Mast Cells, Histamine, and IL-6 Regulate the Selective Influx of Dendritic Cell Subsets into an Inflamed Lymph Node

Wojciech Dawicki, Dunia W. Jawdat, Nong Xu and Jean S. Marshall

*J Immunol* 2010; 184:2116-2123; Prepublished online 18 January 2010; doi: 10.4049/jimmunol.0803894

http://www.jimmunol.org/content/184/4/2116
Mast Cells, Histamine, and IL-6 Regulate the Selective Influx of Dendritic Cell Subsets into an Inflamed Lymph Node

Wojciech Dawicki, Dunia W. Jawdat, Nong Xu, and Jean S. Marshall

In response to bacterial stimuli, multiple dendritic cell (DC) populations accumulate within the draining lymph node, thus enhancing opportunities for effective T cell–DC interaction. DC subpopulations, such as plasmacytoid, CD8+, and CD11b+ subsets, have distinct roles in determining the nature of the immune response. The mechanisms whereby individual DC subpopulations are mobilized and the extent to which these processes are linked to increases in overall lymph node cellularity have not been determined. In the current study, the mechanisms of DC subset mobilization to the draining auricular lymph node were examined after intradermal injection of Staphylococcus aureus-derived peptidoglycan. Using mast cell–deficient mice and local mast cell reconstitution, plasmacytoid and CD8+ DC responses were shown to be mast cell dependent, whereas the CD11b+ DC response was not. A histamine H2 receptor-dependent, CXCL9-independent pathway controlled the selective influx of both plasmacytoid and CD11b+ DC into the lymph node, but not lymph node cellularity. In contrast, IL-6 was important for the mobilization of CD8+ and CD11b+ DC. TNF and IL-1 receptor were dispensable for plasmacytoid, CD11b+, and CD8+ DC responses. These findings provide novel opportunities for the selective mobilization of specific DC subsets to lymph nodes and demonstrate critical roles for both histamine and IL-6 in this process. The Journal of Immunology, 2010, 184: 2116–2123.

Multiple DC subpopulations can be found within the lymph node, but their individual functions in the establishment of immunity and tolerance have not been fully elucidated. In response to local infection or inflammatory insults, the number of DCs in the draining lymph node increases. Five major conventional DC subpopulations and one plasmacytoid DC (pDC) population have been identified in lymph nodes draining the skin. Conventional DCs that express CD11c can be divided into migratory DCs that enter the lymph node through the lymphatics and lymphoid tissue-resident DCs that are derived from precursors that enter the lymph node from the blood. CD11b-expressing migratory DCs in lymph nodes draining the skin are primarily Langerhans cells and dermal DCs. These cells reside in the epidermis and dermis, take up Ags, and then migrate into the lymph node. The lymphoid tissue-resident DCs can be further subdivided into CD8+ CD11b- and CD8+ CD11b+ groups, which collect and present Ags in the lymph nodes after entering from the blood. CD8+ DCs have a high capacity for cross-presentation of extracellular Ags on MHC class I, making them particularly important for CD8 T cell responses. In addition, conventional DCs, CD11c+ blood-derived pDCs are found in the lymph node. Activated pDCs produce large quantities of type I IFNs and are capable of Ag capture and presentation.

Mast cells have been implicated in the process of DC mobilization and lymph node activation. Activation of these sentinel cells leads to the production of soluble mediators, such as histamine, TNF, and IL-6. Histamine H2 receptor-dependent, CXCL9-independent, and IL-6-dependent pathways control the selective influx of both plasmacytoid and CD11b+ DC into the lymph node, but not lymph node cellularity. In contrast, IL-6 was important for the mobilization of CD8+ and CD11b+ DC. TNF and IL-1 receptor were dispensable for plasmacytoid, CD11b+, and CD8+ DC responses. These findings provide novel opportunities for the selective mobilization of specific DC subsets to lymph nodes and demonstrate critical roles for both histamine and IL-6 in this process.
with food and water provided ad libitum. All experiments were approved by the animal research ethics board of Dalhousie University (Halifax, Nova Scotia, Canada).

**Abs and reagents**

Anti-mouse CD8 (clone 53-6.7), CD11b (clone M1/70), CD11c (clone N418), CD19 (MB19-1), B220 (clone RA3-6B2), and isotype control Ab were purchased from BioLegend (San Diego, CA). Anti-mouse pDC Ag-1 (PDCA-1) was purchased from Miltenyi Biotec (Auburn, CA). Anti-mouse CD90 (clone 53-2-1), anti-mouse Ly6c, and streptavidin-PerCP were purchased from BD Biosciences (San Jose, CA). *Staphylococcus aureus* PGN was purchased from Sigma-Aldrich (Oakville, Canada).

