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Mast Cells Have a Pivotal Role in TNF-Independent Lymph Node Hypertrophy and the Mobilization of Langerhans Cells in Response to Bacterial Peptidoglycan

Dunia M. Jawdat, Geoffrey Rowden, and Jean S. Marshall

Peptidoglycan (PGN) from Gram-positive bacteria, activates multiple immune effector cells. PGN-induced lymph node (LN) hypertrophy and dendritic cell mobilization in vivo were investigated following PGN injection into the skin. Both LN activation and the migration of Langerhans cells (LCs) to draining LN were dependent on the presence of mast cells as demonstrated using mast cell deficient W/Wv mice. However, these responses did not require TLR2, TLR4, or MYD88. TNF-deficient mice exhibited normal increases in LN cellularity but significantly reduced LC migration. In contrast, responses to IgE-mediated mast cell activation were highly TNF dependent. Complement component C3-deficient mice showed decreased LN hypertrophy and abrogated LC migration in response to PGN. These data demonstrate a critical role for mast cells and complement in LN responses to PGN and illustrate a novel TNF-independent mechanism whereby mast cells participate in the initiation of immunity. The Journal of Immunology, 2006, 177: 1755–1762.

Peptidoglycan (PGN)3 is a potent activator of the innate immune system (reviewed in Ref. 1), which stimulates the production of inflammatory cytokines by multiple cell types (reviewed in Ref. 2). Immune responses to PGN can be mediated through interaction with specific pattern recognition molecules, including TLR2 (3–5), nucleotide-binding oligomerization domain (NOD)1 (6, 7), NOD2 (8, 9), and peptidoglycan recognition proteins (10). In addition, PGN administered in vivo can activate the three major complement pathways (11–13). Despite the importance of this molecule in initiating early immune responses, the critical cells and receptor systems involved in PGN-induced lymph node (LN) activation and dendritic cell (DC) mobilization in vivo have not previously been investigated.

Mast cells have been widely studied in allergy, however, they also have a critical role as sentinel cells in host defense (14, 15). Mast cells express a wide range of immune receptors that aid in pathogen recognition, including TLRs, complement receptors, and CD48 (reviewed in Ref. 16). Mast cells are strategically located at host-environment interfaces, such as the skin. They produce a wide range of mediators including histamine, arachidonic acid derivatives, and numerous cytokines and chemokines, which can be selectively released (17, 18). In addition, mast cells are a source of TNF. Mast cells are thus far the only cell type described to have presynthesized TNF, which can be released within minutes of mast cell activation. In addition, they have the capability to newly synthesize and secrete this pivotal cytokine (19, 20). Mast cells have been widely implicated in aspects of host defense such as neutrophil recruitment following Gram-negative bacterial challenge, leading to bacteria clearance (14, 15), T cell activation (21), and LN hypertrophy (22). TNF has been shown to be the major mast cell-derived factor responsible for these processes. However, the role of mast cells in responses to Gram-positive bacteria is less well studied and may involve alternate mechanisms. Murine mast cells have previously been shown to respond to PGN predominantly via a TLR2-dependent mechanism to facilitate neutrophil recruitment. There is also evidence for local mast cell degranulation in the skin following administration of PGN (23).

The innate and adaptive immune responses are closely related and interdependent. Although the role of mast cells in innate immunity is well established, their role in adaptive immunity is not well understood (24). Key processes in the initiation of adaptive immune responses are the mobilization and migration of tissue activated DCs into the draining LN and the activation of the local LN. Together these provide an optimal environment for circulating lymphocytes to come in contact with DCs to initiate an immune response. Langerhans cells (LCs) are a specialized subset of immature DCs found in the epidermis of the skin. They can be identified by their expression of CD207 or “Langerin.” The expression of this marker is lost once they have migrated into the draining LN (25). We have previously shown that the migration of LCs, a type of immature DCs, from the skin into the draining auricular LN in response to a classical IgE-mediated allergic immune stimulus is mast cell dependent (26). We have also shown that histamine, via H2 receptors, is a major mediator in this process.

