Tick Saliva Inhibits Dendritic Cell Migration, Maturation, and Function while Promoting Development of Th2 Responses

Anna Skallová, Giandomenica Iezzi, Franziska Ampenberger, Manfred Kopf and Jan Kopecký

*J Immunol* 2008; 180:6186-6192; doi: 10.4049/jimmunol.180.9.6186

http://www.jimmunol.org/content/180/9/6186

---

**References**

This article *cites 34 articles*, 11 of which you can access for free at:

http://www.jimmunol.org/content/180/9/6186.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Tick Saliva Inhibits Dendritic Cell Migration, Maturation, and Function while Promoting Development of Th2 Responses

Anna Skallová,* Giandomenica Iezzi,† Franziska Ampenberger,† Manfred Kopf,† and Jan Kopecký2,*

Similarly to other blood-feeding arthropods, ticks have evolved immunosuppressive mechanisms enabling them to overcome the host immune system. Although the immunomodulatory effect of tick saliva on several cell populations of the immune system has been extensively studied, little is known about its impact on dendritic cells (DCs). We have examined the effect of *Ixodes ricinus* tick saliva on DC function in vitro and in vivo. Exposure of DCs to tick saliva in vitro resulted in impaired maturation, upon CD40 or TLR9, TLR3 and TLR7 ligation, as well as reduced Ag presentation capacity. Administration of tick saliva in vivo significantly inhibited maturation and early migration of DCs from inflamed skin to draining lymph nodes, and decreased the capacity of lymph node DCs to present soluble Ag to specific T cells. Moreover, saliva-exposed DCs failed to induce efficient Th1 and Th17 polarization and promoted development of Th2 responses. Our data reveal a complex inhibitory effect exerted by tick saliva on DC function. Given the role of DCs as the key instigators of adaptive immune responses, alteration of their function might represent a major mechanism of tick-mediated immune evasion. The *Journal of Immunology*, 2008, 180: 6186–6192.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Grant 524/05/0811 from the Grant Agency of the Czech Republic, by the project of the Basic Research Centre LC06009 from the Czech Ministry of Education, and by the project of the Basic Research Centre LC06009 from the Czech Ministry of Education, and by the research project of Institute of Parasitology Z60220518.

2 Address correspondence and reprint requests to Dr. Jan Kopecký, Institute of Parasitology, Biology Centre AS CR, Branisovska 31, 370 05 České Budějovice, Czech Republic. E-mail address: jan@paru.cas.cz

Abbreviations used in this paper: SGE, salivary gland extract; DC, dendritic cell; LCMV, lymphocytic choriomeningitis virus; CMFDA, 5-chloromethylfluorescein diacetate; DBP, dibutyl phthalate; BMDC, bone marrow-derived DC.

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00
filtered through a 0.22 μm filter (Millipore) and saliva protein concentration was determined using a Bradford reagent (Sigma-Aldrich) before use.

**Tick SGE preparation**

Adult *I. ricinus* ticks were allowed to feed as described above. After 6 days, partially engorged female ticks were removed and their salivary glands were dissected. For each SGE sample, the salivary glands of 20 ticks were pooled, homogenized in 1 ml of PBS by sonication and clarified by centrifugation at 10,000 × g for 10 min. Protein concentration was determined as described above.

**DC isolation and purification**

Spleens or auricular lymph nodes were isolated, minced with scissors, digested in IMDM containing 1 mg/ml collagenase-D (Worthington Biochemical) at 37°C for 1 h, and passed through a 70 μm nylon cell strainer (BD Falcon). DCs were isolated using magnetic beads conjugated with anti-CD11c (N418) Ab and MACS Column separation following the manufacturer’s instructions (Miltenyi Biotec). Purity of isolated DCs (~90% CD11c+ cells) was determined by subsequent FACS analysis.

**SMARTA-2 transgenic CD4+ T cell isolation and purification**

Spleens of SMARTA-2 mice were passed through a 70-μm nylon cell strainer (BD Falcon) and CD4+ T cells were isolated using magnetic beads conjugated with anti-CD4 Ab and MACS Column separation following the manufacturer’s instructions (Miltenyi Biotec). Purity of isolated T cells (~90% CD4+CD82Mϕ) was determined by FACS analysis.

