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*J Immunol* 2010; 184:4470-4478; Prepublished online 10 March 2010;
doi: 10.4049/jimmunol.0902485
http://www.jimmunol.org/content/184/8/4470

Supplementary Material

http://www.jimmunol.org/content/suppl/2010/03/11/jimmunol.0902485.DC1

References

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Identification of the Cellular Sensor That Stimulates the Inflammatory Response to Sterile Cell Death

Hajime Kono,* Dipti Karmarkar,* Yoichiro Iwakura,† and Kenneth L. Rock*

Cell death provokes a robust inflammatory response. We have previously shown that this response is dependent on IL-1α. In this study, we investigate the cellular mechanism used by a host to sense cell death, produce IL-1α and also the role of IL-1β in this response. In almost all cases examined, the IL-1 that stimulated the death-induced inflammatory response came from the host rather than the cell that was dying. In these situations, host bone marrow-derived cells were the key source of the IL-1α that was required for the inflammatory response. Conditional cellular depletion and reconstitution in CD11b promoter-driven diphtheria toxin receptor transgenic mice revealed that host macrophages played an essential role in the generation of the inflammatory response and were the source of the required IL-1α. In addition, we found a role for IL-1β in the death-induced inflammatory response and that this cytokine was generated by both bone marrow-derived and resident host cells. The one exception to these findings was that when dendritic cells were injected into mice, they provided a portion of the IL-1 that stimulated inflammation, and this was observed whether the dendritic cells were live or necrotic. Together, these findings demonstrate that macrophages play a key role as the primary sentinels that are required to sense and report cell death in ways that initiate the inflammatory response. One key way they accomplish this important task is by producing IL-1α that is needed to initiate the inflammatory response. The Journal of Immunology, 2010, 184: 4470–4478.

When cells die in vivo, particularly by necrosis, they stimulate a robust inflammatory response (1,2). Local arterioles dilate, venules leak protein-rich fluid and neutrophils, followed by monocytes, extravasate into the tissues. This reaction to cell death is seen in virtually all vascularized tissues and is so stereotypical that its progression is used forensically to date the time of injury (3).

The inflammatory response to cell injury is important because it has a number of consequences for the host (4–6). On the positive side, the response rapidly delivers the soluble and cellular innate defenses to the site of death, where they may neutralize or sequester the injurious agent, such as an infection. In addition, the recruited cells can also clear cellular corpses and promote tissue repair. On the negative side, the innate defenses are indiscriminate and once mobilized can injure friend and foe alike. As a result, there is often collateral damage to otherwise nondiseased cells resident at the site of inflammation. In situations of sterile cell death, there is often little or nothing that the mobilized innate immune mechanisms can defend against and consequently the net effect of the process may be to do more harm than good. Because of this, the inflammatory response to sterile cell death can exacerbate or even cause disease (7–12).

Despite the importance of the cell death-induced inflammatory response and its universality, its underlying mechanisms are poorly understood. In general terms, it is believed that the immune system generates this response because cell death may be a sign of a dangerous process that is a threat to the host (4–6, 13). To detect cell injury, it is thought that the innate immune system has evolved mechanisms to recognize and respond to molecules that are exposed on or released from dying cells (5, 14). It has been suggested that complement, natural Abs, or various different leukocytes may be the elements of the immune system that are responsible for recognizing cell injury and initiating this inflammatory response (5, 6, 14–23); however, how the immune system actually does this is unresolved. This is an important issue because it is a fundamental response and one that is a potential target for therapeutic intervention.

A recent insight into cell death-induced inflammation was that the cytokine IL-1 played a critical role in this response (24–26). Mice that lack the IL-1 receptor type I (IL-1R1) and therefore the ability to respond to IL-1 failed to generate almost any acute neutrophilic inflammation to dying cells but could respond normally to a microbial stimulus (24). Because the source of IL-1 is cellular, this discovery suggested that some type of cellular element was playing a key role in triggering the inflammatory response to tissue injury. Many different cell types can produce IL-1, and it is not known which of these plays the key role in this sterile inflammatory response (27, 28). Therefore, we undertook the current studies to investigate the nature of this cell and its role in the cell death-induced inflammatory response.

