Mast Cells as Sensors of Cell Injury through IL-33 Recognition

Mattias Enoksson, Katarina Lyberg, Christine Möller-Westerberg, Padraic G. Fallon, Gunnar Nilsson and Carolina Lunderius-Andersson

*J Immunol* 2011; 186:2523-2528; Prepublished online 14 January 2011;
doi: 10.4049/jimmunol.1003383
http://www.jimmunol.org/content/186/4/2523
Mast Cells as Sensors of Cell Injury through IL-33 Recognition

Mattias Enoksson,* Katarina Lyberg,* Christine Möller-Westerberg,* Padraic G. Fallon,† Gunnar Nilsson,*1 and Carolina Lunderius-Andersson*1

In response to cell injury, caused, for example, by trauma, several processes must be initiated simultaneously to achieve an acute inflammatory response designed to prevent sustained tissue damage and infection and to restore and maintain tissue homeostasis. Detecting cell injury is facilitated by the fact that damaged cells release intracellular molecules not normally present in the extracellular space. However, potential underlying mechanisms for the recognition of endogenous danger signals released upon cell injury have yet to be elucidated. In this study, we demonstrate that mast cells, potent promoters of acute inflammation, play a key role in responding to cell injury by recognizing IL-33 released from necrotic structural cells. In an in vitro model of cell injury, this recognition was shown to involve the T1/ST2 receptor and result in the secretion of proinflammatory leukotrienes and cytokines by mouse mast cells. Remarkably, of all of the components released upon necrosis, our results show that IL-33 alone is a key component responsible for initiating proinflammatory responses in mast cells reacting to cell injury. Our findings identify IL-33 as a key danger signal released by necrotic structural cells capable of activating mast cells, thus providing novel insights concerning the role of mast cells as sensors of cell injury. The Journal of Immunology, 2011, 186: 2523–2528.

The ability to recognize and respond to cell injury is fundamental to the survival of all animal species. Upon cell injury, endogenous danger signals, so-called damage-associated molecular patterns, are released by necrotic cells, including heat shock proteins (1), high-mobility group box 1 (HMGB1) (2, 3), uric acid (4), and cytokines of the IL-1 family: IL-1α (5, 6) and, as recently suggested, IL-33 (7, 8). Such endogenous danger signals are recognized by various immune cells that initiate inflammatory processes (9). The requirements made on the specialized cells that respond to tissue damage are numerous. In the first place, such sentinel cells must be prepositioned in tissues, allowing a rapid response. Secondly, these cells must possess the capability to produce and secrete selective mediators required for the induction of an acute inflammatory response involving vascular changes and the recruitment of leukocytes. Mast cells possess these important characteristics (10). These long-lived cells are present in all tissues, especially numerous at sites exposed to the external environment (11, 12), and rapidly produce and secrete a variety of signal substances upon activation, including histamine, proteases, eicosanoids, chemokines, and cytokines (13). Taken together, these properties make mast cells ideal first-hand responders to tissue damage/cell injury, capable of initiating and orchestrating an inflammatory response.

As mast cells have previously been shown to recognize and respond to exogenous danger signals (also called pathogen-associated molecular patterns) such as LPS, zymosan, and peptidoglycan (11, 14), we hypothesized that mast cells also play an important role in the recognition of endogenous danger signals, such as IL-33, thereby contributing cell injury responses.

IL-33 is a novel cytokine of the IL-1 family that has previously been shown to induce Th2-associated cytokines (15), as well as induce release of proinflammatory mediators in mouse bone marrow-derived mast cells (BMMCs) (16–20) and human mast cells (21, 22), in which IL-33 also promoted maturation (21), enhanced mast cell survival, and increased mast cell adhesion to fibronectin (22). In addition, IL-33 is involved in tryptase (mouse mast cell protease–6) regulation in BMMCs (23). Importantly, IL-33 seems to be preferentially released from necrotic cells (24). For instance, IL-33 has been shown to be released from endothelial cells following mechanical injury (7). On the contrary, release of bioactive IL-33 from apoptotic cells has not been demonstrated. Instead, IL-33 has been shown to be inactivated during apoptosis (24).