**DC exposure to tick saliva in vitro**

Purified spleen DCs were cultured in 96-well plates, at a concentration of 1 × 105 cells per well, in 100 μl IMDM supplemented with 10% heat-inactivated FCS, 50 μM 2-ME, 100 μg/ml penicillin, and 100 U/ml streptomycin (all from Sigma-Aldrich) in the presence or absence of the indicated concentrations of tick saliva. The corresponding volume of 80 μM pilocarpine (final concentration in well ~2 μM) was included as an additional control treatment. The DCs were activated by the addition of anti-CD40 mAb (10 and 2 μg/ml), CpG (100 and 10 nM) (Microsynth AG), imiquimod (10 and 2 μg/ml), or polyI:C (20 and 5 μg/ml) (both from InvivoGen). After 24 h of incubation, cells were stained with FITC-labeled B-7-2, PE-labeled anti-MHC class I, and biotin-labeled anti-I-A^d^ followed by streptavidin-allophycocyanin (all from eBioscience) and analyzed by flow cytometry (FACSCalibur, BD Biosciences) and FlowJo software (Tree Star). Dead cells were excluded from the analysis by propidium iodide staining.

**CD4+ T cell priming and polarization in vitro**

Purified DCs (1 × 10^5) and SMARTA-2 transgenic CD4+ T cells (5 × 10^5) were cultured as described above with the presence of a low (10 nM), an intermediate (100 nM), or a high (1000 nM) dose of the gp61–80 of the LCMV glycoprotein, and in the presence of absence of different stimuli and the tick saliva concentrations, as described above. After 48 h, specific T cell proliferation was assessed by measuring IL-2 concentration in culture supernatants by standard sandwich ELISA. T cell polarization was evaluated on day 5 of culture, upon restimulation with titrated numbers of purified DCs and 1 × 105 naive SMARTA-2 transgenic CD4+ T cells as described above. Specific T cell proliferation was assessed after 48 h by determination of IL-2 production in culture supernatants by standard sandwich ELISA. T cell polarization was determined on day 5 of culture by intracellular cytokine staining as described above. Alternatively, expanded cells were restimulated for 24 h in the presence of freshly purified spleen DCs and 1 μM gp61–80, and cytokine content in culture supernatants was determined using standard sandwich ELISA.

**DC exposure to tick saliva in vivo**

Eight-wk-old C57BL/6 mice were injected intradermally in the ears with 5 × 10^5 naive SMARTA-2 transgenic CD4+ T cells in the presence of the indicated tick saliva concentrations. Pilocarpine (final concentration in well ~2 μM) was used as an additional control treatment, because a corresponding pilocarpine contamination has been determined in tick saliva. The DCs were activated by the addition of anti-CD40 mAb, CpG, imiquimod, or polyI:C. After 24 h of incubation, cells were stained with fluorochrome-labeled mAbs specific for B7-2 (BD Biosciences) and FlowJo software (Tree Star). Dead cells were excluded from the analysis by propidium iodide staining.

**Cocultures of in vivo stimulated DCs with naive CD4+ T cells**

Mice were repeatedly injected with tick saliva, SGE, or PBS, and painted with acetone-DBP mixture as described above. Immediately before skin painting, each mouse was intradermally injected into the ears with 2 μg of the gp61–80 of the LCMV glycoprotein. Three days after treatment, draining lymph nodes were dissected and DCs were isolated as described above. Lymph node DCs isolated from naive mice were used as a negative control. Titrated numbers of purified DCs were cultured with 5 × 10^5 naive SMARTA-2 transgenic CD4+ T cells as described above. Specific T cell proliferation was assessed after 48 h by determination of IL-2 production in culture supernatants by standard sandwich ELISA. T cell polarization was determined on day 5 of culture by intracellular cytokine staining as described above. Alternatively, expanded cells were restimulated for 24 h in the presence of freshly purified spleen DCs and 1 μM gp61–80, and cytokine content in culture supernatants was determined using standard sandwich ELISA.

**FIGURE 1.** *I. ricinus* tick saliva inhibits DC maturation in vitro. Purified spleen DCs were cultured in 96-well plates (1 × 10^5 cells/well), in the presence or absence of the indicated tick saliva concentrations. Pilocarpine (final concentration in well ~2 μM) was used as an additional control treatment, because a corresponding pilocarpine contamination has been determined in tick saliva. The DCs were activated by the addition of anti-CD40 mAb, CpG, imiquimod, or polyI:C. After 24 h of incubation, cells were stained with fluorochrome-labeled mAbs specific for B7-2 (A), MHC class I (B), and MHC II (C), and analyzed by flow cytometry. Data are expressed as the mean fluorescence intensity of live CD11c+ cells ± SEM. *p < 0.05. Data are representative of three repeat experiments.
Materials and Methods

Statistical analysis

Data were analyzed by ANOVA followed by Scheffé Post Hoc test, using Statistica v.7.1 software. The parametric assumptions of the data were verified using Kolmogorov-Smirnov test. The homogeneity of variances was tested by the Levene test. Data without normal distribution were transformed by use of the $X^2 = \log(X)$ formula. The results were considered statistically significant when $p < 0.05$.