**Induction of local responses to PGN**

Mice were anesthetized, and each mouse ear pinna was injected with 2.5 μg PGN in 20 μl saline or, as a control, saline alone. Eighteen hours post-injection, mice were euthanized, and the draining auricular lymph nodes were harvested.

**Local mast cell reconstitution**

Bone marrow-derived mast cells (BMMCs) were generated from C57BL/6 mice as described previously (23). After at least 5 wk of culture and when the culture was >80% mast cells, 5 × 10^4 mast cells were injected intradermally into the ear pinna. Reconstituted mice were used in experiments 10–12 wk post-reconstitution (24). At the time of harvest, the ear skin was analyzed histologically to ensure the effectiveness of the reconstitution.

**Protein array**

BMMCs were stimulated with 100 μg/ml S. aureus PGN (Sigma-Aldrich) in RPMI 1640 containing 1% FCS, further supplemented with 1% mouse plasma. After 24 h, the supernatants were harvested by centrifugation. Cytokine production was assayed using RayBio Mouse Cytokine Ab Array III (Norcross, GA) following the manufacturer’s protocol. Image analysis was performed using Quantity One Software (Bio-Rad, Hercules, CA). The list of cytokines and chemokines assessed on this array was: Axl, B lymphocyte chemokine, CD30L, IFN-γ, IL-10, IL-12 p70, IL-15, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, keratinocyte-derived cytokine, leptin, leptin receptor I, TNF receptor II, thrombopoietin, VCAM-1, and vascular endothelial growth factor.

**Flow cytometry**

Auricular lymph nodes were individually minced with a scalpel and passed through a 100-μm cell strainer (BD Biosciences). Lymph node cells were stained with Abs against CD11c, CD19, CD90, CD8 and CD11b, or CD19 and PDCA-1. To determine the frequency of CD8+ and CD11b+ DCs, we first gated on CD11c+, CD90low (Fig. 1A), that drained ear pinnae that had received a single injection of saline or PGN. Lymph node cells were stained with Abs against CD11c, CD90, CD8 and CD11b, or CD19 and PDCA-1. To determine the frequency of CD8+ and CD11b+ DCs, we first gated on CD11c+ and CD90low cells (Fig. 1A) and subsequently gated on either CD8+ or CD11b+ (Fig. 1B). CD90 staining was included to ensure that the CD8+ DC gate did not include T cells. Plasmacytoid cells were defined as PDCA-1+ and CD19− (Fig 1C). CD19 staining was unnecessary to exclude CD19+ cells that nonspecifically bound to PDCA-1 or irrelevant isotype-matched Abs. To further confirm that PDCA-1+CD19− cells were pDCs, their expression of B220 and Ly6c was analyzed (Fig. 1D, 1E).

**Inhibitor treatment**

Montelukast, naproxen (Cayman Chemicals, Ann Arbor, MI), disodium cromoglycate, ranitidine, and pyrilamine (Sigma-Aldrich) were dissolved in PBS. To inhibit histamine receptors H1 and H2, pyrilamine (50 μg/kg) and ranitidine (25 μg/kg) were injected i.p. 90 min prior to PGN injections (4, 25, 26). To inhibit PG synthesis, the nonselective cyclooxygenase (COX)-1 and COX-2 inhibitor naproxen (25 mg/kg), was injected i.p. 60 min prior to PGN activation (27). To block the cysteinyl leukotriene 1 (cysLT1) receptor, montelukast (25 mg/kg) was injected i.p. 60 min prior to PGN administration (28). To block skin mast cell degranulation, disodium cromoglycate (40 mg/kg) was injected i.p. 60 min prior to PGN administration. As a control, equal volumes of diluent were injected in parallel groups of animals.

**Measurement of CXCL9 in the lymph node**

Auricular lymph nodes draining the site of injection from three mice were harvested, flash-frozen in PBS, and weighed. Lymph nodes were then thawed and sonicated on ice. The volumes were adjusted for lymph node weight, and the CXCL9 level was determined by ELISA using CXCL9 DuoSet Abs and a chemokine standard purchased from R&D Systems (Minneapolis, MN). ELISA detection was performed using an ELISA Amplification System purchased from Invitrogen (Burlington, CA).

