repeated infestations.

Antigenic extract

Salivary glands were dissected from female ticks that had been fed for 5 days on rabbit. Antigen extracts were prepared following the procedure described previously.[19] Briefly, salivary glands were homogenized in ice-cold extraction buffer consisting of 50 mM phosphate-buffered saline (PBS) at pH 7·4, 5 mM phenylmethylsulphonyl fluoride (PMSF) and 10 mM ethylenediamine tetraacetic acid (EDTA). Extracts were centrifuged at 16000 *g* for 30 min at 4°. The supernatant was dialysed overnight in 25 mM, pH 7·4, and centrifuged again under the same conditions. Protein concentration was determined using the Coomassie blue method. The dyalisat was finally sterilized through a 0·2 μm millipore filter and stored at −20° until used.

Cell culture

Mice were killed 9 days after infestation. Axiliary and brachial lymph nodes were removed. The basic experimental method for the salivary gland antigen T-cell proliferation assays was adapted from procedures described previously.[20] 4×10^5 lymph node cells per well were cultivated in 100 μl of complete culture medium containing RPMI-1640 (Gibco, Basel, Switzerland), supplemented with 10% fetal calf serum (v/v), 2 mM L-glutamin, 1 mM sodium pyruvate, 1 mM non-essential amino acids (Sigma, St Louis, MO), 0·05 mM mercaptoethanol, 100 U/ml penicillin/streptomycin (Gibco) and 25 μg/ml Fungizone (Gibco). After 96 hr incubation with or without 2·5 μg/well of salivary gland extract, 0·1 μCi/well of [^{3}H]thymidine (Amersham Int., Amersham, UK) was added 18–24 hr before harvesting the cells. Tritiated thymidine incorporation was determined by liquid scintillation counting. Results show the means of quadruplicate salivary gland extract-stimulated wells ±SD. Means of quadruplicate unstimulated wells were previously subtracted (net c.p.m.).

For culture supernatant collection, 1×10^6 lymph node cells in 100 μl of complete culture medium were stimulated with or without 4 μg/well of concanavalin A (Con A; Sigma). Supernatants were removed 24 hr after stimulation and stored at −75° until used for IL-4 and IFN-γ determination.

IL-4- and IFN-γ-specific ELISA

Enzyme-linked immunosorbent assay (ELISA) cytokine tests were performed according to the critical observation method reported previously.[21–23] The production of IL-4 and IFN-γ was measured using two rat monoclonal antibodies (mAb) for each cytokine (Pharmingen, Lugano, Switzerland). The Pharmingen mouse cytokine ELISA protocol was adapted for this work. Briefly, two micro-ELISA plates (Dynatech M-129 A; Dynatech, Embrach-Embraport, Switzerland) were coated with 100 μl of purified mAb R4-642 (4 μg/ml) for the IFN-γ plate and 100 μl of mAb clone BVD4-1D11 (4 μg/ml) for the IL-4 plate. Monoclonal antibody dilutions were made with 0·1 M sodium carbonate buffer (pH 8·2) and incubated at 4° overnight. Plates were washed twice and unoccupied sites were saturated for 2 hr with 200 μl of 10% FCS in PBS, pH 7·4. Plates were then washed four times and 100 μl of cell culture supernatants, pooled from 10 cell culture wells, were allowed to incubate overnight at 4°. Specific recognition of IFN-γ and IL-4 molecules was detected with biotinylated mAb clone XMG1.2 for IFN-γ, and biotinylated mAb clone BVD6-24G2 for IL-4. One-hundred microlitres of each mAb (2 μg/ml) was added per plate and incubated for 45 min at room temperature. Plates were washed again and 100 μl of avidin–peroxydase (Sigma), diluted 400-fold in PBS–10% FCS, was allowed to incubate for 30 min at room temperature. An enzymatic colour reaction was generated using 2,2′-Azino-bis-[3-ethylbenzthiazoline-6-sulphonic acid] (ABTS; Sigma) substrate and measured with an ELISA spectrophotometer (Dynatech) 30 min after incubation at 405 nm. Results show the mean of quadruplicate wells and error bars indicate the variation of individual values from the mean. Dilutions of IL-4 or IFN-γ recombinant proteins (rIL-4, rIFN-γ; Pharmingen) were used as positive test controls. Experiments were repeated at least five times and representative data are shown.

RESULTS

In vitro proliferation of tick-specific lymphocytes

Nine days after being infested with nymphal *I. ricinus* ticks, BALB/c mice developed a specific immune response. At this time, all ticks were engorged and had been detached from their hosts for 2–3 days. Tick salivary gland extracts induced *in vitro* lymphocyte proliferation of axillary and brachial lymph nodes draining the tick fixation site (net c.p.m. = 38381 ± 5678). Cells from the opposite site did not proliferate (net c.p.m. = 414 ± 81), showing the regionalization of the immune response.

We observed a dramatic increase *in situ* of the size of axillary and brachial lymph nodes localized near the tick attachment site compared to similar lymph nodes localized at the non-infested side (data not shown).

In vitro IL-4 and IFN-γ production

Nine days after the beginning of the first infestation, cells from the lymph nodes draining the site of tick fixation produced high levels of IL-4 (largely > 8 ng/ml) when stimulated with Con A. Cells from the opposite site incubated with Con A and cells incubated without Con A (controls) did not produce IL-4 (Fig. 1a).

In the same conditions, cells from the draining lymph nodes produced low levels of IFN-γ (approximately 12 U/ml). Cells from the opposite site stimulated with Con A and control cells did not produce IFN-γ (Fig. 1b).

