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Mast Cell-Dependent Down-Regulation of Antigen-Specific Immune Responses by Mosquito Bites¹

Nadya Depinay,² Fériel Hacini,² Walid Beghdadi, Roger Peronet, and Salaheddine Mécheri³

While probing host skin to search for blood vessels, the female *Anopheles* mosquito delivers *Plasmodium* parasites in the presence of saliva. Saliva from various blood-feeding vectors which contains several pharmacologically active components is believed to facilitate blood feeding as well as parasite transmission to the host. Recently, we found that mosquito saliva has the capacity to activate dermal mast cells and to induce local inflammatory cell influx. Our main objective in the present work is to investigate whether saliva, through mosquito bites, controls the magnitude of Ag-specific immune responses and whether this control is dependent on the mast cell-mediated inflammatory response. Using a mast cell knockin mouse model, we found that mosquito bites consistently induced MIP-2 in the skin and IL-10 in draining lymph nodes, and down-regulate Ag-specific T cell responses by a mechanism dependent on mast cells and mediated by IL-10. Our results provide evidence for new mechanisms which may operate during *Plasmodium* parasite transmission by mosquito bites. *The Journal of Immunology*, 2006, 176: 4141–4146.

To set-up animal models for *Plasmodium* infection, a majority of investigators use i.v. or intradermal routes for sporozoite delivery. To study the immunological mechanisms that take place at the onset of the infection which occur in the skin, we believe that these routes of transmission are inadequate. Natural disease transmission requires infectious mosquito bites through which sporozoites are delivered into the skin in the presence of *Anopheles* mosquito saliva. It has been suggested that saliva may play a crucial role as a factor associated with enhancement of sporozoite infectivity (1). There is now compelling evidence that saliva has a profound effect on pathogen transmission. Indeed, *Plasmodium berghei* sporozoites delivered into mice through mosquito bites were more infectious than when sporozoites were injected i.v., suggesting a facilitating role of saliva for parasite transmission. A better knowledge of the immunomodulatory properties of saliva may certainly contribute to a better understanding of the mechanisms of *Plasmodium* transmission. In the skin, mosquito saliva molecules enter in close contact with mast cells, considered now as potent sentinels of the innate immune system often found intimately connected to dendritic cells in the dermis (2). We feel that these early cellular events initiated by mosquito bites are central in shaping the host immune response against the *Plasmodium* parasite.

Reactions to mosquito bites result, depending on mosquito species, in cutaneous reactions varying from small papules to large pruritic swellings. But most of the pharmacological and immunomodulatory properties of mosquito saliva remain elusive. Several studies have focused on the immunogenic and allergenic (3) or

antihemostatic (4) activities of mosquito salivary components. Recently, saliva from *Anopheles stephensi* has been shown to contain a high m.w. glycoprotein endowed with an intense neutrophil chemotactic activity which contributes to the inflammatory reaction through the accumulation of neutrophils at the site of the mosquito bite (5). Mosquito bites can elicit both immediate (6) as well as delayed hypersensitivity reactions (7). Because these reactions are highly dependent on mast cells, mosquito saliva very likely contain components with immunomodulatory potential which act through mast cell activation. As mast cells are major producers of TNF- α and MIP-2/IL-8 (8, 9), these cells have been shown to be providers of central mediators recruiting polymorphonuclear (PMN)⁴ cells during delayed hypersensitivity reactions (10). Accordingly, T cell-dependent PMN cell recruitment was dramatically reduced in mast cell-deficient *Kit W/Kit W^{-v}* mice (11). Currently, the role of mast cells in mosquito saliva-induced inflammatory response is not fully understood and remains to be elucidated. Recently, we demonstrated that mosquito bites induce dermal mast cell degranulation leading to fluid extravasation and PMN cell influx (12). This mast cell activation was not mediated by IgE because it occurs in naive mice. This inflammatory response did not occur in mast cell-deficient *W/W^v* mice, unless these were reconstituted specifically with mast cells. Furthermore, mosquito bites cause a substantial increase of TNF- α concentrations within draining lymph nodes (LN) (12). The aim of the present study was to investigate the cytokine changes occurring at skin sites that have received mosquito bites, and in the draining LNs, and the relevance of mast cells in these processes. We also examined to what extent saliva-induced mast cell activation alters a T cell-mediated immune response in a model of delayed hypersensitivity reaction. Our results suggest that mosquito saliva components down-regulate Ag-specific immune responses by a mechanism that is mast cell dependent. These findings identify new mechanisms that may operate during *Plasmodium* parasite transmission and may provide new opportunities to control malaria disease.