In this study, we have examined the role of mast cells as sensors of cellular injury. The hypothesis that an important function for mast cells is to quickly respond to cell injury was proposed already 50 y ago (25). Despite the fact that mast cells are recognized as important inflammatory cells (10, 13), the mechanism for this hypothesis has not been deciphered. In this report, by monitoring responses of mast cells treated with cell-free supernatant from necrotic cells, we provide a mechanism for this hypothesis.

Materials and Methods
Experimental animals, isolation, and in vitro stimulation of cells

Bone marrow was isolated from C57BL/6 wild-type, MyD88−/− (26), TLR1−/− (27), TLR2−/− (28), TLR4−/− (29), TLR5−/− (30), TLR6−/− (31), TLR7−/− (32), TLR8−/− (33) and TLR9−/− (34), A20R−/− (35),
A2AR−/− (36), and T1/ST2−/− (37) mice, and BMMCs were prepared as described earlier (38). The purity of the BMMCs obtained was routinely ≥95%, as assessed by toluidine blue staining. In a typical experiment, 10⁷/ml BMMCs were seeded into each well of 48-well plates and exposed to various amounts of necrotic cell supernatant (see further below) for 0.5 or 24 h, after which the supernatants from each culture were collected. The total volume per well was 200 μl. As a positive control for mast cell activation, ionomycin (Sigma-Aldrich) was used. For inhibition studies, cells were pretreated with 10 μM SB203580 (a p38 inhibitor) for 30 min prior to the addition of necrotic cell supernatant or rL−33 (Alexis Biochemicals).

Preparation of necrotic cell supernatant

Supernatant was collected from mouse embryonal fibroblasts (MEFs), HMGB1−/− MEFs, smooth muscle cells, keratinocytes, monocytes, splenocytes, mixed neuronal cells, and BMMCs rendered necrotic by repeated freeze-thawing. In brief, the cells were resuspended in PBS at a density of 20 × 10⁶, subjected three repeated freeze-thawing (cycling between −80 and 37°C four times), and then centrifuged at 13,000 rpm for 10 min and the supernatant collected.

Monitoring the responses of mast cells

Histamine release was determined using an immunomassay kit (ImmunoTech; Beckman Coulter). Secreted cysteinyl leukotrienes were quantified using an enzyme immunoassay kit (Amer sham Biosciences) and cytokines/chemokines with commercial ELISA kits (Biosource International and R&D Systems) and LumineX (Millipore).

Knockdown of IL-33 expression by MEFs with small interfering RNA

To transiently silence IL-33 expression by MEFs, these cells were transfected with IL-33 small interfering RNA (siRNA) or, as a control, nonspecific siRNA (Dharmacon), harvested 24 h later, and rendered necrotic for the preparation of necrotic supernatant as described above. To validate IL-33 knockdown, expression of the corresponding mRNA was analyzed by quantitative PCR (qPCR) and supernatant levels of the protein itself by ELISA. qPCR was performed in an iCycler (Bio-Rad) using the following primers: IL-33 sense: 5′-TCC TTG CTT GCC AGT ATC CA-3′ and IL-33 antisense: 5′-TGC TCA ATG TGT CAA CAG ACG-3′.

Western blotting

Following treatment of BMMCs with 10 ng/ml rL−33 or necrotic cell supernatant, levels of phosphorylated p38 and total p38 protein were determined by standard procedures (38), utilizing Abs directed against phospho-p38 MAPK (Thr180/Tyr182) and p38 MAPK, respectively (both from Cell Signaling Technology). IL−33 was detected in necrotic cell supernatant utilizing an anti-mouse IL−33 Ab (Ab3626; R&D Systems), followed by a donkey anti-goat IgG-HRP secondary Ab (Santa Cruz Biotechnology).

Statistical analysis

The Mann–Whitney and one-way ANOVA (Kruskal-Wallis) tests were employed for statistical analyses, with a p value <0.05 being considered statistically significant.

