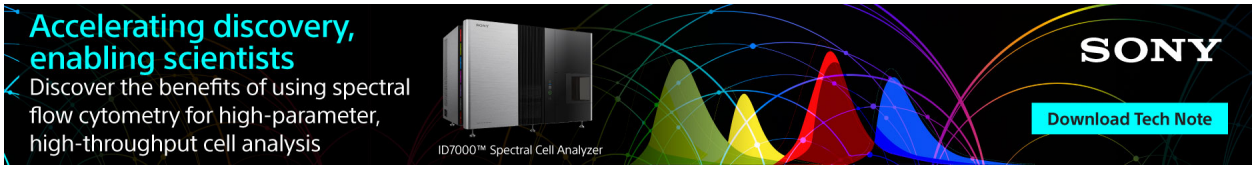

The Journal of Immunology

This information is current as of August 9, 2022.

Cutting Edge: Distinct Toll-Like Receptor 2 Activators Selectively Induce Different Classes of Mediator Production from Human Mast Cells

Jeffrey D. McCurdy, Timothy J. Olynych, Lauren H. Maher and Jean S. Marshall

J Immunol 2003; 170:1625-1629; ;
doi: 10.4049/jimmunol.170.4.1625
http://www.jimmunol.org/content/170/4/1625

References This article **cites 27 articles**, 13 of which you can access for free at:
http://www.jimmunol.org/content/170/4/1625.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2003 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Downloaded from http://www.jimmunol.org/ by guest on August 9, 2022

THE JOURNAL OF IMMUNOLOGY

CUTTING EDGE

Cutting Edge: Distinct Toll-Like Receptor 2 Activators Selectively Induce Different Classes of Mediator Production from Human Mast Cells[1,2]

Jeffrey D. McCurdy, Timothy J. Olynych,* Lauren H. Maher,* and Jean S. Marshall[3]*†*

***Mast cells play a critical role in host defense against bacterial infection. Murine mast cells produce cytokines in response to bacterial peptidoglycan and LPS via Toll-like receptor (TLR) TLR2- and TLR4-dependent mechanisms. The expression of TLRs by human mast cells and responses to known TLR activators was examined. Human mast cells expressed mRNA for TLR1, TLR2, and TLR6 but not TLR4. Bacterial peptidoglycan and yeast zymosan were potent inducers of GM-CSF and IL-1β and also induced substantial short-term cysteinyl leukotriene generation. In contrast, a synthetic triacylated lipopeptide induced short-term degranulation but failed to induce cysteinyl leukotriene production. The TLR4 activator* Escherichia coli *LPS did not induce a GM-CSF, IL-1β leukotriene, or degranulation response. These data demonstrate highly selective production of different classes of mast cell mediators in response to distinct TLR activators of potential importance to the host response to bacterial or fungal pathogens. The Journal of Immunology,* 2003, 170: 1625–1629.**

Mast cells have long been known to contribute to allergic diseases and chronic inflammatory processes and, more recently, have been shown to play an important role in host defense against bacterial infections (1–4). Mast cells are well equipped to participate in these reactions based on their strategic tissue distribution and ability to selectively produce a wide range of proinflammatory mediators. Mast cells are a potent source of preformed, granule-associated mediators, newly synthesized lipid mediators, as well as a large number of cytokines and chemokines. Human mast cells produce the NF-κB-regulated cytokines, GM-CSF and IL-1β, which have been implicated in the mobilization, recruitment, and enhanced survival of effector cells, such as neutrophils, at the site of infections (5). Cysteinyl leukotriene production by mast cells is of particular interest because of their potent bronchoconstrictive and chemotactic effects (6).

Toll-like receptors (TLR)[4] are a family of pattern recognition receptors known to play an important role in host defense (reviewed in Ref. 7): TLR2 is critical for responses to bacterial-derived lipopeptides (8–10) and peptidoglycan (PGN) (11, 12) as well as zymosan (13), the cell wall component of yeast, while TLR4 is the major signaling molecule for most types of LPS (7). Ligand specificity for a number of TLR2 activators is thought to require heterodimerization with additional TLR molecules, TLR1 and TLR6 (14, 15). Studies suggest that TLR2/TLR6 heterodimers mediate responses to PGN or zymosan, while TLR1/TLR2 heterodimers mediate responses to the synthetic tripalmitoyl lipopeptide $Pam_3Cys\text{-}Ser\text{-}(Lys)_4$ (Pam_3Cys) in the mouse (14, 15). Distinct TLR molecules are capable of inducing differential cellular responses (16, 17). Rodent mast cells as well as murine mast cell lines produce several cytokines, including IL-6 and TNF-α, in response to activation with the TLR activators LPS and PGN (18–21). However, human mast cell responses to TLR activators are poorly defined.