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⁴ Abbreviations used in this paper: PMN, polymorphonuclear; BMDC, bone marrow-derived mast cell; DTH, delayed-type hypersensitivity; LN, lymph node.

Materials and Methods

Animals

Female C57BL/6, 6–8 wk old, were purchased from Roger Janvier. Mast cell-sufficient (WBB6F1-+/+) (+/+) and control congenic mast cell-deficient (WBB6F1-W/W^v) (W/W^v) mice were purchased from The Jackson Laboratory and were raised in our animal facility. All animal care and experimentation were conducted in accord with the Pasteur Institute animal care and use committee guidelines.

A. stephensi (sda 500 strain) was maintained at 26°C with 75% relative humidity under a 12-h photoperiod. Adult mosquitoes were provided a 10% sugar solution, and females were blood-fed on anesthetized rabbits biweekly. Larvae were fed on Friskies Cat Chow (Purina).

Exposure of mice to mosquito bites

Mice were anesthetized by i.p. injection of ketamine (600 mg/kg) and xylazine (20 mg/kg) and were placed on top of mosquito cages to allow for biting through the mesh. Mosquito bites were focused on footpads. To allow exposure to saliva with minimal bleeding of animals (maximum 20 biting mosquitoes/mouse), mosquito feeding was disrupted every 2–3 min, and *Anopheles* that had taken blood meal were systematically counted. Based on the number of mosquitoes that have actually taken their blood meal, the mean of biting mosquitoes was determined as 17 ± 2 /mouse. For the sake of homogeneity from one experiment to another, the overall period of exposure to mosquito bites was 20 min. All mice were naive before exposure to mosquito bites.

Preparation of bone marrow-derived mast cells (BMMC) and mast cell reconstitution

BMMC from C57BL/6 mice were prepared as described by Razin et al. (13) and modified by us. After 3 wk of culture using RPMI 1640 supplemented with 10% FCS (American Type Culture Collection) and in the presence of 3 U/ml rIL-3 (AbCys), the cells were harvested after 21 days of culture and consisted of 98% pure mast cells as assessed by toluidine blue staining. Consistent with our previous reports, nonspecific esterase staining, immunofluorescence staining for Mac-1, NLDC-145, and B220 cell surface Ag indicated that mast cell preparations were not contaminated with macrophages, dendritic cells, or B cells, respectively.

W/W^v mice were reconstituted locally with mast cells by injecting intradermally in the footpad skin 2×10^6 cultured mast cells 1 wk before exposure to mosquito bites. In some experiments, W/W^v mice were systemically reconstituted with mast cells by i.v. injection of 2×10^6 cultured BMMC 6 wk before exposure to *A. stephensi* mosquito bites.

Delayed-type hypersensitivity (DTH) reaction

Immediately after exposure or not of mice to mosquito bites, OVA (50 µg) comprised in 25 µl of saline was emulsified with an equal volume of CFA and injected s.c. in both sides of the base of the tail. DTH reactions were elicited in groups of six mice 7 days after the immunization by challenging mice with 30 µl of 20 mg/ml aggregated OVA injected s.c. in the left hind footpad while the right hind footpad was injected with the same volume of saline. Footpad thickness was measured at 24 and 48 h after challenge using a skin thickness gauge. The extent of swelling was measured by subtracting values given by saline-injected footpads from those of Ag-injected footpads. Aggregated OVA was prepared by heating a 2% solution of OVA at 70°C for 1 h. After cooling, the precipitate was washed and resuspended in the original volume of saline.

To assess the role of MIP-2 and IL-10 in the modulation of DTH response by mosquito bites, 250 µg of rabbit anti-mouse MIP-2, anti-mouse IL-10, or control rabbit IgG (provided by S. L. Kunkel, Department of Pathology, University of Michigan Medical School, Ann Arbor, MI) was injected twice into the peritoneal cavity of naive mice 24 and 3 h before sensitization with OVA and exposure of mice to mosquito bites.

Ag-specific T cell responses and cytokine analysis

Popliteal LN cells were harvested and resuspended in RPMI 1640 medium supplemented with 10% FCS. The T cell stimulation assay was conducted in flat-bottom 96-well plates using RPMI 1640 supplemented with 5% (v/v) FCS (ATGC), 5×10^{-5} M 2-ME, 2 mM L-glutamine, 0.1 mM non-essential amino acids (Flow Laboratories), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Eurobio). Cells were exposed to 300 µg/ml OVA in a final volume of 1 ml/well (3×10^6 leukocytes), a concentration found to be optimal in preliminary assays. After a 48-h incubation at 37°C, supernatants were removed, frozen, and later assayed for cytokine determination. Supernatants were analyzed for their IFN-γ and IL-10 contents by ELISA according to the manufacturer's instructions (R&D Systems).

