Check for updates

INFECTION AND IMMUNITY, May 2009, p. 1881–1887 Vol. 77, No. 5
0019-9567/09/$08.00+0 doi:10.1128/IAI.00030-09
Copyright © 2009, American Society for Microbiology. All Rights Reserved.

Langerhans Cell Deficiency Impairs *Ixodes scapularis* Suppression of Th1 Responses in Mice▽

Diana L. Vesely,[1] Durland Fish,[2] Mark J. Shlomchik,[3] Daniel H. Kaplan,[4] and Linda K. Bockenstedt[1]*

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520[1]; Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06520[2]; Departments of Laboratory Medicine and Immunobiology, Yale University School of Medicine, New Haven, Connecticut 06520[3]; and Department of Dermatology, Center for Immunology, University of Minnesota, Minneapolis, Minnesota 55455[4]

Received 8 January 2009/Returned for modification 9 February 2009/Accepted 28 February 2009

***Ixodes scapularis* ticks transmit a number of human pathogens, including the Lyme disease spirochete *Borrelia burgdorferi*. *I. scapularis* suppresses host immunity in the skin to promote feeding and systemically skew T-helper (Th)-cell differentiation toward Th2 cells in secondary lymphoid organs. Although components of tick saliva are known to influence Th-cell polarization, the mechanism whereby tick feeding in the skin modulates regional and systemic Th-cell responses is unknown. In this study, the role of the epidermal Langerhans cell (LC) subset of skin dendritic cells in tick-mediated Th1/Th2-cell immunomodulation was assessed. Mice deficient in LCs (Langerin-DTA mice) exhibited enhanced lymph node (LN) concanavalin A (ConA)-induced Th1 responses after tick infestation in comparison to results for uninfested Langerin-DTA or wild-type (WT) mice, whereas effects on Th2-cell production of interleukin 4 were more variable. Nonetheless, the altered T-cell response did not impact tick feeding or refeeding. Gamma interferon production by ConA-stimulated LN cells of both WT and LC-deficient mice was enhanced by as much as fourfold after *B. burgdorferi*-infected-tick feeding, indicating that immunomodulatory effects of tick saliva were not able to attenuate the Th1 immune responses induced by this pathogen. Taken together, these findings show a requirement for LCs in the tick-mediated attenuation of Th1 responses in regional lymph nodes but not in the spleens of mice and show that the presence of a pathogen can overcome the Th1-inhibitory effects of tick feeding on the host.**

Blood-feeding arthropods are responsible for a significant proportion of all vector-borne infections, which number in the hundreds of millions annually worldwide (35, 36). In the United States, Lyme disease, due to infection with the spirochete *Borrelia burgdorferi*, is the most common arthropod-transmitted disease, with more than 20,000 cases reported in 2007 alone (3a, 32). *Ixodes scapularis* is the primary vector for *B. burgdorferi* in North America, and it also transmits several other pathogens associated with emerging infectious diseases, including *Anaplasma phagocytophilum* and *Babesia microti*. Each of these pathogens has established a life cycle that requires their sequential passage between a vertebrate host and ticks, underscoring the importance of tick feeding in maintaining these pathogens in nature.

Interaction between the tick and the blood meal host has a significant impact on the host immune system and has been shown to enhance pathogen transmission (24). *I. scapularis* ticks must remain attached to their hosts for several days to acquire their blood meals and consequently have developed strategies to avoid immune rejection by the host (8, 11, 18, 25, 26, 31, 34, 39). *I. scapularis* saliva has pharmacologic properties that allow feeding to occur without host detection of the tick, which vector-borne pathogens like *B. burgdorferi* have exploited to establish infection and survive in the mammalian host (25–27, 34). Proteomic analysis of tick saliva has revealed antihemostatic, anti-inflammatory, and immunomodulatory components, which are thought to subdue the host cutaneous immune response and promote ingestion of the blood meal (18, 26, 34). In addition to effects on local immunity, the feeding of *I. scapularis* ticks on mice has been reported to result in systemic immune deviation of T-helper (Th) responses toward the anti-inflammatory Th2 type, as assessed by mitogen (concanavalin A) stimulation of lymphocytes harvested from the spleens 7 to 10 days after tick placement (30, 40–42).

Although many of the effects of tick feeding have been linked to components of tick saliva (9, 10, 26, 34, 38), the mechanism(s) whereby tick feeding leads to systemic modulation of Th-cell cytokine production, distant from the tick bite in the skin, remains poorly understood. Dendritic cells in the skin, including epidermal Langerhans cells (LCs) and dermal dendritic cells (DCs), are key antigen-presenting cells that prime T-cell responses in regional draining lymph nodes after skin disruption. LCs in particular perform a sentinel function by detecting foreign antigens in the skin and migrating to the lymph nodes, where they are thought to interact with T cells and influence the Th-cell response through specific cytokine production (28). Although they were originally thought to play a seminal role in the priming of T-cell responses, more-recent studies have revealed that they can also modulate T-cell priming by other antigen-presenting cells (14–17, 20, 28, 37). Disruption of E-cadherin attachments of LCs to keratinocytes in

* Corresponding author. Mailing address: Section of Rheumatology, Department of Internal Medicine, Yale University School of Medicine, TAC S-520, 300 Cedar Street, New Haven, CT 06520. Phone: (203) 785-2453. Fax: (203) 785-7053. E-mail: Linda.Bockenstedt@yale.edu.
▽ Published ahead of print on 9 March 2009.

Downloaded from http://iai.asm.org/ on January 21, 2021 by guest

the skin can induce LC migration in an immature state in which they can induce tolerance in the host, in part through the secretion of cytokines that promote Th2 responses (12). LCs appear to suppress the development of Th1 responses, as has been shown by the increase in the type IV hypersensitivity reaction to the contact allergen 2,4-dinitrofluorobenzene in mice genetically deficient in epidermal LCs (Langerin-DTA mice) (14). Additionally, studies using a mouse model of skin allograft rejection demonstrated that an absence of LCs leads to graft rejection, suggesting that LCs play a regulatory role in skin graft acceptance (23). In this study, we evaluated the role of LCs in the modulation of regional and systemic T-cell responses by tick feeding. Our results implicate LCs in tick-feeding-induced suppression of Th1 responses in the regional draining lymph nodes but not in modulation of systemic splenic Th-cell responses.

