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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 LNs 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 aberrant 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) 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/10ScNJ matched with C57BL/10J, complement component 3 (C3)-deficient B6.129S4-C3 matched with C57BL/6 mice, TNF-deficient B6; 129S6-TNF mice matched with B6.129S2 mice (The Jackson Laboratory) and TLR2-deficient mice on a C57BL/6 background (a gift from D. 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 congenic WBB6F1 (American Type Culture Collection) in 25 μl of PBS or diluent control (as stated). 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.

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
PGN-induced LN activation is mast cell-dependent
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 pinna. After 18 h, the draining auricular LNs were excised, and the total number of cells was observed in the LN draining the PGN-injected site compared with LN draining the saline injected site. 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).

Examining the role of histamine receptors
To determine the role of histamine H1 and H2 receptors in the PGN-mediated response, PGN or control diluent were injected in to the ear pinnae 90 min after either H1 antagonist pyrilamine (50 mg/kg), the H2 antagonist cimetidine (50 mg/kg) (Sigma-Aldrich) or diluent control were injected i.p. Eighteen hours later mice were sacrificed and LNs were harvested and responses were evaluated as described above.

Mediators analysis
TNF, GM-CSF, IL-1β, leukotriene C4 (LTC4), and CCL3 (MIP-1α) production was examined in cell-free supernatants harvested after 4 h or 18 h incubation of BMMCs with TNP-BSA or PGN in the presence of fresh or heat inactivated (56°C for 30 min) normal mouse plasma at 37°C. For TNP-BSA treatments, some groups of cells were previously sensitized by 24–48 h incubation with monoclonal IgE anti-TNP. GM-CSF and CCL3 content of supernatants was measured via a commercial ELISA kit (R&D Systems). TNF and IL-1β contents of supernatants was measured via an “in-house” ELISA as previously described (29) using the following Abs: polyclonal goat anti-mTNF (R&D Systems) for coating, anti-mouse TNF mAb biotin-labeled (Perbio Science) for detection, monoclonal rat IgGlanti-mouse IL-1β for coating (R&D Systems), and anti-mouse IL-1β Ab biotin-labeled for detection, respectively. LTC4 production was assessed 20 min following BMMC incubation with or without the above mentioned activators using an ELISA, as previously described by Volland et al. (30).

Degranulation and β-hexaminidase assay
BMMCs in HEPES-Tyrode’s buffer were incubated with the above-mentioned activators for 20 min at 37°C, both supernatants and pellets were analyzed for β-hexaminidase according to the method of Schwartz et al. (31).
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 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 mice (Fig. 3). Following local mast cell reconstitution of W/W 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 7 days mice were challenged with TNP-BSA i.v. and after a further 24 h the draining auricular LNs were excised and the total LN cellularity was determined. In wild-type mice, a significant increase in the total number of 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).

PGN-induced LC migration is mast cell dependent

To determine the role of TNF in PGN-induced LN hypertrophy, TNF-deficient mice and their wild-type controls were injected with PGN into the right ear pinna and saline into the left ear pinna. After 18 h, the cellularity of the draining auricular LN was determined. TNF-deficient and control mice had a similar increase in LN cellularity in response to PGN (Fig. 2a).

To compare this response, with the role of TNF in LN responses 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 7 days mice were challenged with TNP-BSA i.v. and after a further 24 h the draining auricular LNs were excised and the total LN cellularity was determined. In wild-type mice, a significant increase in the total number of cells was observed in the LN draining the IgE-injected site compared with LN draining the saline injected site. This IgE/Ag induced response was completely abrogated in TNF-deficient mice (Fig. 2b).

PGN-induced LC migration and is TLR2, TLR4, and MyD88 independent

PGN has previously been shown to activate immune effector cells through TLR2. The contributions of specific TLRs and the adapter
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. After 14 days, mice were injected with TNP-BSA i.v., and 24 h later, the draining LN tissues were harvested and Langerin-positive DC were counted using anti-Langerin/CD207 flow cytometry. All numbers represent the mean value ± SEM (n = 5/group). *represents p < 0.05; **represents p < 0.001. # represents p < 0.01 when comparing PGN injected side of TNF-deficient mice compared with the PGN injected side in wild-type control mice after subtraction of the baseline numbers of cells observed in nodes from the saline-injected side of each mouse strain.

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, alternate, or mannos 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

FIGURE 4. PGN induced LC migration is partially TNF independent while IgE/Ag induced LC migration is TNF dependent. a, TNF-deficient mice and their normal control mice received a saline injection as a control in one ear pinna and PGN injection in the contralateral ear. After 18 h, draining LN tissues were harvested, and Langerin-positive DC were counted. b, TNF-deficient mice and controls received a saline injection as a control in one ear pinna and anti-TNP IgE injection in the contralateral ear pinna. After 14 days, mice were injected with TNP-BSA i.v., and 24 h later, the draining LN tissues were harvested and Langerin-positive DC were counted using anti-Langerin/CD207 flow cytometry. All numbers represent the mean value ± SEM (n = 5/group). *represents p < 0.05; **represents p < 0.001. # represents p < 0.01 when comparing PGN injected side of TNF-deficient mice compared with control animals (Fig. 5).