Figure 2 shows the evolution of IL-4 and IFN-γ production by lymph node cells draining the tick fixation site. The assays were carried out 9 days after the beginning of the first and the third infestation. At any time control cells did not produce either IL-4 or IFN-γ. When stimulated with Con A the IL-4 levels remained high from the first until the third infestation

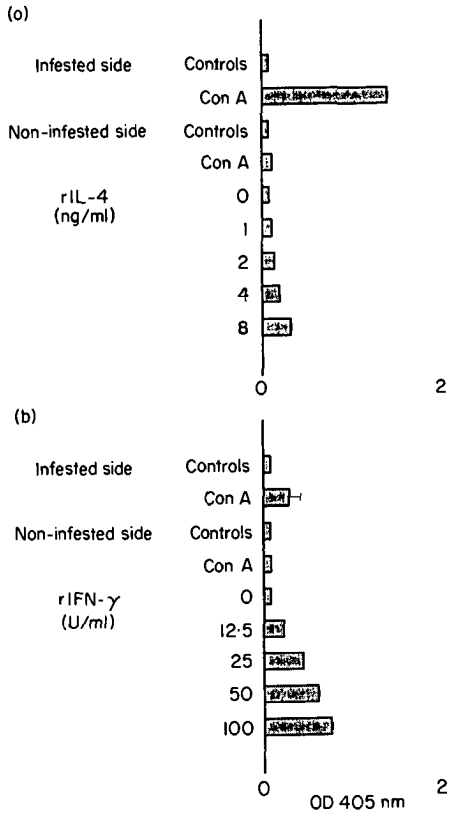

Figure 1. Measurement of IL-4 and IFN-γ by specific ELISA. Comparison between a pool of axillary and brachial lymph node cells from the infested side of mice, and similar cells from the non-infested side. Mice were infested with 15 nymphal *I. ricinus* ticks and lymph node cells were removed 9 days after tick infestation. 1×10^6 lymphocytes were stimulated *in vitro* during 24 h with Con A (4 μg/well) or without (controls). (a) IL-4 data. (b) IFN-γ data. Results show the mean of quadruplicate wells and error bars indicate the variation of individual values from the mean. Serial dilutions of IL-4 and IFN-γ recombinant protein were used as positive test controls.

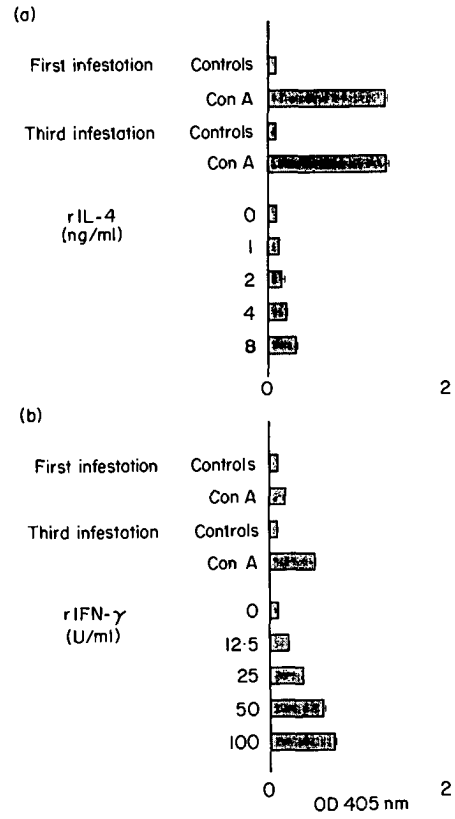

Figure 2. Measurement by specific ELISA of IL-4 and IFN-γ evolution from the first to the third infestation. A pool of axillary and brachial lymph node cells from two groups of mice each infested once and three times with 15 nymphs of *I. ricinus* tick were used. 1×10^6 cells were stimulated during 24 hr with Con A (4 μg/well) or without (controls). (a) IL-4 data. (b) IFN-γ data. Results show the mean of quadruplicate wells and error bars indicate the variation of individual values from the mean. Serial dilutions of IL-4 and IFN-γ recombinant protein were used as positive test controls.

(Fig. 2a). IFN-γ production was very low after the first infestation (approximately 12 U/ml) but increased after the third infestation (approximately 50 U/ml) (Fig. 2b).

DISCUSSION

Recent investigations have indicated that two memory cell types (specific for the antigen and specific for the antigen entry site) may be generated during antigen introduction into the host.[24,25] The existence of antigen-specific T lymphocytes can be proven by *in vitro* stimulation of draining lymph node cells with the same antigen that has been used previously for sensitization.[20]

In this report we have demonstrated the regionalization of the immune response. Axillary and brachial lymph node cells from mice infested with *I. ricinus* nymphal ticks can be stimulated with tick salivary gland extracts *in vitro*. Specific memory cell types in the lymph nodes are associated with the antigen entry site. The importance of the antigen entry site on the subsequent development of effector cells is poorly understood. It has been shown that *in vitro* stimulation of lymphocytes recovered from lymph nodes that receive afferent lymphatic drainage from mucosal tissues produce less IL-2 and IFN-γ than IL-4 and IL-5.[24]

In our study the high level of IL-4 measured after Con A stimulation may be attributed to the previous sensitization by tick salivary immunogens of specific T-helper lymphocytes in the draining lymph nodes. They were not necessarily responsible for IFN-γ production, which may have been due to antigen entry site memory.

In the human, repeated activation in skin or skin-associated peripheral lymph nodes may reinforce the cutaneous lymphocyte-associated antigen (CLA) expression on T cells functionally associated with skin, and thus enhance the efficiency of these cells by preferentially focusing their recirculation in the skin or related sites.[26] In BALB/c mice infested with *I. ricinus* nymphs, it has been reported recently that only the number of IFN-γ mRNA-positive cells increased during repeated infestations at the tick-binding site, compared to IL-4 mRNA-positive cells.[11] IFN-γ is a pivotal stimulator of

lymphocyte migration into the skin and a major mediator of lymphocyte recruitment in DTH.[27]

The antigen-processing cells seem to play a foremost role for differentiation into either Th1 or Th2 cells.[28] Epidermal Langerhans' cells are essential for mammalian hosts to generate a specific anti-tick immune response.[29,30] In tick-infested BALB/c mice they probably generate epitopes that induce selective responsiveness of Th2-like cells. It has been reported that pretreatment of mice with aqueous antigen induces selective unresponsiveness in Th1-like cells, which secrete IL-2 and IFN-γ, but not in Th2-like cells, which release essentially IL-4 and IL-5.[31] A decrease of specific IgG2a and IgG2b in mice pretreated with aqueous antigen has also been demonstrated. The aqueous nature of immunogens in tick saliva could also induce this type of polarized cytokine pattern.