In the current study, we examined the mechanism of LN hypertrophy and LC migration in response to Staphylococcus aureus-derived PGN. Our results demonstrate critical roles for both mast cells and complement activation but not TLR2, TLR4, or MyD88 and demonstrate a novel TNF-independent pathway for mast cell-mediated LN hypertrophy.
Materials and Methods

Mice

C57BL/6 mice, genetically mast cell-deficient WBB6F1-W/W (W/W) matched with congenic WBB6F1-+/+ (+/+) mice, TLR4-deficient C57BL/10ScSnJ matched with C57BL/10J, complement component 3 (C3)-deficient B6SJL-Py/Py-J matched with C57BL/6 mice, TNF-deficient B6; 129S6-TNF mice matched with B6129S7F2 mice (The Jackson Laboratory) and TLR2-deficient mice on a C57BL/6 background (a gift from Dr.S. Akira, Osaka, Japan), MyD88-deficient mice and matched C57BL/6 controls were used. All mice were 6–10 wk of age and housed under specific pathogen-free conditions with food and water provided ad libitum. All experiments were approved by the animal research ethics board of Dalhousie University.

Mast cell reconstitution

Bone marrow-derived mast cells (BMMCs) were generated from the bone marrow of WBB6F1-+/+ (+/+) mice according to the method of Tertian et al. (27), a minimum of 98% pure mast cells were used. W/W mice were locally reconstituted as previously described (28). Cells derived from draining auricular LN excised after 24 h.

Histology

Carnoy’s fixed, paraffin-embedded ear pinna sections from W/W mice with and without prior local mast cell reconstitution were stained with Alcian blue and Safranin O. The numbers of granulated mast cells were counted in 10 randomly selected microscopic fields (at ×1000 magnification) per section.

Induction of local skin responses to bacterial products

Mice received an intradermal (i.d.) injection into the right ear pinna of 2.5 μg purified Staphylococcus aureus derived PGN (Fluka BioChemika distributed by Sigma-Aldrich) in 25 μl of saline and 25 μl of diluent in the left ear pinna. After 18 h mice were sacrificed, and their draining auricular LNs were excised. A LN-derived single cell suspension was prepared, counted, washed, and resuspended in RPMI 1640 medium. Either cytocentrifuge preparations were made or flow cytometric analysis of the LN-derived single cell suspension was performed as described below. For experiments using locally mast cell reconstituted W/W mice both ear pinnae were injected with PGN.

Statistical methods

Following confirmation of appropriate data distribution, differences between left and right LNs were evaluated using a paired Student’s t test. Differences in responses between animal groups were compared using an unpaired Student’s t test.

Results

To determine the role of mast cells in PGN-induced LN activation, mast cell deficient W/W mice and mast cell containing +/+ mice were injected with PGN into the right ear pinna and saline into the left ear pinna. After 18 h, the draining auricular LNs were excised, and the total LN cellularity was determined. In mast cell containing +/+ mice a significant increase in the total number of cells was observed in the LN draining the PGN-injected site compared with LN draining the saline injected site. In contrast, no increase in the total number of cells was observed in the LN draining the PGN-injected site compared with saline injected sites in W/W mice (Fig. 1). In keeping with previous studies (22), the majority of the increase in total LN cellularity observed could be accounted for by changes in the numbers of CD3-positive T cells (data not shown).

To determine whether mast cells are critical for inducing LN hypertrophy in response to PGN, mast cell deficient W/W mice were locally reconstituted by i.d. injection with BMMCs in the right ear only. After a 10-wk resting period, to allow mast cells to mature in vivo, mice were injected with PGN into both ear pinnae. After 18 h, the ear pinna draining auricular LNs were excised and the total cellularity was determined. A significant increase in the total number of cells was observed in the mast cell reconstituted site compared with nonreconstituted site confirming the requirement of mast cells to induce LN hypertrophy in response to PGN. Ear pinna skin was routinely harvested to confirm effective and consistent mast cell reconstitution. For example, in a representative experiment the mean numbers of cells with metachromatic granules (mast cells) were found to be 5.0 ± 0.4 cells per field (n = 5), while the equivalent mast cell numbers in unreconstituted control ears of W/W mice were 0.08 ± 0.1 cells per field (n = 5).
PGN-induced LC migration is mast cell dependent