In the current study, human mast cells are demonstrated to express a distinct profile of TLRs compared with murine mast cells and other leukocytes. Differences in TLR expression are reflected by selective mast cell stimulation by TLR activators. Furthermore, distinct TLR2 activators are shown to induce differential mediator production including, the novel selective induction of substantial cysteinyl leukotriene generation and cytokine production without evidence of classical degranulation.

Materials and Methods

Reagents

Escherichia coli LPS (serotype O55:B5), zymosan from *Saccharomyces cerevisiae*, and PGN, purified from *Staphylococcus aureus*, were purchased from Sigma (St. Louis, MO). *S. aureus* PGN contained <0.0025 ng/μl endotoxin as assessed using the *Limulus* amebocyte lysate test from Sigma. Synthetic lipopeptide ($Pam_3Cys\ SerLys_4$) was obtained from Prof. Dr. G. Jung University of Tubingen (Tubingen, Germany).

Departments of *Microbiology and Immunology and †Pathology, Dalhousie University, Halifax, Nova Scotia, Canada

Received for publication August 26, 2002. Accepted for publication December 11, 2002.

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

[1] This work was supported by the Canadian Institutes for Health Research.

[2] A portion of this work has been presented in abstract form by J. McCurdy and J. S. Marshall at "Experimental Biology 2002", New Orleans, LA, April 20–24, 2002.

[3] Address correspondence and reprint requests to Dr. Jean S. Marshall, Department of Pathology, Sir Charles Tupper Medical Building, Dalhousie University, Halifax, Nova Scotia, B3H 4H7 Canada. E-mail address: Jean.Marshall@Dal.ca.

[4] Abbreviations used in this paper: TLR, Toll-like receptor; PGN, peptidoglycan; CBMC, cord blood-derived mast cell; MyD88, myeloid differentiation factor 88; BMMC, bone marrow-derived mast cell.

Copyright © 2003 by The American Association of Immunologists, Inc.

0022-1767/03/$02.00

Downloaded from http://www.jimmunol.org/ by guest on August 9, 2022

Mast cells

Cord blood-derived mast cells (CBMC) were obtained by long-term culture of cord blood progenitor cells as previously described (22), using a modification of the method described by Iikura et al. (23). Following 5–8 wk, mast cells were determined by morphologically and by the presence of metachromatic granules (toluidine staining). Only >95% of pure mast cells was used in experiments except for short-term β-hexosaminidase release studies where >90% mast cell purity was sometimes used. Representative CBMC cultures were also analyzed by flow cytometry for the mast cell marker c-*kit* and the monocyte marker CD14. Less than 2% of the cells expressed CD14 ($n = 6$) while >95% of the cells expressed c-*kit* ($n = 8$). Mast cells were placed in mast cell growth medium devoid of PGE_2 for >16 h before use.

The human basophilic/mast cell line KU812 (24) was grown in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS (Life Technologies).

Cytokine analysis

IL-1β, IL-6, RANTES, and GM-CSF activation with various TLR activators were examined in supernatants harvested after 24 h, from CBMC cells following activation with various TLR activators. Cytokines were analyzed via "in-house" ELISA as previously described (22). Sensitivity of the IL-6 and IL-1β assay was 1.95 pg/ml, that of RANTES was 30 pg/ml, and that of GM-CSF was 3 pg/ml.