Materials and Methods

Reagent and Abs

Abs against Ly-6G (clone 1A8), CD11b (M1/70) were obtained from BD Bioscience (San Jose, CA). Abs against F4/80 (BM8), MHC-class II (M5/114.15.2), CD11c (N418) were obtained from eBioscience (San Diego, CA). Anti-7/4 Ab was purchased from Serotec (Oxford, U.K.). The 7-AAD
was obtained from Molecular Probes (Eugene, OR). Recombinant mouse MIP-2 was from Peprotech (Rocky Hill, NJ). Diphtheria toxin A (DTA) was obtained from Calbiochem (San Diego, CA). Acetaminophen was purchased from Sigma-Aldrich (St. Louis, MO).

Animal and cell lines

Wild-type (WT) C57BL/6, WT FVB/N, IL-1–deficient mice (29), CD11b-diphtheria toxin receptor (DTR) mice on a FVB background (30, 31) were purchased from The Jackson Laboratory (Bar Harbor, ME). CD11b-DTR on a C57BL/6 background was obtained from Dr. Dan R. Littman (New York University Medical School, New York, NY) (32). IL-1α, IL-1β, and IL-1β double-deficient mice of C57BL/6 background were described previously (33). Irradiation bone marrow chimera was prepared as described (34). All animal protocols were approved by the University of Massachusetts animal care and use committee. EL4 cells were maintained in RPMI-1640 with 10% FCS and antibiotics. J2-virus immortalized macrophage (MP) cell line (a gift from Dr. Douglas Go- lenbock, University of Massachusetts Medical School, Worcester, MA) was maintained in DMEM with 10% FCS. All the cultured cell lines are tested negative for mycoplasma (Lanza, Basel, Switzerland).

Preparation of necrotic cells

Bone marrow–derived dendritic cells (BMDCs) were obtained from cultures of bone marrow cells of C57BL/6 or IL-1–deficient mice with IL-4 (10 ng/ml), Invitrogen, San Diego, CA) and GM-CSF (5 ng/ml, Invitrogen) in Hybridoma culture medium for 7 d. A single-cell suspension of liver was obtained by collagenase type IV treatment as previously described (35). To induce necrosis from thermal injury, BMDCs, liver or EL4 cells were washed five times with PBS, then resuspended in PBS at 10 million cells/50 μl, and then heat shocked at 45°C for 10 min, followed by 37°C incubation for 5 h; this resulted in necrosis (7-aminonactamycin Di-propidium internal positive cell) of the viable cell.

To induce mechanical necrosis, brain, liver, or heart from C57BL/6 or IL-1–deficient mice were weighed and added with five times of weight of PBS and subjected to mechanical injury by a motor-driven tissue tesseract, followed by nitrogen cavitation for 10 min at 500 pounds per square inch. Similarly, mechanical necrosis was induced in EL4 and BMDCs by nitrogen cavitation for 10 min at 500 pounds per square inch. After thermal injury, there were no viable cells observed by microscopy with trypan blue staining. After mechanical injury, no viable cells were seen by microscopy and when analyzed by flow cytometry typically 99.9% of the cells were disrupted into fragments.

The resulting necrotic cell suspensions were used for experiments without any clarification (i.e., containing all released cellular components and debris).

Neutrophil recruitment to peritoneal cavity

Mice were injected i.p. with 5 million necrotic BMDCs in 500 μl PBS, 30 million of necrotic EL4 cells in 150 μl PBS, 150 μl liver homogenate, or 500 μl heart. The amount of the necrotic DCs, brain, liver, and heart was equivalent to WT tissues (Fig. 1). Additionally, there was no reduction in inflammatory activity is meaningful is uncertain because it was not observed with necrotic heart from IL-1αβ–double-deficient mice, as is described next). Similarly, there was no reduction in inflammation to liver cells from IL-1α–/– mice that were made necrotic by thermal injury (Supplemental Fig. 1). Because dying cells could also release IL-1β that might contribute to inflammation, we also examined tissues from IL-1αβ–double-deficient animals. The proinflammatory activity of brain, liver, and heart was equivalent to WT tissues (Fig. 1D–F).

