Role for nuclear interleukin-37 in the suppression of innate immunity

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Contributed by Charles A. Dinarello, January 3, 2019 (sent for review December 13, 2018; reviewed by Diana Boraschi and Theoharis C. Theoharides)

The IL-1 family member IL-37 broadly suppresses innate inflammation and acquired immunity. Similar to IL-1α and IL-33, IL-37 is a dual-function cytokine in that IL-37 translocates to the nucleus but also transmits a signal via surface membrane receptors. The role of nuclear IL-37 remains unknown on the ability of this cytokine to inhibit innate inflammation. Here, we compared suppression of innate inflammation in transgenic mice expressing native human IL-37 (IL-37Tg) with those of transgenic mice carrying the mutation of aspartic acid (D) to alanine (A) at amino acid 20 (IL-37D20ATg). The mutation D20A prevents cleavage of caspase-1, a step required for IL-37 nuclear translocation. In vitro, peritoneal macrophages from IL-37Tg mice reduced LPS-induced IL-1β, IL-6, TNFα and IFNγ by 40–50% whereas in macrophages from IL-37D20ATg mice this suppression was not observed, consistent with loss of nuclear function. Compared with macrophages from IL-37Tg mice, significantly less or no suppression of LPS-induced MAP kinase and NFκB activation was also observed in macrophages from IL-37D20ATg mice. In vivo, levels of IL-1β, IL-6, and TNFα in the lungs and liver were markedly reduced during endotoxemia in IL-37Tg mice but not observed in IL-37D20ATg mice. However, suppression of innate inflammation remains intact in the IL-37D20A mice once the cytokine is released from the cell and binds to its receptor. These studies reveal a nuclear function for suppression of innate inflammation and are consistent with the dual function of IL-37 and a role for caspase-1 in limiting inflammation.

IL-37 | caspase-1 | mutation | inflammation | suppression

IL-37 is unique compared with other members of the IL-1 family (1, 2). Unlike the proinflammatory members of the family, IL-37 broadly reduces innate inflammation and acquired immune responses (3, 4). There are five isoforms of IL-37 in humans; IL-37b has the most complete number of exons and is the isoform expressed in transgenic mice in the present study. IL-37 isoform d has the same antiinflammatory properties observed for IL-37b and also uses SMAD3 for suppressing inflammation (5), an observation reported in 2010 (6). In human peripheral blood mononuclear cells, a knock-down of IL-37 results in increased production of LPS- as well as IL-1β–induced cytokines (6), supporting a role for endogenous IL-37 in suppression of innate inflammation. Mice transgenic for human IL-37 (IL-37Tg) are protected against LPS-induced systemic inflammation (6), chemical colitis (7), metabolic syndrome (8), spinal cord injury (9), and suppressed immune responses following challenge by specific antigen (10). Not unexpectedly, IL-37 affects host defense (11).

In addition to studies in IL-37 transgenic mice, the extracellular function of IL-37 using recombinant IL-37 inhibits inflammation in vitro and in vivo in wild-type (WT) mice (12–18). Recombinant IL-37 improves insulin sensitivity and reduces inflammatory cytokine production in adipose tissue (13), ameliorates joint and systemic inflammation (15), limits the metabolic costs of chronic inflammation, which promotes exercise tolerance (14), and promotes intestinal immune homeostasis (19). Although the functions of recombinant IL-37 require the IL-18 receptor (IL-18Rα) and the antiinflammatory coreceptor IL-1R8 (formerly Single IgG IL-1 Related Receptor) and although molecular proximity studies of the heterotrimeric complex have been reported (12, 20), direct surface binding of IL-37 to these receptors has not been demonstrated.

IL-37 is a dual-function cytokine that translocates to the nucleus (21). In mouse macrophages stably transfected with human IL-37, ∼20% of IL-37 translocates to the nucleus, which is associated with decreased inflammatory cytokine production (6, 21, 22). However, in the presence of a peptide caspase-1 inhibitor, there is no translocation to the nucleus and no reduction in LPS-induced cytokines (21). Mutation of the aspartic acid at the caspase-1 recognition and cleavage site at position 20 to alanine (IL-37D20A) results in resistance to caspase-1 cleavage and abolishes the nuclear localization (22). In the mouse RAW macrophage line, transfection of IL-37D20A results in a loss of the suppression of cytokine production (22).

It remains unknown to what extent nuclear IL-37 plays a role in the antiinflammatory function of IL-37. To this end, we generated a transgenic mouse expressing human IL-37D20A (IL-37D20ATg). The advantage of using the D20A mutation is that endogenous caspase-1 is present in the cells for processing of IL-1β and IL-18 as well as other caspase-1 functions and that only the intracellular processing of IL-37 is prevented for nuclear translocation. Therefore, the IL-37D20ATg mouse allows one to

Significance

Interleukin-1 family members are highly inflammatory, but member IL-37 is unique in broadly suppressing inflammation and specific immunity. IL-37 is a dual-function cytokine by binding in the nucleus and to cell surface receptors. We generated an IL-37 transgenic mouse carrying the aspartic acid (D) to alanine (A) mutation at amino acid 20 (D20A), which prevents the nuclear translocation of IL-37. In transgenic mice expressing native IL-37, inflammatory cytokines are reduced during systemic endotoxemia, but, in mice expressing the D20A mutation, protection is lost, consistent with a nuclear function of IL-37. Nevertheless, IL-37D20A released from cells binds to its receptors and initiates suppression of innate inflammation. These studies reveal a nuclear function of IL-37 in vivo.

Author contributions: S.L., J.A.-A., R.L.-V., and C.A.D. designed research; S.L., J.A.-A., C.P.N., I.W.T., T.A., P.B., and C.A.D. performed research; S.L., C.P.N., B.E.P., P.B., and C.A.D. contributed new reagents/analytic tools; S.L., J.A.-A., C.P.N., R.L.-V., and C.A.D. analyzed data; and S.L. and C.A.D. wrote the paper.

Reviewers: D.B., Institute of Protein Biochemistry; and T.C.T., Tufts University School of Medicine.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1821111116/-/DCSupplemental.