RT-PCR

Total RNA was isolated using TRIzol reagent (Life Technologies). RNA samples were treated with DNase I (Life Technologies). Primers used for PCR amplification of reverse-transcribed RNA samples for MD-2, myeloid differentiation factor 88 (MyD88), β-actin, TLR1, TLR2, TLR4, and TLR6 were purchased from Research Genetics (Huntsville, AL), and sequences were as follows: 1) human MD-2: sense, 5′-GCACTCATCCGATGCAAGT-3′; antisense, 5′-GTTGTATTGACAGTCTCTCC-3′; 2) MyD88: sense, 5′-GGGAGGAGATGGACTTTGAGT-3′; antisense, 5′-GCAATAGACCAGACACAGGT-3′; 3) TLR1: sense, 5′-TTCTGGCACTTCCTTGAAGG-3′; antisense, 5′-GTCTCCAACTCAGTAAGGTG-3′; 4) TLR2: sense, 5′-GCCAAAGTCTTGATTGATTGG-3′; antisense, 5′-TTGAAGTTCTCCAGCTCCTG-3′; 5) TLR4: sense, 5′-TGGATACGTTTCCTTATAAG-3′; antisense, 5′-GAAATGGAGGCACCCCTTC-3′; and 6) TLR6: sense, 5′-ATCAGAACTCACCAGAGGTC-3′; antisense, 5′-CATGAGGACACAGCATGTGT-3′. Thirty-four PCR cycles were used.

Short-term mediator release and β-hexosaminidase assay

CBMC (0.5×10^6/ml) in modified HEPES-Tyrode's buffer, supplemented with 1% FBS as a source of soluble CD14 and LPS-binding protein, were activated with the positive control A23187 (0.5 mM) or various TLR activators. After a 20-min incubation, supernatants and pellet fractions were each analyzed for β-hexosaminidase according to the method of Schwartz et al. (25).

Cysteinyl leukotriene assay

CBMC (1×10^6/ml) were incubated for a predetermined optimal time of 20 min at 37°C with activating agents or controls in RPMI 1640 medium supplemented with 1% FCS and 10 ng/ml stem cell factor. Supernatants were harvested by centrifugation, snap frozen, and later analyzed by an enzyme immunoassay using a commercial kit (Amersham, Montreal, Quebec, Canada), which detected cysteinyl leukotrienes including leukotriene C_4 and its degradation products D_4 and E_4. The sensitivity of this assay was <30 pg/ml.

Immunohistochemistry

Nasal polyp tissues were obtained, after informed consent, from subjects requiring routine polypectomy. Only tissues from subjects who had not received topical steroid therapy in the 3 wk before surgery were used. Frozen tissue sections (5 μm) were stained with Alcian blue (pH 1.0). Endogenous peroxidase activity was quenched by treatment with 0.5% H_2O_2. Slides were then blocked with normal human serum and stained using anti-TLR2 polyclonal antisera (Santa Cruz Biotechnology, Santa Cruz, CA) or normal goat IgG at the same dilution (DAKO, Carpinteria, CA). Ab binding was detected using biotinylated swine anti-goat Ab (Cedarlane Laboratories, Hornby, Ontario, Canada) and a commercial development system.

Statistics

Comparison of data sets was performed using ANOVA and Dunnett's post-test.

Results and Discussion

TLR mRNA expression by CBMC

It has been previously reported that murine mast cells express mRNA for a number of TLRs including: TLR2, TLR4, TLR6, and TLR8 (18, 20). To determine the human mast cell expression of TLRs, CBMC and the mast cell/basophil line KU812 were analyzed for TLR mRNA expression by RT-PCR. Prominent PCR products of the appropriate size were expressed for TLR1, TLR2, and TLR6 but not TLR4 by both CBMC and KU812 (Fig. 1*A*). In comparison, the human macrophage cell line U937 expressed bands for all TLRs examined, including TLR4. All cells examined expressed mRNA for the adapter molecules MyD88 and MD-2. Immunohistochemical analysis was used to assess the protein expression of TLR2 by human tissue mast cells. The majority of nasal polyp human mast cells, staining positive with Alcian blue, at low pH, also expressed low levels of TLR2 (Fig. 1, *B* and *C*). TLR2-positive mast cells were also observed in human lung tissue (data not shown). The staining pattern for TLR2 was consistent with surface expression on mast cells.

