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Short communication

NLRP12 is a neutrophil-specific, negative regulator of *in vitro* cell migration but does not modulate LPS- or infection-induced NF-κB or ERK signalling

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ABSTRACT

NOD-like receptors (NLR) are a family of cytosolic pattern recognition receptors that include many key drivers of innate immune responses. NLRP12 is an emerging member of the NLR family that is closely related to the well-known inflammasome scaffold, NLRP3. Since its discovery, various functions have been proposed for NLRP12, including the positive regulation of dendritic cell (DC) and neutrophil migration and the inhibition of NF-κB and ERK signalling in DC and macrophages. We show here that NLRP12 is poorly expressed in murine macrophages and DC, but is strongly expressed in neutrophils. Using myeloid cells from WT and *Nlrp12*$^{-/-}$ mice, we show that, contrary to previous reports, NLRP12 does not suppress LPS- or infection-induced NF-κB or ERK activation in myeloid cells, and is not required for DC migration *in vitro*. Surprisingly, we found that *Nlrp12* deficiency caused increased rather than decreased neutrophil migration towards the chemokine CXCL1 and the neutrophil parasite *Leishmania major*, revealing NLRP12 as a negative regulator of directed neutrophil migration under these conditions.

1. Introduction

NOD-like receptors (NLR) are cytosolic proteins that recognise a variety of danger signals and trigger diverse defensive mechanisms. Some NLRs (*e.g.* NOD1 and NOD2) induce proinflammatory gene expression by initiating NF-κB and MAPK signalling pathways. Other NLRs (*e.g.* NLRP1, NLRP3, and NLRC4) initiate the assembly of large cytosolic signalling platforms called inflammasomes, which drive caspase-1 activation and the maturation and secretion of key proinflammatory cytokines, IL-1β and IL-18. Recently, some NLRs (*e.g.* NLRC3, NLRC5 and NLRX1) were proposed to function as inflammation suppressors by inhibiting proinflammatory signalling pathways (Allen, 2014). Such studies have contributed to a developing paradigm that a third key function of the NLR family is to suppress inflammatory responses.

NLRP12 is closely related to the well-characterised inflammasome scaffold, NLRP3 (Schroder and Tschopp, 2010), but its molecular function remains a matter of debate. Several studies suggest that NLRP12 forms an inflammasome or regulates inflammasome function. Mutations in NLRP12 are associated with a genetic disease, familial cold autoinflammatory syndrome 2 (FCAS2), which closely resembles FCAS1 caused by gain-of-function NLRP3 mutations, and is also alleviated by anti-IL-1 receptor therapy (Borghini et al., 2011; Jéru et al., 2008). Further, NLRP12 was reported to contribute to IL-1β and IL-18 production in response to *Yersinia pestis* (Vladimer et al., 2012), and IL-1β production during malaria-associated sepsis (Ataide et al., 2014). Several reports implicate NLRP12 in the suppression of NF-κB and ERK signalling pathways in murine macrophages, dendritic cells (DCs), or human THP-1 monocytic cells (Allen et al., 2012; Lich et al., 2007; Williams et al., 2005; Zaki et al., 2013, 2011). Other reports suggest that NLRP12 drives cell migration in murine neutrophils and DC

Abbreviations: BMDC, bone marrow-derived dendritic cells; BMDM, bone marrow-derived macrophages; BMN, bone marrow neutrophils; DC, dendritic cells; NLR, NOD-like receptor; PEC, peritoneal elicited cells.

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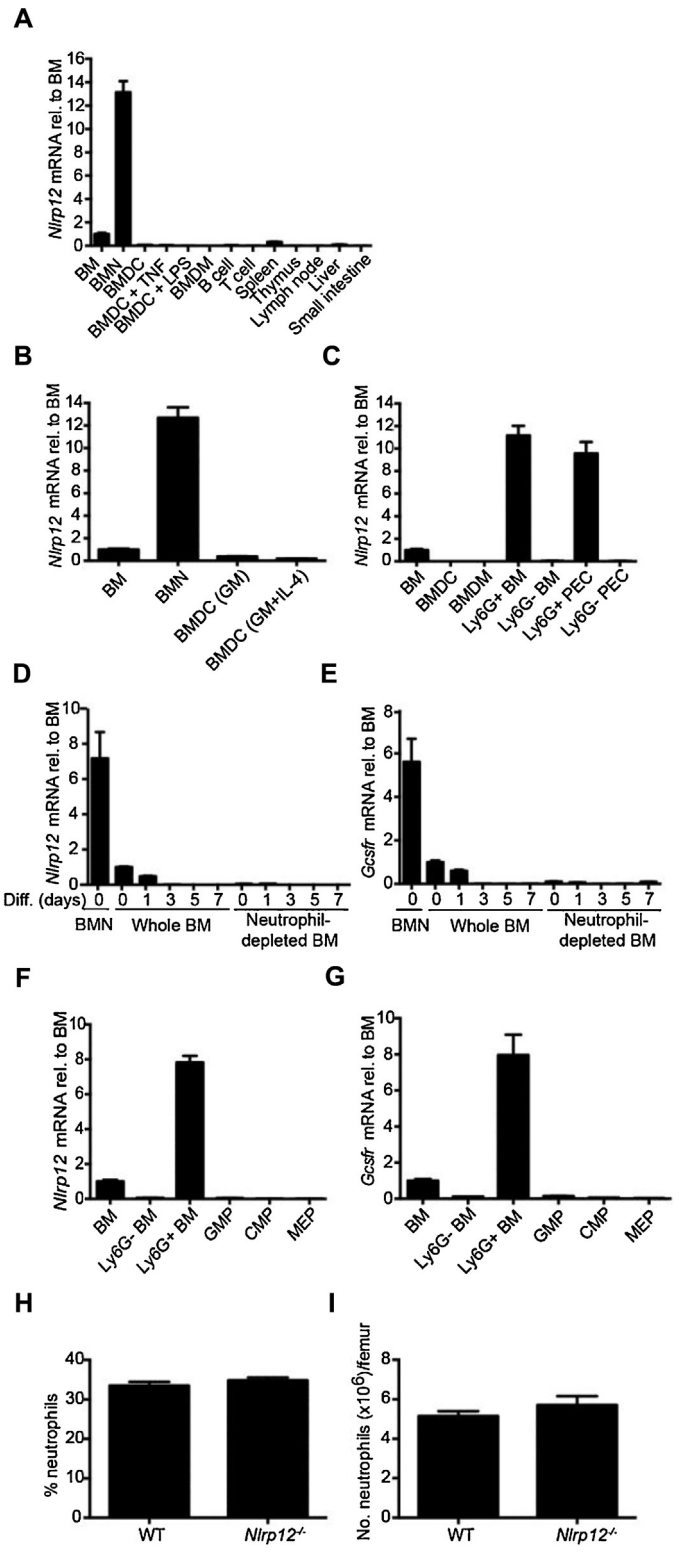