Published online February 21, 2019.

solely characterize the role of nuclear IL-37 on responses in vitro and in vivo.

Results

The D20A Mutation in IL-37 Abolishes Nuclear Translocation in Peritoneal Macrophages from IL-37D20ATg Mice. As shown in Fig. 1, IL-37 is present in the nucleus of primary peritoneal macrophages from transgenic mice expressing native IL-37 but absent in the nucleus of macrophages from IL-37D20ATg mice. This finding is consistent with our previous study in cell lines expressing IL-37D20A (22). As shown, cytosolic IL-37 remains intact in the same macrophages. Both cytosolic and nuclear forms of IL-37 are present in macrophages from mice expressing native IL-37. Cells from WT mice lacking the IL-37 gene do not contain IL-37 (Fig. 1) (6, 23).

The D20A Mutation in IL-37 Abolishes the Suppressive Effect of IL-37 on LPS-Induced IL-1β, TNFα, IL-6, and IFNγ in Peritoneal Macrophages. To determine whether the mutation of the caspase-1 cleavage site prevents the antiinflammatory function of IL-37 in primary cells, peritoneal macrophages from WT, IL-37Tg, and IL-37D20ATg mice were incubated with LPS. As shown in Fig. 2*A*, LPS-induced IL-1β and TNFα production was decreased by 45% and 38%, respectively, in macrophages from IL-37Tg mice, but this suppression was significantly less in macrophages from IL-37D20ATg mice, where only 22% and 10% suppression was observed for IL-1β and TNFα (Fig. 2*A*). Similarly, in comparison with macrophages from WT mice, LPS-induced IL-6 production decreased by 37% in macrophages from IL-37Tg mice, but this suppression was diminished in macrophages from IL-37D20ATg mice where only 9% suppression was present. LPS-induced IFNγ was also attenuated in the same cells carrying the D20A mutation, from 45% suppression in cells of IL-37Tg to only 10% suppression in cells from IL-37D20ATg (Fig. 2*A*).

Effect of the D20A Mutation of IL-37 on LPS-Induced MAPK Activation. We next examined the intracellular kinase activities in response to LPS stimulation in peritoneal macrophages from WT, IL-37Tg, and IL-37D20ATg mice. Exposure to LPS dramatically increased the phosphorylation of ERK and p38 MAPK compared with unstimulated cells from WT mice (Fig. 2*B*). As shown, LPS-induced MAPK phosphorylations were markedly reduced in cells from IL-37Tg mice. In contrast, these phosphorylations in macrophages from IL-37D20ATg were at nearly the same level as in cells from WT mice and were significantly higher than in cells from IL-37Tg mice (Fig. 2*B* and *SI Appendix*, Fig. S1). A densitometric quantitative analysis of both phospho-ERK and p38 signals normalized to the β-actin control is shown in *SI Appendix*, Fig. S1. These in vitro data reveal functional properties of nuclear IL-37 in primary cells.

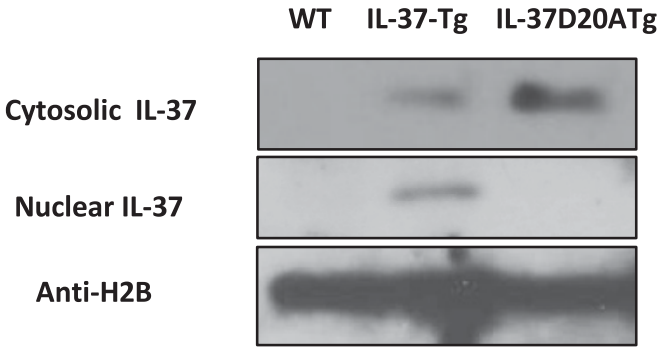

Fig. 1. IL-37 protein expression in WT, IL-37Tg, or IL-37D20ATg strains. Thioglycollate-elicited peritoneal macrophages from WT, IL-37Tg, or IL-37D20ATg mice were collected, centrifuged, and lysed with homogenizing buffer. Cytosolic and nuclear fractions were separated for Western blotting of IL-37 (see *Materials and Methods*). Nuclear H2B protein content was used as loading control. These are representative images of two independent experiments.

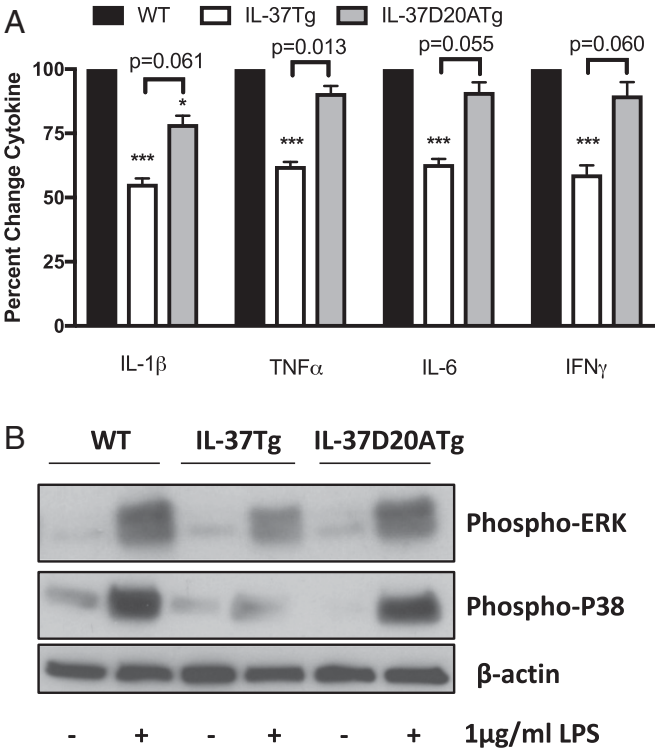

Fig. 2. Loss of suppression of MAP kinase signaling in cells from IL-37D20ATg mice. (*A*) LPS-induced IL-1β and TNFα as well as LPS-induced IL-6 and IFNγ production in peritoneal macrophages from WT, IL-37Tg, or IL-37D20ATg mice. Mean ± SEM percent change of LPS-induced cytokine production with WT is set at 100; $N \geq 6$. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with WT macrophages. The bracket indicates the *P* value comparing cells from IL-37Tg or IL-37D20ATg mice. (*B*) Western blotting of phospho-ERK and phospho-p38 MAP kinases in peritoneal macrophages with or without LPS stimulation. The cells were stimulated with or without 1 μg/mL of LPS for 30 min before the cell lysates were prepared. These are representative images of three independent experiments.