Detection of cytokine mRNA by RT-PCR

Samples from skin were kept frozen until mRNA extraction. Tissues were disrupted using a Polytron (Brinkmann Instruments) and homogenized in 350 µl of RLT buffer (Qiagen). RNA was extracted by a Qiagen kit and cDNA preparations were conducted following standard procedures using oligo dT and 10 U of superscript enzyme (Invitrogen Life Technologies). Quantitative PCR was performed with the GeneAmp 7000 (Applied Biosystems) as indicated by the manufacturer. Primers and probes for the quantitative PCR assay of cytokines used are listed below: IL-12, sense, 5'-AACTTGAGGGAGAAGTAGGAATGG-3', and antisense, 5'-GGAAGCACGGCAGCAGAA-3'; IFN-γ, sense, 5'-TGGCTCTGCAGGATTTTCATG-3', and antisense, 5'-TCAAGTGGCATAGATGTGGAAGAA; TNF-α, sense, 5'-TGG GAG TAG ACA AGG TAC AAC CC-3', and antisense, 5'-CATCTTCTCAAAATTCGAGTGACAA-3'; MIP-2, sense, 5'-CTTGAGAGTGGCTATGACTTCTGTCT-3', and antisense, 5'-TGACTTCAAGAACATCCAGATCTT-3'; IL-4, sense, 5'-ACAGGAGAAGG GACGCCAT-3', and antisense, 5'-GAAGCCCTACAGACGAGCTCA-3'; IL-10, sense, 5'-GGTTGCCAAGCCTATCGGA-3', and antisense, 5'-ACCTGCTCCACTGCCTTGCT-3'; IL-13, sense, 5'-AGACCAGACTCCCTGTGCA-3', and antisense, 5'-TGGGTCTGTAGATGGCATTG-3'; and β-actin, sense, 5'-AGAGGGAAATGGTGCCTGAC-3', and antisense, 5'-CAATAGTGACCTGGCCGT-3'.

Statistical analysis

The paired two-tailed Student *t* test was used with *p* = 0.05 taken as the level of significance.

Results

Selective induction of MIP-2 in skin and IL-10 in LN following mosquito bites

In a previous study, we demonstrated that noninfectious mosquito bites induce a local inflammatory response characterized by mast cell degranulation and rapid leukocyte infiltration followed by leukocyte sequestration in draining LNs (12). In the present work, we analyzed the pattern of cytokines and chemokines that are induced in skin and in lymph nodes following mosquito bites. As shown in Fig. 1, the CXC chemokine MIP-2 and the cytokine IL-10 measured by RT-PCR were selectively increased in the skin and in the LNs, respectively. In the skin, TNF-α does not seem to be strongly induced indicating that intracellular stores of TNF-α in mast cells are predominantly released by mosquito saliva as demonstrated previously (12). Only a minor increase of IL-13 and IL-4 mRNA were observed while no IFN-γ nor IL-12 could be detected (data not shown). In a previous report, MIP-2 production was found to be exclusively produced in ear tissues undergoing hapten-specific DTH response and that MIP-2 required for PMN recruitment during DTH response was dependent on the presence of mast cells (10). Furthermore, the T cell-dependent MIP-2 mRNA expression in ear tissue of *KitW/KitW^v* mice was of minor relevance for PMN recruitment (10). We addressed the same question with regard to the mast cell-associated MIP-2 induction in the model of mosquito bites. To address this issue, we reconstituted *KitW/KitW^v* mice with in vitro-cultured BMMCs exclusively at the skin site selected for mosquito bites (8, 14). After local reconstitution with BMMCs, the skin tissues contained important amounts of MIP-2 mRNA (Fig. 1) while no detectable MIP-2 could be detected in the skin of *KitW/KitW^v* mice exposed to mosquito bites. In vivo induction of MIP-2 during mosquito bites was strictly dependent on the presence of mast cells. In LNs, among various cytokines tested, IL-10 mRNA was predominantly induced (Fig. 1). IL-10 mRNA was detected at 8 h after mosquito bites and the amount of mRNA reached the maximum level at 24 h, and fell back to normal by 48 h (data not shown). Other cytokines including IL-4, IFN-γ, IL-12, and TNF-α were only marginally induced. TNF-α was represented mainly as a protein as a result of mosquito bite-induced translocation of mast cell-derived TNF-α from the skin to LNs (data not shown and Ref. 12).