MATERIALS AND METHODS

Mice. The FVB strain of mice deficient in epidermal Langerhans cells, Langerin-DTA transgenic mice, were produced as described previously (14). Langerin-DTA mice contain an attenuated form of diphtheria toxin under the control of genomic bacterial artificial chromosome elements that restrict expression to epidermal LCs. These mice lack LCs but continue to express dermal DCs that bear the langerin marker (5, 14). Nontransgenic littermates were used as wild-type (WT) controls. Six- to twelve-week-old Langerin-DTA and WT mice were used for all experiments. Mice were housed in filter-frame cages and administered food and water ad libitum according to Yale University animal care and use guidelines. Mice were euthanized by carbon dioxide asphyxiation. The Yale animal care and use committee approved all studies involving mice.

Tick infestation of mice. All experiments were performed with either uninfected nymphal *I. scapularis* ticks or nymphal *I. scapularis* ticks experimentally infected with *Borrelia burgdorferi* strain N40 as described previously (2). Ticks were maintained in a laboratory-reared tick colony at the Yale University School of Medicine. Mice were mildly anesthetized with ketamine (100 mg/kg of body weight) and xylazine (10 mg/kg) prior to tick infestation. Five nymphal ticks were placed in each ear canal of the mice (10 ticks/mouse), after which mice were placed in individual cages. Ticks were allowed to feed to repletion, the duration of which varies from 3 to 5 days, and detach naturally into a water bath. Ticks were collected from the water bath on a daily basis for up to 1 week. In experiments evaluating tick feeding patterns, engorged ticks were collected, rinsed in water, and allowed to air dry before being weighed using a Mettler balance. For repeated tick feedings, groups of 10 uninfected nymphal ticks were fed on groups of WT and Langerin-DTA mice (2 mice per group) every 21 days for a total of 3 separate infestations. Ticks were collected and weighed as described above.

Measurement of T-cell cytokine production. Spleens and regional lymph nodes (superficial cervical, deep cervical, mediastinal, axillary, and brachial) were harvested from Langerin-DTA and WT mice on day 7 after tick infestation and triturated between the frosted ends of two sterile glass slides to create cell suspensions. After red blood cell lysis, splenocytes from the four to five individual mice of each experimental group were pooled and resuspended in Click's medium supplemented with 10% fetal bovine serum, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml of penicillin, and 100 μg/ml of streptomycin (complete medium). One-ml triplicate aliquots of 5×10^{6} cells/ml were added to 24-well tissue culture plates (Costar polystyrene no. 3524) and stimulated with 2 μg/ml concanavalin A (ConA) or left unstimulated. Lymphocytes from four to five individual mice of each experimental group were pooled, aliquoted at 5×10^{5} cells in 0.2 ml of complete medium in triplicate into 96-well tissue culture plates (Costar polystyrene no. 3595), and stimulated with 2 μg/ml ConA or left unstimulated. Culture supernatants from both lymph nodes and splenocytes were harvested after 48 h of incubation and stored frozen at −80°C until cytokine measurement. Cytokines from the lymph node and spleen culture supernatants were quantified using cytokine-specific enzyme-linked immunoabsorbant assay (ELISA) kits for mouse interleukin 2 (IL-2), gamma interferon (IFN-γ), IL-4, and IL-10 (eBioscience, San Diego, CA) according to the manufacturer's instructions. ELISA assays that compared the effects of *B. burgdorferi*-infected ticks and uninfected ticks on cytokine production were performed in the same 96-well ELISA plate.

Statistical analysis. Statistical significance was evaluated using the Graphpad Prism, version 4.0, software program for the Macintosh (GraphPad Software, Inc.). One-way analysis of variance (ANOVA) with a Newman-Keuls multiple-comparison posttest was used to evaluate statistical significance. When the overall P value for ANOVA was <0.05, the Newman-Keuls multiple-comparison posttest was performed to determine significance between individual groups. Differences at P values of <0.05 in the posttest were considered significant.

RESULTS

LCs attenuate Th1-cell responses in the lymph nodes after tick feeding. *I. scapularis* tick feeding on mice has been shown to modulate ConA-induced T-cell cytokine production in lymph nodes and spleens, with a reduction in the Th1 cytokine IFN-γ and an increase in the Th2 cytokine IL-4 (30, 42). To determine whether the observed modulation was mediated in part by LCs, we assessed the effects of tick infestation on cytokine responses in WT and Langerin-DTA transgenic mice (Fig. 1 and 2). As expected based on previous studies with BALB/c and C3H/HeN mice (30, 42), tick feeding suppressed ConA-induced IFN-γ production by lymph node (Fig. 1a) and splenic (Fig. 2a) T cells of FVB mice. The degree of suppression in lymph nodes depended on mouse gender; female FVB mice had greater ConA-induced responses at baseline in lymph nodes than male mice. However, in five independent experiments, we found higher levels of ConA-induced IFN-γ in lymph node cultures from Langerin-DTA mice infested with ticks than in similarly stimulated lymph node cells from tick-infested WT mice (Fig. 1a). Although tick feeding on WT mice suppressed the production of IFN-γ by splenic T cells, the absence of LCs had no effect on this response (Fig. 2a). Tick feeding on WT mice did not attenuate IL-2, but an absence of LCs led to a statistically significant increase in production of this cytokine after ConA stimulation of lymph node cells in the females (Fig. 1b) ($P < 0.05$).

The Th2 cytokine IL-4 was not detected in uninfested WT or Langerin-DTA lymph node (Fig. 1c) or splenic (Fig. 2b) T-cell culture supernatants after ConA stimulation. Tick feeding on these mice increased IL-4 production in both mouse groups, consistent with results reported in previous publications (30, 41, 42), but LC deficiency did not affect IL-4 levels. The Th2 cytokine IL-10 could also be detected in ConA-stimulated lymphocytes only after tick feeding, but no significant differences were found in the presence or absence of LCs (Fig. 1d).