In BALB/c mice infested with nymphal *I. ricinus* ticks, we have observed an increase of B lymphocyte reactivity after *in vitro* lipopolysaccharide (LPS) stimulation of lymph node cells compared to uninfested mice (unpublished results). Absence of IgG antibody production is common, but some BALB/c infested mice can produce low levels of IgG antibodies.[7,19] T-helper induction of IL-4 promotes polyclonal B-specific lymphocyte production but IFN-γ prevents their differentiation to plasmocytes capable of generating specific IgG antibodies.[32,33] However, high levels of IL-4 may induce inhibition of IgG production by immunoglobulin switching to IgE antibodies.[34,35] IFN-γ enters late into the process of B-cell differentiation. It may induce the inhibition of immunoglobulin transcription, through an as yet unknown mechanism, or act post-transcriptionally. It may also function by increasing the threshold for precursor differentiation in response to LPS. Some authors have hypothesized that IFN-γ is an essential regulator of polyclonal B-cell responses in normal homeostasis.[36]

The immune response balance in BALB/c mice infested with *I. ricinus* nymphs does not induce acquired resistance due to no or very weak IgG antibody production, high levels of IL-4, low levels of IFN-γ, light DTH and significant ITH (compared to DTH).[8] In conclusion, the probable strong Th2 polarization of the cytokine profile in this host–parasite system can be associated with susceptibility to tick infection.

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REFERENCES

1. Mosmann T.R., Schumacher J.H., Street N.F. *et al.* (1991) Diversity of cytokine synthesis and function of mouse $CD4^+$ T cells. *Immunol Rev* **123,** 209.
2. Mosmann T.R. & Coffman R.L. (1989) TH1 and TH2: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 7, 145.
3. Murray J.S., Madri J., Pasqualini T. & Bottomly K. (1993) Functional CD4 T cell subset interplay in an intact system. *J Immunol* **150,** 4270.
4. Firestein G.S., Roeder W.D., Laxer J.A. *et al.* (1989) A new murine $CD4^+$ T cell subset with an unrestricted cytokine profile. *J Immunol* **143,** 518.
5. Snapper C.M., Finkelman F.D. & Paul W.E. (1988) Differential regulation of IgG1 and IgE synthesis by interleukin 4. *J Exp Med* **167,** 183.
6. Ushio H., Watanabe N., Kiso Y., Higuchi S. & Matsuda H. (1993) Protective immunity and mast cell and eosinophil responses in mice infested with larval *Haemaphysalis longicornis* ticks. *Parasite Immunol* **15,** 209.
7. Borský I., Hermánek J., Uhlír J. & Dusbábek F. (1994) Humoral and cellular immune response of BALB/c mice to repated infestations with *Ixodes ricinus* nymphs. *Int J Parasitol* **24,** 67.
8. Mbow M.L., Christe M., Rutti B. & Brossard M. (1994) Absence of acquired resistance to nymphal *Ixodes ricinus* ticks in BALB/c mice developing cutaneous reactions. *J Parasitol* **80,** 81.
9. van Loveren H. & Askenase P.W. (1984) Delayed-type hypersensitivity is mediated by a sequence of two different T cell activities. *J Immunol* **133,** 2397.
10. van Loveren H., Kato K., Meade R. *et al.* (1984) Characterization of two different Ly-1^+ T cell populations that mediate delayed-type hypersensitivity. *J Immunol* **135,** 2402.
11. Mbow M.L., Rutti B. & Brossard M. (1994) IFN-γ, IL-2 and IL-4 mRNA expression in the skin and draining lymph nodes of BALB/c mice repeatedly infested with nymphal *Ixodes ricinus* ticks. *Cell Immunol* **156,** 254.
12. Ramachandra R.N. & Wikel K.S. (1992) Modulation of host-immune responses by ticks (Acari: Ixodidea): effect of salivary gland extracts on host macrophages and lymphocyte cytokine production. *J Med Entomol* **29,** 818.
13. Betz M. & Fox B.S. (1991) Prostaglandin E_2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J Immunol* **146,** 108.
14. Roper R.L. & Phipps R.P. (1992) Prostaglandin E_2 and cAMP inhibit lymphocyte activation and simultaneously promote IgE and IgG1 synthesis. *J Immunol* **149,** 2984.
15. Ribeiro J.M.C., Evans P.M., MacSwain J.L. & Sauer J. (1992) *Amblyomma americanum*: characterization of salivary prostaglandins E_2 and $F_{2\alpha}$ by RP-HPLC/bioassay and gas chromatography-mass spectrometry. *Exp Parasitol* **74,** 112.
16. Farrar M.A. & Schreiber R.D. (1993) The molecular cell biology of interferon-γ and its receptor. *Annu Rev Immunol* **11,** 571.
17. Holter W. (1992) Regulation of interleukin 4 production and of interleukin 4-producing cells. *Int Arch Allergy Immunol* **98,** 273.
18. Graf J.F. (1978) Copulation, nutrition et ponte chez *Ixodes ricinus* L. (Ixodoidea: Ixodidae) lè partie. *Bull Soc Entomol Suisse* **51,** 89.
19. Rutti B. & Brossard M. (1989) Repetitive detection by immunoblotting of an integumental 25-kDa antigen in *Ixodes ricinus* and a corresponding 20-kDa antigen in *Rhipicephalus appendiculatus* with sera of pluri-infested mice and rabbits. *Parasitol Res* **75,** 325.
20. Corradin G., Etlinger H.M. & Chiller J.M. (1977) Lymphocyte specificity to protein antigens. I. Characterization of antigen-induced *in vitro* T cell-dependent proliferative response with lymph node cells from primed mice. *J Immunol* **119,** 27.
21. Prulin F.H., To S.E., Winston R. & Rodman T.C. (1991) Caveats and suggestions for the ELISA. *J Immunol Meth* **137,** 27.
22. Chirmule N., Oyaizu N., Kalyanaraman V.S. & Pahwa S. (1991) Misinterpretation of results of cytokine bioassays. *J Immunol Meth* **137,** 141.
23. le Moal M.A., Motta I. & Truffa-Bachi P. (1989) Improvement of an ELISA bioassay for the routine titration of murine interferon-gamma. *Res Immunol* **140,** 613.
24. Daynes R.A., Araneo B.A., Dowell T.A., Huang K. & Dudley D. (1990) Regulation of murine lymphokine production *in vivo*. III. The lymphoid tissue microenvironment exerts regulatory influences over T helper cell function *J Exp Med* **171,** 979.
25. Kroemer G., Cuende E. & Martínez-A. C. (1993) Compartmentalization of the peripheral immune system. *Adv Immunol* **53,** 157.
26. Picker L.J., Treer J.R., Ferguson-Darnell B. *et al.* (1993) Control of lymphocyte recirculation in man. II. Differential

regulation of the cutaneous lymphocyte-associated antigen, a tissue-selective homing receptor for skin-homing T cells. *J Immunol* **150,** 1122.

