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

Nadya Depinay, Fériel Hacini, Walid Beghdadi, Roger Peronet and Salaheddine Mécheri

*J Immunol* 2006; 176:4141-4146; doi: 10.4049/jimmunol.176.7.4141
http://www.jimmunol.org/content/176/7/4141

**References** This article cites 25 articles, 12 of which you can access for free at:
http://www.jimmunol.org/content/176/7/4141.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
Mast Cell-Dependent Down-Regulation of Antigen-Specific Immune Responses by Mosquito Bites

Nadya Depinay, Fériel Hacini, Walid Beghdadi, Roger Peronet, and Salaheddine Mécheri

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.

The Journal of Immunology

Copyright © 2006 by The American Association of Immunologists, Inc.

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

1 This work was supported by the Institut Pasteur Strategic Horizontal Program on Anopheles.

2 N.D. and F.H. contributed equally to this work.

3 Address correspondence and reprint requests to Dr. Salaheddine Mécheri, Unité des Réponses Précoces aux Parasites et Immunopathologie, Institut Pasteur, 28 rue du Docteur Roux, Paris 75724 Cedex 15, France. E-mail address: smecheri@pasteur.fr

4 Abbreviations used in this paper: PMN, polymorphonuclear; BMMC, 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) (W/W) 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 ± 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 report, 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 mice were reconstituted locally with mast cells by injecting intradermally in the footpad skin 2 × 10⁶ cultured mast cells 1 wk before exposure to mosquito bites. In some experiments, W/W mice were systemically reconstituted with mast cells by i.v. injection of 2 × 10⁶ 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 at 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 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 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 injected footpads.

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’-AACCTGAGGGAAGTTGAAATGG-3’, and antisense, 5’-GGA AGACCGGCAAGAA-3’; IFN-γ, sense, 5’-TGAGCTGCAGGAT TTCTCAT-3’, and antisense, 5’-TCAAGTGCGCATGATGGAAGA AGA-3’, TNF-α, sense, 5’-TTGGGAGTAGAACTCTTTCT-3’, and antisense, 5’-TACTTCTCTCAAAATTTGAGTACA-3’; IL-4, sense, 5’-ACAGGAGAAGGAGCTTCAAGG-3’, and antisense, 5’-GAAGCCTCAGACAGGCTC-3’; IL-10, sense, 5’-GTTGCGAAACCTTTATGGA-3’, and antisense, 5’-ACCTGCTCAGCTTTCTTCT-3’; IL-12, sense, 5’-AGACCGACCTCGTGTCA-3’, and antisense, 5’-TGGTCTCTGTAGATGGCATTG-3’, and β-actin, sense, 5’-AGAGGGAATGTTCTGCAG-3’, and antisense, 5’-CAATAGTGCAGGCGCT-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 following 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 predominately 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 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 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 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).
pendent experiments were performed and the means were calculated using the uninfected animal as a calibrator. Three inde-

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 ~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 ~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 unbiten 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 μg/ml OVA concent-

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 unbiten 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.
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/Wv, and mast cell-reconstituted W/Wv (W/Wv-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/Wv 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/Wv-R mice exposed or not to mosquito bites. DTH responses were significantly reduced in W/Wv-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/Wv-R mice were consistently weaker than in +/+ mice, probably because of the incomplete reconstitution of W/Wv mice by mast cells (~40%). Collectively, these data indicate that DTH responses are regulated by mast cells and, most interestingly, that 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/Wv, and mast cell-reconstituted W/Wv (W/Wv-R) mice were exposed or not to mosquito bites at the sensitization phase with OVA. LN cells harvested...
IFN-γ constitutively by mast cells completely or partly restored IL-10 and mast cell-reconstituted W/Wv mice. This was not the case of IFN-γ induced by mosquito bites, which was found to be tightly controlled by mast cells. We previously reported that mosquito bites induce local mast cell degranulation elicited by salivary components. We reported earlier that mosquito bites induce IFN-γ expression because decreased production by mosquito bites obv-
served in W/Wv mice could not be reproduced in mast cell-reconstituted W/Wv mice. These data suggest that mosquito bites downmodulate 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/Wv 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 IL-10 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 saliva (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 func-
tion 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 demonstrated significantly reduced 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/Wv mice and re-
constitution 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/Wv mice. This was not the case of IFN-γ production because decreased production by mosquito bites ob-
served in +/+ mice could not be reproduced in mast cell-reconstituted W/Wv mice. These data suggest that mosquito bites downmodulate the DTH response by a mechanism that is dependent on mast cells and is mediated by IL-10.

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 super-
natants. As shown in Fig. 6, LN cells from bitten +/+ mice pro-
duced less IFN-γ and more IL-10 than mice not exposed to mos-
quito 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/Wv mice and re-
constitution 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/Wv mice. This was not the case of IFN-γ production because decreased production by mosquito bites ob-
served in +/+ mice could not be reproduced in mast cell-reconstituted W/Wv mice. These data suggest that mosquito bites downmodulate the DTH response by a mechanism that is dependent on mast cells and is mediated by IL-10.
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 naïve 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 naïve 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.

Acknowledgments

We thank Dr. Stephen Kunkel for his generous gift of anti-IL-10 and anti-MIP-2 Abs. We also thank all the members of the Centre d’élevage, de production et d’infection des Anopheles, at the Institut Pasteur for providing us with Anopheles mosquitoes. 

Disclosures

The authors have no financial conflict of interest.

References