The above results implied that the IL-1 driving the sterile inflammatory response was coming from cells in the host. To directly test this point, we injected necrotic EL4 cells i.p. into WT or IL-1–deficient mice and again quantified the resulting influx of neutrophils into the peritoneum. Injection of necrotic brain, and liver from IL-1α–/– mice (killed by mechanical injury) stimulated as much neutrophilic inflammation as did the same tissues from WT animals (Fig. 1A, 1B). Similarly, inflammation to necrotic heart from IL-1α–/– mice was only modestly less than that to the same tissue from WT animals (Fig. 1C) (and whether this small reduction in inflammatory activity is meaningful is uncertain because it was not observed with necrotic heart from IL-1αβ–double-deficient mice, as described above). Similarly, there was no reduction in inflammation to liver cells from IL-1α–/– mice that were made necrotic by thermal injury (Supplemental Fig. 1). Because dying cells could also release IL-1β that might contribute to inflammation, we also examined tissues from IL-1αβ–double-deficient animals. The proinflammatory activity of brain, liver, and heart was equivalent to WT tissues (Fig. 1D–F).

Statistical analyses

Data are reported as means ± SEs. Statistical analysis in each independent experiment was performed with an unpaired, two-tailed Student t test. One-way ANOVA and Dunnett's multiple comparison post test were used to compare the means of separate groups with the control group. In some cases, Bonferroni's multiple comparison post test was used to compare among multiple groups. p < 0.05 was considered statistically significant.

Results

The source of IL-1 in the cell death-induced inflammatory response: release from dying cells or production by the host?

We have previously reported that IL-1α was essential for the acute neutrophilic inflammatory response stimulated by sterile cell death; however, the source of this cytokine was not known. It is possible that IL-1α comes from a pool of preformed cytokine released from dying cells, as recently suggested for BMDCs (36). Alternatively, IL-1α could be produced by cells in the host that recognize and respond to dying cells. To evaluate the role of these different mechanisms, we performed several experiments.

To examine the role of IL-1 from dying cells, we injected i.p. buffer or a variety of primary necrotic cells from WT or IL-1α–deficient animals and quantified the resulting influx of neutrophils into the peritoneum. Injection of necrotic brain, and liver from IL-1α–/– mice (killed by mechanical injury) stimulated as much neutrophilic inflammation as did the same tissues from WT animals (Fig. 2A, 2B). Similarly, inflammation to necrotic heart from IL-1α–/– mice was only modestly less than that to the same tissue from WT animals (Fig. 2C) (and whether this small reduction in inflammatory activity is meaningful is uncertain because it was not observed with necrotic heart from IL-1αβ–double-deficient mice, as is described above). Similarly, there was no reduction in inflammation to liver cells from IL-1α–/– mice that were made necrotic by thermal injury (Supplemental Fig. 1). Therefore, dying cells could also release IL-1β that might contribute to inflammation, we also examined tissues from IL-1αβ–double-deficient animals. The proinflammatory activity of brain, liver, and heart was equivalent to WT tissues (Fig. 2D–F).

The above results implied that the IL-1 driving the sterile inflammatory response was coming from cells in the host. To directly test this point, we injected necrotic EL4 cells i.p. into WT or IL-1–deficient mice and again quantified the resulting influx of neutrophils into the peritoneum. The dead EL4 cells stimulate strong neutrophilic inflammation in WT mice (Fig. 2A), as we have previously reported (24). In contrast, the neutrophil response to the injection of the dead cells into IL-1–deficient mice was markedly reduced. The neutrophilic inflammatory response was completely inhibited in mice lacking both IL-1α and IL-1β or the IL-1RI (Fig. 2A). These responses were also substantially reduced, although not to background, in mice lacking just IL-1α or IL-1β (Fig. 2A). Similar results were obtained regardless of whether the EL4 cells were killed by mechanical or thermal injury (Supplemental Fig. 2).

