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Immune Complexes Inhibit IL-1 Secretion and Inflammasome Activation

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IgG immune complexes have been shown to modify immune responses driven by APCs in either a pro- or anti-inflammatory direction depending upon the context of stimulation. However, the ability of immune complexes to modulate the inflammasome-dependent innate immune response is unknown. In this study, we show that IgG immune complexes suppress IL-1α and IL-1β secretion through inhibition of inflammasome activation. The mechanism by which this inhibition occurs is via immune complex ligation of activating FcγRs, resulting in prevention of both activation and assembly of the inflammasome complex in response to nucleotide-binding domain leucine-rich repeat (NLR) P3, NLRC4, or AIM2 agonists. In vivo, administration of Ag in the form of an immune complex during priming of the immune response inhibited resultant adaptive immune responses in an NLRP3-dependent model of allergic airway disease. Our data reveal an unexpected mechanism regulating CD4+ T cell differentiation, by which immune complexes suppress inflammasome activation and the generation of IL-1α and IL-1β from APCs, which are critical for the Ag-driven differentiation of CD4+ T cells. The Journal of Immunology, 2014, 193: 5190–5198.

Immunoglobulin G–Ag immune complexes have numerous effects on the host immune system driven by their signaling through FcγR. Signals from FcγR can be either activating or inhibitory in nature. Signaling resulting in activation of the innate immune system leads to enhanced phagocytosis, the induction of Abs-dependent cellular cytotoxicity, degranulation, as well as profound modifications in cytokine production by macrophages, dendritic cells, and monocytes (1). Immune complexes have been shown to upregulate IL-10 production while concurrently suppressing IL-12 p40 generation (2–5). These changes induced in innate immune cytokine production have a major influence on the generation of an Ag-specific adaptive response and have been shown to repress Th1 responses while enhancing Th2 responses (2, 6).

IL-1α and IL-1β are closely related cytokines that both signal through the IL-1R1. As potent proinflammatory cytokines, the secretion of each is tightly regulated. IL-1β secretion is dependent upon the activation of caspase-1, which is activated as part of a multiprotein complex called the inflammasome (7, 8). In contrast, IL-1α processing and secretion can proceed in either an inflammasome-dependent or -independent manner depending on the particular agonist (9). Upon activation, the nucleotide-binding domain leucine-rich repeat (NLR) containing family members (NLRP1, NLRP3, and NLRC4) and the PYHIN family member (AIM2) can form an inflammasome complex, which typically contains an NLR (or AIM2), the adapter protein apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC), and the cysteine protease caspase-1. Activation of the inflammasome requires a two-step process; signal one, or priming, occurs in response to either microbial or endogenous danger signals (10, 11). Priming results in the generation of pro–IL-1α, pro–IL-1β, and pro–IL-18 and also readies the inflammasome for activation through an unknown mechanism. Signal two can be provided via numerous stimuli, resulting in the activation of the specific inflammasome complex and ultimately caspase-1 activation.

In this study, we demonstrate that the ligation of FcγR by IgG immune complexes during priming inhibits the assembly and activation of the inflammasome complex. This in turn results in the markedly diminished production of IL-1α and IL-1β. We further show that in vivo aluminum hydroxide (alum)–driven adaptive immune responses require the presence of both IL-1α and IL-1β and that IgG immune complex–mediated suppression of IL-1α and IL-1β results in the inhibition of effector CD4+ T cell responses.

Materials and Methods

Mice

CS7BL/6N and CD45.1 (B6Ly5.2Cr) mice were obtained from the National Cancer Institute mouse repository (Frederick, MD). The generation of Nlrps1a−/−, Casp1−/−, Il1r1−/−, Il1a−/−, Il1b−/−, Fceir1g−/−, and Fcgr2b−/− mice has been described previously (12–19). OT-II (B6.Cg-Tg(TcrαTcrβ)H25Cbn/J) transgenic mice (20) were purchased from The Jackson Laboratory (Bar Harbor, ME). All protocols were approved by the Institutional Animal Use and Care Committees at the University of Iowa.
Immune complexes

IgG-opsonized SRBC (elG) were produced as described previously using a rabbit anti-SRBC Ab (Rockland, Gilbertsville, PA) (5). For IgG–Ova immune complexes, chicken egg OVA (Ova; Grade V; Sigma-Aldrich, St. Louis, MO) and goat anti-Ova IgG (MP Cappel, Santa Ana, CA) were mixed at a 1:32 (µg Ova/µg IgG) ratio and incubated for 30 min at room temperature. To produce IgG-opsonized Candida albicans, log growth-phase C. albicans (3–5 × 10^7 yeast/ml) was incubated with 0.5 mg/ml rabbit anti-
C. albicans polyclonal IgG (Thermo Scientific) for 40 min at 4°C. The IgG-opsonized C. albicans was washed and resuspended in Dulbecco’s PBS.