The role of mast cells in LC migration out of the skin and into the draining LNs, in response to S. aureus derived PGN, was then determined. Mast cell deficient W/W^v mice and mast cell containing +/+ mice were injected with PGN into the right ear pinna and saline into the left ear pinna. After 18 h, the numbers of Langerin-positive (CD207) cells within the draining auricular LNs were determined. In mast cell containing +/- mice, a significant increase in the total number of cells was observed in the LN draining the PGN-injected site compared with control side. In contrast, no increase in the number of Langerin-positive cells were observed in the LN draining the PGN-injected site compared with saline injected sites in mast cell-deficient W/W^v mice (Fig. 3). Following local mast cell reconstitution of W/W^v mice in one ear the LC migration response was restored (Fig. 3).

TNF dependence of LC migration

Using a similar experimental design, the TNF-dependence of the LC migration response was determined. TNF-deficient mice had a small but significant increase in the number of Langerin-positive cells in the LN draining the PGN-injected site compared with saline injected site (Fig. 4a). This response was only 31.5% of the response in TNF containing control animals, demonstrating an important role for TNF in this process.

An IgE/Ag mediated system of local mast cell activation has previously been used to demonstrate the importance of mast cell activation in the induction of LC migration (26). Here, we also determined the role of TNF in LC migration following IgE-mediated mast cell activation. TNF deficient mice and their wild type controls were injected with anti-TNP IgE into the right ear pinna and saline into the left ear pinna. After 14 days, mice were challenged with TNP-BSA i.v. After 24 h, the draining auricular LNs were excised and the numbers of Langerin-positive cells were determined. In wild type mice a significant increase in the number of Langerin-positive cells was observed in the LN draining the IgE-injected site compared with LN draining the saline injected side. This highly mast cell-dependent response (26) was completely abrogated in TNF-deficient mice (Fig. 2b).

FIGURE 2. PGN-induced LC migration is mast cell dependent. Genetically mast cell-deficient W/W^v mice, and their mast cell containing congenic +/+ mice, received a saline injection as a control in one ear pinna and PGN (100 ng/ml) injection in the contralateral ear pinna. W/W^v mice reconstituted with mast cells in one ear pinna only (W/W^v + MC) received PGN injection into both ear pinnae. After 18 h, the numbers of Langerin-positive DC were determined. Mast cell deficient W/W^v mice and mast cell containing +/+ mice were injected with PGN into the right ear pinna and saline into the left ear pinna. After 18 h, the numbers of Langerin-positive cells within the draining auricular LNs were determined. In mast cell containing +/- mice, a significant increase in the total number of cells was observed in the LN draining the PGN-injected site compared with control side. In contrast, no increase in the number of Langerin-positive cells were observed in the LN draining the PGN-injected site compared with saline injected sites in mast cell-deficient W/W^v mice (Fig. 3). Following local mast cell reconstitution of W/W^v mice in one ear the LC migration response was restored (Fig. 3).

FIGURE 3. PGN induced LC migration is mast cell dependent. Genetically mast cell-deficient W/W^v mice, and their normal congenic +/- mice received a saline injection as a control in one ear pinna and PGN injection in the contralateral ear pinna. After 18 h, draining LN tissues were harvested, and total LN cells were counted. After 14 days, mice were injected with TNP-BSA i.v., and 24 h later, the draining LN tissues were harvested and total cells were counted. All numbers represent the mean value ± SEM (n = 5). ** represents p < 0.01; *** represents p < 0.001. Representative results of three similar independent experiments.
molecule MyD88 were therefore examined. TLR2-deficient mice (3), MyD88-deficient mice (32) with the appropriate control mice (C57BL/6), TLR4-deficient C57BL10ScNJ mice, and their appropriate control C57BL/10J were injected with PGN into the right ear pinna and saline into the left ear pinna and draining LNs examined. The PGN-induced increases in total LN cellularity were not reduced in TLR2, TLR4, or MyD88-deficient animals when compared with control animals (Fig. 5a). TLR2, TLR4 and MyD88-deficient mice also all had significant increases in the number of Langerin-positive cells in the PGN-injected side compared with saline injected side, with the TLR2–/– mice having a significantly (p < 0.001) greater response to PGN then the C57BL/6 controls (Fig. 5b).