**Basophil maturation and stimulation**

Bone marrow cells from C57BL/6 mice were grown in complete media (10% FBS, RPMI 1640) supplemented with IL-3 (5 ng/ml) for 10 d. Cells were then stained with anti-CD117, anti-FcRl, and anti-CD90 Abs to facilitate sorting of CD117+ FcRl+CD90+ cells with an FACSArria cell sorter (BD Biosciences). Sorted cells were incubated at 10^6 cells/100 μl in RPMI 1640 plus 1% v/v fresh mouse plasma, with and without PGN, for 30 min at 37°C. Supernatants and pellets were boiled for 20 min and snap frozen. Histamine was assayed using a histamine ELISA (BD Biosciences) according to the manufacturer’s protocol.

**Statistical analysis**

Differences between the left and right lymph nodes were evaluated using a paired Student t test. Differences between different groups of animals were evaluated using an unpaired Student t test. A p value of < 0.05 was considered significant.

**Results**

**PGN increases the number of CD8+ DC, CD11b+ DC, and pDC in the draining lymph node**

The lymph node is the site of T cell priming and thus a critical location for DC function. Using flow cytometry, we enumerated CD8+ DCs, CD11b+ DCs, and pDCs in individual auricular lymph nodes that drained ear pinnae that had received a single injection of saline or PGN. Lymph node cells were stained with Abs against CD11c, CD90, CD8 and CD11b, or CD19 and PDCA-1. To determine the frequency of CD8+ and CD11b+ DCs, we first gated on CD11c+ and CD90low cells (Fig. 1A) and subsequently gated on either CD8+ or CD11b+ (Fig. 1B). CD90 staining was included to ensure that the CD8+ DC gate did not include T cells. Plasmacytoid cells were defined as PDCA-1+ and CD19− (Fig 1C). CD19 staining was unnecessary to exclude CD19+ cells that nonspecifically bound to PDCA-1 or irrelevant isotype-matched Abs. To further confirm that PDCA-1+CD19− cells were pDCs, their expression of B220 and Ly6c was analyzed (Fig. 1D, 1E).

**FIGURE 1.** Identification of DC subpopulations from auricular lymph nodes. CD8+ DCs and CD11b+ DCs were identified as CD11c+ and CD90low (A) that were also CD8+ and CD11b+ (B), respectively. pDCs were identified as CD19+ and PDCA-1+ (C). To further confirm that the CD19+ and PDCA-1+ were pDCs, they were also stained for B220 (D) and Ly6c (E). B represents events that fall within the gate in A. D and E represent events that fall within the gate in C. Representative results of two similar independent experiments.
majority of this increase was due to CD90⁺ T cells and CD19⁺ B cells (Table I). Therefore, PGN injection not only drove the increase of CD8⁺ DCs, CD11b⁺ DCs, and pDCs, but also the increase in T cells and B cells within the lymph node. Interestingly, the number of CD8⁺ DC, T cells, and B cells, when comparing cell numbers between nodes draining ear pinnae of individual mice, increased by a mean of 2.2-, 2.6-, and 3.0-fold, respectively. This was very similar to the 2.7-fold increase in total lymph node cellularity. In contrast, the number of CD11b⁺ DCs and pDCs increased by 4.0- and 4.3-fold, respectively. This led us to consider the existence of different mechanisms for the recruitment of individual DC subsets.

Mast cells are required for the selective recruitment of pDC and CD8⁺ DC into the lymph node following the injection of PGN

Mast cells have been shown to be essential for the migration of Langerhans cells to the lymph node (5) in response to PGN. To determine the importance of mast cells in the accumulation of other DC subpopulations, we compared the numbers of CD8⁺ DCs, CD11b⁺ DCs, and pDCs in the lymph node following PGN injection in WT and mast cell-deficient c-kit⁺/− mice. The mast cell-deficient mice had a significant (p < 0.05) increase in the number of CD11b⁺ DCs and pDCs, but not in CD8⁺ DCs following PGN activation (Fig. 2A–C), suggesting a role for mast cells in the PGN-mediated accumulation of CD8⁺ DC. The increased number of pDCs following PGN treatment was significantly (p < 0.05) abrogated in the mast cell-deficient mice when compared with WT controls, suggesting a role for mast cells in the mobilization of this subset. The total number of cells in the lymph nodes that drained the sites of PGN injection was also significantly decreased in the mast cell-deficient mice (Fig. 2D) compared with WT mice.