Similarly, a substantial component of the neutrophilic inflammatory response to a necrotic primary tissue (liver) was also dependent on IL-1 production from the host (Fig. 2B). These results are consistent with our findings that IL-1–deficient cells stimulate robust neutrophilic inflammation and indicate that for many dying cells much if not all of the IL-1 driving the sterile inflammatory response is coming from cells of the host.

The one exception we observed was with DCs. Similar to Eigenbrod et al. (36), we found that injection of necrotic BMDCs...
from IL-1α−/− mice stimulated less inflammation than the ones from WT mice, (Fig. 3A). Similar results were observed whether the BMDCs were killed by thermal (Fig. 3A) or mechanical injury (Supplemental Fig. 3). We also found that the inflammatory response to IL-1α−/−β−/− DCs was reduced to a similar degree (Fig. 3B). In both of these situations, the inflammatory responses to the IL-1−/− deficient DCs were still well above the buffer-injected background. This implied that the host was also producing some of the required IL-1. Indeed, we found that the inflammatory response to necrotic DCs was partially reduced (~50%) in IL-1−/− deficient hosts (Fig. 3C).

The finding that necrotic IL-1+/+ DCs were much more inflammatory than IL-1−/− ones implied that the DCs must have been making IL-1 before they were killed. Indeed, we found that injection of live WT DCs elicited inflammation to a similar degree to necrotic ones and that this was reduced if the live DCs lacked the ability to produce IL-1 (Fig. 3D, 3E). This contrasts with other cell types where necrotic cells are strongly proinflammatory but live ones are not (Fig. 3F).

FIGURE 1. Requirement of IL-1 released from dying cells for neutrophil recruitment. A–C, Necrotic brain homogenate (A), liver homogenate (B), or heart homogenate (C) from C57BL/6 (WT) or IL-1α−/− mice were injected i.p. into C57BL/6 mice. Total neutrophil number in the peritoneal cavity was measured 14 h post-injection. D–F, Total neutrophil number in the peritoneal cavity of C57BL/6 mice in response to i.p. injected necrotic brain homogenate (D), liver homogenate (E), or heart homogenate (F) derived from C57BL/6 (WT) or IL-1α−/−β−/− mice. All data are combined results of three or more experiments and represented as means ± SEM (n = total number of mice from the multiple experiments for each group). PBS groups, WT mice received i.p. PBS. *p < 0.05, NS, versus WT group.

Does the cell death-induced IL-1 generated in the host come from parenchymal and/or BMDCs?

We next examined the nature of the cells in the host that are producing the IL-1 needed for the neutrophilic inflammatory response to cell death. Many different cell types can potentially make this cytokine (27). To begin to elucidate which cell lineages were the source of IL-1 in this inflammatory response, we generated chimeric mice whose bone marrow and/or radioresistant parenchyma cells lacked functional IL-1 genes and challenged them i.p. with necrotic EL4 cells.

Neutrophilic inflammation to dead cells was significantly reduced in radiation chimeras whose bone marrow lacked either functional IL-1α or IL-1β genes (Fig. 4A). The neutrophil recruitment was slightly further attenuated in mice whose bone marrow lacked the ability to make both IL-1α and IL-1β (Fig. 4A). Although the inhibition of the inflammatory responses was quite marked, it was not as complete as was observed in mice lacking these cytokines in all cells; this suggested that IL-1 from radioresistant cells might also be contributing to responses, albeit in a minor way.