The D20A Mutation in IL-37 Reverses the Inhibitory Effect of IL-37 on LPS-Induced NFκB Activation. Because LPS and cytokines activate NFκB signaling through MAPK (24–26), we next carried out flow cytometry analysis to detect changes in NFκB signaling in peritoneal macrophages from WT, IL-37Tg, and IL-37D20ATg mice. The peritoneal macrophages were labeled as $CD11b^{+}CD3^{-}$ (*SI Appendix*, Fig. S2*A*). LPS stimulation induced a significant increase of NFκB activation in WT macrophages over the spontaneous NFκB signal. The percentage of p-NFκB positive cells from spontaneous condition compared with the LPS-induced condition in WT $CD11b^{+}CD3^{-}$ cells are 24.0 ± 1.4% to 33.7 ± 0.9%, $P = 0.004$ (*SI Appendix*, Fig. S2*B*). As shown in Fig. 3, although both spontaneous and LPS-induced NFκB activation are significantly reduced in macrophages from IL-37Tg mice compared with cells from WT mice, the reduction was absent in cells from IL-37D20ATg mice. The difference in LPS-induced NFκB activation between cells from IL-37Tg and IL-37D20ATg mice is similar to the difference observed in LPS-induced cytokine production and MAPK activation (Fig. 2 and *SI Appendix*, Fig. S1).

We also compared NFκB activation in cultured splenocytes from WT, IL-37Tg, and IL-37D20ATg mice. Considering the complexity of spleen cellular composition, we selectively studied two distinct cell populations in the spleen (*SI Appendix*, Fig. S3*A*): the $CD11b^{-}/CD3^{+}$ T cell population and the $CD11b^{+}/CD3^{-}$ myeloid population (27). There was no clear increase in LPS-induced NFκB activation over the spontaneous NFκB signal in either

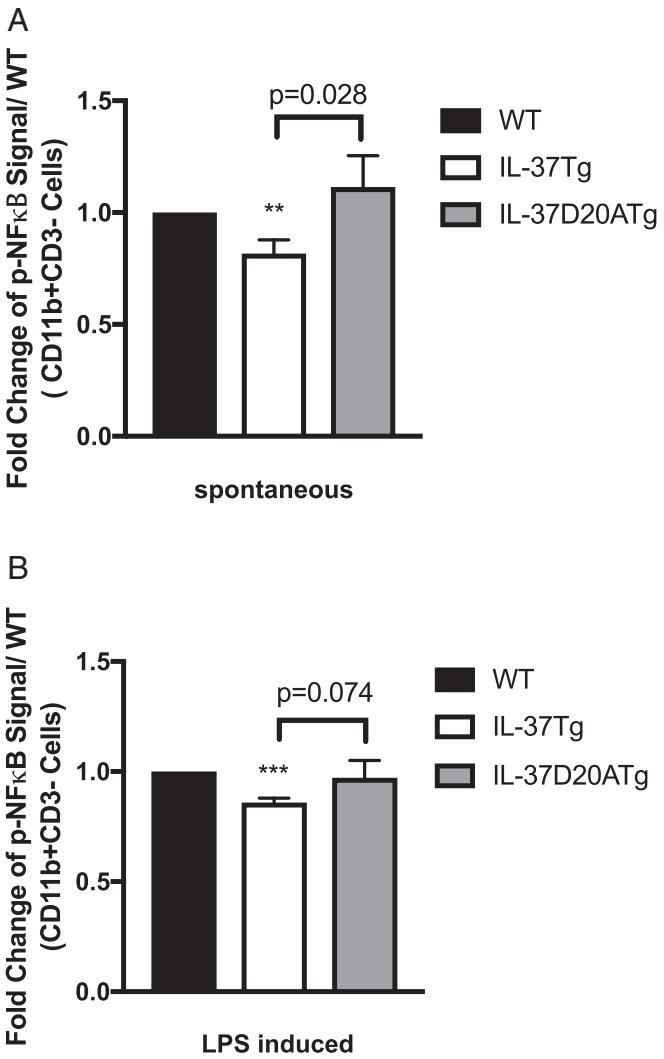

Fig. 3. Flow cytometry analysis of intracellular NFκB activation in macrophages from WT, IL-37Tg, or IL-37D20ATg mice. Cells were stimulated with or without 1 μg/mL of LPS for 30 min before the cells were collected for flow cytometry. (*A*) Statistical analysis of p-NFκB signal in $CD11b^+CD3$ peritoneal macrophages from WT, IL-37Tg, or IL-37D20ATg mice. Spontaneous p-NFκB signal in WT cells was set as 1. (*B*) Statistical analysis of LPS-induced p-NFκB signal in $CD11b^+CD3^-$ peritoneal macrophages from WT, IL-37Tg, or IL-37D20ATg mice. The p-NFκB signal in LPS-stimulated WT cells (LPS induced) was set as 1; mean ± SEM of three independent experiments; $^{**}P < 0.01$ or $^{***}P < 0.001$ compared with WT macrophages. The *P* values between samples from IL-37-Tg and IL-37D20ATg mice are indicated by the brackets.

$CD11b^+/CD3^-$ or $CD11b^-/CD3^+$ cells. In addition, no significant changes were observed in either spontaneous or LPS-induced NFκB signal in the $CD11b^+CD3^-$ cells or spontaneous NFκB signal among the $CD11b^-CD3^+$ cells from WT, IL-37Tg, and IL-37D20ATg mice (*SI Appendix*, Fig. S3 *B* and *C*, *Left*). However, a significant decrease in LPS-induced NFκB activation was found in $CD11b^-CD3^+$ cells from IL-37Tg mice, compared with the p-NFκB signal in cells from WT mice (*SI Appendix*, Fig. S3*C*, *Right*). The inhibition on LPS-induced NFκB activation in cells from IL-37Tg mice remained intact in cells from IL-37D20ATg mice.