To further confirm the role of mast cells in the recruitment of DCs into the lymph node, we locally reconstituted the mast cell-deficient mice prior to PGN activation. Mast cells were derived from the bone marrow of C57BL/6 mice and injected into one ear pinna, whereas the other ear was injected with diluent as a control. Ten to twelve weeks later, this resulted in mice that had one ear pinna with mature mast cells and the other without mast cells. PGN was injected in both ears, and 18 h later, the draining lymph nodes were harvested. The presence of mast cells significantly (p < 0.05) increased the number of CD8⁺ DCs and pDCs, but not CD11b⁺ DCs, in the draining lymph node in response to PGN (Fig. 2E–G). The presence of mast cells also significantly increased the total number of cells in the lymph node following PGN activation (Fig. 2H). Taken together, these data demonstrate that mast cells act to selectively promote the accumulation of CD8⁺ DCs, pDCs, and overall lymph node cellularity, but not CD11b⁺ DCs, in response to PGN activation.

PGN-mediated accumulation of CD11b⁺ DCs and CD8⁺ DCs in the lymph node is dependent on IL-6

To identify mast cell-derived protein mediators that could promote the recruitment of DC subpopulations in response to PGN, we analyzed the cytokine production profiles of PGN-activated mast cells compared with controls. BMMCs were activated with PGN and cytokine production determined by cytokine protein array. Twenty-four-hour stimulation led to 1096%, 85%, and 62% increases in IL-6, L-selectin, and eotaxin-2, respectively. The importance of IL-6 in PGN-mediated accumulation of DC subpopulations was therefore determined.

IL-6–deficient (IL-6−/−) and control WT (C57BL/6) mice were examined in parallel to evaluate the role of IL-6 in the accumulation of various DC subsets within the lymph node in response to

![FIGURE 2. Draining lymph node responses to PGN in mast cell-deficient mice. WT C57BL/6 mice and genetically mast cell-deficient c-kit⁺/− mice received a saline injection as a control in one ear pinna and PGN injection in the contralateral ear (A–D). After 18 h, draining lymph nodes were harvested and pDC (A, E), CD8⁺ DC (B, F), CD11b⁺ DC (C, G), and total cell (D, H) numbers were determined. In separate experiments, c-kit⁺/− mice had one ear pinna reconstituted with mast cells 10–12 wk prior to the injection of both ears with PGN (E–H). Bars represent mean ± SEM per draining lymph node. *p < 0.05; **p < 0.01; ***p < 0.001. n = 10–15 from three independent experiments.](http://www.jimmunol.org/)

---

**Table I. Lymph node responses to PGN administration in WT C57Bl/6 mice**

<table>
<thead>
<tr>
<th></th>
<th>Saline (× 10⁶)</th>
<th>PGN (× 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pDC</td>
<td>1.4 ± 0.16</td>
<td>4.7 ± 0.60³</td>
</tr>
<tr>
<td>CD8⁺ DC</td>
<td>0.75 ± 0.07</td>
<td>1.2 ± 0.11³</td>
</tr>
<tr>
<td>CD11b⁺ DC</td>
<td>2.8 ± 0.37</td>
<td>7.4 ± 0.56³</td>
</tr>
<tr>
<td>T cells</td>
<td>1.0 ± 0.11</td>
<td>2.2 ± 0.24³</td>
</tr>
<tr>
<td>B cells</td>
<td>1.7 ± 0.17</td>
<td>4.0 ± 0.38³</td>
</tr>
<tr>
<td>LN cells</td>
<td>3.4 ± 0.24</td>
<td>7.5 ± 0.47³</td>
</tr>
</tbody>
</table>

*Mean number of cells per lymph node ± SE based on 20 mice from four independent experiments.

Denotes a significant increase (p < 0.001) compared with saline control.
PGN activation. In the IL-6−/− mice, injection of PGN into the ear pinna led to a significant (p < 0.05) increase in CD11b+ DCs and pDCs, but not CD8+ DCs (Fig. 3A–C) in the draining lymph node, suggesting that IL-6 plays a selective role in the recruitment of CD8+ DCs into the lymph node. The CD11b+ DC response was also significantly (p < 0.01) lower in IL-6−/− than in WT mice. Both the WT and IL-6−/− mice showed a significant increase in the total number of lymph node cells in response to PGN, but there were significantly (p < 0.05) fewer cells in the lymph node draining the site of PGN activation (Fig. 3D) in IL-6−/− mice compared with WT. These data indicate that IL-6 has an important role in the accumulation of CD11b+ DCs and CD8+ DCs in response to bacterial PGN, as well as a role in regulating changes in overall lymph node cellularity.