27. Issekutz T.B., Stoltz J.M. & Meide P.V.D. (1988) Lymphocyte recruitment in delayed-type hypersensitivity. The role of IFN-γ. *J Immunol* **140,** 2989.
28. Seder R.A. & Paul W.E. (1994) Acquisition of lymphokine-producing phenotype by $CD4^+$ T cells. *Annu Rev Immunol* **12,** 635.
29. Nithiuthai S. & Allen J.R. (1985) Langerhans cells present tick antigens to lymph node cells from tick-sensitized guinea-pigs. *Immunology* **55,** 157.
30. Allen J.R., Khalil H.M. & Wikel S.K. (1979) Langerhans cells trap tick salivary gland antigens in tick-resistant guinea pigs. *J Immunol* **122,** 563.
31. Burstein H.J., Shea C.M. & Abbas A.K. (1992) Aqueous antigens induce *in vivo* tolerance selectively in IL-2 and IFN-γ producing (Th1) cells. *J Immunol* **148,** 3687.
32. Rolink A. & Melchers F. (1993) B lymphopoiesis in mouse. *Adv Immunol* **53,** 123.
33. Reynolds D.S., Boom W.H. & Abbas A.K. (1987) Inhibition of B lymphocyte activation by interferon-γ. *J Immunol* **139,** 767.
34. Siebenkotten G., Esser C., Walb M. & Radbruch A. (1992) The murine IgG1/IgE class switch program. *Eur J Immunol* **22,** 1827.
35. Coffman R.L., Lebman D.A. & Rothman P. (1993) Mechanism and regulation of immunoglobulin isotype switching. *Adv Immunol* **54,** 229.
36. Abed N.S., Chace J.H., Fleming A.L. & Cowdery J.S. (1994) Interferon-γ regulation of B lymphocyte differentiation: activation of B cells is a prerequisite for IFN-γ-mediated inhibition of B cell differentiation. *Cell Immunol* **153,** 356.

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Immunosuppression and cytokine production in mice infested with *Ixodes ricinus* ticks: a possible role of laminin and interleukin-10 on the *in vitro* responsiveness of lymphocytes to mitogens

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SUMMARY

T cells from BALB/c mice infested 9 days before with *Ixodes ricinus* nymphs had a suppressed response to *in vitro* concanavalin A (Con A) stimulation compared to cells from uninfested mice. When laminin (the main component of the extracellular matrix) was used as a coating agent, the Con A response of naive mice was characterized by a decrease in cell proliferation, whereas there was no significant effect on the mitogen response of cells from infested mice. In contrast, an increased response to lipopolysaccharide (LPS) was observed when assaying lymph node cells of infested mice, probably reflecting an increase in B-lymphocyte number or activity. LPS cell stimulation was not modified by laminin. Supernatants of lymph node cells, taken 9 days after the first infestation of mice, stimulated with Con A *in vitro*, contained interleukin-10 (IL-10) but no significant levels of IL-5 as tested by enzyme-linked immunosorbent assay. At this stage of the infestation all T cells reactive with tick antigens generated in lymph nodes that drain the tick fixation site, were $CD4^+$ cells, as determined by $CD4^+$ depletion. With cells taken 9 days after the third infestation an increase of IL-5 and IL-10 was observed. The IL-10 levels were higher than the IL-5. According to these observations, we conclude that the reduction of T-cell proliferation in response to Con A observed in lymph node cells from infested mice, may be due to the combined effect of laminin interaction with T lymphocytes during migration and IL-10 production by these lymphocytes.

INTRODUCTION

It has been demonstrated *in vitro* that lymphocytes from animals infested with ticks showed a decreased proliferation in response to the mitogens concanavalin A (Con A) and, phytohaemagglutinin (PHA).[1,2] However, no effect was observed in lymphocytes stimulated with lipopolysaccharide (LPS), a mitogen specific to B lymphocytes. These observations have led to the conclusion that tick salivary gland components may have an immunosuppressive effect on T lymphocytes. Furthermore, it has been shown that during the polyclonal T-cell stimulation with Con A, both interleukin-2 (IL-2) production and induction of reactivity to IL-2 were accessory cell-dependent events.[3]

Laminin is the main constituent of extracellular matrix, it is involved in many cellular activities including cell adhesion and lymphocyte migration as described previously.[4] *In vivo* treatment with anti-laminin antibodies modified lymphocyte traffic.[5] In addition, activated T lymphocytes were shown to have proteolytic enzymes to degrade components of the basement membrane *in vitro*, including laminin.[6]

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In BALB/c mice infested with *Ixodes ricinus* nymphs, inflammatory cells including neutrophils, eosinophils, basophils, lymphocytes and monocytes infiltrate the dermis at the tick feeding sites.[7] During the inflammatory response, an increase in lymphocyte traffic from draining lymph nodes to the tick attachment sites has been postulated.[8] Conceivably, lymphocytes would then come in contact with extracellular matrix components.

