Cell

Article

Dopamine Controls Systemic Inflammation through Inhibition of NLRP3 Inflammasome

Yiqing Yan,[1,4] Wei Jiang,[1,4] Lei Liu,[1] Xiaqiong Wang,[1] Chen Ding,[3] Zhigang Tian,[1,2,*] and Rongbin Zhou[1,2,*]
[1]Institute of Immunology and the CAS Key Laboratory of Innate Immunity and Chronic Disease, School of Life Sciences and Medical Center, University of Science and Technology of China, Hefei 230027, China
[2]Innovation Center for Cell Biology, Hefei National Laboratory for Physical Sciences at Microscale, Hefei 230027, China
[3]Beijing Proteome Research Center, National Center for Protein Sciences Beijing, Beijing 102206, China
[4]Co-first author
*Correspondence: tzg@ustc.edu.cn (Z.T.), zrb1980@ustc.edu.cn (R.Z.)
http://dx.doi.org/10.1016/j.cell.2014.11.047

SUMMARY

Inflammasomes are involved in diverse inflammatory diseases, so the activation of inflammasomes needs to be tightly controlled to prevent excessive inflammation. However, the endogenous regulatory mechanisms of inflammasome activation are still unclear. Here, we report that the neurotransmitter dopamine (DA) inhibits NLRP3 inflammasome activation via dopamine D1 receptor (DRD1). DRD1 signaling negatively regulates NLRP3 inflammasome via a second messenger cyclic adenosine monophosphate (cAMP), which binds to NLRP3 and promotes its ubiquitination and degradation via the E3 ubiquitin ligase MARCH7. Importantly, in vivo data show that DA and DRD1 signaling prevent NLRP3 inflammasome-dependent inflammation, including neurotoxin-induced neuroinflammation, LPS-induced systemic inflammation, and monosodium urate crystal (MSU)-induced peritoneal inflammation. Taken together, our results reveal an endogenous mechanism of inflammasome regulation and suggest DRD1 as a potential target for the treatment of NLRP3 inflammasome-driven diseases.

INTRODUCTION

The NLRP3 inflammasome is a cytosolic protein complex composed of NLRP3, ASC, and caspase-1, and assembled in response to both microbial infection and endogenous "danger signal" (Davis et al., 2011; Martinon et al., 2009). The activation of NLRP3 inflammasome promotes the maturation and release of several proinflammatory cytokines, such as interleukin-1β (IL-1β) and IL-18, so it plays critical roles in the initiation of inflammation and the development of immune responses (Lamkanfi and Dixit, 2012; Schroder and Tschopp, 2010). However, as excessive and persistent inflammation is quite harmful, NLRP3 inflammasome has been involved in diverse inflammatory diseases, including type 2 diabetes, atherosclerosis, and gout, thus the activation of NLRP3 inflammasome should be tightly controlled (Davis et al., 2011; Lamkanfi and Dixit, 2012). Several regulatory mechanisms have been identified to suppress NLRP3 inflammasome. Type I interferon has been shown to attenuate NLRP3 inflammasome activation via Stat1-dependent manner, while nitric oxide has been identified as another negative regulator of NLRP3 inflammasome activation (Guarda et al., 2011; Mishra et al., 2013). Recently, we have proposed that ω-3 fatty acids can negatively regulate NLRP3 inflammasome activation via G protein coupled receptor 120 (GPR120) and GPR40 (Yan et al., 2013). Although NLRP3 inflammasome has been extensively investigated, its regulatory networks, especially the endogenous mechanisms, still remain elusive.

Dopamine (DA) is a neurotransmitter, which not only can regulate behavior, movement, endocrine, cardiovascular, renal, and gastrointestinal functions, but also functions as an important molecule bridging the nervous and immune systems (Basu and Dasgupta, 2000; Beck et al., 2004; Sarkar et al., 2010). DA receptors are present in almost all immune cell subpopulations (Sarkar et al., 2010). Acting on its receptors, DA or agonists for DA receptors have been reported to modulate the activation, proliferation, and cytokine production in immune cells (Basu and Dasgupta, 2000; Sarkar et al., 2010; Torres-Rosas et al., 2014). In addition, dopamine D2 receptor (DRD2) knockout mice show remarkable inflammatory response in CNS, suggesting that DA and its downstream signaling has an antiinflammatory function (Shao et al., 2013). Consistent with this, the deficiency of DA is tightly associated with immune system abnormalities and CNS inflammation in the progression of Parkinson disease (PD) (Perry, 2012; Wüllner and Klockgether, 2003). Although the antiinflammatory effect of DA and its implication in the pathology of PD are emerging, the mechanisms are still poorly understood.

Here, we demonstrate that DA is an endogenous regulator of inflammasome activation and suggest the DRD1 as a potential target for the treatment of NLRP3 inflammasome-driven diseases.

RESULTS

DA Inhibits NLRP3 Inflammasome Activation

To determine the effect of DA on inflammasome activation, LPS-primed bone marrow-derived macrophages (BMDMs) were pretreated with DA before nigericin challenge. NLRP3-dependent caspase-1 activation and IL-1β maturation by

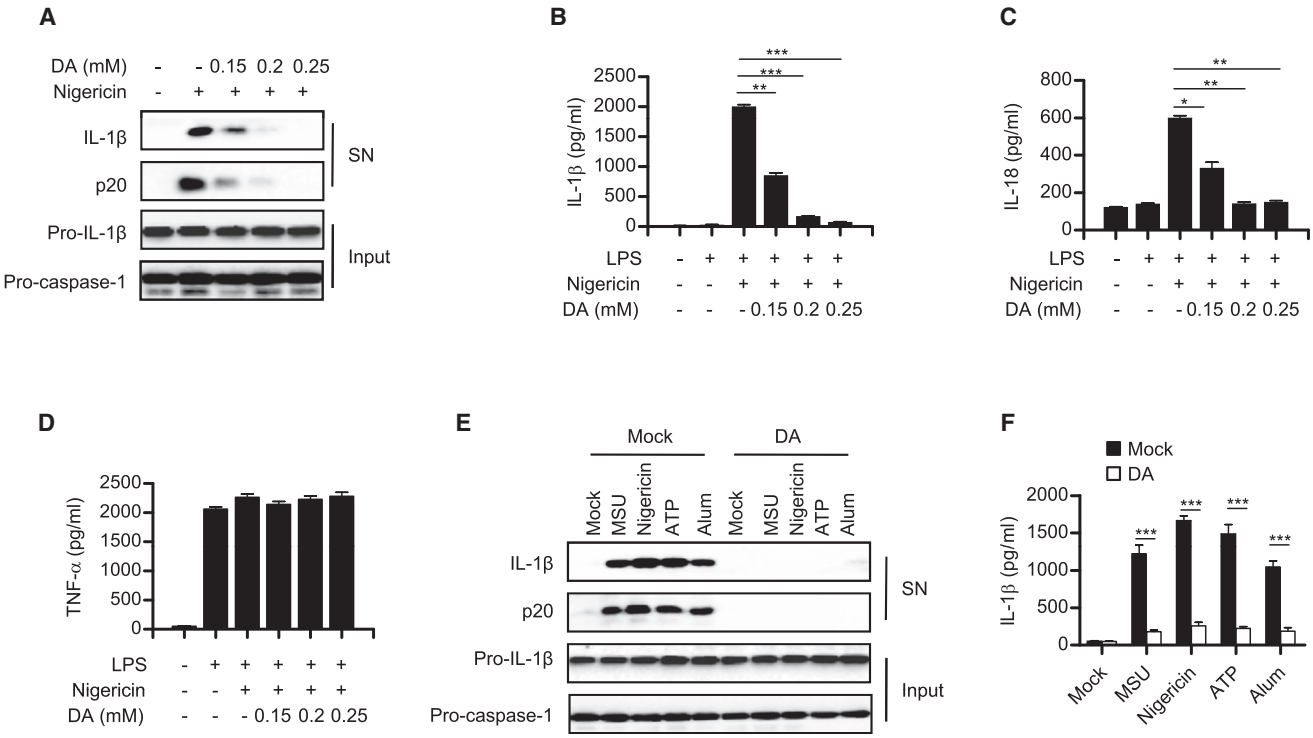

Figure 1. DA Inhibits NLRP3 Inflammasome Activation

(A) Immunoblot analysis of IL-1β and cleaved caspase-1 (p20) in culture supernatants (SN) of LPS-primed BMDMs treated for 3 hr with various doses (above lanes) of DA and then stimulated with nigericin, and immunoblot analysis of the precursors of IL-1β (pro-IL-1β) and caspase-1 (pro-caspase-1) in lysates of those cells (Input).

(B–D) ELISA of IL-1β (B), IL-18 (C), and TNF-α (D) in supernatants from LPS-primed BMDMs treated for 3 hr with various doses (above lanes) of DA and then stimulated with nigericin.

(E) Immunoblot analysis of IL-1β and cleaved caspase-1 (p20) in culture supernatants (SN) of LPS-primed BMDMs treated for 3 hr with DA and then stimulated with MSU, nigericin, ATP, and Alum, and immunoblot analysis of the precursors of IL-1β (pro-IL-1β) and caspase-1 (pro-caspase-1) in lysates of those cells (Input).

(F) ELISA of IL-1β in supernatants from LPS-primed BMDMs treated for 3 hr with DA and then stimulated with MSU, nigericin, ATP, and Alum.

Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001.