Transgenic Mice with the D20A Mutation Are Not Protected by LPS Challenge in Vivo. IL-37Tg mice are protected from LPS-induced systemic endotoxemia (6). To assess the role of nuclear IL-37 in the response to endotoxemia, we subjected WT, IL-37Tg, and IL-37D20ATg mice to i.p. LPS as was performed in mice

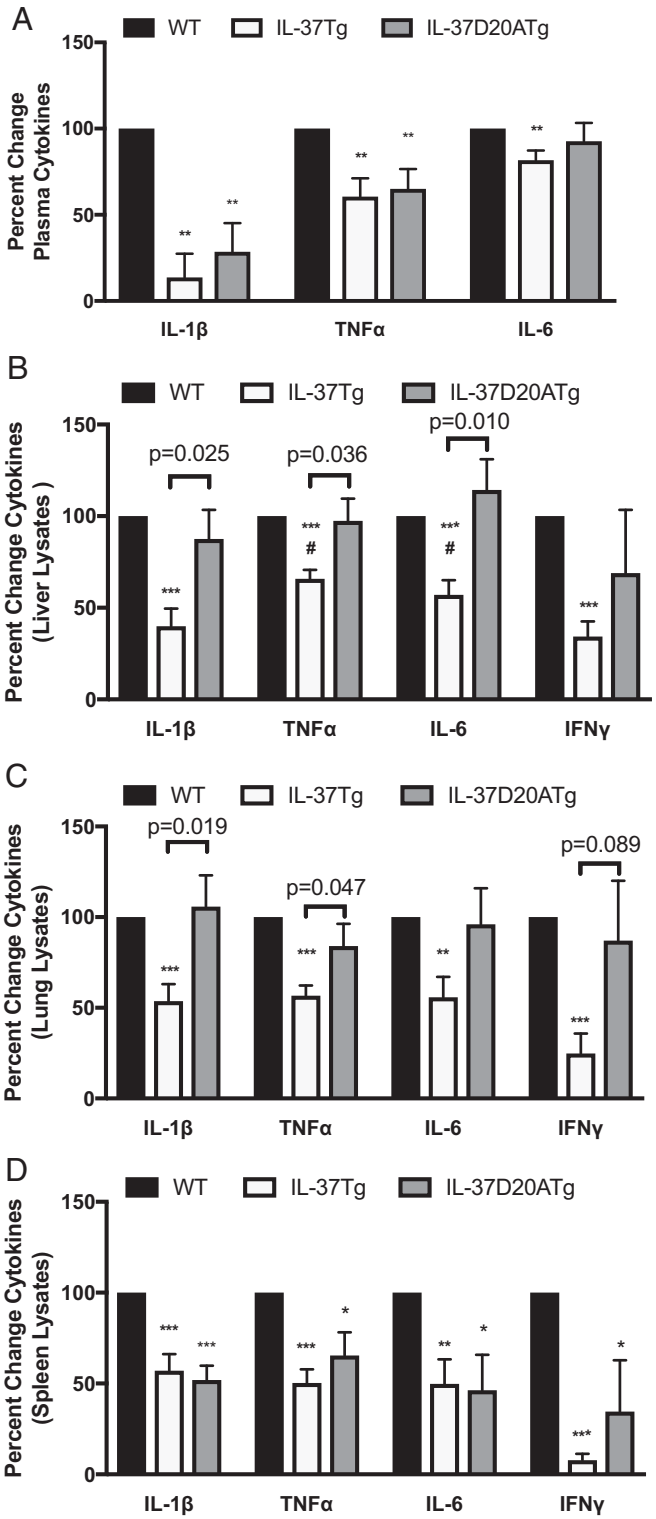

Fig. 4. Function of IL-37 in vivo. WT, IL-37Tg, or IL-37D20ATg mice were challenged with LPS (10 mg/kg) intraperitoneally and then assessed after 20 h. (*A*) Plasma cytokine levels. (*B–D*) Cytokine levels in organ lysates of liver (*B*), lung (*C*) or spleen (*D*). For all, cytokines in WT mice were set at 100% and the mean ± SEM percent change was calculated for IL-37Tg and IL-37D20ATg mice. N ≧ 4 independent experiments for each group of three mice. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with WT samples. The *P* value between samples from IL-37-Tg and IL-37D20ATg mice are indicated with brackets.

expressing native IL-37 or treated with recombinant IL-37 (6, 12). As anticipated, LPS-induced inflammatory responses were substantially lower in IL-37Tg mice compared with WT mice (Figs. 4 and 5). Mice expressing native IL-37 had lower levels of cytokines in the plasma (Fig. 4*A*) and in tissues from the liver, lung, and spleen (Fig. 4 *B*–*D*). However, the reduced inflammation observed in tissues from IL-37Tg mice was not observed in IL-37D20ATg mice (Fig. 4 *B*–*D*). For example, in the tissues, IL-1β, TNFα, IL-6, and IFNγ levels from liver and lung organ lysates (Fig. 4 *B* and *C*) were significantly higher in IL-37D20ATg mice compared with mice expressing native IL-37. However, the inhibition of inflammatory responses by IL-37 remained significantly low in the spleen of IL-37D20ATg mice, with only TNFα and IFNγ increased (Fig. 4*D*). In the plasma of IL-37D20ATg, IL-1β and TNFα levels remained low (Fig. 4*A*). The white blood cell counts are lower in both IL-37Tg and IL-37D20ATg mice compared with the WT mice (*SI Appendix*, Fig. S4*A*). Thus, besides the loss of inhibition on LPS-induced inflammatory responses in the liver and lungs of IL-37D20ATg mice, we observed data in which the suppression of inflammatory responses in IL-37D20ATg mice were the same as in IL-37Tg mice, supporting the concept that the extracellular function of IL-37 remains intact in both strains.