In this study, the effect of laminin on the *in vitro* response of lymph node cells to Con A and LPS from tick-infested and uninfested BALB/c mice was evaluated. In a previous study we demonstrated *in vitro* production of high levels of IL-4 and low levels of interferon-γ (IFN-γ) by lymph node cells from BALB/c mice infested with ticks.[9] In the present report, we have studied the ability of these cells to produce IL-5 and IL-10, two cytokines produced by Th2 cells. IL-5 strongly contributes to eosinophilopoiesis in mice[10] whereas, IL-10 inhibits Th1 cell development and also reduces the *in vitro* T-lymphocyte proliferative response to Con A stimulation.[11]

MATERIALS AND METHODS

Mice and ticks

Eight- to 12-week-old BALB/c female mice were purchased from IFFA-CREDO (Arbresle, France). *Ixodes ricinus* nymphal ticks were reared in our laboratory as previously described.[12]

Infestations

Mice were infested with 15 nymphs placed in a small plastic capsule (15 mm in diameter) glued onto the skin of the mice with a mixture composed of beeswax and collophonium, at the site which is drained by brachial and axillary lymph nodes.[7] Each experiment was done in cohorts of five mice. Mouse flanks were alternated during repetitive infestations and these infestations were interspaced by 10 days.

Tick salivary gland antigens (SGA)

Antigen extracts were prepared following the procedure previously described.[13] Salivary glands were dissected from female ticks that had been fed for 5 days on a rabbit. Salivary glands were homogenized in ice cold extraction buffer consisting of 50 mM phosphate-buffered saline (PBS) at pH 7·4, 5 mM phenylmethylsulphonyl fluoride (PMSF) and 10 mM ethylene diaminetetraacetic acid (EDTA). Antigenic extracts were centrifuged at 16 000 *g* for 30 min at 4°. The supernatant was dialysed overnight in 25 mM PBS pH 7·4 and centrifuged again under the same conditions. Protein concentration was determined using Coomassie Blue method. Sterilized dialysate was finally stored at −20° until use.

Cell culture

Ninety-six well, flat-bottomed plates (Falcon) were coated with 75 μg/well of natural mouse laminin from Engelberth–Holm–Swarm sarcoma (EHS) cells, (Gibco, Basel, Switzerland) at 4° overnight.[14] Plates were then washed four times with RPMI-1640 (Gibco) containing 10% fetal bovine serum (FBS; v/v) (Gibco). During the last washing the remaining sites were blocked with washing buffer for 20 min at room temperature. Mice were sacrificed 9 days after infestation and brachial and axillary lymph nodes were harvested and placed in Hanks' balanced salt solution (HBSS) buffer. Single-cell suspensions were generated and 4×10^5 cells were cultured in a total volume of 100 μl/well in complete culture medium [RPMI-1640 (Gibco), supplemented with 10% FBS (v/v), 2 mM L-glutamine, 1 mM sodium pyruvate, 1% non-essential amino acids (Sigma, St Louis, MO), 5×10^{-5} M mercaptoethanol, 100 U/ml Peni/Strep (Gibco) and 0·25 μg/ml Fungizone (Gibco)] Con A (2 μg/well) and LPS (2 μg/well), (Sigma) were added in designated wells. After a 48-hr incubation of the culture at 37° in saturated atmosphere with 5% CO_2, wells were pulsed with 0·1 μCi/well (37·0 MBq/ml) of tritiated thymidine (Amersham Int, Amersham, UK). Cells were harvested 18–24 hr later and [^{3}H] thymidine (TdR) uptake was determined by liquid scintillation counting. The results were expressed as the mean counts per minute (c.p.m.) for quadruplicate determinations ± SD. Student's *t*-test was performed for statistical analysis of the data. Experiments were repeated at least three times and data of a representative experiment are shown.

For cell culture supernatant collection, 1×10^6 lymph node cells in 100 μl of complete culture medium were stimulated with or without 4 μg/well of Con A (Sigma). Supernatants were removed 24 hr after stimulation and stored at −75° until use for IL-5 and IL-10 determination.

Lymph node T CD4$^+$ cells depletion

Single-cell suspensions of axillary and brachial lymph nodes were generated as described above. Cells were incubated 30 min at 4° with 0·25 μg/10^6 cells of monoclonal antibody (mAb) (IgG2a) rat anti-mouse L3T4/CD4 (Pharmingen, AMS, Lugano, Switzerland). Monoclonal antibodies were diluted in PBS pH 7·4 (0·15 M NaCl, 0·01 M sodium phosphate) with 1% FBS. Cells were washed three times with HBSS and again incubated with Dynabeads M-450 sheep anti-rat IgG (Dynal, Milan Analytica, La Roche, Switzerland) diluted in PBS : 1% FBS (ratio 40 : 1) for 45 min at 4°. The CD4$^+$ T lymphocytes were then depleted using Magnetic Particle Concentrator MPC-1 (Dynal). The remaining suspended cells were removed and used as the CD4-depleted-cell suspension.

For the SGA T-cell proliferation assays, lymph node cells were cultured at 4×10^5 cells per well for 96 hr with or without 2·5 μg/well of SGA in 100 μl of complete culture medium. The proliferative response was determined as described above. Results show the mean of quadruplicate SGA-stimulated wells ± SD with the means of quadruplicate unstimulated wells previously subtracted (net c.p.m.). Culture supernatants were prepared and removed as described above.

IL-5 and IL-10-specific ELISAs

The production of IL-5 and IL-10 was measured using two rat monoclonal antibodies for each cytokine (Pharmingen) in an antigen capture enzyme-linked immunosorbent assay (ELISA). Micro-ELISA plates Dynatech M-129 A (Dynatech, Embrach–Embraport, Switzerland) were coated with 100 μl of purified mAb clone TRFK5 (4 μg/ml) for IL-5 and 100 μl of mAb clone JS5-2A5 (4 μg/ml) for IL-10. Monoclonal antibodies were diluted with 0·1 M sodium carbonate buffer (pH 8·2) and incubated at 4° overnight. After successive washings for 2 hr, unoccupied sites were blocked with 200 μl of 10% FBS in PBS pH 7·4, plates were washed four times and 100 μl of cell culture supernatants, pooled from ten cell culture wells were incubated overnight at 4°. Specific recognition of IL-5 and IL-10 molecules was detected with biotinylated mAb clone TRFK4 for IL-5 and biotinylated mAb clone SXC-1 for IL-10. The secondary antibody was added at 100 μl (4 μg/ml) per well and incubated 45 min at room temperature. Plates were washed again and 100 μl of avidin-peroxidase (Sigma) diluted 400-fold in PBS 10% FBS was allowed to incubate for 30 min at room temperature. An enzymatic colour reaction was generated using 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) (Sigma) substrate and measured at 405 nm with an ELISA spectrophotometer (Dynatech, Switzerland) 30 min after incubation. Results show the mean of quadruplicate wells. Serial dilutions of IL-5 or IL-10 recombinant proteins (Pharmingen) were used to generate the standard curve. Experiments were repeated at least three times and data of a representative experiment are shown.