See also Figure S1.

nigericin were indeed inhibited by DA in a dose-dependent manner (Figures 1A and 1B and Figure S1A available online). Similarly, DA also inhibited nigericin-induced production of IL-18, another NLRP3-inflammasome-dependent cytokine (Figures 1C and S1B). However, the production of TNF-α, an inflammasome-independent cytokine, was not affected by DA in this condition (Figure 1D), suggesting that DA inhibited IL-1β production via affecting inflammasome activation. The physiological DA levels found in extracellular fluid surrounding neural synapses are ~1 μM (Basu et al., 2001; Chakroborty et al., 2008), but in our study, the DA concentration required for inflammasome inhibition is over 100 μM, which exceeds the physiological concentration of DA. We speculated that the high DA concentration needed for inflammasome inhibition might be due to the instability of DA. Actually, the half-life of DA is <2 min in plasma and is even much shorter in mouse brain tissues (Rouge-Pont et al., 2002), possibly because dopamine can be broken down into inactive metabolites by a set of enzymes, such as monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT) (Youdim et al., 2006). To test this possibility, we changed the DA treatment protocol from one single treatment with high dose to multiple treatments with low dose. The results showed that one single treatment with 45 μM or 90 μM DA could not inhibit nigericin-induced IL-1β production, while treatment with 1.5 μM or 3 μM DA for 30 times (time interval is 5 min) inhibited nigericin-induced IL-1β production significantly, although the total doses were identical (Figure S1C). Moreover, although DA could not inhibit nigericin-induced IL-1β production at the doses under 10 μM, it really could suppress IL-1β production in the presence of MAO and COMT inhibitors at these doses (Figure S1D). In addition, the DA treatment had no effect on cell viability (Figure S1E). Taken together, these results indicate that DA has the potential to inhibit caspase-1 activation and IL-1β secretion.

To test whether DA only affect nigericin-induced NLRP3 inflammasome activation, we examined other NLRP3 agonists. The results showed that DA could inhibit caspase-1 cleavage and IL-1β secretion induced by all examined agonists, including

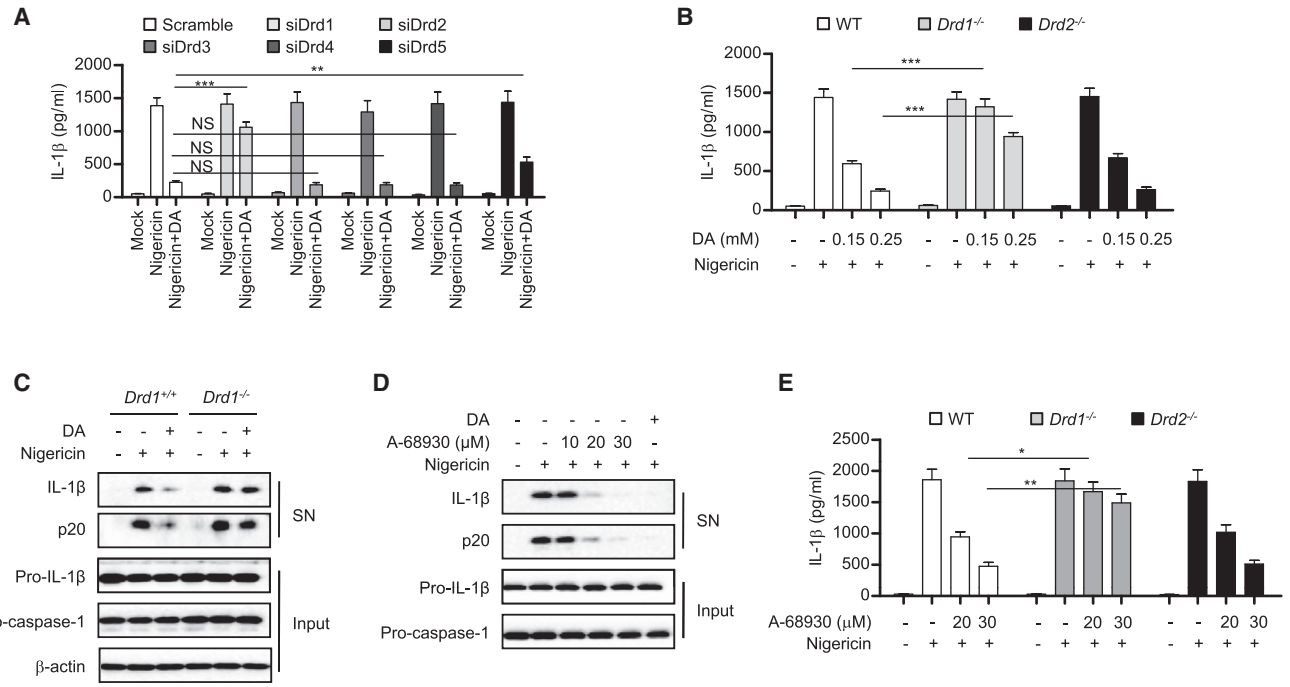

Figure 2. DA Inhibits NLRP3 Inflammasome Activation via DRD1

(A) ELISA of IL-1β in supernatants from LPS-primed BMDMs transfected with control siRNA with a scrambled sequence or *Drd1-Drd5*-specific siRNA as indicated, treated for 3 hr with DA and stimulated with nigericin.

(B) ELISA of IL-1β in supernatants from LPS-primed BMDMs of *Drd1*$^{-/-}$ and *Drd2*$^{-/-}$ mice treated for 3 hr with DA and stimulated with nigericin.

(C) Immunoblot analysis of IL-1β and cleaved caspase-1 (p20) in culture supernatants (SN) of LPS-primed BMDMs from *Drd1*$^{-/-}$ mice treated for 3 hr with DA and then stimulated with nigericin, and immunoblot analysis of the precursors of IL-1β (pro-IL-1β), caspase-1 (pro-caspase-1) and β-actin in lysates of those cells (Input).

(D) Immunoblot analysis of IL-1β and cleaved caspase-1 (p20) in culture supernatants (SN) of LPS-primed BMDMs treated for 3 hr with various doses (above lanes) of A-68930 and then stimulated with nigericin, and immunoblot analysis of the precursors of IL-1β (pro-IL-1β) and caspase-1 (pro-caspase-1) in lysates of those cells (Input).

(E) ELISA of IL-1β in supernatants from LPS-primed BMDMs of *Drd1*$^{-/-}$ and *Drd2*$^{-/-}$ mice treated for 3 hr with A-68930 and then stimulated with nigericin.

Data are means ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001.

See also Figure S2.

MSU, Alum, and ATP, similar to nigericin (Figures 1E and 1F), suggesting that DA is a potent and broad inhibitor for NLRP3 inflammasome activation. Moreover, DA pretreatment had minimal effect on poly (dA:dT) transfection-induced AIM2 inflammasome activation or *Salmonella typhimurium* (*Salmonella*) infection-induced NLRC4 inflammasome activation (Figures S1F–S1I). Taken together, these results demonstrate that DA specifically inhibits NLRP3 inflammasome activation and subsequent IL-1β production.

DA Inhibits NLRP3 Inflammasome Activation via DRD1 Signaling

Next, we investigated the mechanisms underlying the inhibitory activity of DA on NLRP3 inflammasome activation. DA exerts its effects by binding to the activating receptors located on the surface of cells. There are at least five subtypes of dopamine receptors identified, termed DRD1–DRD5, and all of them can be detected in immune cells, including macrophages and dendritic cells (McKenna et al., 2002; Meredith et al., 2005; Ricci et al., 1999). To determine which receptor was involved in DA-induced NLRP3 inflammasome inhibition, these receptors were silenced by small interfering RNA (siRNA) in BMDMs, respectively (Figures S2A and S2B). Knockdown of *Drd1* in BMDMs significantly suppressed the inhibitory effect of DA on inflammasome activation, while knockdown of *Drd2*, *Drd3*, or *Drd4* in BMDMs had no effect (Figure 2A). In addition, knockdown of *Drd5* in BMDMs had a little bit of an effect on DA-induced inflammasome inhibition (Figure 2A**)**. These results suggest that DA inhibits NLRP3 inflammasome activation primary through DRD1. To further confirm this, we tested the role of DA in *Drd1*$^{-/-}$ cells. The results showed that the inhibitory effect of DA on nigericin-induced IL-1β secretion in BMDMs was inhibited completely or partially when *Drd1* was absent, depending on the doses of DA (Figures 2B and 2C). We also examined whether the agonist of DRD1 could inhibit inflammasome activation. First, we found that the agonist of DRD1 A-68930 inhibited nigericin-induced IL-1β secretion significantly, while it had no effect on cell viability, similarly to DA (Figures 2D and S2C), and the agonists of DRD2, DRD3, or DRD4 had mild or no effect on NLRP3 inflammasome activation (Figure S2D). In addition, A-68930-induced NLRP3

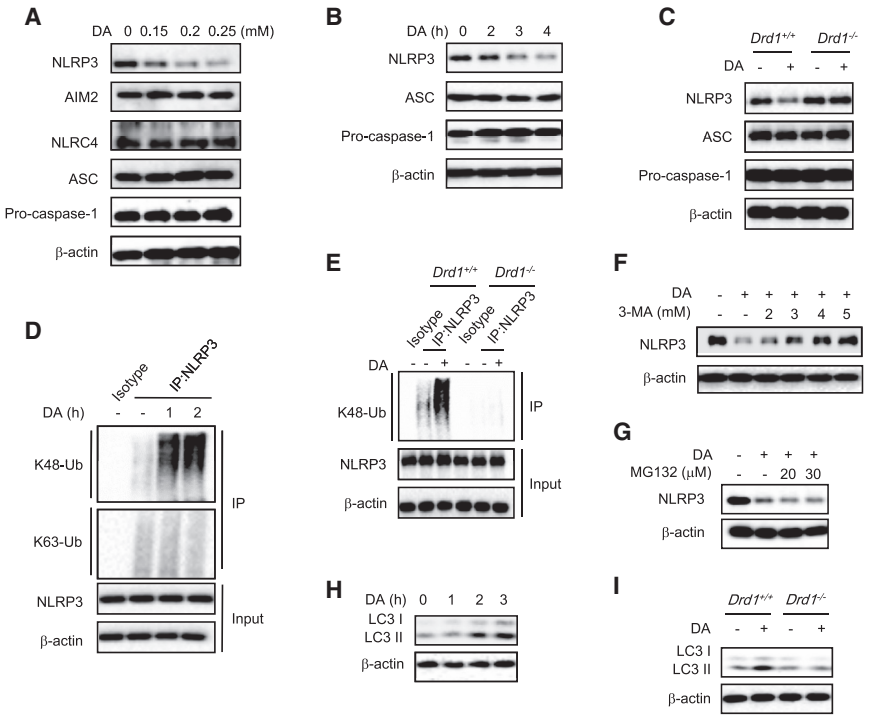

Figure 3. DRD1 Signaling Promotes NLRP3 Ubiquitination and Degradation to Inhibit NLRP3 Inflammasome

(A) Immunoblot analysis of NLRP3, AIM2, NLRC4, ASC, Pro-caspase-1, and β-actin from LPS-primed BMDMs treated for 3 hr with various doses of DA.