Results

BMMCs exhibit a potent proinflammatory response to treatment with necrotic cell supernatant

When cells die in a necrotic fashion following cell injury, cell content is released into the extracellular space. Certain intracellular molecules released in this way function as endogenous danger signals, alerting the immune system, causing it to mount an inflammatory response. We hypothesized that mast cells can sense such warning signals, thereby participating in the initiation of an inflammatory response through release of proinflammatory mediators. To test this hypothesis, we rendered fibroblasts necrotic by repeated freeze-thawing cycles. The cell-free necrotic supernatant isolated from MEFs was subsequently transferred to a culture of C57BL/6 BMMCs and the responses of the latter monitored. When incubated in this manner with supernatant from cultures containing as many or twice as many necrotic MEFs, the BMMCs did not degranulate (i.e., did not release histamine) (Fig. 1A), but released leukotrienes (Fig. 1B) and secreted the proinflammatory cytokines IL-6 (Fig. 1C) and TNF-α (Fig. 1D), thus exhibiting a potent inflammatory response. In contrast, supernatants from MEFs rendered apoptotic through gamma-radiation did not generate IL-6 secretion by BMMCs (data not shown). Similar results were obtained with BMMCs prepared from BALB/c mice (data not shown). IL-4, IL-10, IL-13, and MCP-1 were not secreted in this system (data not shown). No IL-6 secretion was detected in supernatants from cells treated for 0.5 h with necrotic cell supernatant (data not shown). As a positive control, cells were treated with 1 μM ionomycin, which induced histamine release (45.5 ± 8.2% of total, n = 5, measured 30 min post-treatment) and IL-6 release (39,980 ± 2,836 pg/ml, n = 5, measured 24 h post-treatment) (data not shown).

Our next attempt aimed at identifying the molecular signals responsible for activating the mast cells. Our initial results led us to suspect the involvement of HMGB1, a well-characterized danger signal known to be released from necrotic cells (3) and activate macrophages (39). To investigate the role of HMGB1 in our system, BMMCs were subsequently exposed to the necrotic supernatant from MEFs isolated from HMGB1−/− mice (40). Under these conditions, the BMMCs secreted levels of IL-6 that did not differ significantly from the response of wild-type MEFs to necrotic cell supernatant (Fig. 2A). Next, to examine the possible involvement of uric acid, another danger signal released from damaged cells (4), the MEFs were rendered necrotic after culture in the presence of allopurinol (4), an inhibitor of uric acid production. Once again, no difference in BMMC activation compared with untreated necrotic supernatant was observed (Fig. 2A). Moreover, the use of BMMCs from A2AR−/− and A3R−/− mice, deficient in the adenosine A2A and A3 receptors, respectively, still gave the same levels of IL-6 secretion (Fig. 2B).

Together, these findings revealed that the inflammatory response of mast cells to factors released by necrotic fibroblasts does not involve the known danger signals HMGB1, uric acid, or adenosine.

 Mast cell responses to necrotic cell supernatant are mediated through the adaptor protein MyD88

Because several exogenous danger signals (including LPS and zymosan), as well as certain endogenous danger signals (41, 42),
act through TLRs, for which MyD88 acts as an adaptor protein, we reasoned that responses to necrotic supernatant perhaps also could be mediated through a receptor using MyD88. To investigate this, BMMCs were prepared from MyD88−/− mice and treated with necrotic supernatant. MyD88−/− BMMCs were found to lack the normal IL-6 response to necrotic cell supernatant completely (Fig. 2C), indicating that the observed IL-6 production was MyD88 dependent. To pinpoint the upstream receptor of MyD88 that responds to necrotic cell supernatant, BMMCs were derived from TLR1−/−, TLR2−/−, TLR4−/−, TLR5−/−, TLR6−/−, TLR7−/−, TLR8−/−, and TLR9−/− BMMCs (2:1 ratio) (O) was determined. During BMMC treatment, 10⁶ cells/ml were used in a total volume of 200 μl. The values presented are means ± SEM (n = 3–5). The experiments were repeated at least three times. *p < 0.05, **p < 0.01 in comparison with untreated control cells (Unstim) (A) or wild-type cells (C). For statistical analyses, one-way ANOVA (A) and Mann–Whitney (B–D) test were applied.

