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Immune Complexes Inhibit IL-1 Secretion and Inflammasome Activation

John R. Janczy,*†§ Ceren Ciraci, †‡ Stefanie Haasken, † Yoichiro Iwakura, †‡ Suzanee L. Cassell,*,†§ and Fayyaz S. Sutterwala*,†§

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 (NLIR) 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: 000–000.

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Abbreviations used in this article: alum, aluminum hydroxide; ASC, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; BAL, bronchoalveolar lavage; BMDC, bone marrow–derived macrophage; e, unopsonized SRBC; eG, IgG-opsonized SRBC; LN, lymph node; MOI, multiplicity of infection; NLR, nucleotide-binding domain leucine-rich repeat; WT, wild-type.

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C57BL6/N and CD45.1 (B6Ly5.2Cr) mice were obtained from the National Cancer Institute mouse repository (Frederick, MD). The generation of Nlrp3−/−, Csgp1−/−, Il10−/−, Rilr1−/−, Il1a−/−, Il1b−/−, Fcer1g−/−, and Fcgr2b−/− mice has been described previously (12–19). OT-II [B6.129S2-Tcrb+−/−×C57BL6.129S2-Tcrb+−/−] 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 (eIgG) 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 (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 × 10³ yeasts/ml) was incubated with 0.5 mg/ml rabbit anti-G. 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 ng/ml LPS (Invivogen, San Diego, CA), LPS, and immune complexes or 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/ml silica (Min-U-Sil-5; U.S. Silica), C. albicans FC20 strain at a multiplicity of infection (MOI) of 10:1 for 6 h, Francisella tularensis LVS strain at an MOI of 30:1 for 9 h. Supernatants were collected and assayed for 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. Lymph nodes (LNs), lungs, blood, and bronchoalveolar lavage (BAL) fluid 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, and trypan blue and rhodamine-phalloidin-stained cell nuclei were visualized.

T cell restimulation and proliferation

Cells from the draining LNs were cultured with 10 μM Ova for 72 h, and supernatants were collected and analyzed by ELISA. Ab pairs for IL-17A, IL-13, and IL-4 were from eBioscience. Flow cytometric analysis of intracellular cytokines. Ova-restimulated LN cells were incubated for 4 h in the presence of 3 μg/ml brefeldin A and 2 μM monensin (eBioscience). Cells were fixed and permeabilized using Fixation/Permeabilization buffer (eBioscience) and stained with anti-CD3, -CD4, -IL-13 (eBioscience), and –IL-17A (BD Biosciences). Flow cytometric analysis was performed on a BD Biosciences LSR II and data analyzed with FlowJo software (Tree Star, Ashland, OR).

For proliferation analysis, splenic CD4+ T cells from OT-II transgenic mice were prepared by positive selection using CD4 Miltenyi beads per the manufacturer’s instructions (Miltenyi Biotec, San Diego, CA). CD4+ T cells were labeled with 2.5 μM CFSE (Invitrogen) for 5–7 min at 37°C; 3 × 10⁶ labeled CD4+ T cells were transferred i.v. into CD45.1 congenic mice prepared, and restimulated with alum/eIgG-Ova as described above and T cell proliferation assessed at day 3 postimmunization by flow cytometry on an Accuri C6 flow cytometer (BD Biosciences).

Immunoblotting

Electrophoresis of lysates was performed using the NuPAGE system (Invitrogen) according to the manufacturer’s protocol. To detect caspase-1, IL-1β, ASC, NLRP3, and GAPDH, rabbit polyclonal anti-mouse caspase-1 p10 Abs (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit polyclonal anti-mouse IL-1β Abs (Millipore, Billerica, MA), rabbit polyclonal anti-mouse ASC Abs (Enzo Life Sciences, Farmingdale, NY), mouse monoclonal anti-mouse NLRP3 Abs (Enzo Life Sciences), and mouse monoclonal anti-mouse GAPDH (Calbiochem) were used, respectively.

