Immunology 1996 **87** 259–263

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

F. GANAPAMO, B. RUTTI & M. BROSSARD *Institute of Zoology, Department of Immunology, University of Neuchâtel, Neuchâtel, Switzerland*

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]

Received 12 July 1995; revised 22 September 1995; accepted 27 September 1995.

Correspondence: Prof. M. Brossard, Institute of Zoology, University of Neuchâtel, Emile-Argand 11, CH-2007 Neuchâtel, Switzerland.

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 ($p < 0{\cdot}01$) (*). 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 ($p < 0{\cdot}01$).

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).

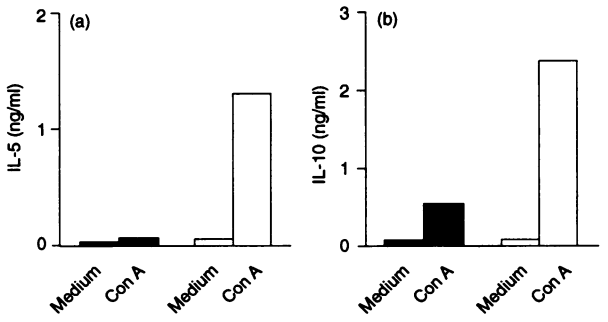

Figure 1. Measurement by specific ELISA of IL-5 and IL-10 evolution from the first to the third infestation. A pool of axillary and brachial lymph node cells from two groups of mice infested once and thrice with 15 nymphs of *I. ricinus* ticks each were used. One million cells were stimulated during 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.

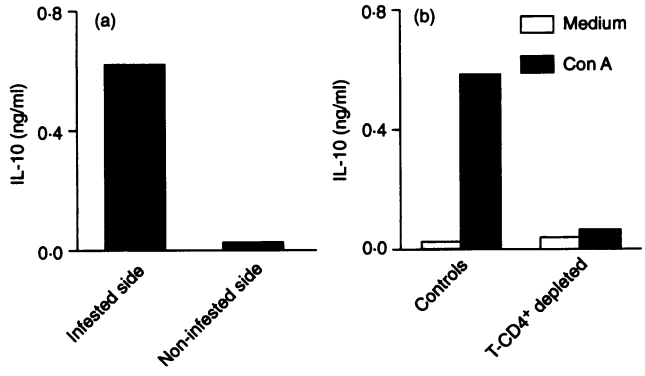

Figure 2. Regionalization of the immune response and contribution of $CD4^+$ T lymphocytes to IL-10 production by lymph node cells from infested mice. Cells were removed 9 days after the first infestation and incubated 24 hr with Con A or cell culture medium only (1×10^6 per well). (a) *In vitro* production 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.

ACKNOWLEDGMENTS

This work is part of the PhD thesis of Frédéric Ganapamo and was supported by the Swiss National Science Foundation, grant number 31-37652. 93 and by a scholarship of the Swiss Confederation. We would like to thank Drs W. T. Golde and M. L. Mbow from CDC (Fort Collins, Colorado, USA) for their critical review of the manuscript and Dr F. Erard from Ciba-Geigy (Basel, Switzerland) for helpful discussions.

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.