In parallel, we measured cytokine levels in the peritoneal lavage fluid, bone marrow cells, and splenocyte cultures from the LPS-challenged mice. As shown in *SI Appendix*, Fig. S4*B*, there was significantly less IL-6 in the peritoneal lavage of IL-37Tg mice compared with WT mice, but no significant difference was detected between IL-37Tg and IL-37D20ATg mice. In addition, we noticed a trend of lower spontaneous TNFα and IL-6 production by either bone marrow cells or splenocytes of the IL-37Tg mice compared with WT mice. In general, cells from IL-37D20ATg mice produce more cytokines than cells from IL-37Tg mice, although these differences did not achieve statistical significance (Fig. 5, *Left*). When the cells were further challenged with LPS ex vivo, significantly less TNFα and IL-6 production was observed in cells from IL-37Tg mice; however, the suppression was absent in cells from IL-37D20ATg mice (Fig. 5, *Right*). These data are consistent with the data from LPS-stimulated cell cultures of thioglycollate-elicited peritoneal macrophages from WT, IL-37Tg, and IL-37D20ATg mice (Fig. 2*A*).

Effects of D20A Mutation in Naive Primary Bone Marrow Cells. As shown in Fig. 5, LPS-induced cytokines from primary spleen and bone marrow cells of LPS-challenged IL-37D20ATg mice revealed a near-complete absence of suppression that is observed in cells from mice expressing native IL-37. We next studied fresh naive bone marrow cells from WT, IL-37Tg, and IL-37D20ATg mice to assess LPS-induced cytokine release at early (6 h) and late (24 h) hours of incubation. After 6 h, there was no suppression of TNFα, IL-6, and KC released from cells of IL-37D20ATg mice compared with IL-37Tg mice (Fig. 6). After 24 h, the TNFα, IL-6, and KC levels from cells expressing native IL-37 remained low compared with cells from WT mice; however, cells from IL-37D20ATg mice released less cytokines (or at a similar level) than cells expressing native IL-37 (Fig. 6).

Discussion

The advantage of the IL-37D20A mutation is that endogenous caspase-1 is present in cells of the entire mouse, enabling the in vivo function of nuclear IL-37 in LPS-induced systemic inflammation. The IL-37D20ATg mouse confirmed that caspase-1 cleavage at the aspartic acid 20 is required for nuclear translocation. The whole-animal studies provide insights into the evolutionary significance of IL-37 as a functional suppressor of inflammation in the nucleus before the appearance of receptors on cell surfaces. Functional IL-37 in the nucleus is supported by several lines of evidence. First, compared with macrophages from IL-37Tg mice, peritoneal macrophages from IL-37D20ATg mice lost the ability to suppress LPS-induced cytokine production (Fig. 2*A*). In addition, the inhibition of LPS-stimulated MAPK activation or NFκB activation was also diminished in macrophages from IL-37D20A mice (Figs. 2*B* and 3). Importantly, when mice were challenged with LPS in vivo, the protection in IL-37D20ATg mice was lost (Fig. 4). Moreover, LPS-induced cytokine production is also diminished or significantly reduced in cultured bone marrow cells and splenocytes from the IL-37D20ATg mice (Fig. 5). Together, the data confirm that nuclear translocation of IL-37 is crucial for the antiinflammatory function of IL-37 in vitro and in vivo.

However, IL-37D20A mice still exhibit antiinflammatory properties under certain conditions. As presented in Figs. 4 and 5 as well

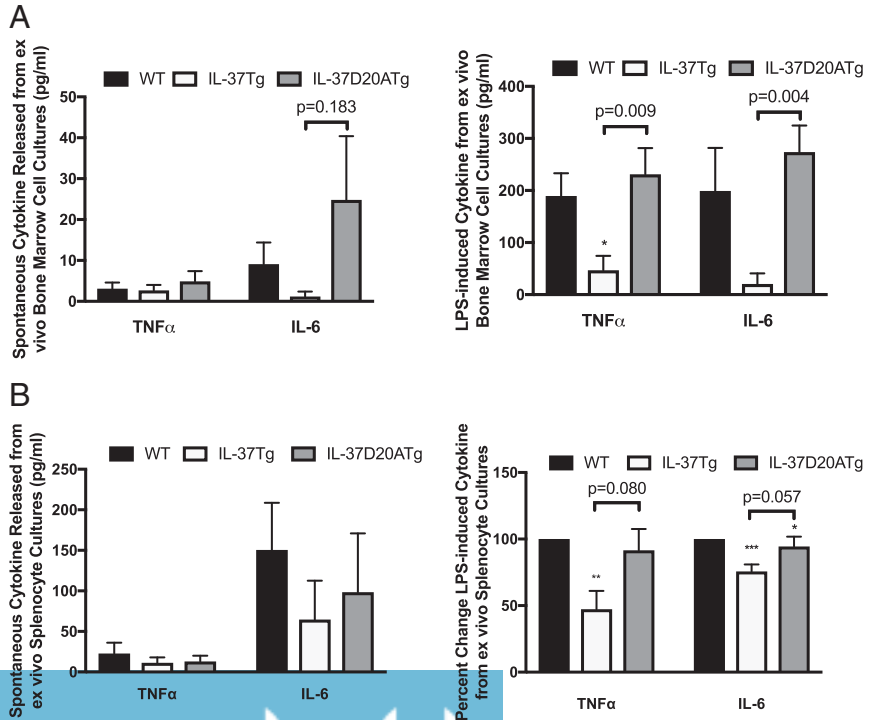

Fig. 5. Ex vivo spontaneous and LPS-induced TNFα and IL-6 released from bone marrow and splenocyte cultures of LPS challenged WT, IL-37Tg or IL-37D20ATg mice. Mice received 10 mg/kg LPS i.p., and, 20 h later, bone marrow cells and splenocytes were collected, seeded in flat-bottom wells, and cultured for 20 h. (*A*) (*Left*) Spontaneous TNFα and IL-6 concentrations in the supernatants from unfractionated bone marrow cells cultured ex vivo. (*Right*) LPS (1 μg/mL)-induced TNFα and IL-6 in the supernatants of unfractionated bone marrow cells. (*B*) (*Left*) Spontaneous TNFα and IL-6 levels released from splenocyte cultures. (*Right*) LPS (1 μg/mL)-induced cytokines. For all, $N \geq 4$ independent experiments; mean concentrations ± SEM of cytokines for *A* and *Left* of *B*. For *Right* of *B*, mean ± SEM percent change was calculated for comparison with cytokine levels in cells from WT mice which were set as 100. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with WT samples. The *P* values between cytokine levels from IL-37Tg and IL-37D20ATg mice are indicated with brackets.