TNF and IL-1β have been shown to be important mast cell-derived cytokines that mediate the trafficking of Langerhans cells into the lymph node (5–9). We evaluated the role of these cytokines in the recruitment of individual DC subpopulations into the lymph node in response to PGN. The number of each DC subpopulation as well as total lymph node cellularity after PGN injection in TNF−/− or IL-1R−/− and WT mice was not significantly different (Table II), suggesting that these cytokines are not individually required for the PGN-mediated influx of cells into the lymph node. TLR2 has been implicated as a pattern recognition receptor in responses to PGN (29) and is expressed by mast cells (30). We used TLR2−/− mice to evaluate the role of this molecule in the lymph node response to PGN injection. The absence of TLR2 did not inhibit either the increase in total lymph node cellularity or the increase of any of the DC subsets we examined (Table II). In keeping with previous reports (5), some enhancement of lymph node responses to PGN was observed in TLR2-deficient animals.

PGs, cysLT1 receptor, and mast cell degranulation are dispensable for PGN-induced accumulation of DC in the lymph node

Mast cell activation can lead to the production of several arachidonic acid metabolites including cysLTs and PGs. These lipid mediators have been shown to promote DC activation and migration (10–14). Mice were treated with the nonselective COX-1 and COX-2 inhibitors naproxen or montelukast to prevent the synthesis of PGs (27). Mice treated with naproxen did not have a significant reduction in the PGN-mediated accumulation of CD8+ DC, CD11b+ DC, and pDC (Table III), suggesting PG production is dispensable for the accumulation of these DC subpopulations. Separate groups of mice were treated with montelukast, which blocks the cysLT1 receptor, to evaluate the role of cysLT1 in DC accumulation within the lymph node. Montelukast treatment (28) failed to decrease the PGN-mediated accumulation of DC subpopulations within the lymph node (Table III), suggesting that cysLT1 receptor binding is not essential for this process. Neither naproxen nor montelukast had any significant impact on PGN-induced changes in total lymph node cellularity. Disodium cromoglycate is a widely used mast cell stabilizing agent that we employed to evaluate the importance of mast cell degranulation in lymph node responses to PGN. Disodium cromoglycate pretreatment of the mice did not reduce the lymph node cellularity response or the responses of DC subsets, with the exception of the CD8+ DC (Table III).

Histamine, through the binding to the H2 receptor, promotes PGN-induced accumulation of DC subsets in the lymph node

Mast cells activated in vivo with PGN undergo degranulation and therefore release histamine (31). Histamine can affect Langerhans cell migration into the lymph node (4), so we reasoned that it might be involved in the mast cell-mediated accumulation of other DC subsets within the lymph node. To determine if histamine was an important mediator in the lymph node response to PGN, we blocked histamine binding to its receptors H1 or H2 with pyrilamine or ranitidine, respectively. The pretreatment of mice with pyrilamine (26) resulted in no significant differences in any of the DC subpopulations examined (Fig. 4A–C) or lymph node cellularity (Fig. 4D) compared with PBS-treated mice. H2 receptor blockade with ranitidine resulted in significant (p < 0.05) inhibition of PGN-mediated pDC and CD11b+ DC accumulation (Fig. 5A, 5C), without affecting CD8+ DC or total lymph node cellularity (Fig. 5B, 5D). Together, this suggests that the selective increases of CD11b+ DCs and pDCs induced by PGN, but not overall changes in lymph node cellularity, are mediated by histamine binding to the H2 receptor.

pDC and CD8+ DC responses in mast cell-deficient animals are not further inhibited by ranitidine treatment

To examine the mechanism of DC responses in mast cell-deficient mice and the source of histamine mediating such responses, we examined the ability of ranitidine to further inhibit DC responses in c-ki-Wsh/Wsh mice. None of the evaluated DC responses were significantly inhibited by ranitidine treatment in mast cell-deficient animals. In ranitidine-treated mast cell-deficient mice, PGN injection increased the number of CD8+ DCs and pDCs from 1.4 × 104 to 1.9 × 104 and 1.2 × 104 to 3.2 × 104, respectively, whereas in (control) PBS-treated mice, the number of CD8+ DCs and pDCs increased from a mean of 9.9 × 103 to 1.6 × 104 and from 1.2 × 103 to 3.3 × 103 DCs per node, respectively. The CD11b+ DC response to PGN, which was significantly inhibited by ranitidine treatment in control mast cell-containing animals (Fig. 5; p < 0.05), remained intact when the experiments were performed in mast cell-deficient mice (CD11b+ DC numbers per node; ranitidine-treated mast cell-deficient animals, saline control node 3.8 × 104, PGN-treated node 9.2 × 104). Diluent-treated mast cell-deficient animals saline control node 4.1 × 104, PGN treated node 10.7 × 104).