**PGN-induced LC migration is complement component C3 dependent**

To examine the contribution of complement activation we used C3-deficient mice. Lack of this early complement component renders mice unable to activate complement via the classical, alternative, or mannose binding pathways. Both C3-deficient mice and controls were treated with PGN in one ear pinna and evaluated as described above. Notably, the increase in draining LN total cellularity was significantly reduced following PGN injection in C3-deficient mice compared with control mice (Fig. 6a). Unlike their congenic controls, C3-deficient mice did not show a significant increase in the number of Langerin-positive cells in the LN draining the PGN injected ear pinna compared with the saline injected site (Fig. 6b).

To confirm that C3-deficient mice were capable of mounting a normal LN hypertrophy and LC response, these mice and controls were injected with anti-TNP IgE into the right ear pinna and saline into the left ear pinna. After 7 days, mice were challenged with TNP-BSA i.v. After an additional 24 h, the draining auricular LNs were excised. Both groups of mice had a significant increase in total cellularity of LN draining the IgE-injected side compared with LN draining the saline injected side (Fig. 6c), although this increase was attenuated in C3-deficient mice. The LC increases observed as a result of IgE-mediated mast cell activation were unchanged in C3-deficient mice compared with their controls (Fig. 6d).

**Histamine receptors H1 and H2 do not have a major role in the response to PGN**

LC migration in response to IgE-mediated mast cell activation has been previously shown to be histamine-dependent and was inhibited by >90% following treatment with the H2 receptor antagonist cimetidine (26). Therefore, C57BL/6 mice were pretreated with pyrilamine (H1 antagonist) 50 mg/kg, cimetidine (H2 antagonist) 50 mg/kg, or diluent control i.p. 90 min before PGN and saline ear pinna injections. After 18 h, the draining auricular LNs were examined. Neither the increase in the total cellularity of LNs nor the migration of LC into the nodes was reduced by antihistamine receptors treatment (Fig. 7).

**The Langerin-positive cells observed in the skin draining LN have undergone maturation**

To examine the maturational status of LC within auricular nodes following PGN treatment, LN cells were stained with the maturation marker CD86. The proportion of CD11c+ cells that were...
CD86⁺ remained high (>80%) in all experimental groups. TLR2, TLR4, Myd88, C3, and TNF-deficient mice all demonstrated similar proportions of CD86⁺/Langerin-positive cells within the nodes following saline or PGN treatment of the ear pinnae (Table I).

**Langerin-positive cell increases are associated with increases in CD11c⁺ cells within the LNs**

The proportion of CD11c⁺ LN DC that were Langerin-positive and the overall number of CD11c⁺ cells/node were also determined (Table I). C57BL/6 mice, TLR2, TLR4, and Myd88-deficient mice showed significant increases in the number of CD11c⁺ cells in the LN draining PGN-injected sites, with the TLR2⁻/⁻ mice having a significantly (p < 0.05) greater response to PGN than the C57BL/6 mice. In contrast, C3-deficient mice showed significantly (p < 0.05) lower numbers of CD11c⁺ cells in the LN draining PGN-injected sites. C57BL/6 mice injected with diluent, pyrilamine, or cimetidine had similar responses to control C57BL/6 mice. TNF-deficient mice and their congenic controls both showed similar increases in the number of CD11c⁺ cells in LN draining PGN injection sites. The number of CD11c⁺ cells/node were also determined in response to IgE-mediated mast cell activation, C3-deficient mice showed a small but significant increase in the number of CD11c⁺ cells, while TNF-deficient mice showed no significant increase in the number of CD11c⁺ cells in the IgE-injected side compared with saline.