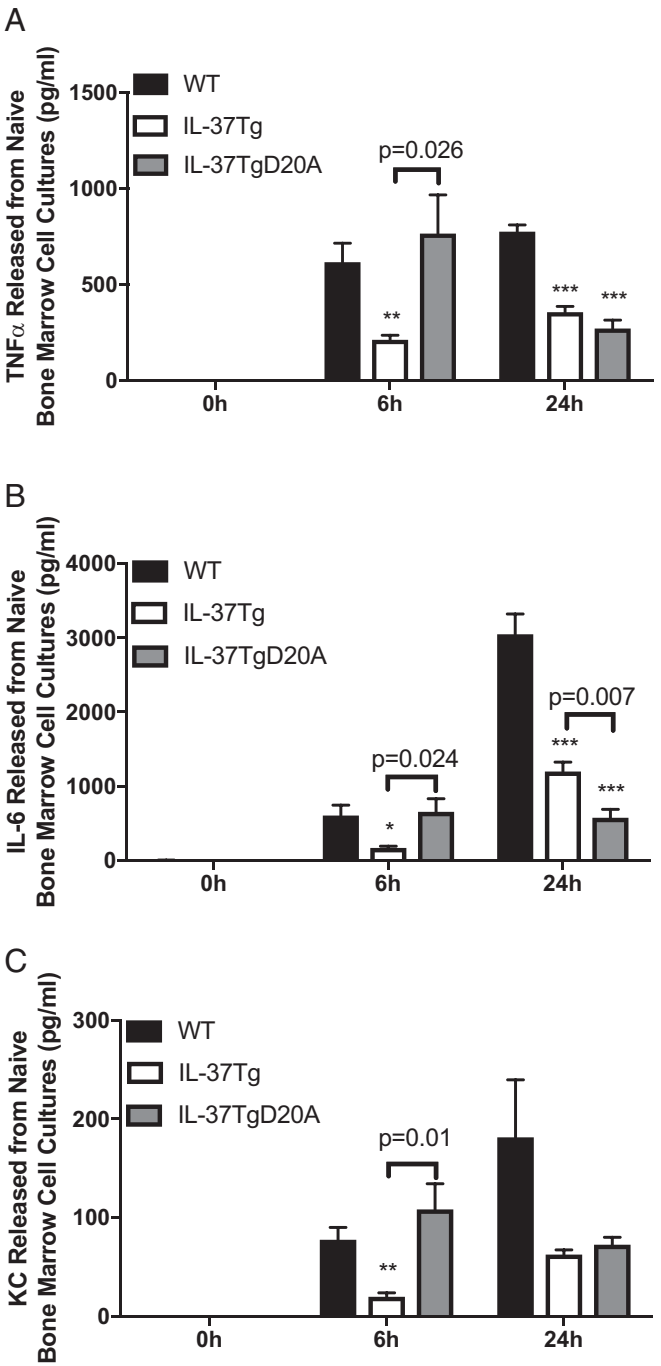

Fig. 6. Time course of LPS-induced bone marrow culture release of TNFα, IL-6, and KC from naive WT, IL-37Tg, and IL-37D20ATg mice. Bone marrow cells were collected, seeded on 24-well plates, and allowed to adhere for 2 h at 37 °C, and nonadherent cells were removed. Adherent cells were cultured in parallel wells with or without 1 μg/mL of LPS. After 6 h, the supernatants were removed from one set of cultures, and, after 24 h, the supernatants were removed from the second set of cultures. (*A*) TNFα, (*B*) IL-6, and (*C*) KC; mean ± SEM of five mice per group. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ compared with WT bone marrow cells. The *P* values between cytokine levels from IL-37Tg and IL-37D20ATg mice are indicated with brackets.

as in *SI Appendix*, Figs. S3 and S4, some protection to LPS-induced inflammatory responses by IL-37 in vivo remains in plasma (IL-1β and TNFα), spleen organ lysates (IL-1β and IFNγ), and cell cultures (spontaneous TNFα and IL-6), as well as peritoneal fluid lavage (IL-6). These findings are consistent with the extracellular function of IL-37 (12). IL-37 is active outside the cell, since endogenous IL-37 is neutralized with anti–IL-37 in IL-37Tg mice challenged with LPS in vivo and recombinant IL-37 in vitro (4, 12, 14, 22).