(B) Immunoblot analysis of NLRP3, ASC, Pro-caspase-1, and β-actin from LPS-primed BMDMs treated for different time points of DA.

(C) Immunoblot analysis of NLRP3, ASC, Pro-caspase-1, and β-actin from LPS-primed BMDMs of *Drd1*$^{-/-}$ mice treated for 3 hr of DA.

(D) LPS primed-BMDMs were treated with DA (0.2 mM). Immunoblot analysis of K48-Ub and K63-Ub proteins in cell lysates immunoprecipitated with NLRP3 antibody.

(E) LPS primed-BMDMs from *Drd1*$^{-/-}$ mice were treated with DA (0.2 mM) for 1 hr. Immunoblot analysis of K48-Ub protein in cell lysates immunoprecipitated with NLRP3 antibody.

(F) Immunoblot analysis of NLRP3 and β-actin in cell lysates from LPS-primed BMDMs treated with different doses of 3-MA for 30 min and then stimulated with DA (0.2 mM) for 3 hr.

(G) Immunoblot analysis of NLRP3 and β-actin in cell lysates from LPS-primed BMDMs treated with different doses of MG132 for 30 min and then stimulated with DA (0.2 mM) for 3 hr.

(H) Immunoblot analysis of LC3 and β-actin in cell lysates from LPS-primed BMDMs treated for different time points of DA (0.2 mM).

(I) Immunoblot analysis of LC3 and β-actin in cell lysates from LPS-primed BMDMs of *Drd1*$^{-/-}$ mice treated with DA (0.2 mM) for 3 hr.

See also Figure S3.

inflammasome inhibition was not affected by DRD1 deficiency (Figure 2E).

Although our results showed that DA did not affect LPS-induced TNF-α production when macrophages were treated with DA after LPS (Figure 1D), previous results have shown that DA can inhibit some inflammasome-independent cytokine production, including TNF-α (Basu and Dasgupta, 2000). To examine the role of DRD1 signaling in inflammasome-independent cytokine production, we treated BMDMs with DA before LPS and found that DA could suppress LPS-induced TNF-α production, but the dose was higher than that needed for inflammasome inhibition (Figure S2E). In addition, the effect of DA on LPS-induced TNF-α production were significantly impaired in *Drd2*$^{-/-}$ cells, while slightly reduced in *Drd1*$^{-/-}$ cells, suggesting that DA inhibits LPS-induced TNF-α production primarily via DRD2 (Figure S2E). These results were consistent with a new study in which they reported that *Drd2*$^{-/-}$ mice showed spontaneous inflammation in the brain and DRD2 can suppress the transcription and production of proinflammatory cytokines, including TNF-α (Shao et al., 2013). These results suggest that DA signals through DRD1 to inhibit inflammasome activation and signals through DRD2 to suppress the transcription proinflammatory cytokines.

DA and DRD1 Signaling Promote NLRP3 Ubiquitination and Autophagy-Mediated Degradation

Next, we asked how DA and DRD1 signaling inhibit NLRP3 inflammasome activation. Interestingly, we found that DA treatment induced NLRP3 degradation in a dose- and time-dependent manner (Figures 3A and 3B). DA-induced protein degradation was specific to NLRP3, because the expression of AIM2, NLRC4, ASC, caspase-1, and pro-IL-1β were stable in DA-treated BMDMs (Figures 3A and 3B). Similar to DA, treatment with the DRD1 agonist A-68930 also induced NLRP3 degradation in BMDMs (Figures S3A and S3B). Further study showed that DA-induced NLRP3 degradation was inhibited in *Drd1*$^{-/-}$ macrophages (Figure 3C), suggesting that DA promotes NLRP3 degradation via DRD1 signaling. Consistent with the degradation of NLRP3, treatment with DA induced a K48-linked polyubiquitination of NLRP3 in macrophages (Figure 3D). Importantly, DA-induced NLRP3 polyubiquitination was also impaired in *Drd1*$^{-/-}$ macrophages (Figure 3E).

We then asked whether proteasome or autophagy mediated the degradation of ubiquitinated NLRP3 protein. Our results showed that autophagy inhibitor 3-Methyladenine (3-MA) could suppress DA-induced NLRP3 degradation, while proteasome inhibitor MG132 could not (Figures 3F and 3G), suggesting that ubiquitinated NLRP3 is degraded via autophagy. Indeed, DA treatment induced EGFP-NLRP3 to form big aggregates in HEK293T cells (Figure S3C), which might explain why ubiquitinated NLRP3 is degraded by autophagy, not by proteasome. Consistent with this, DA treatment could induce autophagy in BMDMs via DRD1 signaling (Figures 3H and 3I). Importantly, DA-induced inflammasome inhibition could be rescued by autophagy inhibitor 3-MA (Figure S3D). These results suggest that autophagy mediates DA-induced NLRP3 degradation.

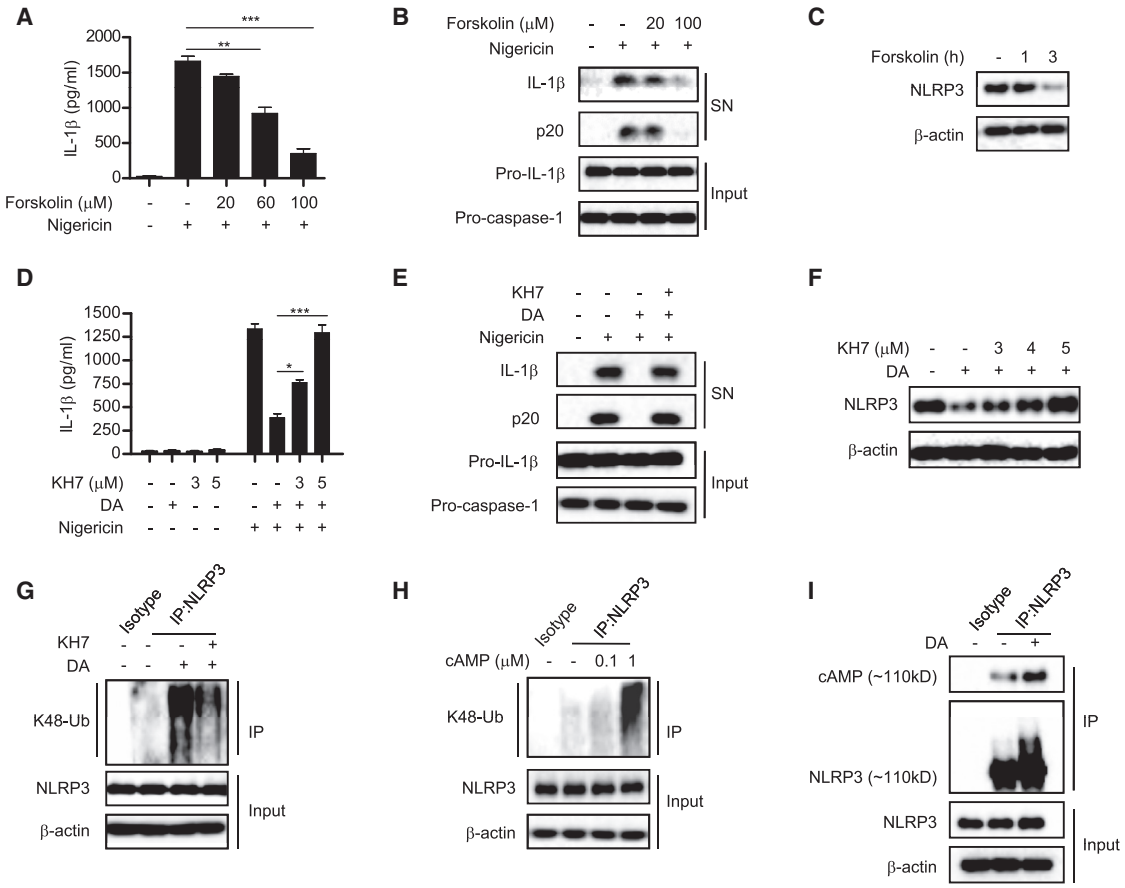

Figure 4. DRD1 Signaling Promotes NLRP3 Ubiquitination and Degradation via cAMP

(A) ELISA of IL-1β in supernatants from LPS-primed BMDMs treated for 3 hr with forskolin and then stimulated with nigericin.