***B. burgdorferi* infection of nymphs induces Th1 responses in lymph nodes in the absence of LCs.** Previous studies with C3H mice have suggested that the Th polarization resulting from tick feeding could facilitate *B. burgdorferi* infection (42). We therefore examined the effects of *B. burgdorferi*-infected ticks on the Th1/Th2 polarization in lymph nodes (Fig. 1) and spleens (Fig. 2) of FVB mice. *B. burgdorferi*-infected-tick feeding led to as much as a fourfold enhancement of ConA-induced IFN-γ production by lymph node T cells for both WT and Langerin-DTA mice relative to that for uninfected ticks, indicating that the presence of this extracellular pathogen overrides any suppressive effects of tick feeding on Th1 responses (Fig. 1a). In male mice, the effect of *B. burgdorferi* infection on tick-mediated modulation of IFN-γ production was specific to the lymph nodes (Fig. 1a), since no significant differences in ConA-induced IFN-γ production by splenocytes were found 7 days after tick placement (Fig. 2a). Since *B.*

Downloaded from http://iai.asm.org/ on January 21, 2021 by guest

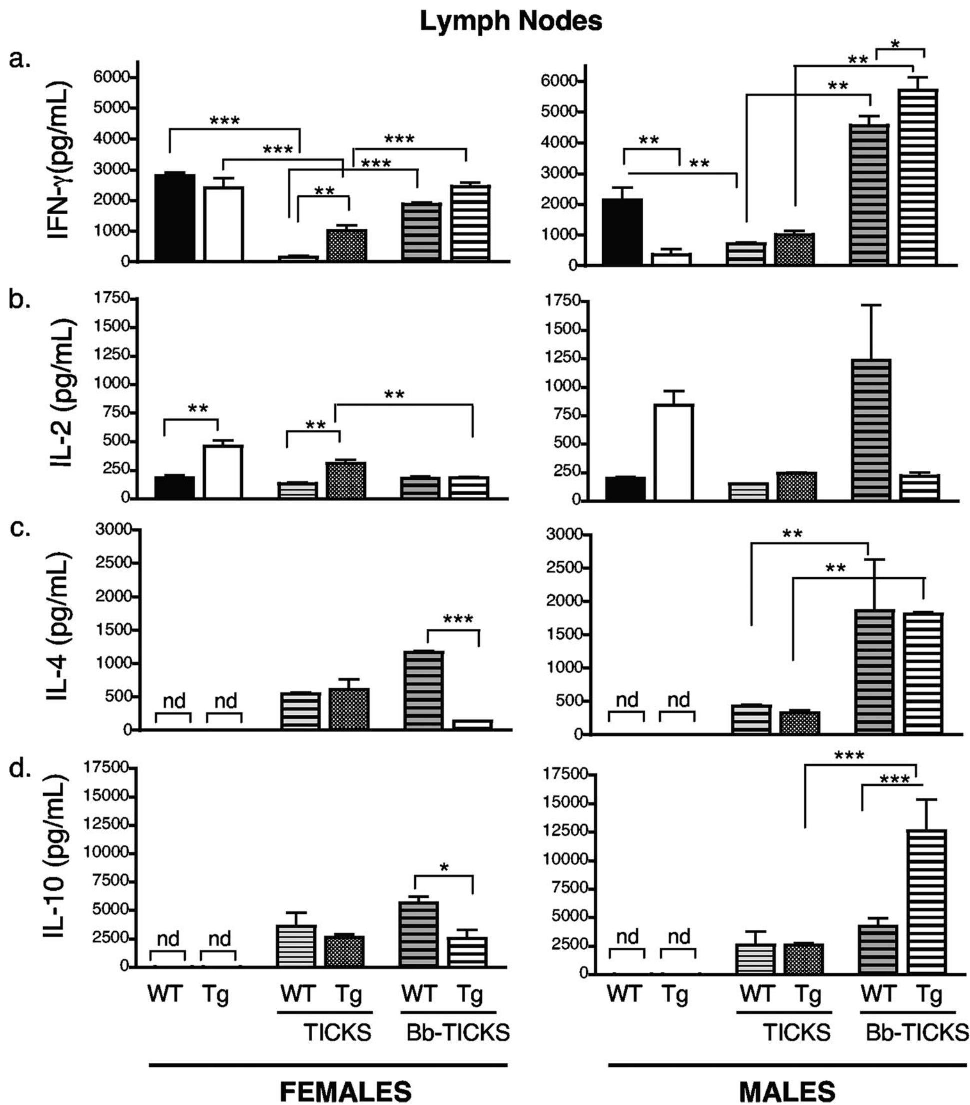

FIG. 1. Absence of LCs alleviates tick-induced suppression of IFN-γ production by lymph node cells, but tick-feeding modulation of IFN-γ cannot be seen in the presence of *B. burgdorferi.* Groups of WT and Langerin-DTA mice (Tg) (four males or four females per group) were either left uninfested or infested with either specific-pathogen-free or *B. burgdorferi*-infected nymphal ticks (10 ticks/mouse). Mice were sacrificed 7 days later, and lymphocytes from the lymph nodes (LNs) were cultured in the presence of 2 μg/ml of ConA for 48 h. IFN-γ, IL-2, IL-4, and IL-10 production was determined by ELISA (a to d). Samples from male and female mice were analyzed in the same ELISA plate. Error bars represent the standard error of the mean for each experimental group. *, differences between indicated groups were calculated by a Newman-Keuls posttest when P values were <0.05 by ANOVA and were denoted with asterisks as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. nd, not detected.