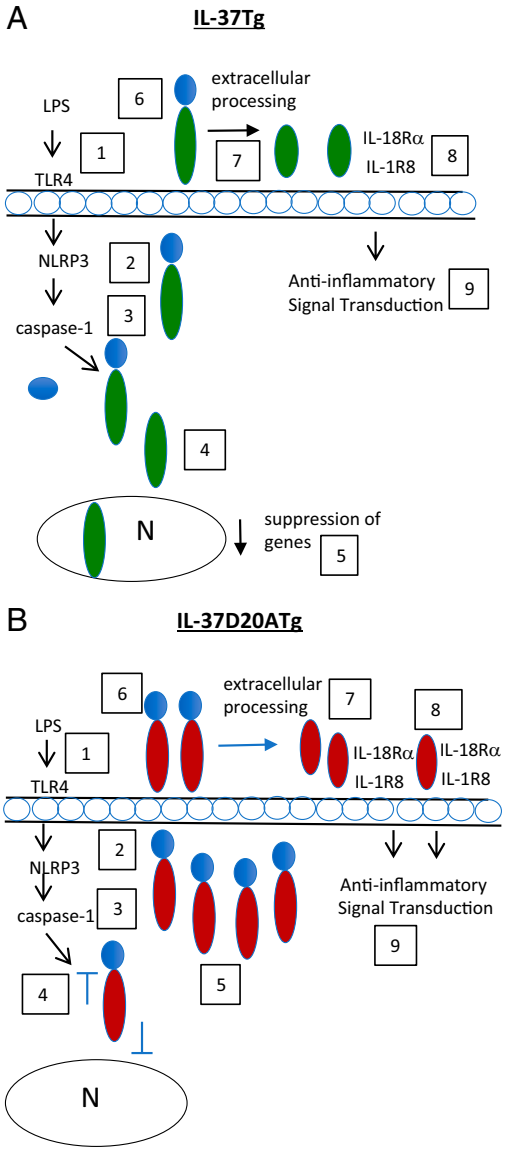

Fig. 7. Dual mechanisms of IL-37–mediated suppression of innate inflammation in IL-37Tg and IL-37D20A transgenic mice. (*A*) The antiinflammatory pathway for native IL-37 expressed in IL-37Tg mice; 1, extracellular LPS binds the TLR4 receptor and initiates inflammatory signaling; 2, synthesis of the IL-37 precursor and other cytokines and activation of NLRP3 and caspase-1; 3, activated caspase-1 cleaves the IL-37 precursor at aspartic acid (D) in position 20; 4, generation of the carboxyl domain of IL-37 (amino acids 20 to 218); 5, IL-37 translocates to nucleus and suppresses the expression of proinflammatory genes activated by LPS as shown in step 1; 6, release of full-length IL-37 into the extracellular space; 7, processing of IL-37 by extracellular enzymes; 8, processed IL-37 binds the cell surface receptors IL-18Rα and IL-1R8; and 9, the IL-37 receptor complex activates antiinflammatory signaling. (*B*) The antiinflammatory pathway for IL-37D20A carrying the caspase-1–resistant mutation expressed in IL-37D20ATg mice; 1, extracellular LPS binds the TLR4 receptor and initiates inflammatory signaling; 2, synthesis of the IL-37 precursor and other cytokines; 3, activation of NLRP3 and caspase-1; 4, the mutated IL-37D20A is resistant to caspase-1 cleavage and does not translocate to nucleus; 5, increased concentrations of the IL-37 precursor inside the cell; 6, release of IL-37 into the extracellular space; 7, processing of IL-37 by extracellular enzymes; 8, processed IL-37 binds the cell surface receptors IL-18Rα and IL-1R8; and 9, the IL-37 receptor complex activates intracellular antiinflammatory signaling.

Throughout these studies, we were aware that, as the level of IL-37D20A protein accumulated intracellularly in the IL-37D20ATg mouse, IL-37D20A could be released from the cell and trigger the cell, as does recombinant IL-37. How the IL-37 precursor is released from the cell in the absence of cell death remains unknown, as there was no increase in LDH in naive bone marrow cells after 24 h of incubation with LPS. Other studies demonstrate that extracellular processing for optimal activity of IL-37b is at or near amino acid 46 (12, 14, 28). In the case of the IL-37D20ATg mouse, there would be more IL-37D20A protein outside the cell compared with native IL-37 in the IL-37Tg mouse, because at least 20% (perhaps more) native IL-37 would be in the nucleus. Therefore, to observe in some tissues as much suppression in the IL-37D20ATg mouse as in the native IL-37Tg mouse was not unexpected (Figs. 4 and 6 and *SI Appendix*, Fig. S5). Indeed, we observed, in the same primary bone marrow cultures from the two strains, similar suppression as shown in Fig. 6. The lack of IL-37–mediated suppression on LPS-induced cytokine production in fresh bone marrow cells of IL-37D20ATg mice was observed after 6 h of LPS stimulation but not after 24 h. This lack of suppression at 6 h is similar to the lack of suppression in peritoneal macrophages from the IL-37D20ATg mice (Fig. 2*A*) and supports the functional role of nuclear IL-37. As depicted in Fig. 7*A*, the intended evolutionary function of IL-37 to limit innate inflammation is maintained with nuclear as well as extracellular functions. In Fig. 7*B*, the intended evolutionary function of IL-37 to limit innate inflammation is also maintained, but without nuclear function.

It is likely that the high-dose LPS challenge in vivo resulted in the release of extracellular IL-37. However, the extent of this release clearly differs in various tissues. For example, the suppression of IL-1β in the lung and liver of native IL-37Tg mice is not observed in IL-37TgD20A mice, consistent with the concept that there is no extracellular IL-37 released. In contrast, there is the same suppression of IL-1β in the spleen, suggesting extracellular IL-37 functions in both strains.

In summary, our studies confirm that failure of caspase-1 cleavage of the intracellular IL-37 precursor prevents translocation of the carboxyl domain of IL-37 into the nucleus, and that results in a loss of the cytokine suppressing functions in vivo and ex vitro. However, the loss of nuclear localization does not abolish the protective effect of IL-37, as the suppression of innate inflammation of IL-37 in the extracellular compartment remains intact. To limit their respective proinflammatory extracellular nature, nuclear IL-1α and nuclear IL-33 function as a sink to limit inflammation (29, 30). In contrast, IL-37 is consistently an antiinflammatory cytokine whether in the nucleus or outside the cell.

Materials and Methods

Generation of IL-37D20ATg Mice. The animal protocols were approved by University of Colorado Animal Care and Use Committee. The IL-37Tg and IL-37D20ATg were generated as follows: IL-37 (isoform b) full sequence with the D to A mutation was released from plasmid pTarget (22) and inserted into the same pIRES vector with CMV promoter used for the generation of IL-37Tg mice (6). Fertilized eggs from C57BL/6 mice were then injected with the pIRES IL-37b or pIRES IL-37bD20A expression plasmid similar as described earlier (6) and implanted into C57BL/6 females. Founders were mated with C57BL/6 WT females or males. The IL-37 transgene for IL-37Tg and IL-37D20ATg was identified by PCR (6).

Age- and gender-matched PCR-negative littermates and WT mice were used as controls. C57BL/6 WT mice were obtained from Jackson Laboratory. Details of other mouse studies are provided in *SI Appendix*.

Cell Cultures, Western Blotting, LPS Model, and Cell Sorting. Details are provided in *SI Appendix*.