In vitro stimulation of bone marrow–derived macrophages

Bone marrow–derived macrophages (BMDM) were generated as described previously (4). BMDM were either left unstimulated or primed with 50 µg/ml LPS (InvivoGen, San Diego, CA), LPS, and immune complexes (LPS and particle control for 3 to 4 h. For studies using Ova or IgG–Ova, BMDM were treated with 1.6 µg/ml Ova equivalents. BMDM were then challenged with 5 mM ATP (Sigma-Aldrich), 50 µg/cm² silica (Min-U-Sil-5; U.S. Silica), C. albicans FC20 strain at a multiplicity of infection (MOI) of 10:1 for 6 h, Pseudomonas aeruginosa PAK strain at an MOI of 1:1 for 6 h, or Francisella tularensis LVS strain at an MOI of 50:1 for 9 h. Supernatants were collected and assayed for IL-1b, IL-1β, IL-18, IL-10, and IL-12 p40. Ab pairs for the IL-1β ELISAs were from R&D Systems. Ab pairs for IL-1α, IL-10, and IL-12 p40 were from eBioscience (San Diego, CA). IL-18 ELISA Ab pairs were from MBL International (Woburn, MA).

Induction and evaluation of airway inflammation

Mice were sensitized on day 0 by i.p. injection with either 2 mg alum (Thermo Scientific) and 20 µg Ova or 2 mg alum and IgG–Ova (20 µg Ova). On days 15, 16, and 17, mice were intranasally challenged with 20 µg Ova in 50 µl PBS. Lung nodes (LNs), lungs, blood, and bronchial lavage fluid (BAL) were harvested on day 19. BAL was performed by delivering 1 ml cold PBS into the airway via a tracheal cannula and gently aspirating the fluid. The lavage was repeated three times. The cells were stained with trypan blue to determine viability, and total nucleated cell counts were obtained using a hemocytometer. Cytospin slides were prepared (Invitrogen) and stained with May-Grinnell, and immunofluorescence was carried out for CD4 and CD8 to determine the percentage of neutrophils, eosinophils, lymphocytes, or mixed inflammatory cell populations. Lung tissues were fixed in 4% paraformaldehyde and permeabilized with 0.5% saponin. Cells were immunostained with rabbit anti-ASC (Enzo Life Sciences) as the primary Ab and FITC-conjugated donkey anti-rabbit IgG (BioLegend), and actin was stained with rhodamine phalloidin (Molecular Probes). Coverslips were mounted on slides using VectaShield with DAPI (Vector Laboratories, Burlingame, CA) and imaged using a confocal microscope (Zeiss 710; Carl Zeiss).

Statistical analysis

Statistical data analysis was done using Prism 5.0a (GraphPad Software, La Jolla, CA). Unless otherwise noted, statistical significance for single comparisons was determined by Student t test; ANOVA with Bonferroni posttest was used for multiple comparisons.

Results

Immune complexes inhibit the secretion of IL-1α, IL-1β, and IL-18 in vitro

Cytokines secreted by APCs are required to instruct the differentiation of CD4+ T cells; to assess if these cytokines were affected by immune complex uptake, BMDM were primed with LPS in the presence of either elG or unopsonized SRBC (e), and the cells were then challenged with the NLRP3 inflammasome agonist silica. As expected, stimulation of LPS-primed BMDM with silica resulted in the secretion of IL-1β and IL-18; however, the presence of elG immune complexes during LPS priming significantly inhibited both IL-1β and IL-18 secretion (Fig. 1A). Consistent with previous studies, immune complexes suppressed LPS-induced IL-12 p40 production while concurrently elevating IL-10 production (Fig. 1A). This inhibition was not specific to the type of immune complex or the NLRP3 agonist as soluble IgG–Ova immune complexes were also capable of inhibiting IL-1β secretion induced by the NLRP3 agonists ATP and alum (Fig. 1B, 1C). Treatment with IgG immune complexes did not appreciably alter IL-6 or TNF-α release (Supplemental Fig. 1A, 1B) (4). Additionally, this inhibition required that IgG be complexed to an Ag as the addition of free IgG failed to inhibit IL-1β secretion (Supplemental Fig. 1C).