burgdorferi remains localized to the skin for up to 48 h after tick detachment (6 to 8 days after tick placement) (33), the absence of effects of *B. burgdorferi* infection on splenic T-cell responses at this time point may be expected. However, as in the experiments with the uninfected ticks, there were differences based on mouse gender. The feeding of *B. burgdorferi*-infected ticks resulted in elevated IFN-γ levels in both the lymph nodes (Fig. 1a) and the spleens (Fig. 2a) of female Langerin-DTA mice compared to results for female Langerin-DTA mice that were infested with pathogen-free ticks ($P < 0.05$ for lymph nodes and spleen) or to results for female WT mice that were infested with *B. burgdorferi*-infected ticks ($P > 0.05$ [lymph nodes] and $P < 0.05$ [spleen]). With the exception of WT males, the feeding of *B. burgdorferi*-infected ticks did not dramatically alter IL-2 levels in the lymph nodes of WT and Langerin-DTA mice compared to those for mice that were infested with pathogen-free ticks (Fig. 1b). Compared to responses induced by uninfected ticks, the feeding of *B. burgdorferi*-infected ticks increased ConA-induced IL-4 levels in the lymph nodes of WT mice of both genders but not Langerin-DTA mice (Fig. 1c). In the spleens, *B. burgdorferi*-infected-tick feeding resulted in diminished ConA-induced IL-4 levels in both the WT and Langerin-DTA mice in comparison to results with feeding of uninfected ticks (Fig. 2b). The feeding of *B. burgdorferi*-infected ticks also resulted in elevated IL-10 levels in the lymph nodes of both WT and Langerin-DTA mice in comparison to results for feeding of uninfected ticks (Fig. 1d). These effects of *B. burgdorferi*-infected tick feeding on the Th2 cytokines IL-10

Downloaded from http://iai.asm.org/ on January 21, 2021 by guest

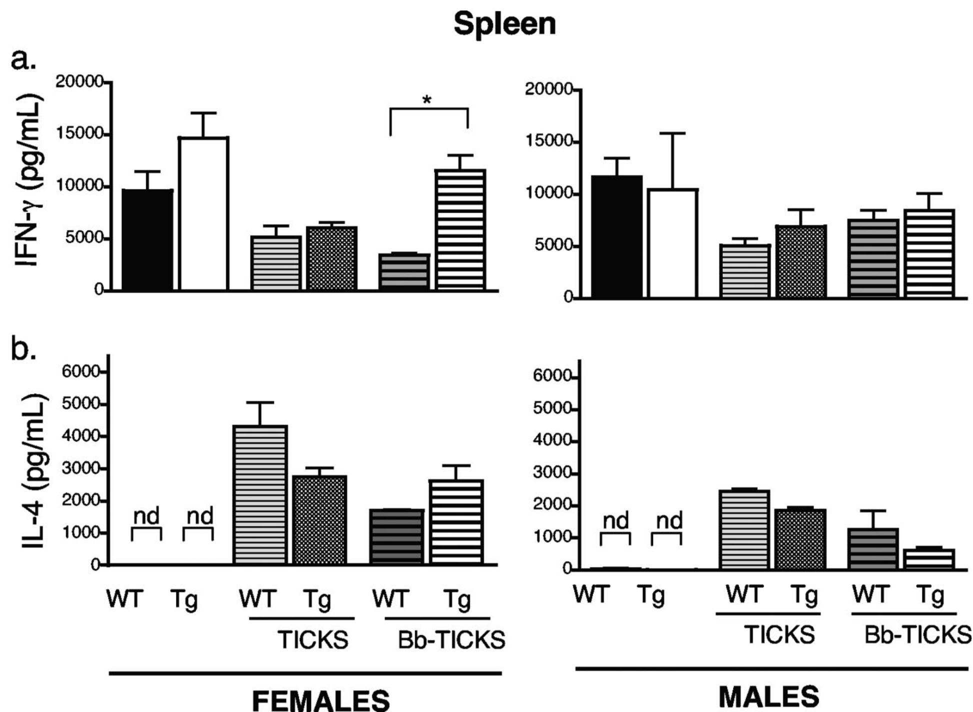

FIG. 2. Absence of LCs does not alter the ability of tick feeding to suppress Th1 responses in the spleen. Groups of WT and Langerin-DTA mice (four female or male mice per group) were infested with either specific-pathogen-free or *B. burgdorferi*-infected (Bb) ticks (10 ticks/mouse). Mice were sacrificed 7 days later, and splenocytes were cultured in the presence of 2 μg/ml of ConA for 48 h. Production of IFN-γ (a) or IL-4 (b) was determined by ELISA. Male and female mice were analyzed in the same ELISA plate. Statistical significance was evaluated with ANOVA and Newman-Keuls multiple-comparison posttest. *, $P < 0.05$ by ANOVA and Newman-Keuls posttest; nd, not detected. Error bars represent the standard error of the mean for each experimental group.

and IL-4 varied depending on gender. For female mice, the feeding of *B. burgdorferi*-infected ticks resulted in significantly lower IL-10 and IL-4 levels for the Langerin-DTA mice than for WT mice (Fig. 1c and d) ($P < 0.05$), whereas for male mice, IL-10 was significantly elevated for Langerin-DTA mice compared to results for WT mice (Fig. 1d) ($P < 0.05$) and no differences in IL-4 were detected (Fig. 1c).

Absence of LCs does not alter tick feeding on the murine host. It has been proposed that the Th modulation observed after tick infestation on mice helps prevent tick rejection, especially after repetitive feedings. We therefore examined whether the absence of LCs alters feeding patterns of specific-pathogen-free or *B. burgdorferi*-infected ticks (Fig. 3). Nymphal ticks feeding on WT and Langerin-DTA mice without previous tick exposure remained attached for similar durations of 3 to 4 days (data not shown), and there were no differences in the percentages of engorged ticks retrieved. The number of ticks that were retrieved from each mouse is shown in Fig. 3a and b, and all of these ticks were engorged. Compared to the average weight of an unfed nymphal tick (0.16 mg), the average weight of the ticks that fed on and detached from the WT and Langerin-DTA mice was significantly greater (approximately 4 mg; $P < 0.001$). Nonetheless, the engorged weights of the ticks after spontaneous detachment from WT and Langerin-DTA mice were not significantly different (Fig. 3a and b) ($P > 0.05$). The bimodal distribution of tick weights is due to the sex of the ticks, since engorged female nymphal ticks are larger than engorged male ticks (6).

