INNATE IMMUNITY

An endogenous caspase-11 ligand elicits interleukin-1 release from living dendritic cells

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Dendritic cells (DCs) use pattern recognition receptors to detect microorganisms and activate protective immunity. These cells and receptors are thought to operate in an all-or-nothing manner, existing in an immunologically active or inactive state. Here, we report that encounters with microbial products and self-encoded oxidized phospholipids (oxPAPC) induce an enhanced DC activation state, which we call “hyperactive.” Hyperactive DCs induce potent adaptive immune responses and are elicited by caspase-11, an enzyme that binds oxPAPC and bacterial lipopolysaccharide (LPS). oxPAPC and LPS bind caspase-11 via distinct domains and elicit different inflammasome-dependent activities. Both lipids induce caspase-11-dependent interleukin-1 release, but only LPS induces pyroptosis. The cells and receptors of the innate immune system can therefore achieve different activation states, which may permit context-dependent responses to infection.

Oxidized phospholipids derived from 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (PAPC), known as oxPAPC, represent one class of DAMPs. OxPAPC is found in dying cells and can reach concentrations of 10 to 100 μM in damaged tissues (4, 5). oxPAPC is an LPS mimic that, depending on context, promotes or inhibits Toll-like receptor 4 (TLR4)–dependent inflammation (6–8). The existence of LPS and a self-derived LPS mimic provides a model to dissect the activities of PAMPs and DAMPs in innate immunity.

If oxPAPC is truly an LPS mimic, then LPS and oxPAPC should exhibit similar activities. We therefore determined whether oxPAPC activates TLR4 in murine bone marrow–derived macrophages (MΦ) and dendritic cells (DCs). LPS, but not oxPAPC, induced TLR4 dimerization and endocytosis, MyD88–IRAK4 interactions (i.e., myddosome formation), and expression of the cytokines interleukin (IL)–6, tumor necrosis factor–α (TNFα), IL-1β, and interferon-β (IFN-β) (Fig. 1, A to C, and fig. S1, A to C). Furthermore, oxPAPC–treated cells contained undetectable viperin or phosphorylated STAT1, both of which were abundant upon LPS treatment (Fig. 1D and fig. S1D). These data indicate that oxPAPC cannot activate TLR4.

Some DAMPs only induce cytokine release from cells previously exposed to microbial products. For example, adenosine triphosphate (ATP) activates IL-1β release from cells primed with TLR ligands (9). We therefore examined IL-1β release from LPS-primed DCs. Interestingly, oxPAPC, similar to ATP, induced the release of cleaved IL-1β from LPS-primed DCs (Fig. 2, A and B, and fig. S2, A and B). oxPAPC also elicited IL-1β release from primed DCs isolated from the spleens of mice (fig. S2D).

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Fig. 1. oxPAPC does not induce TLR4 signaling. (A) MΦs or DCs were treated with LPS or oxPAPC for the indicated time points. TLR4 dimerization and endocytosis were measured by flow cytometry. The line graphs represent means and standard deviations (SDs) of four replicates. (B) MΦs or DCs were treated with LPS or oxPAPC. Cytokine production was analyzed 18 hours later. Means and SDs of four replicates are shown. (C) Myddosome formation in MΦs was assessed at the indicated time points after treatment with LPS or oxPAPC by coimmunoprecipitation (IP) of IRAK4 with MyD88 followed by Western analysis of the proteins indicated. (D) Whole-cell lysates (WCL) were collected and DCs were monitored for STAT-1 phosphorylation and viperin expression after treatment with LPS and oxPAPC. [(C) and (D)] One experiment representative of three is shown.
Oxidation of PAPC to oxPAPC generates a heterogeneous mixture of lipids (fig. S4, A and B). To determine whether alternative sources of oxPAPC have similar activities, we generated oxPAPC enriched in PEIPC [1-palmitoyl-2-(5,6 epoxyisoprostanoyl)-sn-glycero-3-phosphocholine] (fig. S4C), an active component of oxPAPC (10). Like oxPAPC, PEIPC induced IL-1β release from LPS-primed DCs (Fig. 2B).