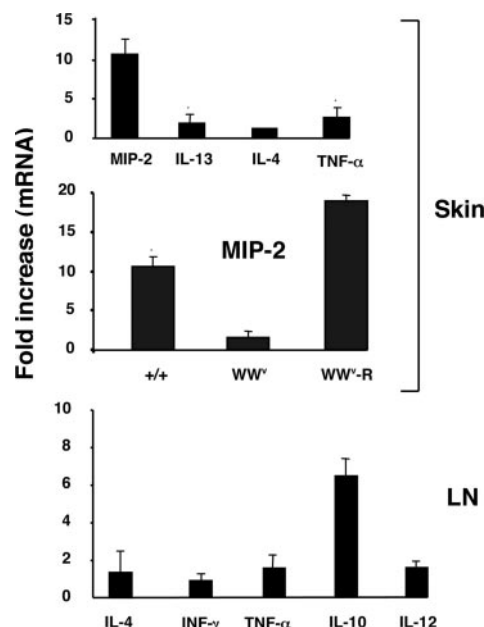


FIGURE 1. Mosquito bites selectively induce MIP-2 in skin and IL-10 in LNs. Real-time RT-PCR was used to measure the changes in mRNA levels of indicated cytokines and chemokines in the skin of C57BL/6 mice, and MIP-2 in the skin of +/+, WW^v, and WW^v-R mice collected 3 h after exposure to mosquito bites. The relative mRNA levels of cytokines genes were measured using real-time RT-PCR in LNs of C57BL/6 mice harvested 24 h after mosquito bites. The expression levels were normalized to the endogenous control gene β -actin, and the relative expression levels were calculated using the uninfected animal as a calibrator. Three independent experiments were performed and the means $\pm$ SD are shown.

Down-regulation of the DTH response by mosquito bites

Naive mice were exposed to mosquito bites during the sensitization phase with OVA. One footpad was challenged 7 days later with OVA and the contralateral footpad with PBS and responses were measured on 2 consecutive days. Results in Fig. 2A show that the normal response as assessed by footpad swelling was measurable at 24 h and became maximal at 48 h in control mice. When mosquito bites were given, however, the DTH response was reduced by $\sim$ 75%. To determine whether footpad swelling reflects the number of leukocytes present within LN, cell counts were measured in individual LN corresponding to OVA- and PBS-challenged footpads 48 h after the elicitation of DTH response. As shown in Fig. 2B, although an increase in the total number of leukocytes was observed in LN from every mouse that had been challenged with OVA, the number of leukocytes was reduced by $\sim$ 50% in LN from mice which received mosquito bites as compared with control mice which were given OVA alone.

Effect of mosquito bites on the cytokine production pattern of Ag-specific LN T cells

In an attempt to determine whether OVA-specific T cell responses reflect DTH reactions, cell suspensions were prepared from individual LN from different mice and incubated or not in the presence of various OVA concentrations. As compared with unbitten mice, IFN- γ , a major Th1 cytokine, was substantially reduced after exposure to mosquito bites (Fig. 3A). In contrast, IL-10, an immunosuppressive cytokine, was produced at a higher level in mice exposed to mosquito bites (Fig. 3B). Depending on the concentration of OVA used for in vitro cell stimulation, a clear tendency for a higher ratio of IL-10 vs IFN- γ was observed in mice treated with mosquito bites (3.6 and 16 for 300 and 100 μ g/ml OVA concen-