To explore the potential role of basophils as a source of histamine in this model, we cultured murine basophils from bone marrow according to established protocols. These basophils were activated for 30 min with PGN in the presence of 1% normal mouse plasma as a source of complement. PGN treatment increased histamine release from basophils by a mean of 19 ± 1.9% above baseline, demonstrating the potential of this cell type to contribute to histamine-dependent responses to PGN.

Histamine H2 receptor blockade does not block CXCL9 production

pDC migration into an inflamed lymph node has been shown to be dependent on β1- and β2-integrins, CXCL9, and E-selectin (32–34). Mast cells produce multiple chemokines and release mediators such as...
as histamine that can induce chemokine production by other cells. We therefore reasoned that mast cell- and histamine-dependent modulation of CXCL9 following PGN activation could explain the mast cell and H2 receptor dependence of pDC recruitment (Figs. 2A, 2E, 5A). To determine if H2 receptor blockade decreased CXCL9 expression in the lymph node, the effect of ranitidine treatment of animals on CXCL9 content in the lymph node was determined. PGN activation led to an increase of CXCL9 in the lymph node at 8 h and 18 h. However, ranitidine did not alter CXCL9 levels in the lymph node (Fig. 6), with or without PGN administration. Therefore, histamine binding to H2 receptor is contributing to pDC recruitment in a CXCL9-independent manner. To further explore the potential role of mast cell-derived CXCL9, we activated BMMC for 6 and 24 h with a range of doses of PGN. No detectable CXCL9 production by mast cells was observed under these conditions (limit of detection, 4 pg/ml; data not shown).

**Discussion**

The presented results demonstrate that mast cells are critical for the efficient recruitment of blood-derived DCs, T cells, and B cells into the lymph node in response to PGN. Examination of the mediators involved revealed distinct pathways that lead to the accumulation of individual cell types. IL-6 was required for the influx of CD8⁺ DCs, CD11b⁺ DCs, T cells, and B cells, but not pDCs, which were distinctly regulated by a histamine H2 receptor-dependent pathway. These findings represent the first demonstration that the recruitment of individual DC subpopulations to the lymph node can be uncoupled from each other as well as from total lymph node cellularity.

PGN administration yields a complex, complement-mediated inflammatory response. Some complement products generated in the context of PGN, such as C3a and C5a (35–37), can induce mast cell degranulation, leading to the release of histamine. Multiple other receptor systems participate in the response to PGN (17–19, 29, 38, 39), allowing PGN to mimic many aspects of the complex response to Gram-positive infection. There is a high level of redundancy in the mechanisms whereby PGN activates early immunity. Dependence of DC subset mobilization and lymph node hypertrophy on IL-6 and histamine receptors indicates a critical role for these molecules within this complex process.

Table II. Draining lymph node cellularity and DC responses to PGN in the absence of TNF, IL-1R, or TLR2

<table>
<thead>
<tr>
<th></th>
<th>pDC (× 10⁶)</th>
<th>CD8⁺ DC (× 10⁶)</th>
<th>CD11b⁺ DC (× 10⁶)</th>
<th>LN Cells (× 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>PGN</td>
<td>Saline</td>
<td>PGN</td>
</tr>
<tr>
<td>WT</td>
<td>1.4 ± 0.26</td>
<td>3.5 ± 0.67</td>
<td>1.1 ± 0.17</td>
<td>1.5 ± 0.22</td>
</tr>
<tr>
<td>TNF⁻/⁻</td>
<td>1.3 ± 0.15</td>
<td>4.0 ± 0.11</td>
<td>1.1 ± 0.18</td>
<td>1.6 ± 0.40</td>
</tr>
<tr>
<td>WT</td>
<td>1.4 ± 0.28</td>
<td>4.6 ± 0.82</td>
<td>0.66 ± 0.11</td>
<td>1.3 ± 0.16</td>
</tr>
<tr>
<td>IL-1R⁻/⁻</td>
<td>1.2 ± 0.13</td>
<td>6.2 ± 1.2</td>
<td>1.0 ± 0.13</td>
<td>1.5 ± 0.17</td>
</tr>
<tr>
<td>WT</td>
<td>1.3 ± 0.14</td>
<td>4.0 ± 0.72</td>
<td>0.86 ± 0.11</td>
<td>1.2 ± 0.19</td>
</tr>
<tr>
<td>TLR2⁻/⁻</td>
<td>1.2 ± 0.21</td>
<td>10.6 ± 3.6</td>
<td>0.88 ± 0.15</td>
<td>1.5 ± 0.32</td>
</tr>
</tbody>
</table>

Data are mean number of cells per lymph node ± SE based on 10–15 mice/group from two to three independent experiments.