Although mice do not develop immunity to tick feeding (19), other animal species (e.g., guinea pigs) develop immunity to repeated tick feeding, which has been shown to be due to the development of cutaneous basophil hypersensitivity to the tick (1, 4). To ensure that LC deficiency did not alter mouse immunity to tick infestation, we measured the duration of tick feeding (data not shown) and weight of engorged ticks that had fed on mice repeatedly used as blood meal hosts (Fig. 3c). Ticks feeding on mice that had previously served as blood meal hosts were still able to feed to repletion over 3 to 5 days, and there were no significant differences in tick weights.

DISCUSSION

Although many of the local effects of tick feeding on the host have been linked to components of tick saliva, the mechanism(s) whereby tick feeding leads to host immunomodulation regionally in the draining lymph nodes and systemically in the spleen remain poorly understood. The purpose of this study was to determine whether the regional and systemic effects of tick feeding on Th-cell dominance require epidermal LCs, which modulate T-cell priming and polarization toward different T-cell subsets in the lymph nodes. Our rationale was based on the fact that LCs are among the first immune components to respond to the penetration of the skin by the tick and therefore may shape the evolving host immune response to the feeding tick. In mice, the tick hypostome penetrates the epidermis and extends into the dermis, where a feeding pit is

Downloaded from http://iai.asm.org/ on January 21, 2021 by guest

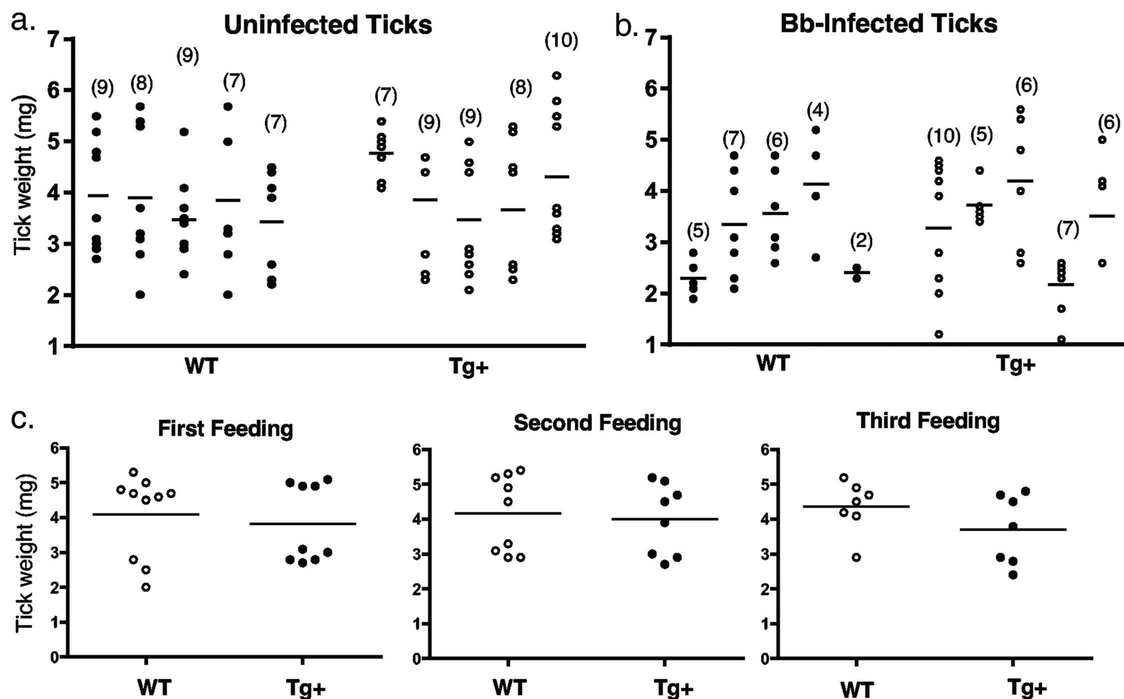

FIG. 3. LC deficiency does not alter tick feeding patterns. Ten uninfected *I. scapularis* nymphal ticks (a) or *B. burgdorferi* (Bb)-infected nymphal ticks (b) were placed on two groups ($n = 5$ [each]) of Langerin-DTA (Tg+) or WT mice and allowed to feed to repletion. Ticks were collected after spontaneous detachment and weighed individually. Numbers in parentheses represent the number of ticks retrieved from each mouse, all of which were engorged. Statistical significance was calculated using ANOVA ($P > 0.05$ [a and b]). (c) Groups of five uninfected nymphs were fed on each of two Langerin-DTA mice and two WT mice every 21 days for a total of three separate and sequential infestations. Nymphs were collected and weighed individually after they naturally detached 3 to 5 days after placement. Weights of ticks that were retrieved and fed to completion are shown. First feeding, ANOVA, $P > 0.05$; second feeding, $P > 0.05$; third feeding, $P > 0.05$.

formed from which the blood meal is eventually ingested. Thus, both epidermal and dermal DCs would be expected to respond to tick infestation. The Langerin-DTA mice used in these studies specifically lack only epidermal LCs in the skin and have normal numbers of dermal DCs, including the recently identified population of langerin-positive (langerin$^+$) dermal DCs, allowing us to differentiate the contribution of epidermal LCs specifically to the immune response to tick feeding (5).

The results reported here support a role for LCs in suppression of Th1-cell cytokine production in regional draining lymph nodes but not in the spleens of mice infested with specific-pathogen-free ticks. This effect appeared to be independent of IL-4 production, since no significant differences were consistently noted in the production of this cytokine in the presence or absence of LCs. These findings are in agreement with previous reports that LCs can suppress Th1-mediated inflammation in the contact model of type IV hypersensitivity (14) and suggest that they play a similar role in the immune response to tick feeding.