**FIGURE 2.** The activation of mast cells by necrotic cell supernatant involves MyD88. Following 24 h of exposure to supernatant from the same number (1:1) or twice as many (2:1) necrotic MEFs, secretion of IL-6 by BMMCs exposed to the supernatant of MEFs treated with allopurinol or isolated from HMGB1−/− mice (A); Aa2AR−/− and AaAR−/− BMMCs (2:1 ratio) (B); MyD88−/− BMMCs (C); and TLR1−/−, TLR2−/−, TLR4−/−, TLR5−/−, TLR6−/−, TLR7−/−, TLR8−/−, and TLR9−/− BMMCs (2:1 ratio) (O) was determined. During BMMC treatment, 10⁶ cells/ml were used in a total volume of 200 μl. The values presented are means ± SEM (n = 3–5). The experiments were repeated at least three times. *p < 0.05, **p < 0.01 in comparison with untreated control cells (Unstim) (A) or wild-type cells (C). For statistical analyses, one-way ANOVA (A) and Mann–Whitney (B–D) test were applied.

**IL-33 present in necrotic cell supernatant activates mast cells through the T1/ST2 receptor**

Because members of the IL-1R family share MyD88-dependent signaling pathways with TLRs, the T1/ST2 receptor (43), a member of the IL-1 family of receptors, was examined next.

Interestingly, we found that mast cells lacking the T1/ST2 receptor failed to secrete IL-6 and released considerably lower levels of TNF-α compared with wild-type cells upon exposure to necrotic cell supernatant (Fig. 3A). In addition, T1/ST2−/− BMMCs also produced substantially lower levels of cysteinyl leukotrienes and LTBrα upon exposure to necrotic supernatant (Fig. 3B). As all observed responses were T1/ST2 dependent, we investigated IL-33 protein levels in necrotic cell supernatant by Western blotting (Fig. 3C) and found full-length pro–IL-33 to be present as a band at ~30 kDa, in line with a previous publication (19). In addition, we observed a band migrating identically to rIL-33, likely representing cleaved IL-33. We next investigated responses of BMMCs treated with the T1/ST2 ligand IL-33. As shown in Fig. 3D, treating BMMCs with IL-33 resulted not only in a dose-dependent release of IL-6 and TNF-α, but also in the release of cysteinyl leukotrienes. Thus, treating BMMCs with IL-33 generated similar responses as BMMCs treated with necrotic supernatant. Taken together, the observed results imply that IL-33 released by necrotic cells induce the production of IL-6, TNF-α, and leukotrienes by mast cells, as production of these mediators was absent or limited in T1/ST2−/− mast cells.

In an attempt to detect further similarities in responses of BMMCs treated with necrotic supernatant and IL-33, we next investigated signaling pathways downstream of T1/ST2 and MyD88. This was investigated by monitoring IL-6 secretion by mast cells preincubated with SB203580, an inhibitor of p38, prior to treatment with necrotic cell supernatant or IL-33. Preincubation with SB203580 reduced IL-6 secretion markedly, both in BMMCs treated with necrotic supernatant and also in BMMCs treated with IL-33 (Fig. 4A). In agreement with these findings, phosphorylation of p38 was detected 5 min after exposure of BMMCs to necrotic supernatant or IL-33 (Fig. 4B).

To provide definite evidence that IL-33 is the molecular component of necrotic cell supernatant that activated the mast cells, expression of this IL by MEFs was silenced with siRNA, and the MEFs were rendered necrotic. The effectiveness of this silencing was validated by qPCR (Fig. 5A) and by determining the levels of IL-33 in the necrotic supernatant (Fig. 5B). When BMMCs were exposed to the supernatant from necrotic MEFs lacking IL-33, secretion of IL-6 was potently attenuated (Fig. 5A). In addition, the supernatant from necrotic structural cells (Fig. 5C), demonstrating that the observed responses of BMMCs treated with necrotic supernatant are dependent almost exclusively on IL-33. In conclusion, our results demonstrate that IL-33 released during necrosis is an important mast cell activator.