ASC speck assay

Immunofluorescence studies were carried out as previously described (23). Briefly, macrophages were seeded on to glass coverslips and challenged as described above. The cells were washed and fixed in 4% paraformaldehyde in PBS. The cells were then blocked and permeabilized in 5% FBS in PBS with 0.5% saponin. Cells were immunostained with rabbit anti-ASC (Enzo Life Science) as the primary Ab and FTTC-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).

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 assay if these cytokines were affected by immune complex uptake, BMDM were primed with LPS in the presence of either eIgG 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 eIgG 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 Atum 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 autolytic 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 eIgG 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..
were LPS primed in the presence or absence of IgG. Expression of NLRP3, or other inflammasome components, BMDM in this 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. The presence or absence of IgG–Ova cells then challenged with either ATP (Fig. 2A) or alum (Fig. 2C) for 6 h. Culture supernatants were harvested 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–Ova and 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 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.

**FIGURE 1.** IgG immune complexes suppress NLRP3 inflammasome activation. (A) BMDM were LPS primed in the presence of e or elG erythrocytes for 4 h and then challenged with silica for 6 h. Cytokine secretion into culture supernatants was measured by ELISA. ***p ≤ 0.001 by Student t test. BMDM were LPS primed in the presence or absence of Ova or IgG–Ova and then challenged with either ATP (B) or alum (C) for 6 h. Culture supernatants were collected and analyzed for IL-1β secretion by ELISA. **p ≤ 0.01 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 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.

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

FcγRs can be either activating (FcyRI, FcyRIII, and FcyRIV) or inhibitory (FcyRIIb). The activating receptors require the ITAM domain for signal propagation (18), whereas the inhibitory FcyRIIb 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 FcγRII-deficient (Fcer1g−/−) or FcγRIIb-deficient (Fcg2b−/−) mice. Immune complexes failed to suppress the secretion of IL-1β in response to silica or ATP in BMDM from Fcer1g−/− mice (Fig. 3A). In contrast, immune complexes mediated suppression of IL-1β secretion remained intact in BMDM from Fcg2b−/− 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 but instead that signaling through the FcγR-chain is required, implicating an activating FcγR in this process.

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.

secretion, whereas soluble activators like ATP induce caspase-1–dependent IL-1α secretion. Consistent with inhibition of caspase-1 activation by IgG immune complexes, we observed a suppression of ATP-induced, but not alum-induced, IL-1α secretion (Fig. 1G, 1H). Taken together, these data suggest that immune complex–mediated suppression of IL-1β secretion and ATP-induced IL-1α secretion is through inhibition of caspase-1 activation.

Immune complexes do not prevent the synthesis of pro–IL-1β, NLRP3, ASC, or caspase-1
FIGURE 2. IgG immune complexes do not affect 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 collected and analyzed for IL-1β 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, NLRP3, 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.

The initiation of phagocytosis is an important function of signaling through the FcγR-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).

Immune complexes inhibit activation of NLRP3, NLRC4, and AIM2 inflammasomes

To determine if inflammasome inhibition by immune complexes is specific to the NLRP3 inflammasome or affects other inflammasomes as well, we used three pathogens, C. albicans, P. aeruginosa, and F. tularensis LVS, that activate the NLRP3, NLRC4, and AIM2 inflammasomes, respectively. Consistent with our observations with other NLRP3 agonists, we observed a marked inhibition of IL-1β secretion in response to C. albicans in BMDM that had been LPS primed in the presence of immune complexes (Fig. 4A). Direct IgG opsonization of C. albicans also resulted in significantly diminished IL-1β secretion from bone marrow–derived dendritic cells as compared with challenge with unopsonized C. albicans (Fig. 4B). Systemic infection of mice with C. albicans 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).