These data demonstrate that human mast cells express a unique profile of TLRs including TLR2 and TLR6, which may suggest a specialized role for in host defense. The lack of TLR4

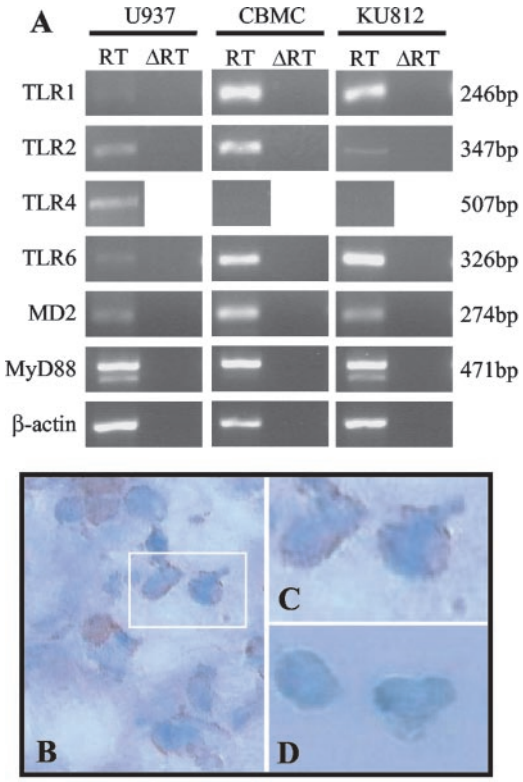

FIGURE 1. Human mast cell TLR expression. *A,* RT-PCR analysis of human mast cell TLRs. The reverse transcription step was omitted in some samples (ΔRT) as a control where primers did not span an intron. RNA isolated from the human macrophage cell line U937 was used as a positive control. Note the strong expression of TLR1 and TLR6 and lack of TLR4. *B–D,* Immunohistochemical analysis of human mast cell TLR2 expression. Alcian blue-stained nasal polyp frozen tissue sections were further stained with anti-TLR2 Ab (note red color) (*B* and *C*), or normal goat IgG (*D*). The data shown are representative of three independent experiments for RT-PCR and two independent experiments for immunohistochemistry.

Downloaded from http://www.jimmunol.org/ by guest on August 9, 2022

expression provides an additional marker to distinguish human mast cells from other myeloid cells.

Selective stimulation of CBMC with TLR2 activators

The responses of human mast cells to a number of classic TLR activators were examined. In initial experiments, CBMC (>95% pure) were stimulated with various TLR activators including: *S. aureus* PGN or *E. coli* LPS. When stimulated with the TLR2 activator *S. aureus* PGN (11, 12) for 24 h, CBMC produced significant levels of GM-CSF (Fig. 2*A*) and IL-1β (Fig. 2*B*) in a dose-dependent manner. Levels of GM-CSF were 17- to 85-fold higher than unstimulated control cells when CBMC were stimulated with 10 μg/ml PGN (n = 15 CBMC donors). PGN induced substantially higher levels of GM-CSF than IgE-mediated activation (PGN, 5075 $\pm$ 1260% medium control n = 4 and IgE mediated, 350 $\pm$ 191% med control n = 4). These cytokines were not detected in sonicated CBMC samples or in the supernatant following 30-min activation with PGN, suggesting that CBMC do not contain or release substantial preformed stores (data not shown). Levels of GM-CSF remained low after a 6-h incubation (41 $\pm$ 4.9 pg/ml, n = 2) and increased in a time-dependent manner with 430 $\pm$ 39 pg/ml (n = 2) GM-CSF produced following a 12-h incubation. CBMC from six donors did not show a significant IL-6 response to PGN when considered as a group, although cells from some individual donors exhibited a marginal response. In contrast, production of RANTES was consistently enhanced following activation with PGN (mean, 542 $\pm$ 128 pg/ml following treatment with 10 μg/ml PGN compared with 163 $\pm$ 52 pg/ml unstimulated controls, n = 4).

Using conditions shown to be effective for TLR-mediated activation of murine bone marrow-derived mast cells (18), CBMC were activated with a high dose of the known TLR4 activator *E. coli* LPS (5 μg/ml). LPS treatment did not significantly increase GM-CSF (Fig. 2*C*) or IL-1β (Fig. 2*D*) production. The levels of cytokines produced following LPS challenge did not exceed a 2-fold increase compared with unstimulated control cells (n > 4; Fig. 2, *C* and *D*).