Caspase-1 activation involves autocalytotic processing of the 45-kDa procaspase-1 to generate two subunits, p20 and p10. Caspase-1 activation in silica or ATP-stimulated LPS-primed BMDM was detected in immunoblots by the appearance of the p10 cleavage product (Fig. 1D). However, if BMDM were LPS primed in the presence of elG immune complexes, caspase-1 activation was not observed in response to silica or ATP challenge (Fig. 1D). To elucidate the mechanism by which immune complexes block inflammasome activation, we examined the oligomerization of inflammasomes upon receiving an activating signal. Upon activation of the inflammasome, the adapter protein ASC forms cytosolic aggregates, or specks, that colocalize with caspase-1, and these foci can be visualized using confocal microscopy (23, 24). We tested if IgG immune complexes were able to block the formation of ASC specks. BMDM were challenged with ATP following LPS priming in the presence or absence of immune complexes. We observed the formation of ASC specks upon ATP challenge in BMDM that had been LPS primed in the absence of immune complexes (Fig. 1E, 1F). However, ATP-challenged BMDM that had been primed in the presence of immune complexes had a significant reduction in ASC specks (Fig. 1E, 1F). These data indicate that immune complexes block caspase-1 activation and subsequent processing and secretion of IL-1β by preventing NLRP3 inflammasome assembly.

Unlike IL-1β, cleavage and secretion of IL-1α can be either inflammasome-dependent or -independent depending on the NLR agonist (9). In general, crystalline activators of the NLRP3 inflammasome like alum induce caspase-1–independent IL-1α secretion. The caspase-1–independent pathway of IL-1α secretion involves the release of IL-1α from the secretory granules of immune cells (25). In our system, immune complexes prevented IL-1α cleavage and secretion, but not IL-1β secretion, in response to ATP. It is possible that immune complexes inhibit caspase-1 activation downstream of caspase-1 cleavage of IL-1α; however, this needs to be clarified by further studies.

Discussion

The results presented here are consistent with previous studies indicating that immune complexes can block secretion of cytokines and cytokine production by APCs (26). However, the mechanism by which immune complexes block inflammasome activation has only recently been elucidated (24). In this study, we present novel data indicating that immune complexes inhibit inflammasome activation through inhibition of caspase-1 (Fig. 1D, 1E). These findings are consistent with previous studies indicating that immune complexes prevent NLRP3 inflammasome assembly (24).

In this study, we compared the effects of immune complexes on inflammasome activation to the effects of immune complexes on cytokine secretion. Our results show that immune complexes prevent NLRP3 inflammasome activation as indicated by a reduced formation of ASC specks (Fig. 1E, 1F). However, it is possible that immune complexes prevent the secretion of IL-1β (Fig. 1B, 1C) by preventing caspase-1 activation. This hypothesis is consistent with previous studies indicating that immune complexes inhibit caspase-1 activation downstream of caspase-1 cleavage of IL-1α (25).

Conclusion

In conclusion, our data indicate that immune complexes block inflammasome activation and caspase-1–independent IL-1α secretion. The mechanism by which immune complexes block inflammasome activation and caspase-1–independent IL-1α secretion is consistent with previous studies indicating that immune complexes prevent NLRP3 inflammasome assembly (24). However, it is possible that immune complexes prevent the secretion of IL-1β by preventing caspase-1 activation downstream of caspase-1 cleavage of IL-1α; however, this needs to be clarified by further studies.
were LPS primed in the presence or absence of IgG immune complexes. To determine if immune complexes inhibited the expression of NLRP3, or other inflammasome components, BMDM were LPS primed in the presence or absence of Ova or IgG–Ova and then challenged with either ATP (A) or alum (C) for 6 h. Culture supernatants were collected and analyzed for IL-1β secretion by ELISA. **p ≤ 0.001 by Student t test. (D) BMDM were LPS primed in the presence or absence of IgG; macrophages were then challenged with either silica or ATP. Six hours later, cell lysates were analyzed for caspase-1 activation by immunoblot. (E and F) BMDM were LPS primed with or without IgG–Ova and then were challenged with ATP for 1 h; cells were fixed, permeabilized, and stained for ASC. Representative confocal images are shown. Scale bar, 10 μm. (E) Number of speck-positive cells was quantified (F). ***p ≤ 0.001 by one-way ANOVA with Bonferroni posttest. BMDM were LPS primed with or without Ova or IgG–Ova cells were then challenged with ATP and alum. Culture supernatants were harvested and analyzed for IL-1α by ELISA. **p ≤ 0.01 by Student t test. Determinations were performed in triplicate and are expressed as the mean ± SD. Results shown are representative of at least three independent experiments.