In contrast to the effects on IL-1β release, neither ATP nor oxPAPC influenced the abundance of cell-associated IL-1β (Fig. 2B and fig. S2C) or the secretion of TNFα (fig. S2, D and E). Additionally, when DCs were treated simultaneously with LPS/ATP or LPS/oxPAPC (i.e., no priming), IL-1β release was only induced by LPS/oxPAPC (fig. S3B), suggesting differences in how these DAMPs promote IL-1β release. When the phosphocholine variant 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was used, it could not elicit IL-1β release (fig. S3C). In contrast, purified components of oxPAPC (KodiA-PC, POVPc, or PGPC) elicited IL-1β release (fig. S3C). In all cases, LPS-induced TNFα secretion was unaffected (fig. S3C). Individual lipids within oxPAPC therefore promote IL-1β release.

Inflammasomes are cytoplasmic protein complexes that trigger IL-1β release (9). To determine whether IL-1β release is inflammasome-dependent, we examined DCs from apoptosis-associated speck-like protein containing a CARD (ASC) knockout (KO), caspase-1 KO, caspase-1/caspase-11 double (d)KO, or NOD-like receptor family, pyrin domain–containing 3 (NLRP3) KO mice, each of which are defective for inflammasome functions (11, 12). All of these factors were required for oxPAPC-induced IL-1β release (Fig. 2C), whereas no inflammasome regulator was required for LPS-induced TNFα secretion (fig. S2F).

Interestingly, oxPAPC could not elicit IL-1β release from Mφs (fig. S3A). To explain this finding, we considered that DCs are better “primed” than Mφs because they produce more TNFα than Mφs in response to LPS (fig. S2E). However, IFN-γ–treated Mφs were primed as well as DCs, yet they could not respond to oxPAPC (fig. S3D). Transfection of oxPAPC elicited IL-1β release from DCs primed with the TLR2 ligand Pam3CSK, but not Mφs, whereas LPS transfection of Mφs elicited IL-1β release (Fig. 2D). ATP treatments also revealed differences between Mφs and DCs. DCs and Mφs die upon LPS/ATP with similar kinetics but release different amounts of IL-1β (Fig. 2E and fig. S2A) and express different levels of ASC (fig. S3, E and F) but not other inflammasome components (fig. S3F). oxPAPC therefore revealed differences in inflammasome-related activities in bone marrow–derived Mφs and DCs (fig. S5). We do note, however, that populations of DCs and Mφs may exist that exhibit different responses to oxPAPC than those described above.

Further analysis of the mechanisms of inflammasome activation revealed that potassium efflux promoted ATP-induced, but not oxPAPC-induced, IL-1β release (fig. S6, A to C). Additionally, oxPAPC did not alter mitochondrial functions (fig. S6D).

Caspase-11 is an LPS receptor that promotes IL-1β release by noncanonical inflammasomes (13, 14). Interestingly, oxPAPC–transfected IL-1β release was largely abolished in caspase-11 KO DCs (Fig. 3A), whereas ATP-mediated IL-1β release remained intact. TNFα secretion was unaffected by caspase-11 deficiency (fig. S6E). Microscopic analysis revealed that oxPAPC and ATP induced the formation of ASC and caspase-1 containing “specks” in LPS-pretreated DCs (Fig. 3B), albeit with different kinetics (fig. S6F). These structures are recognized as individual inflammasomes (15), and in the specific case of oxPAPC stimulations, speck formation was caspase-11 dependent (Fig. 3B and fig. S6G). Caspase-11 is therefore likely required for oxPAPC-induced IL-1β release because it promotes inflammasome assembly in DCs.