(B) Immunoblot analysis of IL-1β and cleaved caspase-1 (p20) in culture supernatants (SN) of LPS-primed BMDMs treated for 3 hr with various doses (above lanes) of forskolin and then stimulated with nigericin, and immunoblot analysis of the precursors of IL-1β (pro-IL-1β) and caspase-1 (pro-caspase-1) in lysates of those cells (Input).

(C) Immunoblot analysis of NLRP3 and β-actin in cell lysates from LPS-primed BMDMs treated for different time points of forskolin (100 μM).

(D) ELISA of IL-1β in supernatants from LPS primed-BMDMs treated with different doses of KH7 for 30 min before 3 hr DA treatment and then stimulated with nigericin.

(E) Immunoblot analysis of IL-1β and cleaved caspase-1 (p20) in culture supernatants (SN) of LPS primed-BMDMs treated with KH7 (5 μM) for 30 min before 3 hr DA treatment and then stimulated with nigericin.

(F) Immunoblot analysis of NLRP3 and β-actin in cell lysates from LPS primed-BMDMs treated with different doses of KH7 for 30 min before 3 hr DA treatment.

(G) LPS primed-BMDMs were treated with KH7 (5 μM) for 30 min before 1 hr DA treatment. Immunoblot analysis of K48-Ub protein from the cell lysates immunoprecipitated with anti-NLRP3 antibody.

(H) Lysates from LPS primed-BMDMs were treated with different doses of cAMP for 30 min. Immunoblot analysis of K48-Ub protein from the cell lysates immunoprecipitated with anti-NLRP3 antibody.

(I) LPS primed-BMDMs were treated with DA (0.2 mM) for 1 hr. Immunoblot analysis of cAMP and NLRP3 proteins from the cell lysates immunoprecipitated with anti-NLRP3 antibody.

Data are means ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

See also Figure S4.

DRD1 Signaling Induces NLRP3 Ubiquitination via Cyclic AMP-Dependent Manner

We further investigated how DRD1 signaling promotes NLRP3 ubiquitination. DRD1 signaling can stimulate the activity of adenylate cyclase and the production of cyclic AMP (cAMP), which is a second messenger and is important in many biological processes (Beaulieu et al., 2004; Nishi et al., 2011). Recently, cAMP has been proposed to negatively regulate NLRP3 inflammasome activation (Lee et al., 2012), so we examined the role of cAMP in DA-induced inflammasome inhibition. We found that the increase of the cAMP levels with adenylate cyclase (ADCY) activator forskolin inhibited NLRP3 inflammasome activation with a dose-dependent manner (Figures 4A and 4B), consistent with a previous report (Lee et al., 2012). Furthermore, forskolin treatment promoted NLRP3 degradation and ubiquitination in macrophages (Figures 4C and S4A), similar with DA treatment.

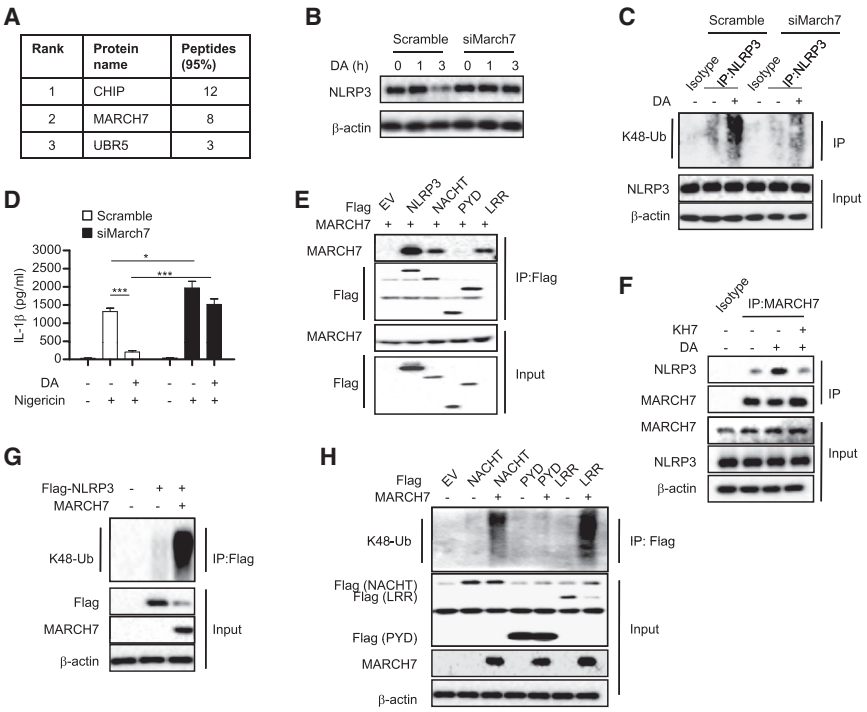

Figure 5. DRD1 Signaling Promotes NLRP3 Ubiquitination through E3 Ligase MARCH7

(A) List of E3 ligase proteins for top peptide hits identified in mass spectrometry study.

(B) Immunoblot analysis of NLRP3 and β-actin in cell lysates from LPS-primed DA-treated BMDMs transfected with siRNA against *March7*.

(C) BMDMs were transfected with siRNA against *March7*. After 48 hr, the cells were primed with LPS and then stimulated with DA (0.2 mM) for 1 hr. Immunoblot analysis of K48-Ub protein from the cell lysates immunoprecipitated with anti-NLRP3 antibody.

(D) ELISA of IL-1β in supernatants from LPS-primed DA and nigericin-treated BMDMs transfected with siRNA against *March7*

(E) Flag-tagged NLRP3 constructs and MARCH7 construct were cotransfected in HEK293T cells. Immunoblot analysis of MARCH7 and Flag proteins in cell lysates immunoprecipitated with anti-Flag antibody. EV, empty vector.

(F) LPS primed-BMDMs were treated with KH7 (5 μM) for 30 min before 1 hr DA treatment. Immunoblot analysis of MARCH7 protein in cell lysates immunoprecipitated with anti-MARCH7 antibody.

(G and H) Flag-tagged NLRP3 construct and MARCH7 construct were cotransfected in HEK293T cells. Immunoblot analysis of K48-Ub protein in cell lysates immunoprecipitated with anti-Flag antibody. Data are means ± SEM, *$p < 0.05$, ***$p < 0.001$. See also Figure S5.

Importantly, DA-induced NLRP3 inflammasome inhibition could be blocked by KH7, an ADCY inhibitor (Figures 4D and 4E), suggesting that DA-induced NLRP3 inflammasome inhibition is cAMP-dependent. Consistent with this, DA-induced NLRP3 degradation and ubiquitination could also be inhibited by KH7 (Figures 4F and 4G). Actually, cAMP also could promote NLRP3 ubiquitination in the lysates of macrophages (Figure 4H). These results indicate that the increase of cAMP in cells can promote NLRP3 ubiquitination and degradation.

Protein kinase A (PKA) and exchange protein activated by cAMP (EPAC) are the two known sensors for intracellular cAMP, so we tested whether they were involved in DA-induced NLRP3 inflammasome inhibition (Gloerich and Bos, 2010). The results showed that inhibition of PKA with H89 did not affect DA-induced NLRP3 inflammasome inhibition or DA-promoted NLRP3 degradation (Figures S4B and S4C). In addition, EPAC agonist also had no effect on NLRP3 inflammasome activation or NLRP3 degradation (Figures S4D and S4E). These results suggest that PKA and EPAC are not the downstream of cAMP to promote NLRP3 ubiquitination. Actually, when NLRP3 were immunoprecipitated and the precipitates were examined with anti-cAMP antibody, a band with the same size of NLRP3 could be detected with anti-cAMP antibody (Figure 4I), suggesting that NLRP3 can directly interact with cAMP, possibly by covalent linkage. Importantly, DA treatment promoted the interaction between cAMP and NLRP3 significantly (Figure 4I). These results indicate that DA-induced cAMP promotes NLRP3 ubiquitination and degradation via binding with NLRP3.

We then examined the role of cAMP in DA-induced autophagy. The results showed that KH7 could inhibit DA-induced autophagy, while forskolin could promote autophagy (Figures S4F and S4G), similar with DA, suggesting that DA-induced autophagy is cAMP-dependent. Similarly, DA-induced autophagy was PKA- and EPAC-independent (Figures S4H and S4I), suggesting that cAMP promotes autophagy without the involvement of its known downstream signaling. Importantly, DA-induced NLRP3 aggregation was inhibited by KH7 (Figure S4J), suggesting that cAMP promotes the formation of NLRP3 aggregates, which could trigger autophagy for its degradation.

Taken together, these results suggest that the binding of cAMP with NLRP3 promotes its ubiquitination and then the ubiquitinated NLRP3 forms aggregates, which are targeted for degradation by autophagy.

DRD1 Signaling Promotes NLRP3 Ubiquitination via E3 Ubiquitin Ligase MARCH7

To identify the E3 ligase that is responsible for DA-induced NLRP3 ubiquitination and degradation, we used mass spectrometry (MS) to identify the E3 ligases associated with NLRP3 and found that E3 ligases CHIP, MARCH7, and UBR5 had the highest number of matched peptides identified by MS in the precipitates (Figure 5A). Further studies showed that knockdown of *Chip* or *Ubr5* by using siRNA did not affect DA-induced NLRP3 degradation in BMDMs (Figures S5A–S5C). However, knockdown of *March7* could rescue DA-induced NLRP3 degradation

in BMDMs (Figures 5B and S5A). Moreover, DA-induced NLRP3 ubiquitination was also impaired when *March7* was silenced (Figure 5C). Consistent with this, the effect of DA on IL-1β secretion depended on MARCH7 (Figure 5D). In addition, forskolin-induced NLRP3 degradation, ubiquitination, and inflammasome inhibition were also rescued by *March7* knockdown (Figures S5D–S5F). These results suggest the importance of E3 ligase MARCH7 in DRD1 signaling and cAMP-induced NLRP3 ubiquitination and inflammasome inhibition.