Results

Tick saliva modulates DC maturation in vitro

We first compared the ability of three different synthetic TLR ligands (CpG, imiquimod, polyIC) or anti-CD40 mAbs to induce DC maturation in the presence or absence of the saliva. The tick saliva significantly affected CD40- or TLRs-mediated DC maturation, as indicated by impaired up-regulation of the MHC class I molecule in all of the stimulatory conditions (Fig. 1B); of the MHC class II molecule, upon exposure to polyIC and anti-CD40 mAb (Fig. 1C); and of the costimulatory molecule B7-2, upon CpG and polyIC stimulation (Fig. 1A). No significant inhibition was observed in DCs cultured in the presence of pilocarpine.

Tick saliva affects DC-mediated proliferation and polarization of naive CD4$^+$ T cells in vitro

Subsequently, we evaluated the consequence of impaired DC maturation on T cell priming. Naive SMARTA-2 T cells were primed by DCs in the presence of stimuli mentioned above, the tick saliva and various Ag doses. As indicated by IL-2 concentration in the supernatants after 2 days of culture, the presence of 10 µg tick saliva strongly reduced the extent of T cell proliferation in response to all Ag doses under all stimulatory conditions. The low dose of the saliva (3 µg) significantly reduced the extent of T cell proliferation in response to low and intermediate (Fig. 2) Ag doses. On the contrary, pilocarpine did not exert any significant effect on T cell activation.

To assess the effect of tick saliva on DC-mediated T cell polarization, expanded T cells were analyzed for cytokine production 5 days after culture. The high dose of imiquimod seemed to be toxic for the cells after 5 days of culture; therefore, it was excluded from the analysis. As compared with controls, significantly lower percentages of IFN-γ producing cells (Fig. 3B)

and higher percentages of IL-4 producing cells (Fig. 3C) were observed in T cell populations generated in the presence of tick saliva, at low (data not shown) and intermediate Ag doses. Moreover, while in the control cultures following stimulation with high Ag doses a fraction of IL-17 producing cells was detectable, Th17 polarization was completely suppressed in the presence of tick saliva (Fig. 3A).

Tick saliva impairs skin DC migration and maturation in vivo

We further studied the effect of tick saliva on DC maturation and migration in vivo. The tick saliva, SGE or PBS were repeatedly injected into the ear skin before induction of skin DC migration by skin painting with an irritant (dybutil phthalate), together with the green fluorescent dye (CMFDA), as previously reported (18).
Draining lymph nodes were collected 24 and 72 h after skin painting and analyzed by flow cytometry for the presence of skin DCs, detectable as CMFDA<sup>+</sup>CD11c<sup>+</sup>CD40<sup>high</sup> cells.

Repeated administration of the tick saliva resulted in a significant decrease in numbers of skin DCs that migrated to draining lymph nodes during the first 24 h after skin painting, as compared with control treatment (21971 ± 6735 vs 5766 ± 1859; p < 0.01).

After 72 h, however, the inhibitory effect on DC migration was no more evident (Fig. 4E). Additionally, when the phenotype of lymph node DCs was analyzed, migratory, as well as resident DCs from saliva- or SGE-treated mice exhibited reduced expression of B7-2 and, at the later time point, also of MHC class II molecules, in comparison with DCs isolated from control mice, possibly reflecting a saliva-mediated inhibition of DC maturation (Fig. 4, A–D).

**Exposure of DCs to tick saliva in vivo affects their Ag presentation and polarization capacity**

To evaluate whether or not the inhibitory effect exerted by tick saliva on DCs in vivo might affect the induction of specific T cell responses, we have analyzed Ag presentation and polarization capacity of DCs exposed to the saliva in vivo. Mice were repeatedly injected intradermally into the ears with tick saliva, SGE, or PBS. After the last injection, the ears of each mouse were painted with a mixture containing green fluorescent dye (CMFDA) and an inflammatory skin irritant (DBP). Draining lymph nodes were collected 24 and 72 h after skin painting, stained, and analyzed by flow cytometry. A and B, Levels of MHC class II expression on resident (CD11c<sup>+</sup>CMFDA<sup>+</sup>) (left) and migratory (CD11c<sup>+</sup>CMFDA<sup>+</sup>) cells (right). C and D, Levels of B7-2 expression on resident (CD11c<sup>+</sup>CMFDA<sup>+</sup>) (left) and migratory (CD11c<sup>+</sup>CMFDA<sup>+</sup>) cells (right).