RESULTS

Laminin effect on lymph node cell response to Con A and LPS

T lymphocytes isolated from axillary and brachial lymph nodes of uninfested mice proliferate less in the presence of Con A when incubated in wells treated with 75 μg of laminin ($p < 0·01$) (Table 1). Laminin made the T-cell response of uninfested mice to Con A equivalent to that of infested mice to Con A alone. However, isolated cells from infested mice when stimulated with Con A, in the presence or absence of laminin, have similar

Table 1. The effect of laminin on *in vitro* responses to Con A and LPS stimulation of lymph node cells from tick-infested and uninfested mice. These data represent the tritiated thymidine incorporation by lymph node cells (mean c.p.m. of quadruplicate wells ± SD)

	Uninfested		Infested	
	Without laminin	Laminin 75 μg/well	Without laminin	Laminin 75 μg/well
Controls	1213 ± 217	1609 ± 526	16778 ± 2650	15924 ± 1559
Con A (2 μg/well)	124729 ± 14229*	88615 ± 13067*‡	93131 ± 18670†‡	96397 ± 8694†
LPS (2 μg/well)	17386 ± 2296§	17705 ± 2933§	79947 ± 17325¶	75118 ± 11509¶

Comparisons between lymph node cell responses from uninfested mice in laminin-coated and uncoated wells showed a significative difference by Student's *t*-test ($p < 0.01$) (*). This laminin effect was not observed in the case of cells from infested mice (†). Similar levels of cell proliferation after Con A stimulation is observed between cells from uninfested mice in laminin-coated wells and cells from infested mice (‡). LPS stimulation was not modified by laminin (§¶) but infested animals showed an increase of LPS reactivity in axillary and brachial draining lymph nodes ($p < 0.01$).

levels of proliferation. Laminin does not completely abrogate the response of lymph node cells from uninfested mice to Con A stimulation.

The response of lymph node cells to LPS was similar in either group of mice. In this case there was an increase of the cellular response to LPS when cells were taken from infested mice, probably due either to an increase in the concentration of B cells or an increase in the sensitivity of these cells in the lymph nodes (Table 1).

T-cell response to salivary gland antigen

Nine days after the first infestation lymphocytes proliferated significantly when stimulated *in vitro* with SGA (net c.p.m. 42879 ± 2142). Depletion of $CD4^+$ T cells abrogated completely the lymph node cell proliferation in response to SGA (net c.p.m. 1323 ± 2069).

IL-5 and IL-10 production by lymph node cells after multiple tick infestations

Data in Fig. 1 show the measurement by specific ELISA of IL-5 and IL-10 evolution from the first to the third infestation. Nine days after the first infestation of mice with ticks, lymph node cells stimulated with Con A released significant levels of IL-10 (0·51 ng/ml, Fig. 1b) whereas IL-5 is undetectable (Fig. 1a). Nine days after the third infestation an increase in IL-5 levels (1·32 ng/ml, Fig. 1a) and IL-10 levels was observed (2·38 ng/ml, Fig. 1b). After one infestation, only cells from lymph nodes which drain the tick-fixation site produced IL-10 (Fig. 2a). Furthermore, IL-10 was exclusively produced by $CD4^+$ T cells as depletion of these cells abrogated IL-10 production by lymph node cells (Fig. 2b).

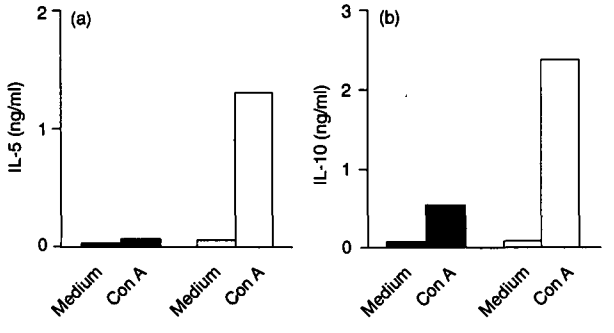

Figure 1. Measurement by specific ELISA of IL-5 and IL-10 evolution from the first to the third infestation. A pool of axillary and brachial lymph node cells from two groups of mice infested once and thrice with 15 nymphs of *I. ricinus* ticks each were used. One million cells were stimulated during 24 hr with Con A (4 μg/well) or without Con A (Medium). Solid bars, first infestation; and open bars, third infestation. Results are expressed as the mean of quadruplicate wells (SD < 10^{-2} not shown).

DISCUSSION

Decrease of the T-lymphocyte response to the mitogen Con A by tick-infested animals has been reported.[1,2] These observations have been attributed to the tick's ability to modulate the immune response of the host. In our work, we demonstrate however that laminin and IL-10 may also play a role in the decrease of the T-cell response to Con A.

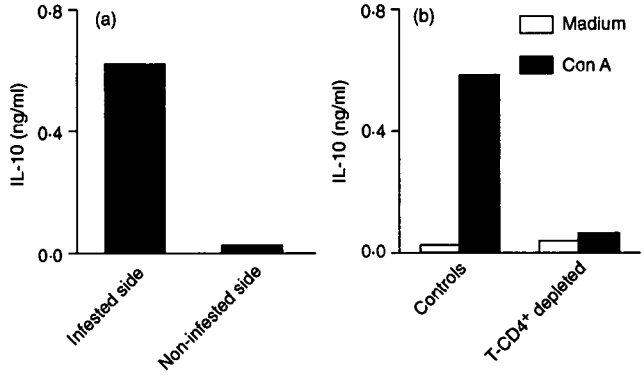

Figure 2. Regionalization of the immune response and contribution of $CD4^+$ T lymphocytes to IL-10 production by lymph node cells from infested mice. Cells were removed 9 days after the first infestation and incubated 24 hr with Con A or cell culture medium only (1×10^6 per well). (a) *In vitro* production of IL-10 by cells from lymph nodes which drain the tick-fixation site compared to cells from similar lymph nodes at the opposite side. (b) *In vitro* contribution of $CD4^+$ lymphocytes to IL-10 production. The treatment of results are the same as previously reported in Fig. 1.