Table III. Contribution of cysLT1 and COX to lymph node responses following PGN injection

<table>
<thead>
<tr>
<th></th>
<th>pDC (× 10⁶)</th>
<th>CD8⁺ DC (× 10⁶)</th>
<th>CD11b⁺ DC (× 10⁶)</th>
<th>Total LN Cells (× 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>PGN</td>
<td>Saline</td>
<td>PGN</td>
</tr>
<tr>
<td>PBS</td>
<td>1.1 ± 0.1</td>
<td>2.1 ± 0.29</td>
<td>0.64 ± 0.08</td>
<td>1.3 ± 0.23</td>
</tr>
<tr>
<td>Montelukast</td>
<td>1.0 ± 0.11</td>
<td>2.2 ± 0.21</td>
<td>0.83 ± 0.09</td>
<td>1.7 ± 0.17</td>
</tr>
<tr>
<td>PBS</td>
<td>1.7 ± 0.26</td>
<td>7.2 ± 1.5</td>
<td>1.2 ± 0.16</td>
<td>2.0 ± 0.26</td>
</tr>
<tr>
<td>Naproxen</td>
<td>1.4 ± 0.26</td>
<td>9.4 ± 2.2</td>
<td>1.3 ± 0.24</td>
<td>2.2 ± 0.34</td>
</tr>
<tr>
<td>PBS</td>
<td>1.2 ± 0.19</td>
<td>9.7 ± 2.2</td>
<td>0.93 ± 0.11</td>
<td>1.8 ± 0.17</td>
</tr>
<tr>
<td>Cromolyn</td>
<td>1.2 ± 0.17</td>
<td>6.4 ± 1.0</td>
<td>0.86 ± 0.10</td>
<td>1.3 ± 0.11</td>
</tr>
</tbody>
</table>

Data are mean number of cells per lymph node ± SE based on 10 mice/group from two independent experiments.

Maest cells have been widely implicated in the process of DC mobilization from the periphery, based largely on studies of Langerhans cells (4, 5). Our findings demonstrate, for the first time, that mast cells can promote the PGN-induced increase in lymph node-resident pDCs and CD8⁺ DCs. Mast cell reconstitution studies confirmed a role for mast cells in directing changes in overall lymph node cellularity as well as the mobilization of pDCs and CD8⁺DCs. However, the CD11b⁺ DC response to PGN is mast cell independent. In the absence of PGN treatment, the numbers of each of the DC subsets examined and overall lymph node cellularity were similar in both mast cell-containing and mast cell-deficient animals. When mast cells were locally reconstituted in the skin of c-kit<sup>Wash/wash</sup> mice, this also did not significantly enhance overall lymph node cellularity compared with nonreconstituted sites in the absence of PGN treatment (data not shown). Taken together, these data suggest that the activation of mast cells by pathogen products is important for the lymph node accumulation of DC subpopulations from the blood.

The absence of mast cells significantly decreased, but did not completely abolish, the DC responses to PGN. Therefore, other cell types are likely responding directly or indirectly to PGN. The sensitivity of the PGN-mediated accumulation of CD11b⁺ DC to histamine blockade, but not to the absence of mast cells, might be explained by the production of histamine by other cell sources. Basophils are a well-recognized source of histamine in mammals. The ability of murine basophils to release histamine in vitro in response to PGN makes them a potential non-mast cell source of this mediator in the context of our current study. In addition, neutrophils and macrophages can also be a source of histamine (40, 41). Because the CD11b⁺ DC subset includes DCs that are entering the lymph node from the skin, these would likely have been exposed to histamine from a variety of cell sources at the site of PGN administration and would therefore not necessarily require mast cell-derived histamine. An alternate interpretation of our findings is that small amounts of histamine released from the low levels of mast cells that remain in the skin of mast cell-deficient mice (42) are sufficient to modulate CD11b⁺ DC, whereas other DC subsets require larger amounts of mast cell-derived histamine.