Immune complexes do not prevent the synthesis of pro–IL-1β, NLRP3, ASC, or caspase-1

We next asked at what step immune complexes acted to interfere with inflammasome activation. Inhibition of IL-1β secretion occurred if immune complexes were added up to 3 h following the addition of LPS, but not if immune complexes were added concurrently with the NLRP3 agonist, suggesting that immune complexes interfered with the priming signal required for inflammasome activation (Fig. 2A). Additionally, we found that immune complex-mediated inhibition of IL-1β secretion was dependent upon the number of immune complexes used as well as the concentration of Ab used to opsonize the target (Fig. 2B, 2C).

The precise mechanism by which priming readies the inflammasome for activation is unknown; however, it has been suggested that upregulation of NLRP3 expression is one factor in the priming process. To determine if immune complexes inhibited the expression of NLRP3, or other inflammasome components, BMDM were LPS primed in the presence or absence of IgG immune complexes for 4 h and cell lysates subjected to immunoblot. Similar amounts of procaspase-1, pro–IL-1β, NLRP3, and ASC were detected in the absence or presence of immune complexes (Fig. 2D), suggesting that inhibition of inflammasome complex component expression is not responsible for immune complex-mediated caspase-1 inhibition.

Inflammasome inhibition by IgG immune complexes requires signaling through the FcRγ-chain

FcγRs can be either activating (FcγRI, FcγRII, and FcγRIV) or inhibitory (FcγRIIb). The activating receptors require the ITAM containing FcγRγ-chain (encoded by the FcεR1a gene) for signal propagation (18), whereas the inhibitory FcγRIIb signals through a cytosolic ITIM motif (25). To determine the contribution of activating and inhibitory FcγR in immune complex–mediated inflammasome inhibition, we used BMDM from FcRγ-deficient (FcεR1a−/−) or FcγRIIb-deficient (FcγRIIB−/−) mice. Immune complexes failed to suppress the secretion of IL-1β in response to silica or ATP in BMDM from FcεR1a−/− mice (Fig. 3A). In contrast, immune complexes mediated suppression of IL-1β secretion remained intact in BMDM from FcγRIIB−/− mice (Fig. 3B). Together, these data suggest that inhibition of inflammasome activation by immune complexes is not mediated by signaling through the inhibitory receptor FcγRIIb and instead that signaling through the FcγRγ-chain is required, implicating an activating FcγR in this process.
The initiation of phagocytosis is an important function of signaling through the FcRγ-chain. To determine if uptake of IgG immune complexes was required to block IL-1β secretion, we plated macrophages on tissue culture plates that had been coated with IgG or Ova. Exposure of macrophages to plate-bound IgG did not inhibit IL-1β secretion in response to silica (Fig. 3C), suggesting that FcγR ligation alone is not sufficient to inhibit inflammasome activation. In contrast, exposure of macrophages to plate-bound IgG did inhibit IL-12p40 production and enhance IL-10 secretion in response to LPS stimulation (data not shown) (4).

**F. tularensis** and **C. albicans** also resulted in diminished serum IL-1β levels compared with mice challenged with *C. albicans* in the presence of Ova alone (Fig. 4C).

IgG immune complexes were also capable of effectively suppressing IL-1β secretion from BMDM challenged with *P. aeruginosa* or *F. tularensis* LVS, which activate the NLRC4 and AIM2 inflammasomes, respectively (Fig. 4D, 4E). Similar to our findings with NLRC3 agonists, we also observed that *P. aeruginosa*-challenged BMDM that had been primed in the presence of immune complexes had a significant reduction in ASC specks (Fig. 4F). These results demonstrate that immune complexes inhibit caspase-1 activation induced by multiple different inflammasomes, suggesting that inhibition occurs at a point discrete from the individual NLR or AIM2 receptors.