Lymphocyte traffic increases with host pathological status.[15] In fact, inflammatory stimuli induce an increase in cell traffic between the inflammatory site and the closest peripheral lymph node. During infestation of BALB/c mice with *I. ricinus* ticks, inflammatory cells migrate via the bloodstream, cross the epithelium of postcapillary venules and infiltrate the skin.[7] Memory and effector T cells that infiltrate the skin do not return to lymph nodes, or do so only rarely.[16,17] Another possibility for the cells to contact basement membrane, and especially laminin molecules, is through the high endothelial venules (HEV);[18] this is the case for homing lymph node cells and naive lymphocytes which have been recruited from other secondary lymphoid organs.[19,20]

Laminin molecules have a high affinity for sulphated glycolipids on the cell surface, some of which are Con A receptors.[21] The $CD4^+$ T lymphocytes are more affected by laminin than other lymphoid cell types.[22] During their activation, integrins such as the family of very late activation antigens (VLA) are modified to ensure the continued protein linkage between the extracellular matrix and the T-cell cytoskeleton.[17,23,24] Activated cells are able to elicit protease activities which can affect the T-cell response to Con A stimulation *in vitro*.[6] Therefore, it may be reasonable to suggest that tick-specific activated lymphocytes are Con A receptor modified and consequently less responsive to this mitogen.[25–27]

A second event which may explain the decrease of responsiveness of lymph node cells to mitogen is the presence of IL-10 in the Con A-stimulated cell supernatants of infested mice. This interleukin inhibits the proliferation of antigen-stimulated Th1 clones and of mitogen stimulated mouse $CD4^+$ and $CD8^+$ T cells.[11] Murine IL-10 is expressed by mouse $CD4^+$ Th2 clones, and other cell types (B, NK, $CD8^+$, monocytes/macrophages).

The lymph node cell response to *in vitro* LPS stimulation is more pronounced in infested mice than in controls. This suggests an increase in either the proportion or activity of B lymphocytes in infested mice lymph nodes. In our model, specific anti-tick IgG antibodies are only occasionally produced.[13] Thus, the specific murine B lymphocytes generated during the first step of sensitization by ticks are probably confined to the lymph nodes and unable to differentiate into plasma cells.[9]

We have recently demonstrated that lymph node cells from BALB/c mice infested with nymphal *I. ricinus* ticks produce high levels of IL-4 and low levels of IFN-γ after Con A stimulation.[9] This suggests a Th2 polarization pattern of cytokine production in this host–parasite system which can be due to the aqueous nature of tick saliva. Previously, Burstein *et al.*[28] have reported that aqueous antigen induces tolerance in Th1-like but not Th2-like helper cells. The detection of IL-5 and IL-10 in this work suggests that the Th2 polarization occurs by tolerizing Th1 cells. The increase in IL-5 levels from the first to the third infestation with ticks could be associated with the evolution of eosinophil infiltration in the skin of BALB/c mice at the second and the third infestation.[7] Furthermore, tick saliva has been found to contain a large number of substances with pharmacological effects such as anti-haemostatic, vasodilatatory or anti-inflammatory/immunosuppressive components.[29] Prostaglandin E_2 (PGE_2), one of the constituents of some tick saliva, inhibits Th1 cells *in vitro* but not lymphokine production by Th2 cells.[30] This immune modulator also reduces T-cell line production of IL-2 *in vitro*.[31] However, recent observations show that the *in vitro* reduction of IL-2 production by murine spleen cells is due to a 5000 molecular weight protein contained in saliva of *I. dammini* ticks rather than to PGE_2.[32]

As recently reported, Th1 cells regulate macrophage activation and immunoglobulin isotype switching to IgG2a and IgG3, isotypes that mediate antibody-dependent cellular cytotoxicity (ADCC) and complement activation.[33] Th1 cells have been demonstrated to activate appropriate host defences against facultative and obligate intracellular pathogens. In some of these infectious diseases, IL-4, IL-10 and IL-13 produced by Th2 cells down-regulate macrophage activation, even in the presence of IFN-γ. Thus, in BALB/c mice infested with *I. ricinus* nymphs we suggest that IL-4 and IL-10 production could facilitate the transmission of these pathogens. The slight delayed type hypersensitivity previously observed in BALB/c mice infested with *I. ricinus* nymphs may be controlled by IL-4 and IL-10 synergy.[7,34]

According to these results, we conclude that contact between lymph node cells and laminin during lymphocyte migration in tick-infested mice and IL-10 production by these lymphocytes are two significant biological phenomena that may induce a decrease in the response of lymph node cells to the mitogen Con A.

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REFERENCES

1. Wikel S.K. (1982) Influence of *Dermacentor andersoni* infestation on lymphocyte responsiveness to mitogens. *Ann Trop Med Parasit* **76**, 627.
2. Barriga O.O., Da Silva S. & Azevedo J.S.C. (1993) Inhibition and recovery of tick functions in cattle repeatedly infested with *Boophilus microplus*. *J Parasit* **79**, 710.
3. Hünig T., Loos M. & Schimpl A. (1983) The role of accessory cells in polyclonal T cell activation I. Both induction of interleukin 2 production and of interleukin 2 responsiveness by concanavalin A are accessory cell dependent. *Eur J Immunol* **13**, 1.
4. Martin G.R. & Timpl R. (1987) Laminin and other basement membrane components. *Ann Rev Cell Biol* **3**, 57.
5. Kupiec-Weglinski J.W. & De Sousa M. (1991) Lymphocyte traffic is modified *in vivo* by anti-laminin antibody. *Immunology* **72**, 312.
6. Mathus T.L., Weerakoon D.K., Shoren D.J. & Cheng H.T. (1993) Degradation of basement membrane by lymphocytes. *FASEB J (Abstract)* **7**, 4816.
7. Mbow M.L., Christe M., Rutti B. & Brossard M. (1994) Absence of acquired resistance to nymphal *Ixodes ricinus* L. ticks in BALB/c mice developing cutaneous reactions. *J Parasit* **80**, 81.
8. Hopkins J. & McConnell I. (1993) Lymphoid cell traffic. In: *Clinical Aspects of Immunology* (eds. P.J. Lachman, S.K. Peters, F.S. Rosen & M.J. Walport), p. 31. Blackwell Scientific Publications, Oxford.
9. Ganapamo F., Rutti B. & Brossard M. (1995) *In vitro* production of interleukin-4 and interferon-γ by lymph node cells from BALB/c mice infested with nymphal *Ixodes ricinus* ticks. *Immunology* **85**, 120.