Infection leads to the recruitment of pDCs and CD8⁺ DCs into the draining lymph node from the blood (32–34, 43). The influx of pDC is dependent on β<sub>1</sub>- and β<sub>2</sub>-integrins, CXCL9 and E-selectin...
Figure 4. Contribution of histamine receptor H1 to PGN-mediated DC accumulation within the draining lymph node. C57BL/6 mice were injected with pyrilamine or PBS 90 min prior to PGN activation. Both groups of mice received a single injection of saline in one ear pinna and PGN in the other. After 18 h, draining lymph nodes were harvested and pDC (A), CD8⁺ DC (B), CD11b⁺ DC (C), and total cell (D) numbers were determined. Bars represent mean ± SEM per draining lymph node. *p < 0.05; **p < 0.01; ***p < 0.001. n = 10 from two independent experiments.

Figure 5. Contribution of histamine receptor H2 to PGN-mediated DC accumulation within the draining lymph node. WT C57/BL6 mice were injected with ranitidine or PBS 90 min prior to PGN activation. Both groups of mice received a single injection of saline in one ear pinna and PGN in the other. After 18 h, draining lymph nodes were harvested and pDC (A), CD8⁺ DC (B), CD11b⁺ DC (C), and total cell (D) numbers were determined. Bars represent mean ± SEM per draining lymph node. *p < 0.05; **p < 0.01; ***p < 0.001. n = 15 from three independent experiments.

Histamine, by binding to H2 receptor, dampens IFN-γ production, and Th1 priming ability, whereas H2 receptor has a suppressive effect on Th1 production, enhances IL-10, and promotes the development of IL-10-producing T cells and Th2 cells (51–53). Histamine, by binding to H2 receptor, dampens IFN-γ and TNF production in pDCs (50). Our study reveals that histamine, through the H2 receptor, also enhances the number of pDCs and CD11b⁺ DCs in the lymph node in response to a major pathogen product. H2 receptor blockade increases the susceptibility to gastrointestinal bacterial and parasitic infection due to the increase in stomach pH (54). Our results add to the growing number of reports demonstrating additional immunomodulatory effects of H2 receptor blockade. The lack of effect of ranitidine treatment on DC responses in mast cell-deficient mice confirms the importance of the mast cell as a source of histamine for pDC responses. In addition, this also suggests that the modest histamine-dependent component of the CD11b⁺ DC response in mast cell-containing mice may be partly mast cell-dependent, although the majority of the CD11b⁺ DC response to PGN response is mast cell independent. Alternatively, there may be compensatory mechanisms present in e.g., mice deficient in the recruitment of specific DC subsets, T cells, and B cells can be uncoupled. The absence of mast cells dampens the accumulation of CD8⁺ DCs, pDCs, T cells, and B cells, but not CD11b⁺ DCs. Blockade of histamine receptor H2 can selectively decrease the number of CD11b⁺ DCs and pDCs without significantly affecting the recruitment of specific DC subsets, T cells, and B cells.

Figure 6. Contribution of histamine receptor H2 to PGN-mediated CXCL9 production in the draining lymph node. WT C57/Bl6 mice were injected with ranitidine or PBS 90 min prior to PGN activation. Both groups of mice received a single injection of saline in one ear pinna and PGN in the other. After 8 or 18 h, draining lymph nodes were harvested, weighed, and sonicated, and the amount of CXCL9 in the sonicate was measured by ELISA. Bars represent mean ± SEM CXCL9 per mg of lymph node of three independent experiments consisting of three animals per condition. *p < 0.05.
CD8+ DCs, total lymph node cellularity, or the level of CXCL9. This therefore reveals a novel pathway for the control of pDC and CD11b- DC influx into the lymph node. In comparison, IL-6 deficiency decreases the influx of CD8+ DCs, CD11b+ DCs, T cells, and B cells without affecting pDC accumulation. Taken together, these observations demonstrate that individual DC subpopulations in the lymph node can be selectively modulated by changes in the mediator milieu. Modifying the mobilization of selected DC subsets will provide opportunities to better examine their roles in the regulation of T cell immunity, provide opportunities for vaccine adjuvant development, and allow novel approaches for modulation or prevention of T cell-mediated disease.

Acknowledgments

We thank Dr. Robert Liwski and Dr. Ian Haidl for critical review of this manuscript and Yisong Wei for excellent technical assistance.

Disclosures

The authors have no financial conflicts of interest.

References