Tick saliva has been shown to prime DCs to induce Th2-biased immune responses in vitro and in vivo (21), and prostaglandin E2 (PGE_2) has been identified as a component of tick saliva responsible for DC priming in vitro (3, 29). LCs, in addition to expressing prostanoid receptors, express prostanoid synthases and are themselves a source of PGE_2 (3). It is possible that PGE_2 introduced via tick saliva may act upon LCs or complement their tolerogenic role in the context of tick feeding. Nonetheless, the enhanced IFN-γ production in Langerin-DTA mice upon tick feeding indicates that any PGE_2 introduced at physiological levels as a component of tick saliva cannot compensate for the lack of LCs in vivo.

Overall, our results suggest a tolerogenic role for LCs in the context of tick feeding and are in accord with the report that tick saliva primes DCs to induce Th2-biased immune responses (21). In contrast, Kabashima et al. reported that PGE_2 signaling plays a role in the initiation of antigen-specific immune responses by enhancing the T-cell stimulatory capacity of LCs (13). Using the classic contact hypersensitivity assay and mice depleted of langerin$^+$ cells using diphtheria toxin, this group has shown that PGE_2 acts through langerin$^+$ cells to enhance skin immune responses to the hapten dinitrofluorobenzene. Indeed, it has been debated whether LCs play a tolerogenic or immunostimulatory role. Given the later kinetics of migration of epidermal LCs relative to DCs to the lymph nodes (5), we have suspected that LCs serve to modulate T-cell priming by the dermal DCs. The recent identification of a subset of dermal langerin$^+$ dendritic cells has reconciled the differences noted in contact hypersensitivity between mice depleted of langerin$^+$ cells after development versus those congenitally depleted. Dermal langerin$^+$ dendritic cells, which are distinct from LCs (5), possess immunostimulatory capability and have not been considered when interpreting the results of previous publications. The Langerin-DTA mice employed in our study allowed us to differentiate the specific contribution of LCs to the im-

Downloaded from http://iai.asm.org/ on January 21, 2021 by guest

mune response to tick feeding because they have normal numbers of dermal langerin^{+} DCs and lack only epidermal LCs.

It has been postulated that the immunosuppressive effects of tick saliva could impair the immune response to a tick-transmitted pathogen. We found that tick feeding was unable to attenuate the strong host immune response to *B. burgdorferi*. The feeding of *B. burgdorferi*-infected ticks increased IFN-γ production by ConA-stimulated lymph node cells of both WT and Langerin-DTA mice by as much as fourfold and also elevated lymph node IL-4 production to a lesser extent in the WT mice. There were also no differences in *Borrelia* antigen-specific immune responses in WT and Langerin-DTA mice infected by tick bite, assessed 14 days after tick infestation (data not shown). Although the feeding of *B. burgdorferi*-infected ticks masked the LC-mediated attenuation of Th1 responses seen when uninfected ticks feed, a role for LCs in regulating the Th1/Th2 balance can still be appreciated in female Langerin-DTA mice, where *B. burgdorferi*-infected tick feeding resulted in significantly diminished IL-4 levels compared to those for WT mice. In addition, *B. burgdorferi*-infected ticks increased ConA-induced IL-10 production for both WT and Langerin-DTA mice (Fig. 1d). These findings indicate that the feeding of *B. burgdorferi*-infected ticks masked the LC-mediated attenuation of Th1 responses seen when uninfected ticks fed on mice and that tick feeding is unable to dampen the strong immune response to this pathogen. Neither IL-4 nor IFN-γ is necessary for host resistance to *B. burgdorferi* infection or disease, and we found no differences in pathogen burden at the tick feeding site in LC-deficient and WT mice (data not shown).

Although we observed enhanced Th1 responses after uninfected tick feeding on LC-deficient mice, the altered T-cell responses did not impact tick feeding or refeeding. These findings are consistent with the observation that acquired resistance to tick feeding is mediated by basophils, a cell type not present in mouse skin (1).

We observed dissociation of host immunomodulation between the lymph nodes and spleen following tick feeding, since tick feeding on mice deficient in LCs resulted in enhanced immune responses in the lymph nodes but not the spleen relative to results for WT mice. To our knowledge, this is the first report of dissociation between tick modulation of lymph-node and splenic T-cell responses. It is not surprising that differences between the Langerin-DTA and WT mouse groups upon tick feeding were evident in the lymph nodes, since LCs are known to migrate from the epidermis to the lymph nodes, where they influence Th-cell responses. Although DC subsets, including langerin^{+} DCs, which are distinct from epidermal LCs, are found in the spleen, there is no evidence of epidermal LCs at that site (7). Another interesting observation was the inherent sex-based differences in IFN-γ production in the mice in our study. Female mice, regardless of whether they were WT or Langerin-DTA, tended to produce higher levels of IFN-γ at baseline and after tick feeding than did male mice. Upon feeding of *B. burgdorferi*-infected ticks, significantly lower levels of IL-4 and IL-10 were detected in the lymph nodes of female Langerin-DTA mice than in those of female WT mice, but different results were noted for male mice (Fig. 1c and d). Similarly, upon feeding of *B. burgdorferi*-infected ticks, IFN-γ levels were significantly higher in the spleens of female Langerin-DTA mice than in those of WT females, but there were no differences in the male mice (Fig. 2a). The effect of mouse gender and the functional roles of distinct DC subsets for a pathogen remain unclear. DC subsets other than LCs are thought to prime immune responses in the lymph nodes and spleen, whereas LCs may regulate these DC-primed responses (5, 15).

Similar observations that gender affects immunity have been reported for a mouse model of atherosclerosis, where sex-based differences in levels of Th1/Th2 cytokines correlated with sex-based differences in vascular repair (43), and in a recent study that investigated the immunomodulatory effects of *Ixodes ricinus* tick feeding on immunization with bovine serum albumin-QuilA (22). It is widely thought that the basis for such sex-based differences may be the effects of estrogen on immune responses, but additional, unknown factors may also be important. These findings underscore the need to consider influences of gender on immune responses when interpreting results from experiments utilizing mice of both genders.

In summary, our results demonstrate a role for LCs in the tick-feeding-induced reduction in Th1-cell responses in regional draining lymph nodes. Further studies are in progress to delineate the mechanism whereby tick salivary components may influence LC function to modulate lymph node responses.