We further investigated how MARCH7 promoted NLRP3 ubiquitination. First, we found that overexpressed MARCH7 not only interacted with full-length NLRP3, but also interacted with NACHT or LRR domain, but not with PYD domain, in HEK293T cells (Figure 5E). Second, DA treatment could promote the endogenous interaction between NLRP3 and MARCH7 in BMDMs (Figure 5F), and this effect also depended on cAMP because KH7 could inhibit the interaction between NLRP3 and MARCH7 (Figure 5F). Importantly, overexpressed MARCH7 could promote NLRP3 ubiquitination and degradation in HEK293T cells (Figure 5G). Further studies showed that MARCH7 overexpression could promote the ubiquitination and degradation of LRR domain strongly, but could not induce the degradation of NACHT domain, although MARCH7 had some effect to enhance its ubiquitination (Figure 5H). These results suggest that LRR domain is the key region of NLRP3 for MARCH7-mediated ubiquitination and degradation.

DRD1 Signaling Prevents MPTP-Induced Neuroinflammation via Suppression of NLRP3 Inflammasome

Abundant evidence implicates that immune system abnormalities and neuroinflammation are sustaining and exacerbating reasons for the loss of the dopaminergic neurons and DA deficiency (Brochard et al., 2009; Perry, 2012; Rodrigues et al., 2013; Shao et al., 2013). NLRP3 inflammasome has been reported to be involved in the progression of several human diseases (Davis et al., 2011; Martinon et al., 2009). NLRP3 inflammasome can be activated by aggregated α-Synuclein, which has been suggested directly linked to the pathogenesis of PD (Codolo et al., 2013). To determine the role of NLRP3 inflammasome-driven neuroinflammation in the neurodegeneration of nigral dopaminergic neurons, *Nlrp3*$^{-/-}$ mice were treated with neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The data showed that *Nlrp3*$^{-/-}$ mice were resistant to MPTP-induced loss of nigral dopaminergic neurons compared with their wild-type mice (Figures S6A and S6B). This difference was associated with reduced IL-1β and IL-18 production in serum and impaired caspase-1 activation in brain tissue (Figures S6C and S6D), suggesting that dysregulation of NLRP3 inflammasome contributes to the development of MPTP-induced loss of nigral dopaminergic neurons.

We then want to know whether DA and DRD1 signaling can prevent MPTP-induced neuroinflammation and loss of neurons. In brain, the activated microglias and astrocytes have been regarded as important contributors for the progression of PD (Lucin and Wyss-Coray, 2009). Both microglias and astrocytes express NLRP3 and secrete IL-1β via NLRP3 inflammasome-dependent manner (Alfonso-Loeches et al., 2014; Halle et al., 2008). We first checked whether DA could inhibit inflammasome activation in microglias and astrocytes. The results showed that DA treatment could inhibit nigericin-induced IL-1β production in both microglias and astrocytes, and the inhibitory effects of DA were impaired in *Drd1*$^{-/-}$ cells (Figures 6A and 6B). Moreover, DRD1 signaling also induced NLRP3 degradation in microglias and astrocytes (Figures 6C and 6D). These results suggest that DA and DRD1 signaling can promote NLRP3 degradation to inhibit inflammasome activation in microglias and astrocytes. To further study the in vivo relevance of inflammasome inhibition by DA, *Drd1*$^{-/-}$ mice were administrated with MPTP and the loss of dopaminergic neurons was examined. The results showed that *Drd1*$^{-/-}$ mice displayed more severe loss of nigral dopaminergic neurons compared with wild-type mice when treated with MPTP (Figures 6E and 6F). Consistent with this, *Drd1*$^{-/-}$ mice also showed more inflammasome activation by analyzing IL-1β and IL-18 production or caspase-1 activation (Figures 6G and 6H). However, *Drd1*$^{-/-}$*Nlrp3*$^{-/-}$ mice did not show more severe loss of dopaminergic neurons, IL-1β and IL-18 production, or caspase-1 activation compared with *Drd1*$^{-/-}$mice (Figures S6A–S6D). Moreover, MPTP-treated *Drd1*$^{-/-}$ mice showed more NLRP3 expression and less NLRP3 ubiquitination in the brain tissue compared with *Drd1*$^{+/+}$ mice, suggesting that DRD1 signaling might promote NLRP3 ubiquitination in MPTP-induced neuroinflammation (Figure 6I). These results suggest that DRD1 signaling can prevent neuroinflammation via inhibition of NLRP3 inflammasome by promoting NLRP3 ubiquitination in vivo.

To further study the protective role of DRD1 signaling in neuroinflammation, *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice were treated with DRD1 agonist A-68930 before MPTP treatment. A-68930 administration resulted in a remarkable reduction of dopaminergic neuron damage, IL-1β or IL-18 production, and caspase-1 activation in *Drd1*$^{+/+}$ mice, but not in *Drd1*$^{-/-}$ mice (Figures 6E–6H), indicating that activation of DRD1 signaling can protect MPTP-induced inflammasome activation and dopaminergic neuron damage in vivo.

DA and DRD1 Signaling Mitigate LPS-Induced Systemic Inflammation and MSU-Induced Peritoneal Inflammation by Inhibition of NLRP3 Inflammasome

In addition to function as neurotransmitter in the brain, DA also functions as a local chemical messenger in several periphery parts of the body, including blood, intestine, and kidney (Beaulieu and Gainetdinov, 2011). Importantly, DA is a "first line" agent for septic shock as a vasopressor over the past two decades (De Backer et al., 2010). Because septic shock is an inflammatory disorder, we asked whether DA could suppress the inflammatory responses via inhibition of NLRP3 inflammasome in septic shock. The *Nlrp3*$^{+/+}$ or *Nlrp3*$^{-/-}$ mice were treated with DA when challenged with LPS to induce septic shock, and the results showed that DA treatment resulted in significant reduction of serum IL-1β or IL-18 production in *Nlrp3*$^{+/+}$ mice but had no effect in *Nlrp3*$^{-/-}$ mice (Figures 7A and 7B). In addition, DA treatment had only mild effect on serum TNF-α production, which was inflammasome-independent (Figure 7C). These results suggest that DA can inhibit LPS-induced NLRP3 inflammasome activation in vivo.

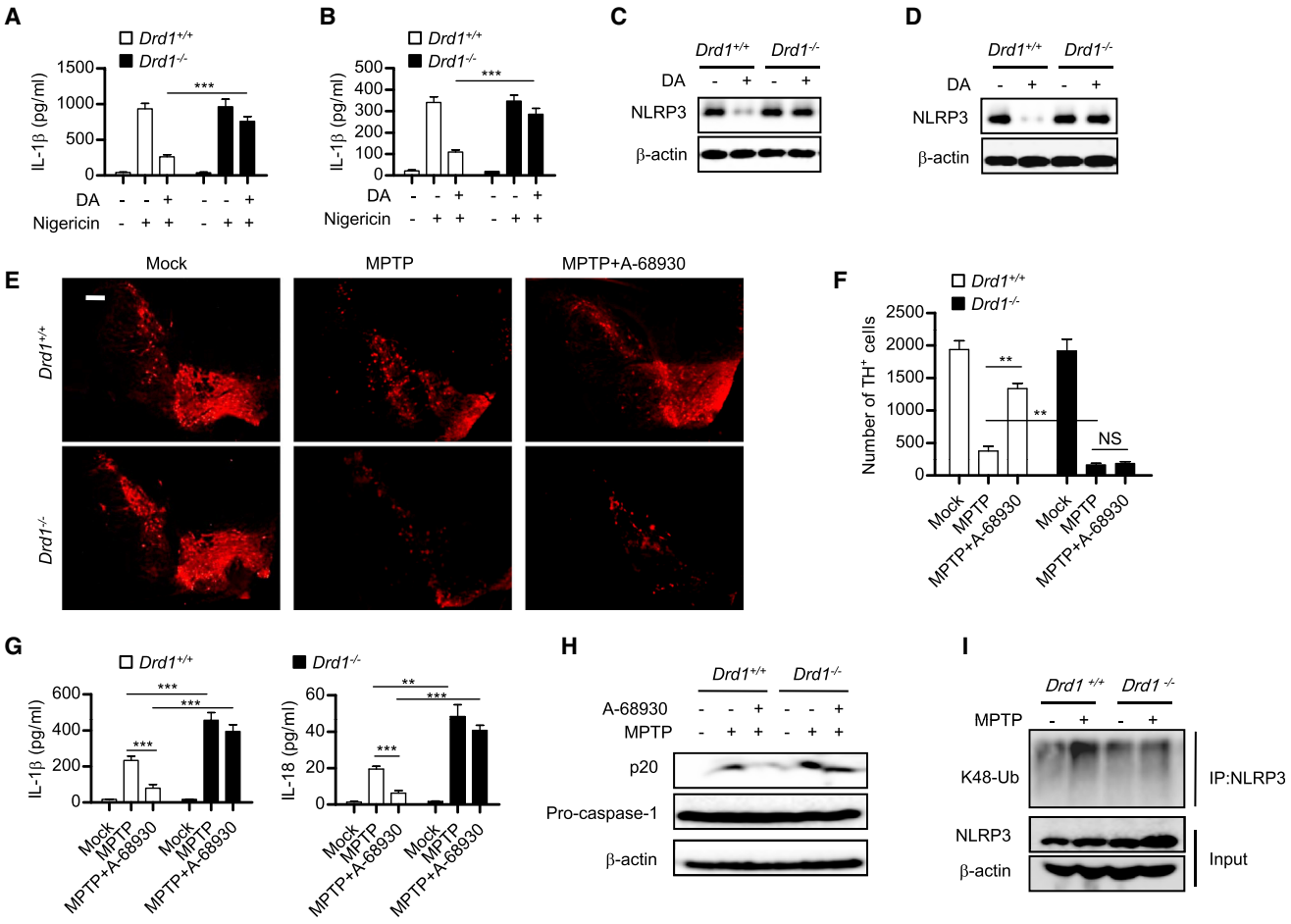

Figure 6. DRD1 Signaling Prevents Neuroinflammation via Suppression of NLRP3 Inflammasome

(A) ELISA of IL-1β in supernatants from LPS-primed microglias of *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice treated with DA (0.2 mM) for 3 hr and stimulated with nigericin.