**IL-33 is released from necrotic cells of structural but not hematopoietic origin**

We next investigated the possibility that necrotic structural cells other than fibroblasts, such as keratinocytes, neuronal cells (astrocytes), and smooth muscle cells, some of which express IL-33 at relatively high levels, also release factors that activate mast cells. The supernatant from cultures of above-mentioned necrotic structural cells could indeed induce IL-6 secretion by BMMCs (Fig. 5D), whereas corresponding necrotic supernatant prepared from cells of hematopoietic origin (splenocytes, monocytes, and BMMCs) had no effect. Moreover, the necrotic supernatant from structural cells contained higher levels of IL-33 (Fig. 5E). Together, our data suggest that IL-33 released upon cell injury mainly derives from damaged structural cells.

**Discussion**

In this study, we investigated the hypothesis that mast cells are important sensors of cell injury, as they display qualities important
MAST CELLS AS SENSORS OF CELL INJURY

FIGURE 3. The proinflammatory responses activated by mast cells upon exposure to necrotic cell supernatant are mediated by IL-33. After exposure for 24 h to supernatant from the same number (1:1) or twice as many (2:1) necrotic MEFs, secretion of cytokines IL-6 and TNF-α (A) by wild-type and T1/ST2+/− MBCMs were measured. After exposure for 30 min, release of cysteinyl leukotrienes and LTB4 (B) by these same BMMCs was determined. IL-33 protein was investigated in necrotic cell supernatant and compared with rIL-33 using Western blotting (C). D, Release of IL-6, TNF-α, and cysteinyl leukotrienes were measured in BMMCs treated with 0, 10, or 100 ng/ml IL-33. During BMMC treatment, 10^6 cells/ml were used in a total volume of 200 μl. The values shown are means ± SEM (n = 5). For statistical analysis, the Mann–Whitney U test was applied. The experiments were repeated at least three times. **p < 0.01 in comparison with wild-type cells (A, B) or untreated cells (D).

to first-hand responders to tissue damage. By monitoring the responses of mast cells treated with cell-free supernatant from necrotic cells, we demonstrate that IL-33 is a key alarmin released by necrotic structural cells. We show that mast cells are activated by IL-33 released from necrotic cells to initiate a potent proinflammatory response, suggesting that mast cells make important contributions to early cell injury responses.

During necrosis, cell integrity is compromised, resulting in the release of several different danger signals. To investigate whether mast cells could respond to endogenous danger signals released from necrotic cells, we rendered MEFs necrotic and treated mast cells with the supernatant from these cells. The use of necrotic supernatant from lysed cells rather than purified individual danger signals better represents physiological conditions, allowing for a more credible in vitro model of cell injury. Our initial experiments revealed that BMMCs treated with supernatant from necrotic MEFs exhibited a potent inflammatory response, manifested by IL-6 and TNF-α secretion and leukotriene release. However, this response was not accompanied by degranulation, indicating solely de novo production of the released mediators. It has been shown earlier that HMGB1 is an important danger signal released by dying cells and that HMGB1−/− cells have a greatly reduced capacity to induce inflammation compared with wild-type necrotic cells (3). However, in our hands, BMMCs treated with necrotic supernatant obtained from HMGB1−/− MEFs secreted IL-6 at similar levels as BMMCs treated with necrotic supernatant from wild-type cells. This is in agreement with a study performed by Chen et al. (5), in

FIGURE 4. Mast cell responses to necrotic cell supernatant and IL-33 are mediated through a p38-dependent pathway. A, IL-6 release was determined in wild-type BMMCs pretreated with or without 10 μM selective p38 inhibitor SB203580 for 30 min prior to treatment with necrotic supernatant (2:1 ratio) or 10 ng/ml IL-33. B, Levels of phosphorylated p38 (p-p38) in wild-type BMMCs following treatment with necrotic cell supernatant (2:1 ratio) or 10 ng/ml IL-33 for the time periods indicated were examined by Western blotting. During BMMC treatment, 10^6 cells/ml were used in a total volume of 200 μl. The values shown are means ± SEM (n = 5). For statistical analysis, the Mann–Whitney U test was applied. The experiments were repeated at least three times. **p < 0.01 in comparison with cells not exposed to SB203580.