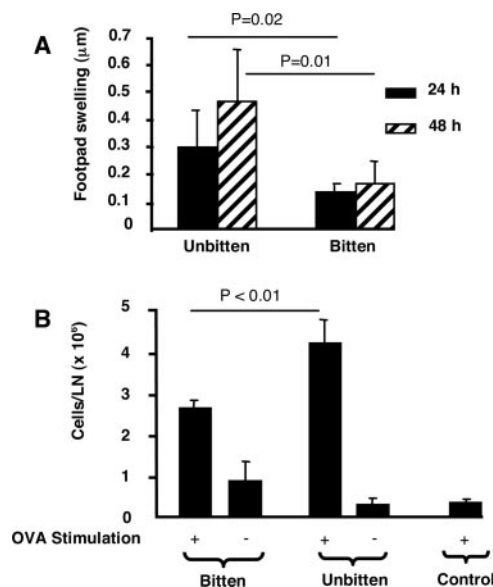


FIGURE 2. Down-regulation of the DTH response by mosquito bites. **A**, Naive C57BL/6 mice were subjected or not to mosquito bites and immediately after were given s.c. injections of 50 μ g of OVA emulsified in CFA in both sides of the base of the tail. DTH reactions were induced 7 days later by s.c. injection of aggregated OVA in the left hind footpad. The right one was injected with saline. Footpad thickness was assessed 24 and 48 h later by subtracting values given by saline-injected footpads. Values represent the mean $\pm$ SD of the swelling response seen in groups of mice five to seven in number. **B**, LNs from the same mice were harvested 48 h after the elicitation of the DTH response and cells were counted. Represented are the numbers of cells in LNs corresponding to the OVA-challenged (+) and PBS-challenged (–) footpads. Values represent the mean $\pm$ SD of lymph node cells seen in various groups of mice (five to seven mice per group).

trations, respectively) as compared with untreated mice (1 and 0.1 for 300 and 100 μ g/ml OVA concentrations, respectively). These data are consistent with footpad swelling and LN cellularity and support the hypothesis that mosquito bites are associated with skewed IL-10 response and down-regulation of the DTH response.

Enhancement of DTH response by abrogating IL-10 is restrained by mosquito bites

To evaluate the role of mosquito bite-induced IL-10 in regulating DTH response, experiments were designed to determine the effects of the abrogation of IL-10 on the DTH responses developed by mice exposed or not to mosquito bites. Previous studies showed that IFN- γ is predominantly associated with the DTH response and that IL-10 is an important regulator of this DTH (15). Because mosquito bites down-regulate the DTH response by inducing IL-10, abrogation of IL-10 may represent an approach to test whether the DTH response in bitten mice may be less affected or less sensitive to anti-IL-10 treatment as compared with that developed by unbitten mice. To test this hypothesis, experiments were initiated to determine the effects of IL-10 neutralization on the mosquito bite-induced DTH response as determined by the measurement of IFN- γ mRNA in footpad skin 48 h after OVA challenge. As shown in Fig. 4, a reduced amount of IFN- γ was observed in the skin of mice exposed to mosquito bites. When neutralizing anti-IL-10 Ab was administered before sensitization with OVA, a 20-fold increase of IFN- γ was obtained. This anti-IL-10-induced increase of IFN- γ expression was much less pronounced in mice exposed to mosquito bites (4-fold increase). Although occurring with a lower magnitude, these variations were also observed in control footpads

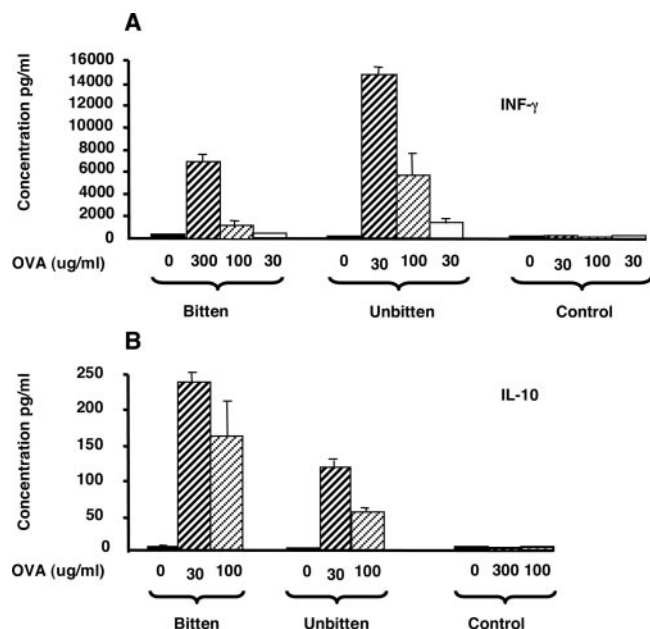


FIGURE 3. Regulation by mosquito bites of cytokine responses during the DTH reaction. LN cells from mice treated according to the same protocol as in Fig. 2, were incubated in triplicate in the presence of various concentrations of OVA and the supernatants were harvested 48 h later and tested for their content in IL-10 (B) and INF- γ (A) measured by ELISA. Differences between values given by different groups of mice corresponding to the same OVA concentrations were all significant with $0.01 < p < 0.005$.

which received PBS (Fig. 4). These data indicate that a stronger control was exerted by mosquito bites on IFN- γ expression very likely because more IL-10 was produced in this condition. Similar experiments have addressed the role of MIP-2 in the regulation of IFN- γ response. Pretreatment of mice with anti-MIP-2-neutralizing Ab had a minimal enhancing effect on IFN- γ expression. The level of IFN- γ expression was strongly reduced in mice treated with anti-MIP-2 Ab and which received mosquito bites. This is probably due to excess amounts of MIP-2 induced by mosquito bites which could not be completely neutralized by administered anti-MIP-2.