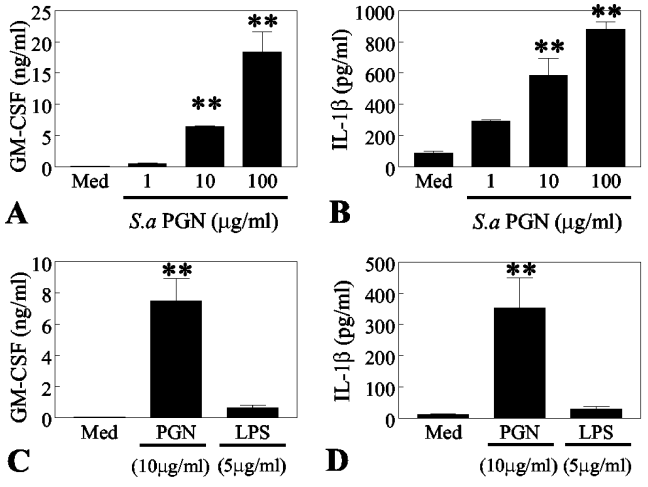

FIGURE 2. GM-CSF and IL-1β production by CBMC in response to TLR activators. CBMC (>95% pure) were stimulated with *S. aureus* PGN (*A* and *B*) or *E. coli* LPS (*C* and *D*) for 24 h. Cell-free supernatants were analyzed for GM-CSF (*A* and *C*) or IL-1β (*B* and *D*) by ELISA. **, $p < 0.01$ compared with medium control. Constitutive cytokine production varied between donors; therefore, representative experiments from CBMC from separate donors are presented. Experiments were conducted in triplicate and expressed as mean values $\pm$ SD. Results are representative of those obtained from six separate donors for PGN activation and three donors for Pam_3Cys.

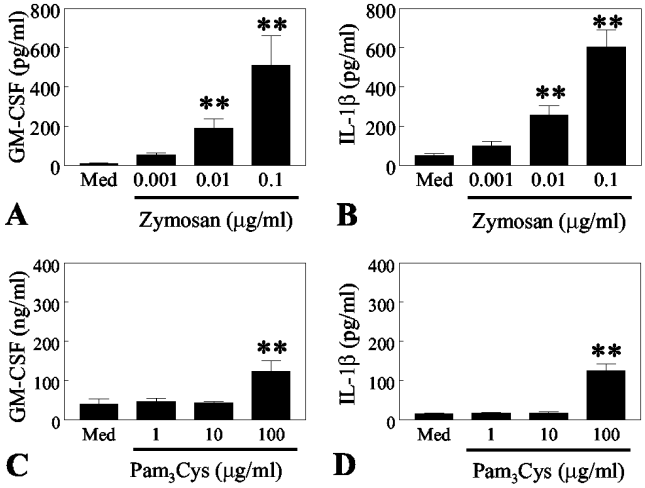

FIGURE 3. GM-CSF and IL-1β production by CBMC in response to TLR activators. CBMC (>95% pure) were stimulated with zymosan (*A* and *B*) or Pam_3Cys (*C* and *D*) for 24 h. Cell-free supernatants were analyzed for GM-CSF (*A* and *C*) or IL-1β (*B* and *D*) by ELISA. **, $p < 0.01$ compared with medium control. Experiments were conducted in triplicate and expressed as mean values $\pm$ SD. Results are representative of those obtained from five donors for zymosan activation and three donors for Pam_3Cys.

The selective responsiveness of CBMC to the TLR2 activator (PGN) but not to the TLR4 activator *E. coli* LPS is consistent with the TLR expression pattern of human mast cells (TLR2 but not TLR4). These findings are notably different from the rodent system whereby bone marrow-derived mast cells were shown to express mRNA for both TLR2 and TLR4 and to respond to both PGN and LPS (18, 20). These results suggest that studies of murine mast cell responses to pathogen products may not be good predictors of human mast cell function.