To directly evaluate the contribution of IL-1 from parenchymal elements, we injected necrotic EL4 cells into mice whose parenchymal, but not BMDCs, lacked IL-1 genes (Fig. 4B). Neutrophilic inflammation was not reduced in mice whose parenchymal elements lacked a functional IL-1α gene. In contrast, in chimeras whose parenchyma lacked functional IL-1β, there was a significantly reduced response. Collectively, these results indicate that BMDCs are a major source of IL-1α (and much of the IL-1β) driving the sterile inflammatory response to dying cells. These findings raise the question of what the precise identity is of the key BMDC(s) needed for the cell death-induced inflammatory response. This is an important question because it addresses the issue of what is the sentinel system that senses and responds to cell death. We hypothesized that this cell would be an MP or DC as these elements are normally resident in tissues and interact with dead cells.

Role of DCs in cell death-induced inflammation

To investigate the role of DCs in the death-induced inflammatory response, we used a transgenic mouse that expressed the human DTR under the control of the CD11c promoter (32). Normally, murine cells are resistant to DT because they lack a receptor to bind and internalize the toxin. However, in this model most DC subsets and the CD11c+ subset of CD8+ T cells are deleted by DTA (32). We treated the CD11c-DTR or WT mice with DTA and then challenged...
The total neutrophil numbers in peritoneal cavity of WT C57BL/6 mice after 14 h i.p. injection of heat-shocked necrotic BMDCs from WT and IL-1α/–/– (A) or IL-1α/–/–β/–/– (B) mice. C, The total neutrophil numbers in peritoneal cavity of WT C57BL/6 or IL-1α/–/–β/–/– mice after 14 h i.p. injection of heat-shocked necrotic BMDCs from WT mice. D, The total neutrophil numbers in peritoneal cavity of WT C57BL/6 mice after 4 h i.p. injection of 5 million of live BMDCs from WT or IL-1α/–/–β/–/– mice. E, The total neutrophil numbers in peritoneal cavity of WT C57BL/6 mice after 4 h i.p. injection of 5 million of live BMDCs from WT or IL-1α/–/–β/–/– mice. F, The total neutrophil numbers in peritoneal cavity of WT C57BL/6 mice after 4 h i.p. injection of live or heat-shocked necrotic EL4 cells. The small amount of inflammation seen after injection of live EL4 is presumably due to a small number of dead cells in the injected suspension. All the data displays are combined results of three or more experiments and represented as means ± SEM (n = total number of mice from the multiple experiments for each group). ***p < 0.001; **p < 0.01; *p < 0.05. NS versus WT group (A–C, E) or same amount of necrosis group (D, F).

Analysis of CD11b+ cells in cell death-induced inflammation

In one set of experiments, we examined the effect on the sterile inflammatory response of conditionally depleting CD11b+ cells by injecting DTA into a transgenic mouse that expresses the DTR under the control of the CD11b promoter (30). In the CD11b-DTR transgenic mice, CD11b+ cells selectively express the DTR and are eliminated on treatment with DTA. Transgenic or non-transgenic mice were injected with or without DTA and then challenged i.p. with buffer or necrotic liver cells. Toxin treatment markedly inhibited the neutrophilic inflammatory response to dead cells in transgenic mice but not WT ones (Fig. 6A). Similar results were obtained injecting necrotic EL4 cells (described further below). The toxin treatment by itself (with no dead cell challenge) did not induce inflammation presumably because the CD11b+ cells that are killed are needed for the inflammatory response, the number of cells that die are too few and/or their mechanism of death does not stimulate inflammation (see below).

Although neutrophils express CD11b, previous studies have shown that these cells are not depleted by DT treatment in the CD11b-DTR transgenic mice, presumably because their levels of CD11b expression (and the DTR) are too low. Nevertheless, to critically test whether neutrophils were present and functional in our experiments, we challenged the CD11b-depleted transgenic mice i.p. with the recombinant chemokine MIP-2 and quantified the influx of neutrophils into the peritoneum. DT treatment did not significantly reduce the MIP-stimulated neutrophil response (Fig. 6B). These results argue that DT depletion of CD11b positive cells is not inhibiting the inflammatory response to dead cells by direct effects on neutrophils or other common components needed for the response, such as vascular endothelium. Data further supporting this conclusion will be presented in sections below.