Role of mast cells in the modulation by mosquito saliva of the Ag-specific immune response

To examine whether the modulation of the DTH response by mosquito bites is dependent on mast cells, OVA-induced DTH reactions were analyzed in $+/+$, W/W^v , and mast cell-reconstituted W/W^v (W/W^v -R) mice that have been exposed or not to mosquito bites. As shown in Fig. 5, mosquito bites markedly reduced the DTH response of $+/+$ mice ($p = 0.01$). In contrast, DTH responses developed by nonreconstituted mast cell-deficient W/W^v mice were not influenced by mosquito bites ($p = 0.3$). To investigate whether the reduced DTH response induced by mosquito bites in $+/+$ mice was under the control of mast cells, footpad swellings were measured in mast cell-reconstituted W/W^v -R mice exposed or not to mosquito bites. DTH responses were significantly reduced in W/W^v -R mice exposed to mosquito bites as compared with control mice ($p = 0.01$). It must be pointed out that in the absence of a mosquito bite, the DTH responses developed by W/W^v -R mice were consistently weaker than in $+/+$ mice, probably because of the incomplete reconstitution of W/W^v mice by mast cells ($\sim 40\%$). Collectively, these data indicate that DTH responses are regulated by mast cells and, most interestingly, that

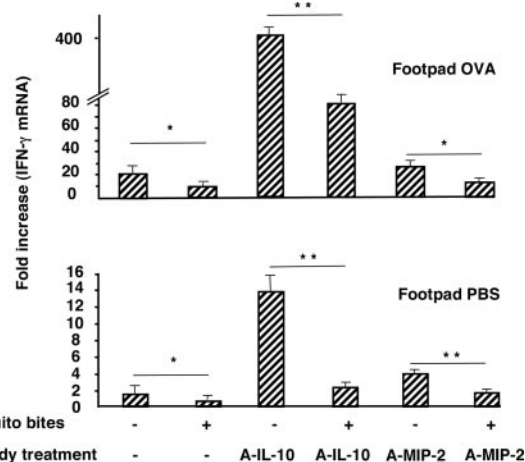


FIGURE 4. Enhancement of DTH response by anti-IL-10 treatment is less pronounced when mice were exposed to mosquito bites. Anti-IL-10 and anti-MIP-2 Abs were injected twice into the peritoneal cavity of naive C57BL/6 mice 24 h and 3 h before sensitization with OVA and exposure of mice to mosquito bites. Skin samples from OVA-, or PBS-challenged footpads were excised 48 h after the DTH response and real-time RT-PCR was used to measure the changes in mRNA levels of IFN- γ . The expression levels were normalized to the endogenous control β -actin gene, and the relative expression levels were calculated using uninfected mice as calibrators. One representative experiment among three is displayed and the means $\pm$ SD are shown. *, $p < 0.01$; **, $p < 0.001$.

mosquito saliva down-regulates T cell-mediated hypersensitivity reactions in a mast cell-dependent manner.

Mast cell-mediated mosquito bite-dependent cytokine regulation during DTH response

To investigate whether the modulation by mosquito bites of cytokine response of OVA-challenged LN cells during DTH response was under the control of mast cells, $+/+$, W/W^v , and mast cell-reconstituted W/W^v (W/W^v -R) mice were exposed or not to mosquito bites at the sensitization phase with OVA. LN cells harvested

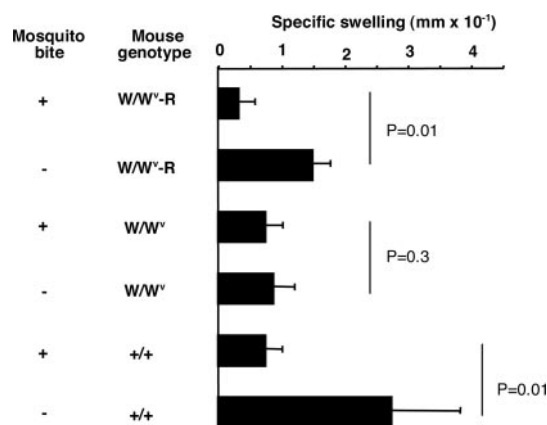


FIGURE 5. Modulation by mosquito bites of DTH reactions in W/W^v and normal mice. Wild-type ($+/+$), mast cell-deficient (W/W^v), and mast cell-reconstituted W/W^v (W/W^v -R) mice were subjected or not to mosquito bites and immediately after were given s.c. injections of $50 \mu\text{g}$ of OVA emulsified in CFA in both sides of the base of the tail. DTH reactions were induced 7 days later by s.c. injection of aggregated OVA in the left hind footpad. The right one was injected with saline. Footpad thickness was assessed by subtracting values given by saline-injected footpads. Values represent the mean $\pm$ SD of the swelling response seen in groups of mice 8–12 in number.