Differential CBMC mediator production by TLR2 activators

CBMC were activated with either the putativeTLR2/TLR6 activators, PGN, and zymosan or the putative TLR2/TLR1 activator Pam_3Cys and analyzed for the short-term release of classical preformed mediators, cytokine production, and cysteinyl leukotriene generation. Stimulation of CBMC for 24 h with zymosan resulted in the production of significant levels of GM-CSF (Fig. 3*A*) and IL-β (Fig. 3*B*) in a dose-dependent manner. Significant cytokine production was observed with doses of zymosan as low as 0.01% (w/v) in all CBMC donors examined, with GM-CSF production at least 15-fold higher than in unstimulated control cells (n = 4). This profile of cytokines is consistent with that produced by CBMC following activation with PGN. Moreover, the levels of GM-CSF and IL-1β produced by CBMC were similar to those produced by freshly isolated peripheral blood leukocytes treated with these activators (data not shown). Stimulation of CBMC with Pam_3Cys resulted in a marginal increase in GM-CSF (Fig. 3*C*) and IL-1β (Fig. 3*D*) at the highest dose of Pam_3Cys examined (100 μg/ml).

CBMC were analyzed for cysteinyl leukotriene production after stimulation for 20 min (optimal) with TLR activators. The TLR2/TLR6 activators PGN and zymosan were potent inducers of cysteinyl leukotriene production whereas Pam_3Cys was

Downloaded from http://www.jimmunol.org/ by guest on August 9, 2022

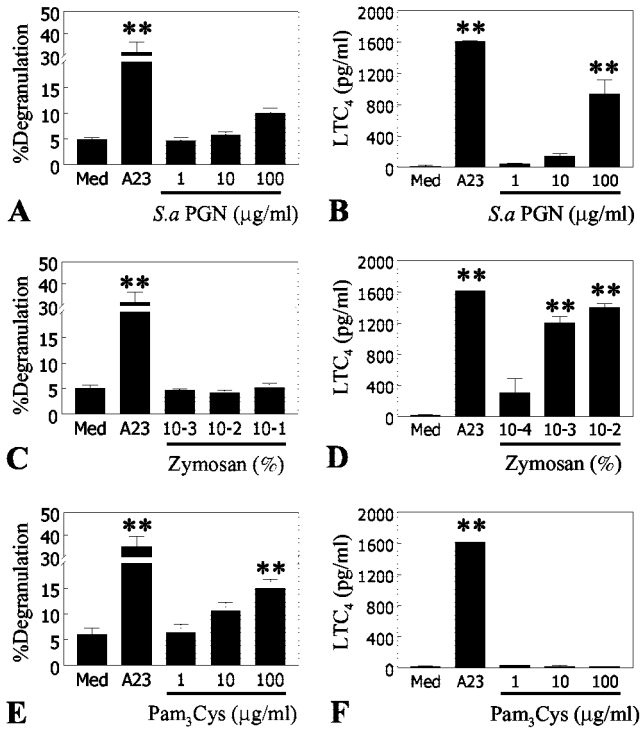

FIGURE 4. Short-term cysteinyl leukotriene generation and β-hexosaminidase release by CBMC in response to TLR2 activators. CBMC were stimulated for 20 min with *S. aureus* PGN (*A* and *B*), zymosan (*C* and *D*), or Pam_3Cys (*E* and *F*) for 24 h. Cell-free supernatants were assayed for cysteinyl leukotriene generation (*B*, *D*, and *F*) or β-hexosaminidase release (*A*, *C*, and *E*). Calcium ionophore A23187 (A23) was used at an optimal dose of 0.5 μM. **, $p < 0.001$ compared with medium control. The data presented are the mean values ± SEM from experiments using CBMC from five separate donors.

ineffective (Fig. 4, *B–E*). Stimulation with either PGN or zymosan resulted in a dose-dependent increase in cysteinyl leukotrienes. The levels of cysteinyl leukotriene generated were similar to other stimuli in human mast cells, including IgE-dependent activation, and were almost equivalent to parallel calcium ionophore (A23187)-induced responses of CBMC.

CBMC were also analyzed for β-hexosaminidase release as a marker of mast cell degranulation. Both PGN and zymosan failed to induce significant levels of CBMC degranulation (Fig. 4, *A* and *C*; $n = 4$). High doses of PGN may have marginally enhanced mast cell degranulation (≈2-fold greater than spontaneous release, $n = 5$); however, these levels failed to reach statistical significance. In contrast, Pam_3Cys induced significant levels of degranulation in a dose-dependent manner (Fig. 4*E*). CBMC degranulation in response to Pam_3Cys was evident at concentrations as low as 10 μg/ml.