FIGURE 5. PGN-induced LN hypertrophy and LC migration is not TLR2, TLR4, or MyD88 dependent. C57BL/6, TLR2-deficient, MyD88-deficient, TLR4-deficient, and WT control 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 cells and Langerin-positive DC cells were counted using anti-Langerin/CD207 flow cytometry. a, mean ± SEM numbers of total cells/draining LN. b, mean ± SEM number of Langerin/CD207-positive DC/draining LN (n = 5). *represents p < 0.05; **represents p < 0.01; ***represents p < 0.001. Representative results of two similar independent experiments.
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-inactivated complement. Mast cell production of major regulators of DC maturation was examined by ELISA, under all activation conditions (Table II). IgE/Ag activation induced substantial degranulation above control sensitized cells (mean = 29.8 ± 6.5%) while PGN in either the presence or absence of active complement did not induce significant degranulation under these conditions (mean = 3.8 ± 5.1%). Similarly, IgE/Ag activated cells produced significantly more TNF (mean = 614.3 ± 86.8 pg/ml) than control cells (mean = 300.2 ± 99.5 pg/ml) while PGN did not induce a TNF response. In marked contrast, PGN in the presence of complement containing plasma induced a significant GM-CSF response \((p < 0.05)\) that was not observed following IgE/Ag activation of the same cell preparations. LTC4 production was observed in response to both PGN and IgE. However, by far the greatest LTC4 response was induced by IgE/Ag activation. PGN in the presence of complement induced significantly more LTC4 \((p < 0.05)\) than when combined with heat-inactivated plasma. The role of complement in enhancing responses to PGN was further confirmed by measurement of the CCL3 response of BMMC to PGN in the presence of heat-inactivated or control mouse plasma. In the presence of active complement, mast cells produced a mean of 487.1 ± 223 pg/ml of this chemokine compared with a baseline level of 86.2 ± 30.8 pg/ml, while in the absence of active complement a mean of only 70.6 ± 31.3 pg/ml was observed \((n = 3)\).

Discussion

The mast cell's contribution to the development of acquired immune responses has not been fully elucidated. Previous studies have demonstrated important interactions with DCs and T cells as well as mast cell dependent induction of LN hypertrophy. Such studies have used models of IgE-mediated mast cell activation and Gram-negative bacterial infection, associated with substantial degranulation and TNF production by mast cells. PGN from Gram-positive bacteria such as S. aureus is also known to activate mast cells in vivo and in vitro (23, 33). However, it is not a potent inducer of human mast cell TNF production, and, in the absence of...
complement, has only moderate effects as a mast cell degranulating agent (34, 35). PGN can activate the immune system through multiple mechanisms including interaction with TLR2 (3, 36, 37), NOD family members (6–9) and activation of the complement pathways (34, 44, 45). TNF and histamine have been implicated in histamine that can be produced following exposure to bacterial products (34, 44, 45). 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 the 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 DCs. 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.

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

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Number of CD11c+ Cells (%)</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 + pyrilamine</td>
<td>155.6 ± 23.5*</td>
<td>54.1 ± 10.7</td>
<td>65.1 ± 4.1</td>
</tr>
<tr>
<td>B6/129SF2</td>
<td>95.6 ± 14.4***</td>
<td>36.2 ± 5.1</td>
<td>60.7 ± 5.5</td>
</tr>
<tr>
<td>TNF+/−</td>
<td>52.8 ± 15.9*</td>
<td>14.8 ± 2.2</td>
<td>54.5 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>37.4 ± 3.7***</td>
<td>12.3 ± 1.7</td>
<td>45.8 ± 1.7</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 < 0.1, and ### p < 0.001. # denotes significantly (p < 0.05) reduced number of CD11c+ cells compared with PGN injected site in B6/129 control animals.

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

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Number of CD11c+ Cells (%)</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>94.3 ± 8.8***</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 view of previous reports (23) of TLR expression by mast cells and PGN activation of murine mast cells via TLR2, we initially considered the role of TLR-mediated signaling in these responses. Surprisingly, when PGN was administered to the skin of TLR2-deficient animals both LN hyperplasia and LC migration were observed. Indeed, there was a trend toward enhanced responses in 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 reveal 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 mean of three experiment ± SEM. All values shown are picograms per milliliter.

Table II. **BMMC mediators, following IgE/Ag or PGN activation in the presence or absence of an active complement (C) cascade**

<table>
<thead>
<tr>
<th>IgE Sensitization</th>
<th>Activator</th>
<th>GM-CSF</th>
<th>IL-1β</th>
<th>TNF</th>
<th>LTC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Heated-plasma alone</td>
<td>&lt;4.1</td>
<td>71.3 ± 5.7</td>
<td>300.2 ± 99.5</td>
<td>112.3 ± 33.8</td>
</tr>
<tr>
<td>+</td>
<td>TNBP-BSA + heated-plasma</td>
<td>&lt;4.1</td>
<td>46.3 ± 0.6</td>
<td>614.3 ± 86.8</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>–</td>
<td>C’ containing plasma alone</td>
<td>5.6 ± 4.3</td>
<td>62.8 ± 8.2</td>
<td>&lt;13.7</td>
<td>158.3 ± 147.2</td>
</tr>
<tr>
<td>–</td>
<td>Heated-plasma alone</td>
<td>&lt;4.1</td>
<td>60.6 ± 9.7</td>
<td>&lt;13.7</td>
<td>210.0 ± 13.6</td>
</tr>
<tr>
<td>–</td>
<td>PGN + C’ containing plasma</td>
<td>46.7 ± 18.1</td>
<td>69.8 ± 11.7</td>
<td>&lt;13.7</td>
<td>407.6 ± 90.2</td>
</tr>
<tr>
<td>–</td>
<td>PGN + heated-plasma</td>
<td>&lt;4.1</td>
<td>70.1 ± 8.0</td>
<td>&lt;13.7</td>
<td>87.5 ± 30.9</td>
</tr>
</tbody>
</table>

* Murine BMMC mediators were examined in cell-free supernatants 4 h (TNF), 18 h (GM-CSF, IL-1β), and 20 min (LTC4) following activation. Each number represents the mean of three experiments ± SEM. All values shown are picograms per milliliter.

References

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