ACKNOWLEDGMENTS. We thank Garret Hedlund, Alexandra Schroder, Nicholas E. Powers, Carlo Marchetti, Dennis de Graaf, and Linda Lang for assistance. This study was supported by National Institutes of Health Grants AI 015614 (to C.A.D.) and AI 128443 (to S.L.), Spanish Ministry of Economy and Competitiveness Grant SAF2016-79774-R, Wings for Life International Foundation, Red de Terapia Celular (TERCEL) (to R.L.-V.), and Deutsche Forschungsgemeinschaft Grant BU 1222/7-1 (to P.B.). J.A.-A. is the recipient of a Formación de Profesorado Universitario (FPU) fellowship awarded by the Spanish Ministry of Science, Innovation and University. S.L. was supported by a Colorado Clinical and Translational Sciences Institute (CCTSI) mentored faculty pilot grant.

1. Boraschi D, Tagliabue A (2006) The interleukin-1 receptor family. *Vitam Horm* 74:229–254.
2. Boraschi D, et al. (2011) IL-37: A new anti-inflammatory cytokine of the IL-1 family. *Eur Cytokine Netw* 22:127–147.
3. Dinarello CA, et al. (2016) Suppression of innate inflammation and immunity by interleukin-37. *Eur J Immunol* 46:1067–1081.
4. Cavalli G, Dinarello CA (2018) Suppression of inflammation and acquired immunity by IL-37. *Immunol Rev* 281:179–190.
5. Zhao M, et al. (2018) IL-37 isoform D downregulates pro-inflammatory cytokines expression in a Smad3-dependent manner. *Cell Death Dis* 9:582.
6. Nold MF, et al. (2010) IL-37 is a fundamental inhibitor of innate immunity. *Nat Immunol* 11:1014–1022.
7. McNamee EN, et al. (2011) Interleukin 37 expression protects mice from colitis. *Proc Natl Acad Sci USA* 108:16711–16716.
8. Ballak DB, et al. (2014) IL-37 protects against obesity-induced inflammation and insulin resistance. *Nat Commun* 5:4711.
9. Coll-Miró M, et al. (2016) Beneficial effects of IL-37 after spinal cord injury in mice. *Proc Natl Acad Sci USA* 113:1411–1416.
10. Luo Y, et al. (2014) Suppression of antigen-specific adaptive immunity by IL-37 via induction of tolerogenic dendritic cells. *Proc Natl Acad Sci USA* 111:15178–15183.
11. van de Veerdonk FL, et al. (2015) Protective host defense against disseminated candidiasis is impaired in mice expressing human interleukin-37. *Front Microbiol* 5:762.
12. Li S, et al. (2015) Extracellular forms of IL-37 inhibit innate inflammation in vitro and in vivo but require the IL-1 family decoy receptor IL-1R8. *Proc Natl Acad Sci USA* 112:2497–2502.
13. Ballak DB, et al. (2018) Interleukin-37 treatment of mice with metabolic syndrome improves insulin sensitivity and reduces pro-inflammatory cytokine production in adipose tissue. *J Biol Chem* 293:14224–14236.
14. Cavalli G, et al. (2017) Interleukin 37 reverses the metabolic cost of inflammation, increases oxidative respiration, and improves exercise tolerance. *Proc Natl Acad Sci USA* 114:2313–2318.
15. Cavalli G, et al. (2017) Treating experimental arthritis with the innate immune inhibitor interleukin-37 reduces joint and systemic inflammation. *Rheumatology (Oxford)* 56:2256.
16. Zeng Q, et al. (2017) Interleukin-37 suppresses the osteogenic responses of human aortic valve interstitial cells in vitro and alleviates valve lesions in mice. *Proc Natl Acad Sci USA* 114:1631–1636.
17. Zhan Q, et al. (2017) IL-37 suppresses MyD88-mediated inflammatory responses in human aortic valve interstitial cells. *Mol Med* 23:83–91.
18. Offenbacher S, et al. (2018) GWAS for Interleukin-1β levels in gingival crevicular fluid identifies IL37 variants in periodontal inflammation. *Nat Commun* 9:3686.
19. Conti P, et al. (2018) Impact of mast cells in mucosal immunity of intestinal inflammation: Inhibitory effect of IL-37. *Eur J Pharmacol* 818:294–299.
20. Nold-Petry CA, et al. (2015) IL-37 requires the receptors IL-18Rα and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. *Nat Immunol* 16:354–365.
21. Sharma S, et al. (2008) The IL-1 family member 7b translocates to the nucleus and down-regulates proinflammatory cytokines. *J Immunol* 180:5477–5482.
22. Bulau AM, et al. (2014) Role of caspase-1 in nuclear translocation of IL-37, release of the cytokine, and IL-37 inhibition of innate immune responses. *Proc Natl Acad Sci USA* 111:2650–2655.
23. Bufler P, et al. (2002) A complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity. *Proc Natl Acad Sci USA* 99:13723–13728.
24. Guha M, Mackman N (2001) LPS induction of gene expression in human monocytes. *Cell Signal* 13:85–94.
25. Beyaert R, et al. (1996) The p38/RK mitogen-activated protein kinase pathway regulates interleukin-6 synthesis response to tumor necrosis factor. *EMBO J* 15:1914–1923.
26. Vanden Berghe W, et al. (1998) p38 and extracellular signal-regulated kinase mitogen-activated protein kinase pathways are required for nuclear factor-kappaB p65 transactivation mediated by tumor necrosis factor. *J Biol Chem* 273:3285–3290.
27. Marchetti C, et al. (2018) OLT1177, a β-sulfonyl nitrile compound, safe in humans, inhibits the NLRP3 inflammasome and reverses the metabolic cost of inflammation. *Proc Natl Acad Sci USA* 115:E1530–E1539.
28. Pan G, et al. (2001) IL-1H, an interleukin 1-related protein that binds IL-18 receptor/IL-1Rrp. *Cytokine* 13:1–7.
29. Cohen I, et al. (2010) Differential release of chromatin-bound IL-1alpha discriminates between necrotic and apoptotic cell death by the ability to induce sterile inflammation. *Proc Natl Acad Sci USA* 107:2574–2579.
30. Bessa J, et al. (2014) Altered subcellular localization of IL-33 leads to non-resolving lethal inflammation. *J Autoimmun* 55:33–41.