(B) ELISA of IL-1β in supernatants from LPS-primed astrocytes of *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice treated with DA (0.2 mM) for 3 hr and stimulated with nigericin.

(C) Immunoblot analysis of NLRP3 and β-actin in cell lysates from LPS-primed microglias of *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice treated with DA (0.2 mM) for 3 hr.

(D) Immunoblot analysis of NLRP3 and β-actin in cell lysates from LPS-primed astrocytes of *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice treated with DA (0.2 mM) for 3 hr.

(E) Immunofluorescent histochemical staining for tyrosine hydroxylase (TH) on the ventral mesencephalon of *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice administrated with MPTP or MPTP plus A-68930. Scale bar represents 500 μM.

(F) Quantitative data of TH expression on the ventral mesencephalon of *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice administrated with MPTP or MPTP plus A-68930.

(G) ELISA of IL-1β and IL-18 in serum of *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice administrated with MPTP or MPTP plus A-68930.

(H) Immunoblot analysis of caspase-1 cleavage (p20), pro-caspase-1, and β-actin of *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice administrated with MPTP or MPTP plus A-68930.

(I) *Drd1*$^{+/+}$ and *Drd1*$^{-/-}$ mice were treated with MPTP. Immunoblot analysis of the lysates of brain tissue immunoprecipitated with anti-NLRP3 antibody.

Data are means ± SEM, $^{**}p < 0.01$, $^{***}p < 0.001$.

See also Figure S6.

To determine whether DA can inhibit LPS-induced systemic inflammation via DRD1 signaling, *Drd1*$^{+/+}$ and *Drd1*$^{-/-}$ mice were treated with LPS, and the results showed that LPS induced more serum IL-1β and IL-18 production, but comparable TNF-α production in *Drd1*$^{-/-}$ mice (Figures 7D–7F). Moreover, DA treatment could suppress LPS-induced serum IL-1β and IL-18 production in *Drd1*$^{+/+}$ mice, but had little effect in *Drd1*$^{-/-}$ mice (Figures 7D and 7E). Importantly, *Drd1* deletion in *Nlrp3*$^{-/-}$ mice could not enhance LPS injection-induced serum IL-1β and IL-18 production (Figures 7G and 7H). Consistent with DRD1 signaling-induced inhibition of NLRP3 inflammasome in vivo, DA treatment in vivo also could induce NLRP3 ubiquitination and degradation in peritoneal macrophage of *Drd1*$^{+/+}$ mice, but not in *Drd1*$^{-/-}$ mice (Figures S7A and S7B). In addition, treatment with autophagy inhibitor 3-MA to inhibit the degradation of NLRP3 could impair DA-induced inflammasome inhibition in LPS-treated mice (Figure S7C). These results indicate that DRD1 signaling can inhibit LPS-induced systemic inflammation via suppression of NLRP3 inflammasome activation.

To further study the protective effect of DRD1 signaling in periphery inflammation, we examined whether DRD1 signaling could suppress MSU-induced peritoneal inflammation, which is NLRP3 inflammasome-dependent (Martinon et al., 2006).

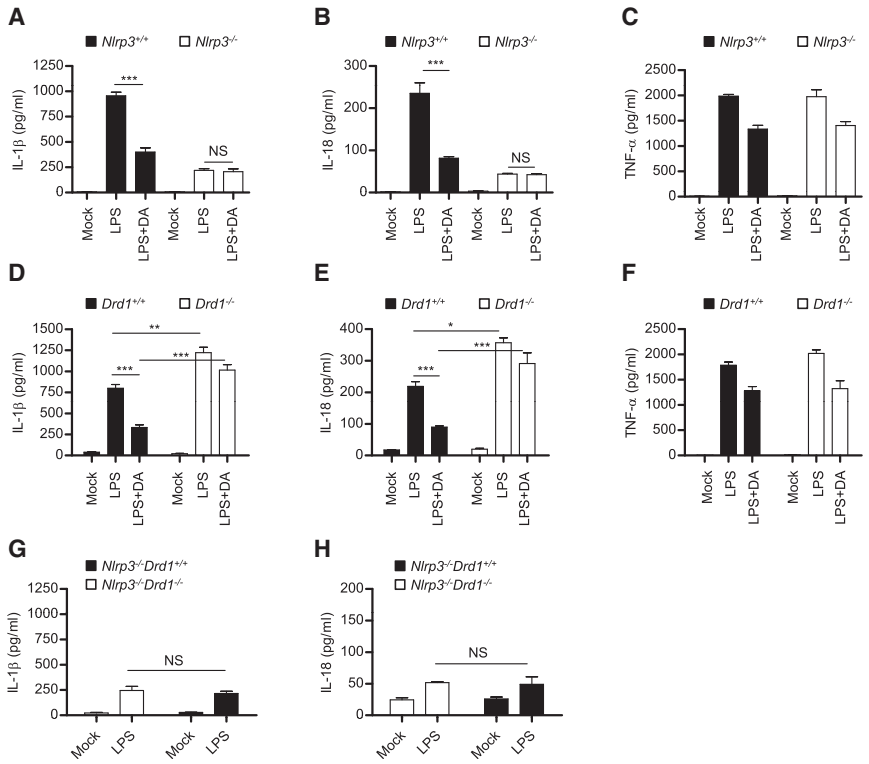

Figure 7. DRD1 Signaling Prevents LPS-Induced Systemic Inflammation and MSU-Induced Peritoneal Inflammation via Suppression of NLRP3 Inflammasome

(A–C) ELISA of IL-1β (A), IL-18 (B), and TNF-α (C) in serum of *Nlrp3*$^{+/+}$ or *Nlrp3*$^{-/-}$ mice intraperitoneally injected with LPS (20 mg/kg of body weight) with or without DA (50 mg/kg of body weight).

(D–F) ELISA of IL-1β (D), IL-18 (E), and TNF-α (F) in serum of *Drd1*$^{+/+}$ or *Drd1*$^{-/-}$ mice intraperitoneally injected with LPS (20 mg/kg of body weight) with or without DA (50 mg/kg of body weight).

(G and H) ELISA of IL-1β (G) and IL-18 (H) in serum of *Nlrp3*$^{-/-}$ mice or *Nlrp3*$^{-/-}$*Drd1*$^{-/-}$ mice intraperitoneally injected with LPS (20 mg/kg of body weight).

Data are means ± SEM, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. NS $p > 0.05$.

See also Figure S7.

The result showed that MSU could induce more IL-1β production and recruit more neutrophils in peritoneal cavity of *Drd1*$^{-/-}$ mice (Figures S7D and S7E). Moreover, DA treatment could inhibit MSU-induced IL-1β production and neutrophil recruitment in *Drd1*$^{+/+}$ mice, but not in *Drd1*$^{-/-}$ mice (Figures S7D and S7E). These results indicate that DRD1 signaling has a negative role in MSU-induced NLRP3 inflammasome activation and peritoneal inflammation in vivo.

DISCUSSION

Increasing evidence has shown that the immune system can be influenced by the nervous system and neurotransmitters (Olofsson et al., 2012; Tracey, 2009). DA not only functions as a neurotransmitter in the brain, but also functions as a local chemical messenger in several periphery parts of the body. A substantial amount of dopamine circulates in the bloodstream, but its functions are not entirely clear. Here, our results demonstrate that DA is an endogenous inhibitor of NLRP3 inflammasome activation, suggesting that DA is a potential antiinflammatory chemical, in addition to a neurotransmitter. The effect of dopamine and its agonists on immune responses including proliferation and cytokine production has been reported. Bergquist et al. (1994) reported that either DA or L-dihydroxyphenylalanine, a precursor of DA, resulted in a dose-dependent inhibition of lymphocyte proliferation and differentiation. Josefsson et al. (1996) reported that DA inhibited the ConA- or LPS-induced proliferation and cytokine production of lymphocytes. Ghosh et al. (2003) reported that DA suppressed anti-CD3-mediated proliferation and cytokine production in activated T cells. Thus, our results and previous studies suggest that DA might play an important role in the communication between nervous system and immune system.

DA exerts its effects by binding to the activating receptors located on the surface of cells. There are at least five subtypes of dopamine receptors that have been identified, termed DRD1–DRD5, and most of them can be detected in immune cells, including macrophages and dendritic cells (McKenna et al., 2002; Meredith et al., 2005; Ricci et al., 1999). Here, our results show that DRD1 plays a primary role and DRD5 plays a minor role, while DRD2, DRD3, and DRD4 have no roles in DA-induced NLRP3 inflammasome inhibition. The different roles of DA receptors could be explained by their different downstream signalings. Activation of D1-like receptors (DRD1 and DRD5) increases intracellular cAMP levels, while activation of D2-like receptors (DRD2, DRD3, DRD4) decreases intracellular cAMP levels (Missale et al., 1998). Consistent with this, our results demonstrate that cAMP played a critical role in DA-induced inflammasome inhibition. Taken together, our results indicate that DA can negatively regulate NLRP3 inflammasome activation via DRD1 signaling and suggest that DRD1 might be a potential target for treatment of inflammatory diseases.