**Comparison of key mediators produced by mast cells in response to PGN or IgE/Ag stimulation**

In view of the marked differences in the apparent mechanism of mast cell dependent responses to IgE/Ag and PGN in vivo we examined the in vitro mediator production of murine BMMCs in response to these stimuli. PGN activation was examined in the presence of mouse plasma containing either active or heat-inacti-
complement, has only modest effects as a mast cell degranulating agent (34, 35). PGN can activate the immune system through multiple mechanisms including interaction with TLR2 (33, 36, 37), NOD family members (6–9) and activation of the complement pathway (11–13).

In the current study, we examined the role of mast cells in the migration of LCs from the skin and into the draining LN as well as the development of LN hypertrophy following i.d. PGN administration. We compared responses to PGN with those observed following IgE/Ag mediated mast cell activation. Using the W/Wv mast cell-deficient mouse model and appropriate local mast cell reconstitutions we demonstrated that mast cells are essential for both LN hypertrophy and LC migration in response to PGN. Previous studies, using LPS administration to mast cell-deficient animals, have demonstrated that there is no fundamental defect in the ability of LCs to migrate or LN hypertrophy to occur in the W/Wv mouse (26). This observation is further supported by efficient responses to PGN following local mast cell reconstitution of W/Wv mice in one ear pinna. Injection of the contralateral mast cell-deficient ear pinna, in the same animals, with PGN yielded little or no response.

In view of previous reports (23) of TLR expression by mast cells in view of previous reports (23) of TLR expression by mast cells, we cannot rule out a potential contribution by TLRs signaling through multiple mechanisms including interaction with TLR2 (3, 36, 37), and PGN activation of murine mast cells via TLR2, we initially considered the role of TLR-mediated signaling in these responses. However, TLR2-deficient animals when compared with matched controls. Similarly, when TLR4- or MyD88-deficient animals were used, both LC migration and LN hypertrophy responses to PGN remained intact. These data suggested that alternate TLRs using MyD88 were also not responsible for the observed mast cell dependent responses. However, we cannot rule out a potential contribution by TLRs signaling through alternate pathways or a role for NOD family members.

PGN is an excellent activator of complement working through the classical, alternate or mannose binding pathways. All of these pathways are ineffective in C3-deficient mice. Using such animals we demonstrated that both LC migration and LN hypertrophy in response to PGN are complement C3 dependent. Mast cells have receptors for a number of complement products including C3a and C5a. These observations raise the possibility that complement products might contribute to the activation of mast cells in the skin following PGN administration. Since C3 deficiency has been reported to be associated with a number of functional defects in other immune effector cells (38, 39) we examined responses to a complement independent, mast cell dependent stimulus in these mice. IgE/Ag mediated activation of mast cells in the skin yielded similar LC responses to those in control C3 containing mice. These results demonstrate that the mast cell’s ability to mobilize LCs is not inhibited in C3-deficient mice, thus confirming the role of complement in the mast cell dependent response to PGN. One potential model for this response would involve PGN activation of complement and subsequent activation of mast cells by complement split products (40) leading to the production of mediators, such as GM-CSF, with the ability to induce maturation and migration of resident LCs. However, since LCs also express complement receptors (41), complement products may also contribute to their maturation and migration directly.

The LN hypertrophy response to IgE/Ag mediated activation was reduced in C3 deficient animals when compared with control animals. This suggests that fundamental defects in this response, associated with C3 deficiency, could contribute to the observed lack of LN hypertrophy response to PGN.

Mast cells produce multiple mediators that may contribute to DC maturation and migration as well as LN hypertrophy responses (17, 18, 42, 43). These include GM-CSF, IL-1β, TNF, LTC4, and histamine that can be produced following exposure to bacterial products (34, 44, 45). TNF and histamine have been implicated in multiple studies of mast cell dependent immune regulation. Histamine has been demonstrated to influence the maturation of DCs as well as the polarization of subsequent T cell responses (46–48).