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 NLRP3 agonists, we also observed that P. aeruginosachallenged 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...
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 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 alum-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 alum-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 alum-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 alum-driven Th2 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

FIGURE 4. IgG immune complexes suppress NLRP3, NLRC4, and AIM2 inflammasome activation. (A) BMDM were LPS primed with or with e or eIgG and then challenged with C. albicans (MOI 10:1) for 6 h. Culture supernatants were collected, and IL-1β was measured by ELISA. (B) Bone marrow–derived dendritic cells were challenged with either unopsonized or IgG–C. albicans (MOI 10:1) for 6 h. Culture supernatants were collected and analyzed for IL-1β secretion by ELISA. **p ≤ 0.01 by Student t test. (C) WT mice were infected i.v. with 2 × 10⁶ C. albicans yeast resuspended in the presence of Ova or IgG–Ova. After 6 h, serum IL-1β levels were determined by ELISA. *p ≤ 0.05 by Mann–Whitney U test (n = 14 mice/group). BMDM were LPS primed with or without e or eIgG and then challenged with either P. aeruginosa PAK strain (MOI 1:1) (D) or F. tularensis LVS (MOI 50:1) (E) for 6 and 9 h, respectively. IL-1β in supernatants was measured by ELISA. (F) BMDM were LPS primed with or without IgG–Ova and then challenged with P. aeruginosa PAK strain (MOI 1:1) for 6 h. Cells were washed, fixed, permeabilized, and stained for ASC. The number of ASC specks was quantified by microscopy. ***p ≤ 0.001 by one-way ANOVA with Bonferroni posttest. Determinations were performed in triplicate and are expressed as the mean ± SD (A, B, and D–F) or mean ± SEM (C). Results shown are representative of at least three independent experiments (A, B, and D–F) or pooled from four independent experiments (C).

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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<sup>-/-</sup> 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 Il1r1<sup>-/-</sup> 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<sup>-/-</sup> and Il1b<sup>-/-</sup> mice compared with wild-type (WT) mice (Fig. 6E, 6F).

**Suppression of CD4<sup>+</sup> 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 vitro 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](http://www.jimmunol.org/)

**FIGURE 6.** Alum-induced Th2 and Th17 responses require the NLRP3 inflammasome and signaling through IL-1R1. (A) II10−/− 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) II10−/− BMDM were LPS primed with or without elG 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−/−, Il1α−/−, and Il1β−/− 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](http://www.jimmunol.org/)

**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.
Immune complexes inhibit inflammasome activation

Immune complexes are 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 (FcγRIlb). Activating FcγRs all signal through the common ITAM-containing FcγR-chain. We show the mechanism by which immune complexes abrogate inflammasome activation depends upon the presence of activating FcγRs, as macrophages from FcγR-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 FcγR-chain for signal propagation (37). Hence, signal propagation through the FcγR-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 requires 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 alum–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 alum-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-κB (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**

The authors have no financial conflicts of interest.

**References**


**Supplemental Figure 1.** IgG immune complexes do not effect LPS-induced TNF-α or IL-6 production. (A, B) BMDM were LPS primed in the presence of IgG-Ova or Ova for 4 h and then challenged with silica for 6 h. Cytokine secretion into culture supernatants was measured by ELISA. (C) BMDM were LPS-primed in the presence or absence of Ova, IgG-Ova and IgG for 4 h and then challenged with silica for 6 h. Culture supernatants were collected and analyzed for IL-1β secretion by ELISA. Determinations were performed in triplicate and are expressed as the mean±SD. Results shown are representative of two (A, B) or three (C) independent experiments. *p≤0.05 by ANOVA with a Bonferroni post-test.
**Supplemental Figure 2.** Immunization with IgG immune complexes suppresses Th2 and Th17 responses. WT mice (n=3 per group) were injected intraperitoneally with either alum/Ova + KLH or alum/IgG-Ova + KLH on day 0; mice were then intranasally challenged with KLH on days 15, 16 and 17. 48 h after the final intranasal challenge mediastinal lymph nodes were collected, pooled and restimulated *in vitro* with KLH protein (0, 100, 200 and 500 µg). 72 h later supernatants were collected and analyzed for IL-13 and IL-17A by ELISA. Determinations were performed in triplicate and are expressed as the mean±SD.