FIGURE 5. IL-33 released from necrotic cells is responsible for the activation of mast cells. siRNA-mediated silencing of the expression of IL-33 in MEFs was validated by qPCR (A) and an ELISA assay (B). C, Following exposure of BMMCs for 24 h to supernatant from twice as many (2:1) MEFs rendered necrotic following treatment with IL-33 or non-targeting siRNA, IL-6 secretion was assayed. D, Following exposure of BMMCs for 24 h to supernatant from twice as many (2:1) cells of the types indicated, IL-6 secretion was determined. E, The levels of IL-33 in the same necrotic cell supernatants were measured by ELISA. During BMMC treatment, 10^6 cells/ml were used in a total volume of 200 μl. In A, a representative experiment is shown. In B–D, the values presented are means ± SEM (n = 4–5). In E, the values are means ± SEM (n = 2–3). The experiments were repeated at least three times. *p < 0.05 in comparison with the cells treated with nontargeting siRNA. For statistical analysis, the Mann–Whitney U test was applied (B, C). SMC, smooth muscle cell.
which it was demonstrated that necrotic HMGB1−/− cells initiated inflammation similarly to necrotic HMGB1+/+ cells in mice. Additionally, in our experimental system, necrotic supernatant from MEFs with inhibited uric acid formation did not significantly reduce the proinflammatory responses in BMMCs, suggesting other components than HMGB1 or uric acid to be responsible for the observed mast cell activation.

Supported by the finding that MyD88−/− BMMCs were unable to secrete IL-6 in response to necrotic supernatant, we also investigated responses to necrotic supernatant by TLR-deficient mast cells. Although TLRs are best known for recognizing exogenous danger signals like LPS, some members of the TLR family have previously been reported to respond to endogenous ligands. For instance, recognition of heat shock protein 60 has been suggested to be TLR4 dependent (44). However, TLR-deficient BMMCs secreted IL-6 at similar levels as wild-type BMMCs when exposed to necrotic cell supernatant, indicating another receptor upstream of MyD88 to be involved. This does not, however, exclude the possibility that the necrotic cell supernatant affects multiple TLR signaling pathways. However, as responses to necrotic supernatant were completely abrogated in T1/ST2−/− BMMCs, we consider it unlikely that necrotic supernatant might affect mast cells by activating a combination of TLRs.

The discovery that T1/ST2−/− BMMCs displayed abrogated IL-6, TNF-α, cysteinyl leukotrienes, and LTB4 secretion in response to necrotic supernatant (Fig. 3A, 3B) led us to speculate that IL-33, the only known T1/ST2 ligand, might be involved in the mast cell’s ability to respond to necrotic supernatant. In agreement with our hypothesis that IL-33 is a principal danger signal released from necrotic cells, it has previously been shown that IL-33 is released upon necrosis rather than during apoptosis (7, 24). Importantly, IL-33 does not seem to require proteolysis for activation, a favorable feature indeed for an alarmin. Lüthi et al. (24) also showed that IL-33 was proteolytically cleaved during apoptosis, thus diminishing its bioactivity. Most importantly, only small amounts of IL-33 were released during apoptosis, whereas induction of necrosis led to IL-33 release (24). In line with this, we show in Fig. 3C that necrotic MEFs release full-length pro–IL-33 as well as cleaved IL-33. The fact that a much higher concentration of rIL-33 was required to elicit IL-6 secretion by BMMCs compared with the concentration we detected in necrotic supernatant by ELISA might thus suggest that pro–IL-33 is more active compared with rIL-33.