NLRP3 inflammasome-mediated IL-1β production is a two-step process: a primary signal that induces pro-IL-1β and NLRP3 synthesis and a secondary signal that activates the inflammasome and the subsequent caspase-1 processing (Bauernfeind et al., 2011). NLRP3 protein expression levels have been shown to be a limiting step in inflammasome activation (Bauernfeind et al., 2009; Franchi et al., 2009). Here, we demonstrate that DA can induce the degradation of NLRP3 protein to inhibit NLRP3 inflammasome and support the recent findings that NLRP3 ubiquitination might play an important role in the regulation of inflammasome activation (Juliana et al., 2012; Py et al., 2013).

Increasing evidence imply that immune dysregulation and neuroinflammation are sustaining and exacerbating reasons for

the loss of the dopaminergic neurons in PD (Brochard et al., 2009; Perry, 2012; Rodrigues et al., 2013; Shao et al., 2013), although the precise mechanisms causing uncontrolled inflammation remain unclear. In this study, we demonstrate that NLRP3 inflammasome is involved in the MPTP-induced loss of the dopaminergic neurons. Moreover, our results show that DA and DRD1 signaling has a protective role for MPTP-induced loss of dopaminergic neurons by suppressing NLRP3 inflammasome activation. Although DRD2 was not involved in DA-induced NLRP3 inflammasome inhibition, a previous report has shown that DRD2 signaling also has a protective role in MPTP-induced loss of the dopaminergic neurons (Shao et al., 2013). It seems DRD2 signaling can affect the transcription of proinflammatory cytokines, including pro-IL-1β, TNF-α, et al., according to Shao et al. (2013). Consistent with this, our study found that DRD2 signaling played a primary role in the inhibitory effect of DA on LPS-induced TNF-α production, while DRD1 only had a minor role. In addition, *Drd2*$^{-/-}$ mice show spontaneous inflammation in the brain, while *Drd1*$^{-/-}$ mice do not (Shao et al., 2013). This suggests that DRD1 only functions to suppress inflammation in "stressed" condition. These results indicate that DRD1 and DRD2 signaling exert a different role in the suppression of inflammation: DRD2 signaling inhibits the transcription proinflammatory cytokines, while DRD1 signaling inhibits the processing and secretion of IL-1β and IL-18 via suppressing inflammasome.

DA is not only an important neurotransmitter in the brain, but also functions as a local chemical messenger in several periphery parts of the body, including blood, intestine, and kidney (Beaulieu and Gainetdinov, 2011). Our results showed that DA treatment in mice suppressed LPS-induced inflammasome-dependent cytokine production, such as IL-1β and IL-18. More importantly, *Drd1*$^{-/-}$ mice are susceptible to LPS-induced systemic inflammation. In addition, our results also showed that DA and DRD1 signaling could suppress MSU-induced IL-1β production and neutrophil infiltration in peritoneal cavity. These results suggest that DA and DRD1 signaling can inhibit systemic or periphery inflammation by inhibition of NLRP3 inflammasome.

Although our results indicate that DA and DRD1 signaling can control both central and periphery inflammation in vivo by inhibition of NLRP3 inflammasome, the DA concentration required for inflammasome inhibition in vitro exceeds the reported physiological concentration of DA (Basu et al., 2001; Chakroborty et al., 2008). To address this issue, we provided results showing that multiple treatments with low dose of DA could inhibit inflammasome activation, while the single treatment with high dose (the same dose with the total dose of multiple treatments) could not. Although this is not enough to prove that the physiological dose of DA can inhibit inflammasome activation, it clearly shows that the instability of DA is an important reason for the requirement of a high dose of DA for inflammasome inhibition. In order to further find out whether the physiological dose of DA can inhibit inflammasome activation, we treated macrophages with physiological dose of DA in the presence of MAO and COMT inhibitors and found that low dose of DA could inhibit inflammasome activation in this condition. These results together suggest that the persistent treatment with physiological dose of DA can inhibit inflammasome activation in vitro. For the in vivo, although the concentration of DA is controlled by MAO and COMT, the dopaminergic neurons can produce DA continuously so the DA levels could be maintained. In addition, we should note that it is hard to measure the levels of DA in vivo because DA is broken very fast. The reported physiological concentration of DA might be much lower than the actual concentration.

Collectively, our results demonstrate that DA and DRD1 signaling can suppress NLRP3 inflammasome activation and identify an endogenous regulatory mechanism for NLRP3 inflammasome-related inflammation. Our results also demonstrate that DA and DRD1 signaling can prevent neurotoxin-induced neuroinflammation, LPS-induced systemic inflammation, and MSU-induced peritoneal inflammation in vivo, suggesting that DA is a restrictor for the development of inflammatory diseases, and DRD1 is a potential target for the treatment for NLRP3-driven inflammatory diseases.

EXPERIMENTAL PROCEDURES

Mice

Nlrp3$^{-/-}$, *Drd1*$^{-/-}$, and *Drd2*$^{-/-}$ mice were described previously (Drago et al., 1994; Kelly et al., 1997; Martinon et al., 2006). *Drd1*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice were crossed to generate *Drd1*$^{-/-}$*Nlrp3*$^{-/-}$ mice. All animal experiments were approved by the Ethics Committee of University of Science and Technology of China.

Cell Preparation and Stimulation

Bone-marrow macrophages were isolated and cultured as described (Yan et al., 2013). Astrocytes were prepared from the striatum of mice at P0, as described (Shao et al., 2013). Microglias were prepared from neonatal mice (age 1–3 days) as described previously (Saura et al., 2003).

For inducing inflammasome activation, 5 × 10^5 cells were plated in 12-well plate overnight and the medium were changed to opti-MEM in the following morning and then the cells were primed with ultra-LPS (500 ng/ml) for 3 hr. After that, the cells were stimulated as described (Yan et al., 2013).

Immunofluorescence

Confocal microscopy analyses were carried out using a Zeiss LSM700. Frozen sections were incubated with the primary antibody for tyrosine hydroxylase followed by incubation with secondary antibody conjugated with Alexa543. Sections were imaged using a cooled CCD (DP72, Olympus) on a microscope (BX53, Olympus).

siRNA-Mediated Gene Silences in BMDMs

BMDMs were plated in 12-well plates (4 × 10^5 cells per well) and then were transfected with 50 nM siRNA using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's guidelines.

MPTP-Induced Neuroinflammation In Vivo

Adult mice (12 weeks) were intraperitoneally given with 20 mg/kg MPTP five times at 2 hr intervals. After 24 hr, the serum samples were collected for ELISA and the brains were dissected and processed for immunohistochemistry or immunoblotting analysis.

LPS-Induced Systemic Inflammation In Vivo

To induce in vivo cytokine secretion, adult mice (12 weeks) were injected intraperitoneally with LPS (20 mg/kg) alone or LPS plus DA (50 mg/kg). After 4 hr, the serum samples were collected and the cytokines were measured by ELISA.

Statistical Analyses

All values are expressed as the mean ± SEM. Statistical analysis were performed with the t test for two groups or one-way ANOVA (GraphPad Software) for multiple groups. P values < 0.05 were considered significant.

See Extended Experimental Procedures for more information.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and seven figures and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2014.11.047.

AUTHOR CONTRIBUTIONS

Y.Y., W.J., L.L., X.W., and C.D. performed the experiments for this work. Y.Y., W.J., and R.Z. designed the research. Y.Y., W.J., C.D., Z.T., and R.Z. wrote the manuscript. R.Z. and Z.T. supervised the project.

ACKNOWLEDGMENTS

We thank Dr. Jurg Tschopp (University of Lausanne) for providing *Nlrp3*$^{-/-}$ mice, Dr. Jiawei Zhou (Shanghai Institutes for Biological Sciences) and Dr. Yuqiang Ding (Tongji University School of Medicine) for providing *Drd1*$^{-/-}$ and *Drd2*$^{-/-}$ mice. This work was supported by National Basic Research Program of China (2014CB910800, 2013CB944904), Natural Science Foundation of China (NSFC) (81330078, 81222040), Doctoral Fund of Ministry of Education of China (20123402120001, 20123402110010), One Hundred Person Project (R.Z.), and the Fundamental Research Funds for the Central Universities (R.Z. and W.J.).

Received: May 7, 2014
Revised: September 21, 2014
Accepted: November 18, 2014
Published: January 15, 2015

REFERENCES

Alfonso-Loeches, S., Ureña-Peralta, J.R., Morillo-Bargues, M.J., Oliver-De La Cruz, J., and Guerri, C. (2014). Role of mitochondria ROS generation in ethanol-induced NLRP3 inflammasome activation and cell death in astroglial cells. Front. Cell. Neurosci. *8*, 216.

Basu, S., and Dasgupta, P.S. (2000). Dopamine, a neurotransmitter, influences the immune system. J. Neuroimmunol. *102*, 113–124.

Basu, S., Nagy, J.A., Pal, S., Vasile, E., Eckelhoefer, I.A., Bliss, V.S., Manseau, E.J., Dasgupta, P.S., Dvorak, H.F., and Mukhopadhyay, D. (2001). The neurotransmitter dopamine inhibits angiogenesis induced by vascular permeability factor/vascular endothelial growth factor. Nat. Med. *7*, 569–574.

Bauernfeind, F.G., Horvath, G., Stutz, A., Alnemri, E.S., MacDonald, K., Speert, D., Fernandes-Alnemri, T., Wu, J., Monks, B.G., Fitzgerald, K.A., et al. (2009). Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J. Immunol. *183*, 787–791.