Data are expressed as the mean fluorescence intensity of live cells ± SEM. *, p < 0.05. E, Absolute number of migratory DCs (CMFDA<sup>+</sup>CD11c<sup>+</sup>CD40<sup>high</sup>) in draining lymph nodes (mean ± SEM), *, p < 0.05. Data are representative of two repeat experiments.
injected into the ears with the tick saliva, SGE, or PBS, as described above. In addition, immediately before skin painting, each mouse was intradermally injected with 2 μg of the specific peptide, gp61–80. Three days after the treatment, titrated numbers of purified draining lymph node DCs were cultured with 5 × 10⁵ naive SMARTA-2 transgenic CD4⁺ T cells as described in the Materials and Methods. On day 5 of culture, cytokine production ability of expanded T cells was assessed by intracellular cytokine staining. A. Intracellular IFN-γ and IL-4 staining. Values represent percentage of positive cells in each quadrant. B and C. Proportions of IFN-γ and IL-4 producing cells, respectively, in the cocultures upon specific restimulation. Data are expressed as the mean proportion of cells in cocultures with indicated DC titer ± SEM. *, p < 0.05.

Discussion
During coevolution with their hosts, ticks, similarly to other blood-feeding arthropods, have evolved mechanisms enabling them to overcome the host immune system and thus successfully finish their blood meal. DCs are key players in the induction of adaptive immune responses and could thus represent privileged targets of
immunomodulatory mechanisms. Surprisingly, however, effects exerted by tick saliva on DC function have not been investigated in detail so far. In this study, we reveal complex modulatory effects of *Ixodes ricinus* tick saliva on DC phenotype and function in vitro and in vivo.

We have demonstrated that exposure of DCs to tick saliva in vitro resulted in impaired maturation, upon CD40 or TLR3, TLR7, and TLR9 ligation, and in reduced Ag presentation capacity. Moreover, priming of naive CD4+ T cells by DCs in the presence of saliva led to preferential development of Th2 effectors, whereas Th1 and Th17 polarization were markedly inhibited. Administration of the tick saliva in vivo significantly diminished DC migration from skin to draining lymph nodes under inflammatory conditions, and decreased the capacity of peripheral lymph node DCs to present soluble Ag to specific T cells. Most importantly, whereas T cells expanded by DCs isolated from control mice differentiated toward Th1, T cells primed by DCs from saliva-treated mice predominantly underwent Th2 polarization.

The tick saliva has been reported to inhibit differentiation and LPS induced-maturation of bone marrow-derived DCs (BMDCs) in vitro (15). More recently, prostaglandin E2 contained in the tick LPS induced-maturation of bone marrow-derived DCs (BMDCs) in vitro (15). More recently, prostaglandin E2 contained in the tick saliva has been found to impair cytokine production by BMDCs as well as their capacity to induce T cell proliferation (16). However, effects of the tick saliva on DC maturation and function in vivo have not been evaluated so far.

By using spleen-derived DCs, a more physiological cell population, we have shown in this study that the tick saliva inhibits DC maturation in vitro in terms of TLR3-, TLR7-, and TLR9-ligation, or CD40 binding. Most importantly, we have demonstrated a significant impact of the tick saliva on DC function in vivo. DC maturation occurs in vivo upon the sensing of "danger signals" including microbial products or signals from damaged tissues by DCs patrolling the periphery. As a consequence, DCs become activated and migrate to regional lymph nodes to initiate specific immune responses (19). We have shown in this study that repeated administrations of tick saliva inhibit early migration of DCs from inflamed skin to draining lymph nodes. The absence of the inhibitory effect on DC migration by SGE might be due to the significant amount of additional proteins of salivary gland cells in the SGE, which are not secreted into the saliva and might probably support DC migration rather than diminish it. However, DCs isolated from skin draining lymph nodes of both, saliva, as well as SGE-treated mice exhibited a less mature phenotype than DCs from control mice, consistent with an inhibition of the maturation induced by inflammatory factors. The inhibitory effect exerted by the saliva may be related to an impaired release of proinflammatory cytokines involved in DC migration and/or maturation. Indeed, tick saliva-mediated inhibition of IL-1 and TNF-α, two cytokines known to promote skin DC migration, as well as DC maturation (20, 21), has been previously reported (4, 22). Although their role in the induction of protective immune response has been questioned recently in the case of viral and protozoan infections (23, 18), it is possible that skin DCs, especially the early migrating dermal DCs (24), may be significant actors in initial transmission of the tick saliva Ags from skin to draining lymph nodes. Thus, it can be expected that the inhibition of DC migration might postpone the development of the specific immune response, consequently facilitating the successful completion of the tick feeding process.