Ag–IgG immune complexes suppress the development of alum-driven Th2 and Th17 immune responses in vivo

To examine the effect of immune complexes on the generation of adaptive immune responses, we used a murine model of allergic airway disease. Mice were immunized i.p. with either Ova or IgG–Ova immune complexes along with the adjuvant alum. Fifteen days later, mice were challenged intranasally with Ova for 3 consecutive d; 48 h later, the extent of airway disease was assessed by histology as well as determining the inflammatory cell composition of the BAL fluid (Fig. 5A). As expected, mice immunized with alum/Ova had

**FIGURE 2.** IgG immune complexes do not effect the synthesis of inflammasome components. (A) BMDM were LPS primed; elgG were added at the indicated time points after LPS treatment. BMDM were then challenged with silica for 6 h. Supernatants were harvested and IL-1β measured by ELISA. (B) BMDM were LPS primed in the presence of increasing numbers of elgG (elgG/BMDM ratios of 10:1, 5:1, 2.5:1, and 1:1). BMDM were challenged with silica for 6 h and culture supernatants collected and analyzed for IL-1β by ELISA. (C) BMDM were primed with LPS in the presence of erythrocytes that were IgG opsonized with decreasing concentrations of anti-SRBC IgG (400, 200, 100, and 50 μg/ml). BMDM were then challenged with silica for 6 h and culture supernatants analyzed for IL-1β release by ELISA. (D) BMDM were LPS primed with or without Ova or IgG–Ova. After 4 h, lysates were collected and analyzed by immunoblot for procaspase-1, pro–IL-1β, NLPR3, ASC, and GAPDH. Determinations were performed in triplicates and are expressed as the mean ± SD. Results shown are representative of at least three independent experiments.

**FIGURE 3.** Signaling through the FcRγ-chain but not FcγRIIb is required for inflammasome suppression. BMDM from *Fcer1g*−/− (A) or *Fcgr2b*−/− (B) were LPS primed with or without elgG and challenged with silica or ATP for 6 h. Culture supernatants were harvested and analyzed for IL-1β by ELISA. (C) BMDM were seeded on tissue-culture plates that had been coated with 2 μg/ml IgG or Ova. The cells were then LPS primed for 4 h and subsequently challenged with silica for 6 h. Culture supernatants were collected, and IL-1β secretion was assessed by ELISA. Determinations were performed in triplicate and are expressed as the mean ± SD. Results shown are representative of at least three independent experiments. *p ≤ 0.05, ***p ≤ 0.001 by Student’s t test.

in the presence of IgG–Ova immune complexes also resulted in diminished serum IL-1β levels compared with mice challenged with *C. albicans* in the presence Ova alone (Fig. 4C).

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Ag–IgG immune complexes suppress the development of alum-driven Th2 and Th17 immune responses in vivo

To examine the effect of immune complexes on the generation of adaptive immune responses, we used a murine model of allergic airway disease. Mice were immunized i.p. with either Ova or IgG–Ova immune complexes along with the adjuvant alum. Fifteen days later, mice were challenged intranasally with Ova for 3 consecutive d; 48 h later, the extent of airway disease was assessed by histology as well as determining the inflammatory cell composition of the BAL fluid (Fig. 5A). As expected, mice immunized with alum/Ova had
an eosinophilic response to intranasal challenge with Ova (Fig. 5B, 5C). In contrast, mice immunized with alum/IgG–Ova had markedly diminished eosinophilic influx into the lungs following intranasal challenge with unopsonized Ova (Fig. 5B, 5C).