ACKNOWLEDGMENTS

We thank Jialing Mao, Ming Li, and Debbie Beck for excellent technical assistance.

This work was funded by NIH NIAMS grant T32 AR07107-26 to D.L.V.; NIH grant R01-AI59529 and the Jockers Award to L.K.B.; grant R01-AR044077 to M.J.S.; and the G. Harold and Leila Y. Mathers Charitable Foundation and USDA-ARS Cooperative Agreement no. 0500-00045-001-01S to D.F.

REFERENCES

1. **Askenase, P. W., B. G. Bagnall, and M. J. Worms.** 1982. Cutaneous basophil-associated resistance to ectoparasites (ticks). I. Transfer with immune serum or immune cells. Immunology **45:**501–511.
2. **Belperron, A. A., and L. K. Bockenstedt.** 2001. Natural antibody affects survival of the spirochete *Borrelia burgdorferi* within feeding ticks. Infect. Immun. **69:**6456–6462.
3. **Benyo, Z., A. Gille, C. L. Bennett, B. E. Clausen, and S. Offermanns.** 2006. Nicotinic acid-induced flushing is mediated by activation of epidermal langerhans cells. Mol. Pharmacol. **70:**1844–1849.

3a. **Bratton, R. L., J. W. Whiteside, M. J. Hovan, R. L. Engle, and F. D. Edwards.** 2008. Diagnosis and treatment of Lyme disease. Mayo Clin. Proc. **83:**566–571.

4. **Brown, S. J., and F. W. Knapp.** 1981. Response of hypersensitized guinea pigs to the feeding of Amblyomma americanum ticks. Parasitology **83:**213–223.
5. **Bursch, L. S., L. Wang, B. Igyarto, A. Kissenpfennig, B. Malissen, D. H. Kaplan, and K. A. Hogquist.** 2007. Identification of a novel population of Langerin+ dendritic cells. J. Exp. Med. **204:**3147–3156.
6. **Cupp, E. W.** 1991. Biology of ticks. Vet. Clin. N. Am. Small Anim. Pract. **21:**1–26.
7. **Douillard, P., P. Stoitzner, C. H. Tripp, V. Clair-Moninot, S. Ait-Yahia, A. D. McLellan, A. Eggert, N. Romani, and S. Saeland.** 2005. Mouse lymphoid tissue contains distinct subsets of langerin/CD207 dendritic cells, only one of which represents epidermal-derived Langerhans cells. J. Investig. Dermatol. **125:**983–994.
8. **Ferreira, B. R., and J. S. Silva.** 1998. Saliva of Rhipicephalus sanguineus tick impairs T cell proliferation and IFN-gamma-induced macrophage microbicidal activity. Vet. Immunol. Immunopathol. **64:**279–293.
9. **Francischetti, I. M., T. N. Mather, and J. M. Ribeiro.** 2005. Tick saliva is a potent inhibitor of endothelial cell proliferation and angiogenesis. Thromb. Haemost. **94:**167–174.
10. **Francischetti, I. M., V. My Pham, B. J. Mans, J. F. Andersen, T. N. Mather, R. S. Lane, and J. M. Ribeiro.** 2005. The transcriptome of the salivary glands of the female western black-legged tick Ixodes pacificus (Acari: Ixodidae). Insect Biochem. Mol. Biol. **35:**1142–1161.