Bauernfeind, F., Ablasser, A., Bartok, E., Kim, S., Schmid-Burgk, J., Cavlar, T., and Hornung, V. (2011). Inflammasomes: current understanding and open questions. Cell. Mol. Life Sci. *68*, 765–783.

Beaulieu, J.M., and Gainetdinov, R.R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol. Rev. *63*, 182–217.

Beaulieu, J.M., Sotnikova, T.D., Yao, W.D., Kockeritz, L., Woodgett, J.R., Gainetdinov, R.R., and Caron, M.G. (2004). Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. Proc. Natl. Acad. Sci. USA *101*, 5099–5104.

Beck, G.Ch., Brinkkoetter, P., Hanusch, C., Schulte, J., van Ackern, K., van der Woude, F.J., and Yard, B.A. (2004). Clinical review: immunomodulatory effects of dopamine in general inflammation. Crit. Care *8*, 485–491.

Bergquist, J., Tarkowski, A., Ekman, R., and Ewing, A. (1994). Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. Proc. Natl. Acad. Sci. USA *91*, 12912–12916.

Brochard, V., Combadière, B., Prigent, A., Laouar, Y., Perrin, A., Beray-Berthat, V., Bonduelle, O., Alvarez-Fischer, D., Callebert, J., Launay, J.M., et al. (2009). Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J. Clin. Invest. *119*, 182–192.

Chakroborty, D., Chowdhury, U.R., Sarkar, C., Baral, R., Dasgupta, P.S., and Basu, S. (2008). Dopamine regulates endothelial progenitor cell mobilization from mouse bone marrow in tumor vascularization. J. Clin. Invest. *118*, 1380–1389.

Codolo, G., Plotegher, N., Pozzobon, T., Brucale, M., Tessari, I., Bubacco, L., and de Bernard, M. (2013). Triggering of inflammasome by aggregated α-synuclein, an inflammatory response in synucleinopathies. PLoS ONE *8*, e55375.

Davis, B.K., Wen, H., and Ting, J.P. (2011). The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu. Rev. Immunol. *29*, 707–735.

De Backer, D., Biston, P., Devriendt, J., Madl, C., Chochrad, D., Aldecoa, C., Brasseur, A., Defrance, P., Gottignies, P., and Vincent, J.L.; SOAP II Investigators (2010). Comparison of dopamine and norepinephrine in the treatment of shock. N. Engl. J. Med. *362*, 779–789.

Drago, J., Gerfen, C.R., Lachowicz, J.E., Steiner, H., Hollon, T.R., Love, P.E., Ooi, G.T., Grinberg, A., Lee, E.J., Huang, S.P., et al. (1994). Altered striatal function in a mutant mouse lacking D1A dopamine receptors. Proc. Natl. Acad. Sci. USA *91*, 12564–12568.

Franchi, L., Eigenbrod, T., and Núñez, G. (2009). Cutting edge: TNF-alpha mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation. J. Immunol. *183*, 792–796.

Ghosh, M.C., Mondal, A.C., Basu, S., Banerjee, S., Majumder, J., Bhattacharya, D., and Dasgupta, P.S. (2003). Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. Int. Immunopharmacol. *3*, 1019–1026.

Gloerich, M., and Bos, J.L. (2010). Epac: defining a new mechanism for cAMP action. Annu. Rev. Pharmacol. Toxicol. *50*, 355–375.

Guarda, G., Braun, M., Staehli, F., Tardivel, A., Mattmann, C., Förster, I., Farlik, M., Decker, T., Du Pasquier, R.A., Romero, P., and Tschopp, J. (2011). Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity *34*, 213–223.

Halle, A., Hornung, V., Petzold, G.C., Stewart, C.R., Monks, B.G., Reinheckel, T., Fitzgerald, K.A., Latz, E., Moore, K.J., and Golenbock, D.T. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat. Immunol. *9*, 857–865.

Josefsson, E., Bergquist, J., Ekman, R., and Tarkowski, A. (1996). Catecholamines are synthesized by mouse lymphocytes and regulate function of these cells by induction of apoptosis. Immunology *88*, 140–146.

Juliana, C., Fernandes-Alnemri, T., Kang, S., Farias, A., Qin, F., and Alnemri, E.S. (2012). Non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activation. J. Biol. Chem. *287*, 36617–36622.

Kelly, M.A., Rubinstein, M., Asa, S.L., Zhang, G., Saez, C., Bunzow, J.R., Allen, R.G., Hnasko, R., Ben-Jonathan, N., Grandy, D.K., and Low, M.J. (1997). Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. Neuron *19*, 103–113.

Lamkanfi, M., and Dixit, V.M. (2012). Inflammasomes and their roles in health and disease. Annu. Rev. Cell Dev. Biol. *28*, 137–161.

Lee, G.S., Subramanian, N., Kim, A.I., Aksentijevich, I., Goldbach-Mansky, R., Sacks, D.B., Germain, R.N., Kastner, D.L., and Chae, J.J. (2012). The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca2+ and cAMP. Nature *492*, 123–127.

Lucin, K.M., and Wyss-Coray, T. (2009). Immune activation in brain aging and neurodegeneration: too much or too little? Neuron *64*, 110–122.

Martinon, F., Pétrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature *440*, 237–241.

Martinon, F., Mayor, A., and Tschopp, J. (2009). The inflammasomes: guardians of the body. Annu. Rev. Immunol. *27*, 229–265.

McKenna, F., McLaughlin, P.J., Lewis, B.J., Sibbring, G.C., Cummerson, J.A., Bowen-Jones, D., and Moots, R.J. (2002). Dopamine receptor expression on

human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. J. Neuroimmunol. *132*, 34–40.

Meredith, E.J., Chamba, A., Holder, M.J., Barnes, N.M., and Gordon, J. (2005). Close encounters of the monoamine kind: immune cells betray their nervous disposition. Immunology *115*, 289–295.

Mishra, B.B., Rathinam, V.A., Martens, G.W., Martinot, A.J., Kornfeld, H., Fitzgerald, K.A., and Sassetti, C.M. (2013). Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1β. Nat. Immunol. *14*, 52–60.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., and Caron, M.G. (1998). Dopamine receptors: from structure to function. Physiol. Rev. *78*, 189–225.

Nishi, A., Kuroiwa, M., and Shuto, T. (2011). Mechanisms for the modulation of dopamine d(1) receptor signaling in striatal neurons. Front. Neuroanat. *5*, 43.

Olofsson, P.S., Rosas-Ballina, M., Levine, Y.A., and Tracey, K.J. (2012). Rethinking inflammation: neural circuits in the regulation of immunity. Immunol. Rev. *248*, 188–204.

Perry, V.H. (2012). Innate inflammation in Parkinson's disease. Cold Spring Harb. Perspect. Med. *2*, a009373.

Py, B.F., Kim, M.S., Vakifahmetoglu-Norberg, H., and Yuan, J. (2013). Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. Mol. Cell *49*, 331–338.

Ricci, A., Bronzetti, E., Mignini, F., Tayebati, S.K., Zaccheo, D., and Amenta, F. (1999). Dopamine D1-like receptor subtypes in human peripheral blood lymphocytes. J. Neuroimmunol. *96*, 234–240.

Rodrigues, M.C., Sanberg, P.R., Cruz, L.E., and Garbuzova-Davis, S. (2013). The innate and adaptive immunological aspects in neurodegenerative diseases. J. Neuroimmunol. *269*, 1–8.

Rouge-Pont, F., Usiello, A., Benoit-Marand, M., Gonon, F., Piazza, P.V., and Borrelli, E. (2002). Changes in extracellular dopamine induced by morphine and cocaine: crucial control by D2 receptors. J. Neurosci. *22*, 3293–3301.

Sarkar, C., Basu, B., Chakroborty, D., Dasgupta, P.S., and Basu, S. (2010). The immunoregulatory role of dopamine: an update. Brain Behav. Immun. *24*, 525–528.

Saura, J., Tusell, J.M., and Serratosa, J. (2003). High-yield isolation of murine microglia by mild trypsinization. Glia *44*, 183–189.

Schroder, K., and Tschopp, J. (2010). The inflammasomes. Cell *140*, 821–832.

Shao, W., Zhang, S.Z., Tang, M., Zhang, X.H., Zhou, Z., Yin, Y.Q., Zhou, Q.B., Huang, Y.Y., Liu, Y.J., Wawrousek, E., et al. (2013). Suppression of neuroinflammation by astrocytic dopamine D2 receptors via αB-crystallin. Nature *494*, 90–94.

Torres-Rosas, R., Yehia, G., Peña, G., Mishra, P., del Rocio Thompson-Bonilla, M., Moreno-Eutimio, M.A., Arriaga-Pizano, L.A., Isibasi, A., and Ulloa, L. (2014). Dopamine mediates vagal modulation of the immune system by electroacupuncture. Nat. Med. *20*, 291–295.

Tracey, K.J. (2009). Reflex control of immunity. Nat. Rev. Immunol. *9*, 418–428.

Wüllner, U., and Klockgether, T. (2003). Inflammation in Parkinson's disease. J. Neurol. *250* (*Suppl 1*), I35–I38.

Yan, Y., Jiang, W., Spinetti, T., Tardivel, A., Castillo, R., Bourquin, C., Guarda, G., Tian, Z., Tschopp, J., and Zhou, R. (2013). Omega-3 fatty acids prevent inflammation and metabolic disorder through inhibition of NLRP3 inflammasome activation. Immunity *38*, 1154–1163.

Youdim, M.B., Edmondson, D., and Tipton, K.F. (2006). The therapeutic potential of monoamine oxidase inhibitors. Nat. Rev. Neurosci. *7*, 295–309.