We have previously observed that the LC response, following IgE mediated mast cell activation, is highly histamine (H2) dependent while LN hypertrophy, in response to the same stimulus, was not. PGN induced LC migration and LN hypertrophy were both profoundly mast cell dependent; however, they were not significantly inhibited by either H1 or H2 receptor blockade. These observations

Table I. *CD11c and CD86 positive cell populations in the draining auricular nodes of various mouse strains 18 h after PGN or saline or 24 h after IgE/Ag or saline injection of the ear pinna*

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Number of CD11c+ Cells (thousands)</th>
<th>% of CD11c+ Cells That Are CD86+</th>
<th>% of Langerin-Positive Cells That Are CD86+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGN</td>
<td>Saline</td>
<td>PGN</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>86.0 ± 10.0***</td>
<td>27.6 ± 4.8</td>
<td>54.1 ± 3.5</td>
</tr>
<tr>
<td>TLR2/−−−</td>
<td>121.8 ± 15.9***</td>
<td>35.0 ± 4.9</td>
<td>63.6 ± 2.8</td>
</tr>
<tr>
<td>MyD88/−−−</td>
<td>45.8 ± 10.3*</td>
<td>16.7 ± 1.8</td>
<td>65.7 ± 3.0</td>
</tr>
<tr>
<td>C57BL/10J</td>
<td>150.5 ± 19.0**</td>
<td>79.0 ± 9.5</td>
<td>66.2 ± 2.9</td>
</tr>
<tr>
<td>TLR4/−−−</td>
<td>135.6 ± 29.0*</td>
<td>62.2 ± 13.2</td>
<td>62.4 ± 1.2</td>
</tr>
<tr>
<td>C3/−−−</td>
<td>11.4 ± 4.2*</td>
<td>5.6 ± 1.5</td>
<td>59.9 ± 6.6</td>
</tr>
<tr>
<td>C57BL/6 + diluent</td>
<td>117.1 ± 34.7*</td>
<td>36.1 ± 8.21</td>
<td>56.3 ± 3.9</td>
</tr>
<tr>
<td>C57BL/6 + pyrimidine</td>
<td>155.6 ± 23.5*</td>
<td>54.1 ± 10.7</td>
<td>65.1 ± 4.1</td>
</tr>
<tr>
<td>C57BL/6 + cimetidine</td>
<td>95.6 ± 14.4***</td>
<td>36.2 ± 5.1</td>
<td>60.7 ± 5.5</td>
</tr>
<tr>
<td>B6/129SF2</td>
<td>52.8 ± 15.9*</td>
<td>14.8 ± 2.2</td>
<td>54.5 ± 2.8</td>
</tr>
<tr>
<td>TNF/−−−</td>
<td>37.4 ± 3.7***</td>
<td>12.3 ± 1.7</td>
<td>45.8 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>IgE</td>
<td>Saline</td>
<td>IgE</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>94.3 ± 8.3***</td>
<td>26.1 ± 4.3</td>
<td>63.8 ± 1.1</td>
</tr>
<tr>
<td>C3/−−−</td>
<td>44.5 ± 2.3*</td>
<td>23.6 ± 3.3</td>
<td>73.7 ± 1.9</td>
</tr>
<tr>
<td>B6/129SF2</td>
<td>60.6 ± 9.1**</td>
<td>14.3 ± 2.6</td>
<td>66.5 ± 1.1</td>
</tr>
<tr>
<td>TNF/−−−</td>
<td>35.4 ± 8.8</td>
<td>28.9 ± 4.3</td>
<td>73.2 ± 2.1</td>
</tr>
</tbody>
</table>

* In each group LN-draining PGN or IgE-injected site was compared with LN draining contralateral saline injected site. *, p < 0.05; **, p < 0.01; and ***, p < 0.001. # denotes significantly (p < 0.05) reduced number of CD11c+ cells compared with PGN injected site in B6/129 control animals.
The mean of three experiment ± SEM. All values shown are picograms per milliliter.

do not rule out a role for histamine in modifying the functional
characteristics of LCs or other DC populations in this model, but
underline the existence of alternate mechanisms of mast cell me-
diated induction of LC migration to the draining LN.