In addition to delayed migration, impaired DC maturation occurring in the presence of the saliva might also contribute to prevent the prompt induction of an efficient immune response. The poor Ag presentation capacity of saliva-exposed DCs might in fact result in suboptimal activation of Ag specific T cells. We have indeed found a significantly reduced proliferation of CD4+ T cells primed by DCs in the presence of the saliva in vitro.

It is known that tick-derived salivary proteins, in particular Salp 15, can directly inhibit T cell activation, through interaction with the coreceptor CD4 (25, 26). However, it has recently been reported that pre-exposure of BMDCs to the tick saliva or saliva components, in particular to prostaglandin E2, impairs the subsequent activation of Ag-specific T cells (16). In line with these reports, our data suggest that inhibition of T cell proliferation by the tick saliva may also be DC mediated. Importantly, T cell proliferation was also reduced on priming by skin draining lymph nodes DCs that had been exposed to the saliva in vivo. The reduced stimulatory capacity of lymph node DCs was most likely a consequence of their incomplete maturation. However, because lymph node DCs acquired Ag in vivo, the possibility that the saliva might also interfere with Ag acquisition by DCs should also be considered.

It has been shown that the tick saliva or SGE diminish the production of Th1-related cytokines and increase the production of Th2-related cytokines (11–14). In this study, we have demonstrated that the preferential induction of Th2 responses by tick saliva is also a consequence of its modulatory effect on DCs. Indeed, exposure of DCs to the saliva in vitro and in vivo inhibited their ability to drive Th1 and Th17 polarization of naive T cells, and resulted in strong Th2 development. The reduced capacity of saliva-exposed DCs to prime Th1 and Th17 cells can be explained by the inhibition of their capacity to release cytokines upon stimulation, as recently reported for BMDCs cells (16). Similarly, we have also found reduced IL-12p70 concentrations in culture supernatants of TLR- or CD40-stimulated spleen DCs in the presence of the saliva (data not shown). Additionally, reduced expression of MHC and costimulatory molecules on saliva-exposed DCs might induce a preferential expansion of Th2 effector cells, as previously reported (27, 28). Tick saliva-induced CD4+ T cell differentiation into Th2 subset, as opposed to Th1 subset, is supposed to be advantageous for the tick. Indeed, Th1-mediated inflammation of host skin would enhance the risk of parasite removal during grooming (29). Moreover, development of a delayed-type hypersensitivity reaction at the tick-attachment site, which can result in death or decreased fitness of the tick, is known to be Th1-mediated (30, 31). Consistently, development of a strong Th2 response against *I. ricinus* nymphs in mice has been shown to be associated with the tolerance of tick bites (32). Additionally, it has been reported that polarization of the immune response toward the Th2 subset creates favorable conditions for the tick-transmitted spirochete *Borrelia burgdorferi* (33). Conversely, inoculation of TNF-α, IFN-γ, and IL-2 mixture during tick feeding has been shown to suppress *B. burgdorferi* transmission by *I. scapularis* (34).

Taken together, our data indicate that the *I. ricinus* tick saliva significantly impairs DC migration and maturation, thereby altering the outcome of DC-CD4+ T cell interaction. Because DCs are major players in initiation and fine-tuning of the adaptive immune response, alteration of their function could be a key mechanism used by the tick to circumvent the host immune system. Moreover, the impact of tick saliva on DC phenotype and especially on the Ag presentation capacity may be subsequently exploited by tick borne pathogens, including the tick-borne encephalitis virus and *Borrelia* spirochetes, to facilitate their transmission and replication in the host.

**Acknowledgments**

We thank Petr Šimek for help with the HPLC, Zdeněk Cimburek for help with the FACS analysis, Jan Erhart for technical assistance, and Kateřina Černá for support and encouragement.
Disclosures
The authors have no financial conflict of interest.

References
32. Gianaparo, F., B. Rutt, and M. Brossard. 1995. In vitro production of interleu-
kin-4 and interleukin-10 by lymph node cells from BALB/c mice infected with nymphal Ixodes ricinus ticks. Immunology 85: 120–124.