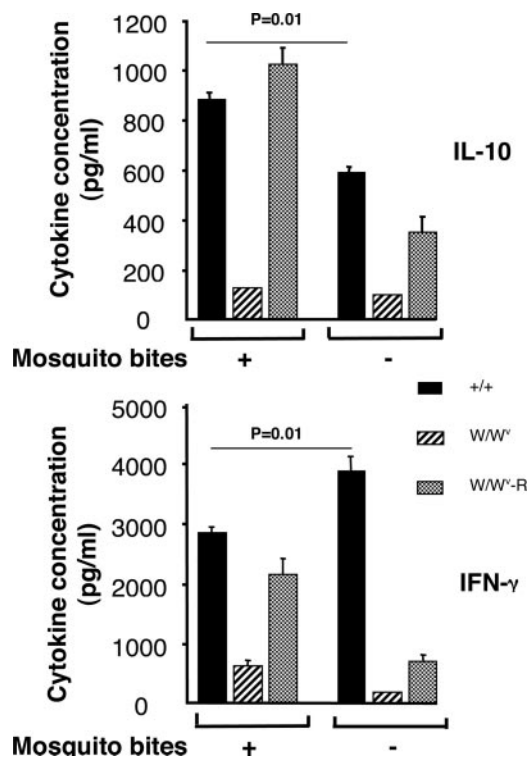


FIGURE 6. Regulation of cytokine response by mosquito bites is dependent on mast cells. Wild-type (+/+), W/W^v, and mast cell-reconstituted W/W^v mice were exposed or not to mosquito bites at the sensitization phase with OVA. LN cells harvested 48 h after challenge with OVA were incubated with OVA and after 48 h of culture, IL-10 and IFN-γ were measured in the culture supernatants by ELISA. Data are representative of two independent experiments and the values represent the mean ± SD from five mice per group.

48 h after challenge with OVA were incubated with OVA for 48 h after which IL-10 and IFN-γ were measured in the culture supernatants. As shown in Fig. 6, LN cells from bitten +/+ mice produced less IFN-γ and more IL-10 than mice not exposed to mosquito bites. This was consistent with the lower DTH response developed by bitten mice as measured by footpad swelling. The role of mast cells in DTH response has been reported earlier (10, 16). In this study, we investigated whether the modulation of cytokine response by mosquito bites was dependent on mast cells. Results shown in Fig. 6 demonstrate a strongly reduced production of both IL-10 and IFN-γ by LN cells from W/W^v mice and reconstitution by mast cells completely or partly restored IL-10 and IFN-γ production, respectively. The regulation of IL-10 production by mosquito bites was found to be tightly controlled by mast cells because increased levels were found in +/+ as well as in mast cell-reconstituted W/W^v mice. This was not the case of IFN-γ production because decreased production by mosquito bites observed in +/+ mice could not be reproduced in mast cell-reconstituted W/W^v mice. These data suggest that mosquito bites down-modulate the DTH response by a mechanism that is dependent on mast cells and is mediated by IL-10.

Discussion

We recently reported that mosquito bites induce dermal mast cell degranulation leading to fluid extravasation and neutrophil influx. This inflammatory response did not occur in mast cell-deficient W/W^v mice, unless these were reconstituted specifically with mast cells (12). In the present study, we addressed the question of whether mosquito bites were able to modulate Ag-specific immune

responses and whether alterations of this immune response were under the control of mast cells. The major findings were that 1) mosquito bites consistently induce MIP-2 in the skin and IL-10 in the draining LNs; 2) Ag-specific T cell responses were reduced in vitro and in vivo by mosquito bites as demonstrated by a DTH response model; and 3) mosquito bites down-regulate the Ag-specific DTH response by a mechanism dependent on mast cells and mediated by IL-10.