Taken together, these data suggest that TLR2 is capable of eliciting distinct mediator responses in human mast cells. This may be occurring either through the use of discrete TLR heterodimers (14, 15) or via alternate signaling pathways. Additional receptors have been identified for zymosan including dectin-1 (26). Further studies are required to determine whether human mast cells express dectin-1 and whether this molecule functions in cooperation with various TLRs.

It is well established that known TLR activators, such as LPS, can induce production of PGs such as PGE_2 via NF-κB-dependent cyclooxygenase 2 generation. However, leukotriene production by human mast cells in response to TLR2 activators has not been previously described. Current models for TLR-mediated signaling do not include a clear mechanism by which leukotriene generation or degranulation would be initiated. A potential mechanism for leukotriene production may involve Rac-1-dependent activation of phosphatidylinositol 3-kinase which has recently been proposed as an important pathway in TLR2 signaling (27). The low yield of CBMC from individual donors and lack of GM-CSF and IL-1β responses to TLR activators in mast cell lines limits the ability to directly address these issues in this system.

Mast cells are well recognized as an important source of leukotrienes in allergic disease. In a number of animal models and disease situations, it has been suggested that local leukotriene generation is dysregulated or may be enhanced by pathogen products under circumstances where there is minimal evidence of mast cell degranulation. LTC_4 and its breakdown products are known to be critical for the bronchoconstriction, edema, and mucous secretion observed in atopic asthma and to be a potent chemoattractant for a number of inflammatory cells as well as dendritic cells. The ability of human mast cells to produce cysteinyl leukotrienes in a degranulation-independent manner in response to TLR activators opens up new possibilities for the mechanisms by which infection exacerbates allergic asthma. The long-term production of RANTES, GM-CSF, and IL-1β by mast cells in response to PGN and zymosan may further enhance host responses to pathogen infection and contribute to the chronicity of inflammatory disease at mast cell-rich sites such as the skin and lung.

Acknowledgments

We thank Yi-Song Wei, Ursula Kadela-Stolarz, and Pat Colp for their excellent technical assistance.

References

1. Galli, S. J., M. Maurer, and C. S. Lantz. 1999. Mast cells as sentinels of innate immunity. *Curr. Opin. Immunol. 11:53.*
2. Malaviya, R., and S. N. Abraham. 2001. Mast cell modulation of immune responses to bacteria. *Immunol. Rev. 179:16.*
3. Echtenacher, B., D. N. Mannel, and L. Hultner. 1996. Critical protective role of mast cells in a model of acute septic peritonitis. *Nature 381:75.*
4. Malaviya, R., T. Ikeda, E. Ross, and S. N. Abraham. 1996. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-α. *Nature 381:77.*
5. Hamilton, J. A. 2002. GM-CSF in inflammation and autoimmunity. *Trends Immunol. 23:403.*
6. O'Byrne, P. M. 2000. Leukotriene bronchoconstriction induced by allergen and exercise. *Am. J. Respir. Crit. Care Med. 161:S68.*
7. Akira, S., K. Takeda, and T. Kaisho. 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat. Immunol. 2:675.*
8. Aliprantis, A. O., R. B. Yang, M. R. Mark, S. Suggett, B. Devaux, J. D. Radolf, G. R. Klimpel, P. Godowski, and A. Zychlinsky. 1999. Cell activation and apoptosis by bacterial lipoproteins through Toll-like receptor-2. *Science 285:736.*
9. Brightbill, H. D., D. H. Libraty, S. R. Krutzik, R. B. Yang, J. T. Belisle, J. R. Bleharski, M. Maitland, M. V. Norgard, S. E. Plevy, S. T. Smale, et al. 1999. Host defense mechanisms triggered by microbial lipoproteins through Toll-like receptors. *Science 285:732.*
10. Hirschfeld, M., C. J. Kirschning, R. Schwandner, H. Wesche, J. H. Weis, R. M. Wooten, and J. J. Weis. 1999. Cutting edge: inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by Toll-like receptor 2. *J. Immunol. 163:2382.*
11. Schwandner, R., R. Dziarski, H. Wesche, M. Rothe, and C. J. Kirschning. 1999. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by Toll-like receptor 2. *J. Biol. Chem. 274:17406.*
12. Yoshimura, A., E. Lien, R. R. Ingalls, E. Tuomanen, R. Dziarski, and D. Golenbock. 1999. Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. *J. Immunol. 163:1.*
13. Underhill, D. M., A. Ozinsky, A. M. Hajjar, A. Stevens, C. B. Wilson, M. Bassetti, and A. Aderem. 1999. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature 401:811.*
14. Takeuchi, O., S. Sato, T. Horiuchi, K. Hoshino, K. Takeda, Z. Dong, R. L. Modlin, and S. Akira. 2002. Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J. Immunol. 169:10.*