10. COFFMAN R.L., SEYMOUR B.W., HUDAK S., JACKSON J. & RENNICK D. (1989) Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* **245,** 308.
11. DING L. & SHEVACH E.M. (1992) IL-10 inhibits mitogen-induced T cell proliferation by selectively inhibiting macrophage costimulatory function. *J Immunol* **148,** 3133.
12. GRAF J.F. (1978) Copulation, nutrition et ponte chez *Ixodes ricinus* L. (Ixodoidea: Ixodidae)-1[e] partie. *Bull Soc Entomol Suisse* **51,** 89.
13. RUTTI B. & BROSSARD M. (1989) Repetitive detection by immunoblotting of an integumental 25-kDa antigen in *Ixodes ricinus* and a corresponding 20-kDa antigen in *Rhipicephalus appendiculatus* with sera of pluriinfested mice and rabbits. *Parasitol Res* **75,** 325.
14. YI-YANG L. & TAK CHENG H. (1992) Basement membrane and its components on lymphocyte adhesion, migration, and proliferation. *J Immunol* **149,** 3174.
15. YEDNOCK T.A. & ROSEN S.D. (1989) Lymphocyte homing. *Adv Immunol* **44,** 313.
16. BERG E.L., GOLDSTEIN L.A., JUTILA M.A. *et al.* (1989) Homing receptors and vascular addressins: cell adhesion molecules that direct lymphocyte traffic. *Immunol Rev* **108,** 5.
17. PICKER L.J., TREER J.R., FERGUSON-DARNELL B., COLLINS P., BERGSTRESSER P.R. & TERTAPPEN L.W.M.M. (1993) Control of lymphocyte recirculation in man II. Differential regulation of the cutaneous lymphocyte-associated antigen, a tissue-selective homing receptor for skin-homing T cells. *J Immunol* **150,** 1122.
18. SPRINGER T.A. (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* **76,** 301.
19. PARROTT D.M.V. & WILKINSON P.C. (1981) Lymphocyte locomotion and migration. *Prog Allergy* **28,** 193.
20. DE SOUSA M., TILNEY N.L. & KUPIEC-WEGLINSKI J.W. (1991) Recognition of self within self: specific lymphocyte positioning and extracellular matrix. *Immunol Today* **12,** 262.
21. ROBERTS D., NAGESWARA R.C., MAGNANI J.L., SPITALNIK T., LIOTTA L.A. & GINSBURG V. (1985) Laminin binds specifically to sulfated glycolipids. *Proc Natl Acad Sci USA* **82,** 1306.
22. HERSHKOVIZ R., MIRON S., COHEN I.R., MILLER A. & LIDER O.T. (1992) Lymphocyte adhesion to the fibronectin and laminin components of the extracellular matrix is regulated by CD4 molecule. *Eur J Immunol* **22,** 7.
23. MIYAKE S., SAKURAI T., OKOMURA K. & YAGITA H. (1994) Identification of collagen and laminin receptor integrins on murine T lymphocytes. *Eur J Immunol* **24,** 2000.
24. WESTON S.A. & PARISH C.R. (1991) Modification of lymphocyte migration by mannans and phosphamannans. Different carbohydrate structures control entry of lymphocytes into spleen and lymph nodes. *J Immunol* **146,** 4180.
25. YAHARA I. & EDELMAN G.M. (1972) Restriction of the mobility of lymphocyte immunoglobulin receptors by Concanavalin A. *Proc Natl Acad Sci USA* **69,** 608.
26. TIMPL R., RHODE H., ROBEY P.G., RENNARD S.I., FOIDART J.M. & MARTIN G.R. (1979) Laminin-A glycoprotein from basement membrane. *J Biol Chem* **254,** 9933.
27. TIMPL R. (1989) Structure and biological activity of basement membrane proteins. *Eur J Biochem* **180,** 487.
28. BURSTEIN H.J., SHEA C.M. & ABBAS A.K. (1992) Aqueous antigens induce *in vivo* tolerance selectively in IL-2 and IFN-γ producing (Th1) cells. *J Immunol* **148,** 3687.
29. TITUS R.G. & RIBEIRO J.M.C. (1990) The role of vector saliva in transmission of arthropod-borne disease. *Parasit Today* **6,** 157.
30. BETZ M. & FOX B. (1991) Prostaglandin E_2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J Immunol* **146,** 108.
31. RIBEIRO J.M.C., MAKOUL G.T., LEVINE J., ROBINSON D.R. & SPIELMAN A. (1985) Antihemostatic, antiinflammatory and immunosuppressive properties of the saliva of tick *Ixodes dammini*. *J Exp Med* **161,** 332.
32. URIOSTE S., HALL L.R., TERLFORD III S.R. & TITUS R.G. (1994) Saliva of the Lyme Disease vector, *Ixodes dammini*, blocks cell activation by nonprostaglandin E_2-dependent mechanism. *J Exp Med* **180,** 1077.
33. LOCKSLEY R.M. (1994) Th2 cells: help for helminths. *J Exp Med* **179,** 1405.
34. POWRIE F., MENON S. & COFFMAN R.L. (1993) Interleukin-4 and interleukin-10 synergize to inhibit cell-mediated immunity *in vivo*. *Eur J Immunol* **23,** 2223.