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**Fig. 2. oxPAPC induces the activation of the NLRP3 inflammasome in DCs.** (A) DCs primed with LPS, followed by ATP or oxPAPC treatment. Cell culture supernatant from DCs subjected to indicated treatments was collected, and processed IL-1β (p17) production was assessed. One experiment representative of three is shown. (B) DCs were treated with LPS alone; were treated with 10, 50, or 120 μM of oxPAPC; or were primed with LPS for 3 hours and then treated with oxPAPC. For this experiment, commercially available oxPAPC and an oxPAPC enriched in PEIPC were used. Eighteen hours after LPS administration, secreted (left panel) and cell-associated (right panel) IL-1β was measured by enzyme-linked immunosorbent assay (ELISA). Means and SDs of four replicates are shown. (C) DCs of the genotypes indicated were treated with LPS alone, were treated with oxPAPC alone, or were primed with LPS for 3 hours and then treated with oxPAPC. Eighteen hours after LPS administration, IL-1β secretion was measured by ELISA. Means and SDs of four replicates are shown. (D) Mφs and DCs were treated with Pam3CSK (P3C) alone, were treated with oxPAPC alone, or were primed with Pam3CSK for 3 hours and then treated with oxPAPC, DOTAP alone, and LPS or oxPAPC encapsulated in DOTAP (N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate). Eighteen hours after P3C administration, IL-1β was measured by ELISA. Means and SDs of four replicates are shown. (E) DCs (left panel) or Mφs (right panel) were primed with LPS for 3 hours and treated with ATP. At indicated time points, IL-1β was measured by ELISA, and cell death was measured by propidium iodide permeabilization assay. Means and SDs of four replicates are shown.

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**REFERENCES AND LINKS**


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Interestingly, multiple TLR ligands primed DCs for oxPAPC responsiveness, as Pam3CSK4-primed DCs induced IL-1\(\beta\) release in response to oxPAPC (fig. S6H) by an NLRP3-, ASC-, and caspase-11-dependent process (fig. S6I). The TLR9 ligand CpG-DNA also primed DCs for oxPAPC responsiveness (fig. S6I). Similarly, oxPAPC, but not DMPC, elicited IL-1\(\beta\) release from an LPS- or CpG-DNA–primed splenic DC line called D1 (fig. S6J). OxPAPC therefore activates multiple DCs upon encounters with diverse TLR ligands. The finding that multiple TLR ligands prime DCs for oxPAPC responsiveness eliminates the possibility that oxPAPC acts as an LPS carrier to caspase-11.

We considered that oxPAPC interacts with caspase-11, like LPS (13). Endogenous caspase-11 (but not caspase-3) was captured from DC or immortal bone marrow–derived MΦ (iMΦ) lysates through interactions with biotin-LPS or biotin-oxPAPC (figs. S4D and S7A and Fig. 3C). Caspase-11 was not captured by the biotinylated NOD2 ligand muramyl dipeptide (MDP) (Fig. 3C). OxPAPC displayed a dose-dependent signal with immobilized catalytically inactive caspase-11(C254A) using surface plasmon resonance (SPR), as did LPS (Fig. 3D). In contrast, DMPC did not bind caspase-11, and oxPAPC did not bind immunoglobulin G (fig. S7B). The dissociation constant (\(K_d\)) between caspase-11 and oxPAPC was calculated as 1.3 \(\times\) \(10^{-6}\) M, whereas the \(K_d\) for interactions with LPS is 3.78 \(\times\) \(10^{-8}\) M (13). Gel filtration chromatography revealed that oxPAPC also promoted caspase-11 oligomerization (Fig. 3E), with monomers eluting at 15.03 ml, dimers at 13.82 ml, and higher-order oligomers earlier.

![Fig. 3. oxPAPC promotes noncanonical inflammasome activation.](http://science.sciencemag.org/)}
Mutation of lysine residues within the caspase-11 CARD (caspase activation and recruitment domain) prevents interactions with LPS (23), as assessed by the ability of biotin-LPS to capture caspase-11 produced in 293T cells (fig. S7C). Interestingly, these mutations did not prevent interactions with biotin-oxPAPC (fig. S7C). Moreover, the isolated caspase-11 catalytic domain (but not the CARD) retained the ability to bind biotin-oxPAPC (fig. S7D). SPR analysis verified these results, because nearly identical affinities of oxPAPC for caspase-11 or the catalytic domain (noted as ΔN59) were calculated (Fig. 3D). LPS could not bind the caspase-11 catalytic domain (Fig. 3D), as expected (23). These data establish that distinct domains within caspase-11 bind LPS and oxPAPC.

The interaction of oxPAPC with the catalytic domain prompted us to examine caspase-11 enzymatic activity. Whereas LPS strongly increased activity of caspase-11 monomers, oxPAPC displayed minimal activity (fig. S7E). We also examined preexisting caspase-11 oligomers, where intrinsic activity is high (fig. S7E). Interestingly, whereas LPS stimulated this activity further, oxPAPC suppressed intrinsic activity (fig. S7E). Moreover, oxPAPC blocked LPS-induced caspase-11 activity in a dose-dependent manner (fig. S7F). These data indicate that LPS promotes, but oxPAPC prevents, caspase-11 activity.