Downloaded from http://www.jimmunol.org/ by guest on August 9, 2022

15. Ozinsky, A., D. M. Underhill, J. D. Fontenot, A. M. Hajjar, K. D. Smith, C. B. Wilson, L. Schroeder, and A. Aderem. 2000. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors. *Proc. Natl. Acad. Sci. USA 97:13766.*
16. Jones, B. W., T. K. Means, K. A. Heldwein, M. A. Keen, P. J. Hill, J. T. Belisle, and M. J. Fenton. 2001. Different Toll-like receptor agonists induce distinct macrophage responses. *J. Leukocyte Biol. 69:1036.*
17. Hirschfeld, M., J. J. Weis, V. Toshchakov, C. A. Salkowski, M. J. Cody, D. C. Ward, N. Qureshi, S. M. Michalek, and S. N. Vogel. 2001. Signaling by Toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. *Infect. Immun. 69:1477.*
18. McCurdy, J. D., T. J. Lin, and J. S. Marshall. 2001. Toll-like receptor 4-mediated activation of murine mast cells. *J. Leukocyte Biol. 70:977.*
19. Leal-Berumen, I., P. Conlon, and J. S. Marshall. 1994. IL-6 production by rat peritoneal mast cells is not necessarily preceded by histamine release and can be induced by bacterial lipopolysaccharide. *J. Immunol. 152:5468.*
20. Supajatura, V., H. Ushio, A. Nakao, K. Okumura, C. Ra, and H. Ogawa. 2001. Protective roles of mast cells against enterobacterial infection are mediated by Toll-like receptor 4. *J. Immunol. 167:2250.*
21. Supajatura, V., H. Ushio, A. Nakao, S. Akira, K. Okumura, C. Ra, and H. Ogawa. 2002. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J. Clin. Invest. 109:1351.*
22. Lin, T. J., T. B. Issekutz and J. S. Marshall. 2000. Human mast cells transmigrate through human umbilical vein endothelial monolayers and selectively produce IL-8 in response to stromal cell-derived factor-1 α. *J. Immunol. 165:211.*
23. Y. Iikura, T. Awaji, G. Tsujimoto, M. Yanagida, H. Uzumaki, G. Takahashi, K. Tsuji, and T. Nakahata. 1996. Selective growth of human mast cells induced by Steel factor, IL-6, and prostaglandin E_2 from cord blood mononuclear cells. *J. Immunol. 157:343.*
24. Kishi, K. 1985. A new leukemia cell line with Philadelphia chromosome characterized as basophil precursors. *Leuk. Res. 9:381.*
25. Schwartz, L. B., K. F. Austen, and S. I. Wasserman. 1979. Immunologic release of β-hexosaminidase and β-glucuronidase from purified rat serosal mast cells. *J. Immunol. 123:1445.*
26. Brown, G., and S. Gordon. 2001. A new receptor for β-glucans. *Nature 413:36.*
27. Arbibe, L., J. P. Mira, N. Teusch, L. Kline, M. Guha, N. Mackman, P. J. Godowski, R. J. Ulevitch, and U. G. Knaus. 2000. Toll-like receptor 2-mediated NF-κB activation requires a Rac1-dependent pathway. *Nat. Immunol. 1:533.*

Downloaded from http://www.jimmunol.org/ by guest on August 9, 2022