Downloaded from http://iai.asm.org/ on January 21, 2021 by guest

11. **Garg, R., I. J. Juncadella, N. Ramamoorthi, Ashish, S. K. Ananthanarayanan, V. Thomas, M. Rincon, J. K. Krueger, E. Fikrig, C. M. Yengo, and J. Anguita.** 2006. Cutting edge: CD4 is the receptor for the tick saliva immunosuppressor, salp15. J. Immunol. **177:**6579–6583.
12. **Jiang, A., O. Bloom, S. Ono, W. Cui, J. Unternaehrer, S. Jiang, J. A. Whitney, J. Connolly, J. Banchereau, and I. Mellman.** 2007. Disruption of E-cadherin-mediated adhesion induces a functionally distinct pathway of dendritic cell maturation. Immunity **27:**610–624.
13. **Kabashima, K., D. Sakata, M. Nagamachi, Y. Miyachi, K. Inaba, and S. Narumiya.** 2003. Prostaglandin E2-EP4 signaling initiates skin immune responses by promoting migration and maturation of Langerhans cells. Nat. Med. **9:**744–749.
14. **Kaplan, D. H., M. C. Jenison, S. Saeland, W. D. Shlomchik, and M. J. Shlomchik.** 2005. Epidermal Langerhans cell-deficient mice develop enhanced contact hypersensitivity. Immunity **23:**611–620.
15. **Kaplan, D. H., A. Kissenpfennig, and B. E. Clausen.** 2008. Insights into Langerhans cell function from Langerhans cell ablation models. Eur. J. Immunol. **38:**2369–2376.
16. **Kissenpfennig, A., S. Henri, B. Dubois, C. Laplace-Builhe, P. Perrin, N. Romani, C. H. Tripp, P. Douillard, L. Leserman, D. Kaiserlian, S. Saeland, J. Davoust, and B. Malissen.** 2005. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity **22:**643–654.
17. **Kissenpfennig, A., and B. Malissen.** 2006. Langerhans cells—revisiting the paradigm using genetically engineered mice. Trends Immunol. **27:**132–139.
18. **Kopecky, J., and M. Kuthejlova.** 1998. Suppressive effect of Ixodes ricinus salivary gland extract on mechanisms of natural immunity in vitro. Parasite Immunol. **20:**169–174.
19. **Levin, M. L., and D. Fish.** 1998. Density-dependent factors regulating feeding success of Ixodes scapularis larvae (Acari: Ixodidae). J. Parasitol. **84:**36–43.
20. **Loser, K., and S. Beissert.** 2007. Dendritic cells and T cells in the regulation of cutaneous immunity. Adv. Dermatol. **23:**307–333.
21. **Mejri, N., and M. Brossard.** 2007. Splenic dendritic cells pulsed with Ixodes ricinus tick saliva prime naive CD4+T to induce Th2 cell differentiation in vitro and in vivo. Int. Immunol. **19:**535–543.
22. **Menten-Dedoyart, C., B. Couvreur, O. Thellin, P. V. Drion, M. Herry, O. Jolois, and E. Heinen.** 2008. Influence of the Ixodes ricinus tick blood-feeding on the antigen-specific antibody response in vivo. Vaccine **26:**6956–6964.
23. **Obhrai, J. S., M. Oberbarnscheidt, N. Zhang, D. L. Mueller, W. D. Shlomchik, F. G. Lakkis, M. J. Shlomchik, and D. H. Kaplan.** 2008. Langerhans cells are not required for efficient skin graft rejection. J. Investig. Dermatol. **128:**1950–1955.
24. **Ramamoorthi, N., S. Narasimhan, U. Pal, F. Bao, X. F. Yang, D. Fish, J. Anguita, M. V. Norgard, F. S. Kantor, J. F. Anderson, R. A. Koski, and E. Fikrig.** 2005. The Lyme disease agent exploits a tick protein to infect the mammalian host. Nature **436:**573–577.
25. **Ribeiro, J. M.** 1995. How ticks make a living. Parasitol. Today **11:**91–93.
26. **Ribeiro, J. M., F. Alarcon-Chaidez, I. M. Francischetti, B. J. Mans, T. N. Mather, J. G. Valenzuela, and S. K. Wikel.** 2006. An annotated catalog of salivary gland transcripts from Ixodes scapularis ticks. Insect Biochem. Mol. Biol. **36:**111–129.
27. **Ribeiro, J. M., and I. M. Francischetti.** 2003. Role of arthropod saliva in blood feeding: sialome and post-sialome perspectives. Annu. Rev. Entomol. **48:**73–88.
28. **Romani, N., S. Ebner, C. H. Tripp, V. Flacher, F. Koch, and P. Stoitzner.** 2006. Epidermal Langerhans cells—changing views on their function in vivo. Immunol. Lett. **106:**119–125.
29. **Sa-Nunes, A., A. Bafica, D. A. Lucas, T. P. Conrads, T. D. Veenstra, J. F. Andersen, T. N. Mather, J. M. Ribeiro, and I. M. Francischetti.** 2007. Prostaglandin E2 is a major inhibitor of dendritic cell maturation and function in Ixodes scapularis saliva. J. Immunol. **179:**1497–1505.
30. **Schoeler, G. B., S. A. Manweiler, and S. K. Wikel.** 2000. Cytokine responses of C3H/HeN mice infested with Ixodes scapularis or Ixodes pacificus nymphs. Parasite Immunol. **22:**31–40.
31. **Schoeler, G. B., S. A. Manweiler, and S. K. Wikel.** 1999. Ixodes scapularis: effects of repeated infestations with pathogen-free nymphs on macrophage and T lymphocyte cytokine responses of BALB/c and C3H/HeN mice. Exp. Parasitol. **92:**239–248.
32. **Schoeler, G. B., and S. K. Wikel.** 2001. Modulation of host immunity by haematophagous arthropods. Ann. Trop. Med. Parasitol. **95:**755–771.
33. **Shih, C. M., R. J. Pollack, S. R. Telford III, and A. Spielman.** 1992. Delayed dissemination of Lyme disease spirochetes from the site of deposition in the skin of mice. J. Infect. Dis. **166:**827–831.
34. **Steen, N. A., S. C. Barker, and P. F. Alewood.** 2006. Proteins in the saliva of the Ixodida (ticks): pharmacological features and biological significance. Toxicon **47:**1–20.
35. **Steere, A. C.** 2001. Lyme disease. N. Engl. J. Med. **345:**115–125.
36. **Steere, A. C., J. Coburn, and L. Glickstein.** 2004. The emergence of Lyme disease. J. Clin. Investig. **113:**1093–1101.
37. **Stoitzner, P., C. H. Tripp, A. Eberhart, K. M. Price, J. Y. Jung, L. Bursch, F. Ronchese, and N. Romani.** 2006. Langerhans cells cross-present antigen derived from skin. Proc. Natl. Acad. Sci. USA **103:**7783–7788.
38. **Titus, R. G., J. V. Bishop, and J. S. Mejia.** 2006. The immunomodulatory factors of arthropod saliva and the potential for these factors to serve as vaccine targets to prevent pathogen transmission. Parasite Immunol. **28:**131–141.
39. **Wikel, S. K.** 1999. Tick modulation of host immunity: an important factor in pathogen transmission. Int. J. Parasitol. **29:**851–859.
40. **Wikel, S. K., and F. J. Alarcon-Chaidez.** 2001. Progress toward molecular characterization of ectoparasite modulation of host immunity. Vet. Parasitol. **101:**275–287.
41. **Wikel, S. K., R. N. Ramachandra, D. K. Bergman, T. R. Burkot, and J. Piesman.** 1997. Infestation with pathogen-free nymphs of the tick *Ixodes scapularis* induces host resistance to transmission of *Borrelia burgdorferi* by ticks. Infect. Immun. **65:**335–338.
42. **Zeidner, N., M. L. Mbow, M. Dolan, R. Massung, E. Baca, and J. Piesman.** 1997. Effects of *Ixodes scapularis* and *Borrelia burgdorferi* on modulation of the host immune response: induction of a Th2 cytokine response in Lyme disease-susceptible (C3H/HeJ) mice but not in disease-resistant (BALB/c) mice. Infect. Immun. **65:**3100–3106.
43. **Zenovich, A. G., A. Panoskaltsis-Mortari, G. J. Caron, A. G. Kolb, R. Fremming, W. D. Nelson, and D. A. Taylor.** 2008. Sex-based differences in vascular repair with bone marrow cell therapy: relevance of regulatory and Th2-type cytokines. Transplant Proc. **40:**641–643.

Editor: S. R. Blanke

Downloaded from http://iai.asm.org/ on January 21, 2021 by guest