One of the cell types that expresses CD11b is a subset of DCs. We therefore investigated whether these cells were depleted by DT
Identification of the CD11b+ cell required for the cell death-induced inflammatory response

To verify that the inhibition of sterile inflammation in the DTA-treated CD11b-DTR mice was due to the loss of a specific cell type we attempted to reconstitute responses in these treated animals by adoptive transfer of WT (DTA-insensitive) cells (Fig. 7). In initial experiments, we injected resident PCs from WT mice into the DTA-treated CD11b-DTR mice. The adoptive transfer of these live cells did not by itself cause any inflammation. However, these transferred cells were able to reconstitute the ability of the DTA-treated transgenic animals to respond to necrotic cells (Fig. 7A).

This result indicates that the inhibition of the sterile inflammatory response in the treated transgenic mice is not due to pleiotropic effects of the DTA treatment.

We next used this adoptive transfer approach to determine the identity of the CD11b+ cell required for the cell death-induced inflammatory response. We found that adoptive transfer of TG-induced PECs, which are primarily MPs, also reconstituted responses (Fig. 7B). Similar results were obtained by transferring F4/80+ MPs purified from the peritoneal exudates (Fig. 7C). Neither of these cell populations caused inflammation by themselves (without dead cell challenge).

The findings above strongly suggested that the CD11b+ cell that is required for the sterile inflammatory response is a MP. However, it is difficult to completely exclude the possibility that the reconstituting activity is coming from some contaminating cell type in the MP populations. To critically test this point, we sought to reconstitute the DTA-treated transgenic mice with a MP population that could have no possible contaminates and for this we used a MP clone immortalized by J2 virus (38) (Fig. 7D). One potential caveat of this approach is that the transgenic mice are on the FVB background, whereas the MP clone is of C57BL/6 origin and therefore genetic differences could in theory stimulate an allogeneic response. However, such responses should take much longer than the 15-h time course of our assay and most importantly, we found that transfer of the immortalized MPs by themselves (with no dead cell challenge) caused no inflammation. Just as we found with purified primary MPs, the MP clone was sufficient to reconstitute the inflammatory response to necrotic cells. These results unambiguously indentify MPs as a key cell participating in the sterile inflammatory response to cell death.

**MPs are required for the production of IL-1α**

In a final set of experiments, we sought to determine whether MPs were the key host cells that were required to produce the IL-1 that drives the sterile inflammatory response. To address this issue we used DTA to deplete the CD11b+ cells from the CD11b-DTR mice and then compared the ability of WT versus IL-1-/- deficient peritoneal MPs to reconstitute the sterile inflammatory response to dead cells. This experimental design again involved the transfer of MPs from C57BL/6 mice (the background of the mutant mice) into the FVB transgenics. However, like the results we observed previously, the transferred cells induced no inflammation by themselves and the WT MPs reconstituted responses to necrotic cells (Fig. 8). In contrast to WT MPs, IL-1α/β double-deficient MPs failed to reconstitute responses. Similar results were obtained using MPs that lacked IL-1α. Surprisingly, IL-1β-deficient MPs did reconstitute responses.

**Discussion**

Although it has long been known that cell death in vivo stimulates a robust acute inflammatory response, the underlying cellular mechanisms that sense cell death and drive the inflammatory response have been poorly understood. This report describes a number of new insights into this process.

One of the major findings in the current report is that the initiation of an acute inflammatory response to sterile cell death requires a CD11bhigh leukocyte that is almost certainly an MP. Supporting this conclusion was the finding that conditionally depleting CD11b+ cells in CD11b-DTR mice inhibited these responses (Fig. 5). The fact that in these same animals inflammation was intact to other stimuli, such as MIP, indicated that the essential CD11b+ cell was operating upstream in the pathway and that there was no negative effect of these depletions directly on neutrophils or other downstream components (e.g., vascular endothelium). This

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
was also shown by the fact that after CD11b cell-depletion, the inflammatory response to dead cells could be reconstituted with CD11b+ leukocytes.