To determine if subsequent adaptive immune responses to the Ag were affected by presentation of Ag within an immune complex, we restimulated mediastinal LN cells from Ova-exposed mice with Ova ex vivo. LN cells from mice immunized with alum/Ova produced IL-13, IL-4, and IL-17A upon Ova restimulation, consistent with an alumin-driven Th2 and Th17 response (Fig. 5D, 5E). Surprisingly, LN cells from mice immunized with alum/IgG–Ova secreted significantly less IL-13, IL-4, and IL-17A upon Ova restimulation, consistent with an alumin-driven Th2 and Th17 response (Fig. 5D, 5E). No difference in IFN-γ production was observed following Ova restimulation of LN cells from mice immunized with alum/Ova or alum/IgG–Ova (data not shown). Ova-specific IgG1 and total IgE Ab induction was also significantly diminished in mice immunized with alum/IgG–Ova compared with alum/Ova (Fig. 5F). Ova-specific IgG2c levels were not different between alum/Ova and alum/IgG–Ova–immunized mice (Fig. 5F).

To determine whether the impaired adaptive response was due to a failure of Ag presenting cells to process or present the IgG–Ova appropriately, we adoptively transferred CFSE-labeled TCR-transgenic OT-II CD4+ T cells into mice immunized with either alum/Ova or alum/IgG–Ova. OT-II CD4+ T cells proliferated normally in both alum/Ova and alum/IgG–Ova–immunized mice, suggesting that the processing and presentation of Ag remained intact (Fig. 5G). We also found that IgG–Ova immune complexes could inhibit Th2 and Th17 immune responses against keyhole limpet hemocyanin, an Ag that was not part of the IgG–Ova immune complex (Supplemental Fig. 2). Taken together, these data demonstrate that Ag–IgG immune complexes suppress the development of both Th2 and Th17 immune responses.

Enhanced IL-10 production induced by immune complexes does not suppress Th2 and Th17 responses

IL-10 is a potent anti-inflammatory mediator that affects a number of cell types and is important in limiting inflammation (26). To determine if the enhanced IL-10 production by BMDM following challenge with immune complexes was required for the inhibition of Th2 and Th17 responses in vivo, we used IL-10–deficient mice. LN cells from Il10−/− mice immunized with alum/IgG–Ova suppressed IL-17A and IL-13 production upon Ova restimulation (Fig. 6A). These data suggest that the enhanced IL-10 production elicited by immune complexes is dispensable for the inhibition of alumin-driven Th2 and Th17 responses. We next asked whether the IL-10 release associated with immune complexes was responsible for the inhibition of IL-1β secretion in vitro. Similar to the findings for the Th2 and Th17 responses, impaired IL-1β secretion remained intact in cells from Il10−/− mice, suggesting immune complex–mediated IL-1β suppression does not require IL-10 (Fig. 6B).

Alum-induced Th2 and Th17 responses require the NLRP3 inflammasome and signaling through IL-1R1

The NLRP3 inflammasome has been implicated in the generation of Th2 and Th17 responses in vivo (27, 28). Furthermore, signaling through the IL-1R1 on CD4+ T cells enhances their expansion and differentiation into Th2 and Th17 effector cells (29, 30). However, the role of NLRP3 inflammasome activation in alum-driven Th17 responses is unclear. To evaluate if the NLRP3 inflammasome was required for Th17 responses in the alum/Ova
In the allergic airway model, we evaluated mice deficient in NLRP3, caspase-1, or IL-1R1. Consistent with the role for IL-1R1 in Th17 differentiation, LN cells from Il1r1^{2/2} mice immunized with alum/Ova had significantly diminished production of IL-17A upon Ova restimulation (Fig. 6C). LNs from mice deficient in either NLRP3 or caspase-1 also had significantly diminished IL-17A production upon Ova restimulation ex vivo (Fig. 6C, 6D). As expected, mice deficient in NLRP3, caspase-1, or IL-1R1 also displayed defective IL-13 responses (Fig. 6C, 6D). Although these data suggest NLRP3-dependent IL-1 production plays an important role in alum-driven Th2 and Th17 responses, the further diminution of IL-17A production in Iilr1^{2/2} mice indicates inflammasome-independent IL-1 production is also likely to contribute to alum-driven Th17 responses in vivo.

Both IL-1α and IL-1β signal through the IL-1R1; however, their individual relevance in driving alum-induced Th2 and Th17 responses is unclear. To assess the separate contribution of each of these cytokines, mice deficient in either IL-1α or IL-1β were immunized with alum/Ova and subsequently challenged intranasally with Ova. Diminished IL-17A and IL-13 production was observed in LN cells restimulated ex vivo with Ova from both Il1a^{−/−} and Il1b^{−/−} mice compared with wild-type (WT) mice (Fig. 6E, 6F).