It has been shown that endothelial cells subjected to mechanical wounding by cell scraping or freeze-thawing also release IL-33 (7). To confirm that IL-33 was the chief component of the necrotic supernatant activating mast cells in our system, we generated necrotic supernatant from MEFs with siRNA-silenced IL-33 expression. In line with our earlier results suggesting IL-33 to be responsible for activating the BMMCs, necrotic supernatant generated from MEFs with silenced IL-33 expression failed to induce IL-6 secretion by BMMCs. Amazingly, IL-33 alone, in a soup of components released by necrotic cells, thus has the capability to alone potently activate mast cells. In support of these results, as shown by us in this paper and also by several earlier studies, IL-33 can induce secretion of IL-6 as well as other cytokines in mast cells (17–20). In addition to confirming that IL-33 can induce cytokine release by BMMCs, we also show, to our knowledge, for the first time that IL-33 can induce release of cysteinyl leukotrienes by mast cells.

Taken together, our findings provide an important link between studies revealing that IL-33 is released upon necrosis and studies demonstrating that mast cells are activated by IL-33 to secrete cytokines. Hence, to our knowledge, our findings provide, for the first time, a plausible mechanism for how mast cells might function as sensors of cell injury. Most remarkably, of all potential activators present in the necrotic cell supernatant, our results show IL-33 to be the sole inducer of cytokine secretion by mast cells, indisputably demonstrating that IL-33 is an important alarmin.

Expression of IL-33 has been described in a variety of tissues (15) and cell types; for instance, in fibroblasts (45), epithelial cells, and endothelial cells (8). Therefore, we addressed the question whether necrotic supernatant generated from other cell types than MEFs would elicit similar responses in BMMCs. These experiments revealed that necrotic supernatant generated from structural cell types (keratinocytes, smooth muscle cells, and mixed neuronal cells) induced IL-6 secretion in BMMCs, whereas necrotic supernatant from cells of hematopoietic origin (BMMCs, monocytes or splenocytes) did not. Moreover, we show that necrotic supernatant from the former cell types correspondingly also contain higher IL-33 levels, in agreement with previous reports (15, 46). Theoretically, should a structural cell die by necrosis, adjacent mast cells will respond to released IL-33 and initiate an inflammatory response.

In summary, using an in vitro model of cell injury, we provide a mechanism for the hypothesis that mast cells are important sensors of cell injury. The present findings indicate that structural cells, such as fibroblasts and keratinocytes, release IL-33 upon injury and that adjacent mast cells respond by producing proinflammatory factors, including IL-6, TNF-α, and leukotrienes. Subsequently, these signals can induce vascular changes, including vasodilatation, increased permeability of the microvasculature, and recruitment of inflammatory cells to the site of injury. Thus, our investigation provides support for the relatively early hypothesis that an important physiological function for mast cells is to act as key sensors of endogenous danger signals released upon cell injury. Moreover, the present demonstration that this response involves recognition of IL-33 by T1/ST2 receptors on the surface of mast cells describes a novel mechanism underlying the role of mast cells as sensors. At the same time, new evidence complementing earlier studies suggesting that IL-33 is a key alarmin released by dying cells is also provided. The responses of mast cells to cell injury are likely to play a highly important role in the initiation of acute inflammation and subsequent healing and, thereby, in the maintenance of tissue homeostasis.

Acknowledgments

We thank Drs. Shizuo Akira (Department of Host Defense, Osaka University, Japan), Andrew McKenzie (Medical Research Council, Laboratory of Molecular Biology, Cambridge, U.K.), Marco Bianchi (Department of Biotechnology, San Raffaele Scientific Institute, Milano, Italy), Bertil Fredholm (Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden), Tobias Bergström (Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden), and Mikael Adner (Department of Environmental Medicine, Karolinska Institute) for providing reagents necessary for these experiments. We also thank Dr. Sara Lind (Department of Medicine, Karolinska Institute) for technical assistance.

Disclosures

The authors have no financial conflicts of interest.

References