Several lines of evidence indicated that the key CD11b-positive leukocyte was a MP. In the CD11b-DTR model in vivo, MPs are one of the principal CD11bhigh cells that are eliminated by DTA. Importantly, inflammatory responses in the peritoneum of CD11b-depleted animals were successfully reconstituted with resident and TG-induced peritoneal leukocytes, both cell populations in which the primary CD11b-expressing cells were MPs (Fig. 7). The fact that purified primary MPs and a pure cultured MP clone could also reconstitute responses in the depleted animals demonstrated that MPs were critical upstream elements in this sterile inflammatory response.

Another important finding in our studies was that BMDCs are the origin of most if not all of the IL-1α that is needed to drive the acute inflammatory response (Fig. 4). Moreover, although WT MPs reconstituted sterile inflammatory responses in mice depleted of CD11b cells, IL-1α-deficient MPs failed to do so (Fig. 8). This demonstrated that MPs are almost certainly the major source of IL-1α needed for the cell death-induced inflammatory response in our models. In one circumstance, part of the initial source of IL-1α can come from the dead cell itself, as first suggested by Eibenbrod et al. (36). However, among the dead cell types we examined, we found that injection of live DCs stimulated IL-1α release. Furthermore, among the dead cell types we examined, we found that injection of live DCs stimulated IL-1α release. This demonstrated that MPs are almost certainly the major source of IL-1α that is needed to drive the acute inflammatory response.

Future studies may determine whether MPs can be used as a model for sterile inflammation and whether they can be used to study the pathogenesis of sterile inflammation.
saw with DCs), there is a substantial component of inflammatory response that is being driven by IL-1 produced by the host.

Another new finding was that IL-1β was also required for the cell death-induced inflammatory response. This had not been seen in earlier Ab-blocking experiments (24) presumably because complete neutralization of this cytokine is notoriously difficult (41) (27). Recently the contributions of IL-1β, along with NLRP3 inflammasomes, to cell death-induced inflammation have been also reported (40, 42). We previously showed that Caspase I-deficient mice did not show a reduction in neutrophil recruitment to necrotic EL4 cells. Although Caspase I plays a major role in many systems in cleaving pro–IL-1β to mature IL-1β, the differences we observe between IL-1β-deficient mice and Caspase I-deficient mice suggest that proteases other than Caspase I can process pro–IL-1β to active IL-1β in vivo, as previously suggested (43). We found that the dominant source of IL-1β in this response was from bone marrow-derived leukocytes, although there was also a contribution from radeo resistant cells in the host (Fig. 4). We detected no contribution directly from dying cells, although again it is possible that this might be seen in situations where the dying cell has been induced to produce high levels of this cytokine.

Our experiments revealed some interesting and surprising findings that merit comment. One result is that there is a strong inhibition of responses in animals deficient in only IL-1α or IL-1β (Fig. 2A, Supplemental Fig. 2). This is surprising because both forms of this cytokine stimulate the same receptor, IL-1R1, and on this basis it would have been predicted that the loss of only one of the two forms of the cytokine would have led to more partial inhibitions. This raises the possibility that IL-1α and IL-1β may somehow stimulate in different ways and that both of these are needed for optimal responses. Another and perhaps more likely explanation is that these two cytokines can stimulate each others expression and as a result might create a positive feedbackloop that is needed to amplify responses (27, 44). This may explain why some of the IL-1β driving the inflammatory response is coming from radeo resistant cells, presumably nonbone marrow-derived cells, in the host (i.e., these “host” cells are responding to the IL-1 produced by MP in and so doing helping to amplify the response).

Another interesting finding is that IL-1β−/− peritoneal MP reconstitute responses in CD11b-depleted mice (Fig. 8). This demonstrates that the IL-1α and IL-1β needed for the inflammatory responses can come from different cells and it is possible that IL-1α stimulates other cells to make IL-1β (33, 45). What the source of IL-1β is in this situation is not defined in our experiments, but it may come from both bone marrow and host cells as suggested by our results with chimeras. It is also possible that under some conditions MP (e.g., different MP subsets or states of activation) would contribute both forms of IL-1.