**Suppression of CD4^+ T cell responses by immune complexes is rescued by exogenous IL-1α or IL-1β**

To ascertain if the suppression of macrophage IL-1α and IL-1β production by immune complexes observed in vivo contributed to the ability of immune complexes to inhibit the generation of Th2 and Th17 responses in vivo, mice were immunized with either alum/Ova or alum/IgG-Ova. Seventy-two hours later, draining LNs were collected, and CFSE dilution was analyzed by flow cytometry. Determinations were performed in triplicate and are expressed as the mean ± SD. Data shown are representative of two (G) or three (C–F) independent experiments, each with a minimum of three mice per group. Cntl., control.
Discussion

Swift and robust innate immune responses are required for the control of microbial pathogens, but a continued or disproportionate innate response can cause collateral tissue damage and lead to autoimmunity. Considering the strong proinflammatory activity of IL-1α and IL-1β and their critical role in the initiation of adaptive immune responses, their processing and secretion must be tightly regulated. In this study, we demonstrate a novel pathway by which inflammasome activation and assembly in macrophages can be modulated: through the internalization of immune complexes. Although previous studies have shown immune complexes can modify innate and adaptive immune responses through regulating the release of IL-10 and IL-12, their impact on inflammasome activation had not been determined. In this study, we show a novel function for immune complexes in which the presence of immune complexes inhibits activation of inflammasomes and thus the release of inflammasome-dependent IL-1α and IL-1β (Fig. 7C).

Figure 6. Alum-induced Th2 and Th17 responses require the NLRP3 inflammasome and signaling through IL-1R1. (A) Il10−/− mice were injected i.p. with either alum/Ova or alum/IgG-Ova on day 0; mice were then intranasally challenged with Ova on days 15, 16, and 17. Forty-eight hours after the final intranasal challenge, mediastinal LNs were restimulated in vitro with Ova protein. Seventy-two hours later, supernatants were collected and analyzed by ELISA for IL-17A and IL-13. *p ≤ 0.05, **p ≤ 0.01 by Student t test. (B) Il10−/− BMDM were LPS primed with or without IgG and then challenged for 6 h with silica or ATP; supernatants were collected and IL-1β measured by ELISA. ***p ≤ 0.001 by Student t test. (C–F) WT, Nlrp3−/−, Il1r1−/−, Casp1−/−, Il1a−/−, and Il1b−/− mice were immunized with alum/Ova as described in (A). Mediastinal LN were restimulated in vitro with Ova protein. Seventy-two hours later, supernatants were collected, and IL-17A and IL-13 was measured by ELISA. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 by one-way ANOVA with Bonferroni posttest (C) or Student t test (D–F). Determinations were performed in triplicate and are expressed as the mean ± SD. Data shown are representative of three independent experiments, each with a minimum of three mice per group.

Figure 7. Immune complex–mediated downregulation of adaptive immune responses is rescued by exogenous IL-1. WT mice were immunized as in Fig. 6 with an additional group receiving rIL-1α (A) or rIL-1β (B). Mediastinal LN were collected and restimulated in vitro with Ova protein. Seventy-two hours later, supernatants were collected and IL-17A and IL-13 measured by ELISA. Determinations were performed in triplicate and are expressed as the mean ± SD. Data shown are representative of three independent experiments each with a minimum of three mice per group. (C) Predicted model depicting the suppression of Th2 and Th17 responses by immune complexes in the context of the adjuvant alum. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 by one-way ANOVA with Bonferroni posttest.
complexes is specific and unique to the innate-activating signal it modifies. These findings of immunologic suppression induced by immune complexes are unexpected, as earlier studies have suggested immune complexes enhance rather than suppress immunogenicity (31–33).

Previous studies suggest that the modulation of IL-10 and IL-12 p40 by IgG–immune complexes occurs at the level of transcription (4). Interestingly, immune complexes do not appear to suppress inflammasome activation in a similar manner, rather immune complexes appear to act at a posttranslational level by preventing inflammasome assembly and subsequent caspase-1 activation. Additionally, the inhibition of inflammasome activation by immune complexes is not specific to the NLRP3 inflammasome but also applies to both the NLRC4 and AIM2 inflammasomes, as the presence of immune complexes inhibited IL-1β release induced by both *P. aeruginosa* and *F. tularensis*. Thus, this novel inhibition of early inflammasome activation by immune complexes appears to apply globally to multiple types of inflammasomes. Importantly, we show that the initial steps of priming occur in cells treated with immune complexes, as determined by the upregulation of inflammasome components, is intact in our immune complex–treated cells. However, the degree of inflammasome inhibition is dependent upon the timing of the addition of immune complexes, by which immune complexes inhibit inflammasome activation only if added prior to the addition of the inflammasome agonist. Hence, inhibition of inflammasome activation by immune complexes occurs following initial priming, but prior to assembly of the inflammasome complex as well as the subsequent cleavage of caspase-1.