TNF has been implicated as a critical cytokine in multiple mast
cell-mediated models of immune modulation and acute inflamma-
tion (14, 15, 21, 22, 49–53). Mast cells have a unique ability to
store and rapidly release TNF during degranulation; in addition,
they can produce newly synthesized TNF in response to a variety
of stimuli (19, 20, 54–56). TNF-deficient mice had a significantly
reduced LC migration response, compared with wild type mice,
following either IgE-mediated or PGN induced mast cell activa-
tion. However, the LC response to PGN was not entirely inhibited
in the absence of TNF, suggesting important contributions by other
mediators. The role of TNF in the migration of other DC subsets
has not been examined in this model, however when CD11c–/–
DCs were evaluated as a whole, similar increases in these cells were
observed following PGN activation in both TNF-containing and
TNF-deficient mice suggesting that not all DC subsets are subject
to the same TNF dependent events in response to PGN. Recent
studies of a murine model of contact hypersensitivity to FITC have
revealed that early recruitment of DCs into LNs draining the skin
and airways is partially mast cell and TNF dependent (49), in
keeping with the results of the current study.

TNF-deficient mice developed a similar level of LN hypertro-
phy as control mice following PGN administration. These results
are in marked contrast to other models of mast cell dependent LN
hypertrophy induced by IgE-mediated mast cell activation (Fig. 4)
or Gram-negative bacterial infection which are highly TNF depen-
dent (22). These data demonstrate the existence of at least two
distinct mechanisms whereby mast cells can contribute to the de-
velopment of LN hypertrophy. A number of mast cell mediators,
including Leukotrienes, IL-6, IL-1β, and GM-CSF, might contrib-
ute to this process. Multiple mechanisms have been described
whereby LN hypertrophy can occur including increased migration
of T cells into the LN as a result of remodeling and other events
enhancing T cell recruitment (22) and decreased emigration of T
cells out of the LN. Mast cells could contribute to these processes
through local mediator production and subsequent lymphatic trans-
port of mediators or activated cells to the draining node.

Mast cell modulation of the maturation of LCs as well as the
development of LN hypertrophy has important implications for the
development of acquired immune responses. Bryce et al. have
demonstrated a role for mast cells and IgE in promoting the de-
velopment of contact hypersensitivity responses (57) in keeping
with recent findings of Suto et al. (49). However, this is not a
consistent finding in all models of contact hypersensitivity (58,
59). The role of mast cells is likely to be highly dependent on the
conditions used to induce responses and the nature and extent of
mast cell mediator production that is initiated. PGN induced LC
migration and maturation may be of particular importance in the
context of atopic dermatitis where >90% of patients have been
demonstrated to be infected with S. aureus. PGN-activated mast
cells could potentially modify the process of immune sensitization
to multiple environmental Ags by enhancing the proportion of ma-
ture LC’s migrating to the draining LNs. Mast cells may impact on
the polarization and effectiveness of T cell responses either
to through well described effects of their mediators on DCs (48, 60)
or through more direct mast cell T cell interactions that have been
recently described (21, 61).

Overall, this study demonstrates that PGN induced LC migra-
tion and LN hypertrophy are highly mast cell and complement
(C3) dependent, although independent of TLR2 or MyD88. The
ability of TNF-deficient animals to undergo LN hypertrophy in
response to PGN indicates a novel TNF-independent mechanism
whereby mast cells can contribute to this process, quite distinct
from the mechanism(s) involved in response to IgE/Ag or Gram-
negative bacterial infection. Modulation of these pathways should
provide novel opportunities to intervene in the earliest states of
development of acquired immune responses to pathogens or
environmental Ags.

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for this study.

Disclosures
The authors have no financial conflict of interest.

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