In separate and unrelated reports, components of *Anopheles saliv* (17) and mast cell products (18, 19) have been reported to exert immunosuppressive activities. We made the hypothesis that mosquito bites may exert their immunosuppressive activity through mast cell degranulation elicited by salivary components. We reported earlier that mosquito bites induce local mast cell degranulation in the skin followed by a rapid inflammatory cell infiltrate (12). TNF-α and MIP-2 were shown to be key inflammatory cytokines primarily produced by activated mast cells (8, 9). Indeed, MIP-2 production by mast cells was found to take place early during contact hypersensitivity reaction (10). Our results support these findings in that mosquito bites induce MIP-2 whose production was dependent on the presence of mast cells. The early induction of MIP-2 in the skin was followed by a unique production of IL-10 in the draining lymph nodes. The proinflammatory activity of MIP-2 on one hand and the immunosuppressive function of IL-10 on the other hand led us to examine the possibility that these cytokines may affect the final outcome of an Ag-specific immune response when this Ag was administered along with mosquito bites. If IL-10 induced by mosquito bites is involved in controlling the magnitude of the immune response, one should see an alteration of the response by mosquito bites. The OVA-specific DTH response, used as a prototype of the T cell-mediated immune response, was found to be drastically inhibited in mice exposed to mosquito bites. This decreased DTH response was reflected both by reduced footpad swelling as well as by diminished leukocyte recruitment in draining LNs. Further analysis of cytokine response of OVA-challenged LN cells in vitro demonstrate significantly reduced IFN-γ (a major cytokine associated with DTH) and elevated IL-10 productions by mosquito bites. These data are compatible with immunosuppressive activity of *Anopheles saliv* on the Ag-specific immune response. This was emphasized by reduced IFN-γ expression by Ag-specific T cells recruited to the skin site of OVA-challenged footpads of mice exposed to mosquito bites. IL-10 was reported to be an important regulator of DTH response in that treatment of sensitized mice with neutralizing anti-IL-10 Ab prolonged the duration of the hypersensitivity reaction beyond the natural course of the response (20). Consistent with these observations, our findings show that administration of anti-IL-10 dramatically enhanced IFN-γ expression (20-fold increase) during DTH response in mice not exposed to mosquito bites. Similar treatment of mice exposed to mosquito bites only increased the IFN-γ response by ~8-fold, suggesting that mosquito bites exert a stronger inhibitory control of DTH response by an IL-10-dependent mechanism. These data provide strong evidence linking IL-10-mediated down-regulation of DTH response to mosquito bites. However, the effector mechanisms remain to be elucidated. In contrast to IL-10, MIP-2 does not seem to exert any substantial effect on IFN-γ response because administration of anti-MIP-2 Ab did not significantly enhance or decrease IFN-γ expression whether mice were exposed to mosquito bites or not.

It is well established that mast cells contribute to the development of DTH reactions (10, 16). In the present work, however, we found that, in a mast cell-dependent manner, mosquito bites inhibit the induction of the DTH response to an immunogenic Ag injected immediately after and at the same site as the mosquito bite. A

similar immunosuppressive effect mediated by mast cells has been previously reported in a contact hypersensitivity model in which UVB light induced mast cell degranulation (21, 22). During the sensitization phase of DTH response, dendritic cells capture the Ag, migrate to draining LNs and undergo a maturation process required for the activation of naive T cells. We speculate that mosquito saliva induces the release of a particular set of inflammatory mediators by activated mast cells that may affect the maturation of adjacent dendritic cells which fail to ultimately elicit fully activated effector T cells. It is known that the ability of dendritic cells to direct the development of naive T cells into Th1, Th2, or regulatory T cells is largely dependent upon the signals that they receive in the peripheral tissues at the time of Ag capture. Histamine, whose mast cells are the major storage site, is an attractive candidate as a dendritic cell modulator especially during early phases of the immune response. Histamine was reported to have immunosuppressive effects, such as inhibition of polymorphonuclear chemotaxis (23) and monocyte IL-12 secretion (24), as well as induction of IL-10 production (25). Interestingly, analysis of cytokine production by cultured LN cells after completion of DTH response indicate that increased production of IL-10 by mosquito bites was correlated with the presence of mast cells. The question remains as to the elucidation of the actual effector event or events underlying the control of IL-10 by mast cells.

In conclusion, we show a direct link between mosquito bite-induced down-regulation of the DTH response and mast cell-dependent IL-10 production. The data further implicate mast cells as an important cell endowed with immunomodulatory properties and suggest that the mechanisms described herein may take place during *Plasmodium* parasite transmission and pathogenicity.

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Disclosures

The authors have no financial conflict of interest.

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