FcγRs can be either activating (FcγRI, FcγRIII, and FcγRIV) or inhibitory (FcγRIib). Activating FcγRs all signal through the common ITAM-containing Fcγ-chain. We show the mechanism by which immune complexes abrogate inflammasome activation depends upon the presence of activating Fcγs, as macrophages from Fcγ-chain–deficient mice are incapable of immune complex–mediated downregulation of inflammasome responses. Interestingly, NLRP3 inflammasome activation by *Schistosoma mansoni* and *C. albicans* requires the C-type lectin receptor dectin-2 (34–36), which couples with the Fcγ-chain for signal propagation (37). Hence, signal propagation through the Fcγ-chain can either be activating or inhibitory for the NLRP3 inflammasome depending on the upstream receptor that initiates the signaling.

Engagement of the IL-1R1 causes a marked expansion of T cells in response to their cognate Ag. Th17 differentiation requirements signaling through the IL-1R1 as cells deficient in the receptor fail to develop into Th17 effectors in an experimental autoimmune encephalomyelitis model (29). Similarly, IL-1R1 signaling has been shown to be required for development of Th2 responses as LN cells from IL-1α– or IL-1β–deficient mice failed to differentiate in vitro into Th2 cells in the presence of IL-4 alone but required the addition of exogenous IL-1α or IL-1β (38). Previous studies have shown a requirement for the NLRP3 inflammasome in the development of alumn–Ag–dependent Th2 responses (27, 28). In this study, we expand on this association and show the NLRP3 inflammasome and IL-1R1 signaling are similarly required for alumn-dependent Th17 responses. Further, the development of specific CD4+ T cell responses is diminished in the absence of either IL-1α or IL-1β, suggesting both are required for full CD4+ T cell effector function. IL-1α is often thought to be interchangeable with IL-1β in terms of function, likely due to the fact that both molecules signal through the IL-1R1 on T cells (39). However, many important differences between IL-1α and IL-1β exist and suggest that their impact on Th cell commitment is more complex than previously appreciated. Engagement of the IL-1R1 on T cells by either IL-1α or IL-1β typically results in downstream signaling through MyD88 to activate NF-kB (40), yet alternative signaling pathways involving the recruitment of PI3K and activation of the protein tyrosine kinase Akt have been described following IL-1R1 ligation (41). Interestingly, the addition of exogenous IL-1α during initial activation in vivo is sufficient to restore both Th2 and Th17 effector cytokine production, whereas addition of exogenous IL-1β is sufficient to restore only Th17 responses. These data concur with studies that show a critical role for IL-1R1 and IL-1α in Th2 sensitization in a house dust mite allergic airway disease model (42). In addition, our studies show that IgG–immune complexes were not able to suppress alum-induced IL-1α production in vitro, consistent with previous findings that alum-induced IL-1α production occurred in an inflammasome-independent manner (9). However, the mechanism by which alum activates the NLRP3 inflammasome in vivo is unclear and may involve additional indirect mechanisms, including the release of extracellular ATP (43).

Our data support a mechanism by which immune complexes block inflammasome activation and, in doing so, prevent the generation of effective adaptive immune responses (Fig. 7C). The presence of immune complexes is a marker for the successful development of an adaptive immune response; thus, the ability of immune complexes to shut off inflammasomes represents a negative-feedback loop. Another example of adaptive immune down-regulation of innate responses is seen by the ablation by CD4+ T effector and memory T cells of inflammasome activation in a CD40/CD40L–dependent manner (44). Together, our data suggest that the presence of an effective adaptive immune response through Ag–Ab immune complexes acts as a negative-feedback loop, shutting off inflammasome activation and hence providing the signal to terminate the inflammatory response.

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**Disclosures**

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