Yet another interesting set of observations concerns the role of DCs in the cell death-induced inflammatory response. Depleting CD11c-positive cells in the CD11c-DTR mouse did not inhibit inflammation to cell death in the peritoneum or liver suggesting that DCs play little role in these responses (Fig. 5). It is possible that a role

**FIGURE 7.** MPs restore inflammatory response to cell death in CD11b-DTR mice. FVB/N or CD11b-DTR mice were injected i.v. with 500 ng DTA and then reconstituted (recon) with naive PCs (A), TG PECs (B), F4/80+ TG PECs (C) or immortalized MP cell line (D). Neutrophil number was determined 14 h after i.p. injection of liver homogenate (A, B) or heat-shocked necrotic EL4 cells (C, D). All data displays are combined results of three or more experiments and represented as means ± SEM (n = total number of mice from the multiple experiments for each group). ***p < 0.001, versus CD11b-DTR group with others.

**FIGURE 8.** IL-1α in MPs is required for reconstituting neutrophil response to cell death in CD11b-DTR mice. FVB/N or CD11b-DTR mice were injected i.v. with 500 ng DTA and then reconstituted (recon) with TG PEC from WT C57BL/6, IL-1α−/−, IL-1β−/−, or IL-1α−/−β−/− mice. Neutrophil number was determined 14 h after i.p. injection stimulated (stim)of heat-shocked necrotic EL4 cells or PBS. Data displays are combined results of three experiments and represented as means ± SEM (n = total number of mice from the multiple experiments for each group). One-way ANOVA and Dunnett’s multiple comparison tests were used to compare the necrotic EL4 stimulated and WT PEC reconstituted CD11b-DTR group with others. ***p < 0.01; *p < 0.05, NS, recon, reconstituted; stim, stimulated.
for these cells was missed because they were not sufficiently de-
pleted; however, we could find only few remaining DCs and the CD11c-DTR experimental system eliminates DC function in other models. Moreover, in independent experiments we found that CD11b cell-depletions which eliminates MPs without any detectable depletion of DCs inhibited inflammatory responses; this again suggested that DCs were not sufficient for responses (Fig. 6C). Therefore, the weight of evidence is that DCs do not play a major role in this sterile inflammatory response, at least in the models we examined.

Based on our findings, we propose that MPs are the initial sensor of cell death and in response produce IL-1 and maybe other mediators that initiate the inflammatory response. Because these cells are resident in tissues, they are strategically placed to carry out this function. Moreover, in this location they are the primary cells that seek out and eat dead cells and therefore are well positioned to be the initial sensor of death. Consistent with this model we had previously found that dead cells directly stimulate MPs to produce IL-1 in vitro (24). However, we cannot fully exclude the possibility that in vivo some other primary mechanism senses cell death and then stimulates MPs to generate IL-1 as a secondary event.

Necrosis (oncrosis) is defined as the cell death characterized by the morphologic changes of swollen cells and organelles, and loss of membrane integrity leading to the release of intracellular contents (2). Cell death due to membrane disruption by mechan-
ical damage is classified as necrosis (46) and many different forms of cell injury can lead to this kind of cell death. Recent studies have revealed that the necrosis induced by deprivation of energy (e.g., ischemia) has highly organized processes (47, 48). Whether all forms of necrosis lead to the same biological outcomes is not known. Although the inflammatory response to both of the heat-
elicitant stimuli, injury to cells, and their mediators that help trigger the acute inflammatory re-

References

The authors have no financial conflicts of interest.

Acknowledgments

We thank Fernando Ontiveros for critical reading of the manuscript and Zuhul Patel, Sharlene Hubbard, Matthew Tanko, and Janice Bellelsle for technical assistance.

Disclosures

The Journal of Immunology