To determine whether caspase-11 activity is required for oxPAPC-induced IL-1β release, we reconstituted caspase-11 KO DCs with wild-type (WT) or catalytic mutant (C254A) caspase-11 or empty vector. LPS elicited IL-1β release from cells expressing WT caspase-11 but not empty vector or mutant caspase-11 (Fig. 3F). These data confirm that caspase-11 activity promotes LPS-induced IL-1β release (17, 18). Interestingly, WT and mutant-reconstituted DCs released IL-1β in response to oxPAPC (Fig. 3F). TNFα release was unaffected under all conditions (fig. S7G). Two modes of caspase-11-mediated IL-1β release therefore exist, with catalytic activity only being necessary for LPS responses.

In addition to caspase-11, oxPAPC-induced IL-1β release requires caspase-1 (Figs. 2C and 3A). Interestingly, independent of caspase-11, biotin-oxPAPC captured endogenous caspase-1 from cell lysates, whereas biotin-LPS could not (fig. S7, H and I). These data support a model whereby oxPAPC and LPS promote inflammasome formation via distinct mechanisms, with oxPAPC specifically forming a caspase-1/11 heterocomplex that may promote IL-1β release. The precise mechanisms that govern oxPAPC-caspase interactions, and how these interactions promote inflammasome activities, await further investigation.

Pyroptosis, another inflammasome-dependent activity (9), is characterized by the loss of plasma membrane integrity and the release of cytoplasmic proteins and organelles. Caspase-11 activity was necessary for transfected LPS to induce pyroptosis, as assessed by lactate dehydrogenase (LDH) release from the cytosol (Fig. 3F). Surprisingly, oxPAPC did not elicit pyroptosis (Fig. 3F). We explored this observation further in WT DCs, where LPS/ATP or transfected LPS induced pyroptosis with differing kinetics (Fig. 4A). Interestingly, although LPS transfection or oxPAPC treatment induced similar amounts of IL-1β release (Fig. 4B), only LPS transfection caused pyroptosis (Fig. 4A and fig. S8A).

To corroborate these observations, we examined plasma membrane integrity of individual cells containing ASC specks. Cells treated with
CD4+ T cells were isolated from draining lymph nodes of WT and caspase-11, and caspase-1/-11 double knockout (dKO) mice were injected subcutaneously with LPS, ovalbumin (OVA), and oxPAPC. The activity of oxPAPC in promoting T cell activation was measured by measuring IL-2, IL-17, and interferon-γ (IFN-γ) secretion. As compared with immunizations with LPS alone, oxPAPC promotes T cell activation in a caspase-11-dependent manner. We speculate that promoting DC hyperactivation may be an immunoregulatory mode of action.

Our analysis also revealed that caspase-11 may be an unusual PRR, which binds PAMPs and DAMPs via distinct domains and has distinct modes of activation. We consider CARD engagement by oxPAPC to be an antimicrobial mode of caspase-11 activation, designed to suppress intracellular bacteria to infiltrating neutrophils after pyroptosis (24). In contrast, catalytic domain engagement by oxPAPC may be an immunoregulatory mode of caspase-11 activation, designed to promote T cell activation, specifically in DCs (fig. S5).

This study therefore provides a mandate to examine whether other PRRs have multiple states of activation.

REFERENCES AND NOTES

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Editor's Summary

**Immune activation in context**

Dendritic cells (DCs) initiate protective immunity upon binding molecules derived from microbes or released from dying cells. Zanoni *et al.* examined how microbial and endogenous signals interact to shape the course of the ensuing immune response (see the Perspective by Napier and Monack). They found that oxPAPC, an oxidized phospholipid released from dying cells, binds to a protein called caspase-11 in DCs, activating an inflammatory program in these cells. Whereas caspase-11 binding to oxPAPC and bacterial lipopolysaccharide causes DCs to produce the cytokine interleukin-1 (IL-1) and undergo cell death, binding to oxPAPC alone triggers DCs to secrete IL-1 and induce strong adaptive immunity. Thus, context-dependent signals can shape the ensuing immune response.

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