Fig. 1. NLRP12 is a granulocyte-restricted NLR.
Nlrp12 mRNA expression was determined by qPCR in C57BL/6 WT mice. (A) Mouse immune cell subsets and tissues. BM, bone marrow; BMN, bone marrow neutrophils; BMDC, bone marrow dendritic cells, untreated or treated overnight with TNF (25 ng/mL) or LPS (50 ng/mL); BMDM, bone marrow-derived macrophages; B cells, $B220^+$ splenocytes; T cells, $CD3^+$ splenocytes. (B) BMDC differentiated using GM-CSF or GM-CSF + IL-4. (C) Purified resting neutrophils (BMN) and inflammatory (peritoneal elicited) neutrophils. $Ly6G^+$ neutrophils and $Ly6G^-$ cells were prepared in parallel from the same pool of BM. Peritoneal elicited cells (PEC) were harvested 4 h after thioglycollate challenge. (D) *Nlrp12* and (E) *Gcsfr* expression in CSF-1-differentiated BM cultures with and without prior neutrophil depletion. (F) *Nlrp12* and (G) *Gcsfr* expression in mature cells and BM progenitors (GMP, granulocyte monocyte progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte erythroid progenitor). All data are mean + SD and are representative of 2–3 independent experiments. (H) Percentage and (I) absolute number of neutrophils in BM WT and $Nlrp12^{-/-}$ mice. Neutrophil percentage is expressed relative to total femur white blood cells. Data are mean + SEM of 3–4 mice.

(Arthur et al., 2010), or MHC class I expression in U937 monocytic cells (Williams et al., 2003). The published functions for NLRP12 in macrophages and DCs are in conflict with other studies indicating that NLRP12 is not expressed in these cell types (Chen et al., 2014; Lord et al., 2009). Here, we aimed to resolve the controversies regarding the expression distribution and function of murine NLRP12. We found that *Nlrp12* is predominantly expressed by neutrophils, and *Nlrp12* deficiency did not affect NF-κB and ERK signalling pathways induced by LPS stimulation, or infection with *Salmonella typhimurium* or *Leishmania major*. In contrast with earlier studies, we found that NLRP12 was not required for, and actually supressed, *in vitro* neutrophil migration towards the chemokine CXCL1 and the parasite *L. major*.

2. Results and discussion

2.1. Murine NLRP12 is predominantly expressed in neutrophils

Previous studies have attributed NLRP12 expression and function to various immune cell subsets. In humans, *NLRP12* expression was reported to be restricted to myeloid cells, including polymorphonuclear cells, monocytes, and DCs (Wang et al., 2002; Williams et al., 2003). Our own studies confirmed high *NLRP12* expression in human polymorphonuclear cells and purified neutrophils, and moderate expression in $CD14^+$ primary monocytes, but we observed only weak expression in monocyte-derived DCs (Chen et al., 2014). Murine *Nlrp12* expression was reported in bone marrow (BM), spleen, neutrophils, and bone marrow-derived DC (BMDC) in one study (Arthur et al., 2010), and in granulocytes, T cells, spleen, liver, small intestine, and mesenteric lymph nodes in another (Zaki et al., 2011). A recent study showed *Nlrp12* expression in bone marrow-derived macrophages (BMDM), neutrophils, lung, and spleen (Vladimer et al., 2012). In order to clarify the expression distribution of *Nlrp12*, we performed a qPCR screen of a wide range of murine cells and tissues and revealed strong expression in neutrophils, with lower expression in BM, spleen, and liver. *Nlrp12* mRNA was barely detectable in BMDM, BMDC, T cells, B cells, thymus, lymph nodes, and small intestine (Fig. 1A). As BMDC were previously reported to express *Nlrp12* (Williams et al., 2003), we tested various DC differentiation conditions, but *Nlrp12* expression remained weak, regardless of the presence or absence of IL-4 during GM-CSF-dependent differentiation (Fig. 1B), or cell maturation with TNF or LPS (Fig. 1A). *Nlrp12* was highly expressed in both resting (bone marrow) and inflammatory (elicited) neutrophils, as compared to BMDC or BMDM (Fig. 1C). Notably, *Nlrp12* was expressed in whole BM, but not in BM depleted of neutrophils ($Ly6G^-$ BM), suggesting that *Nlrp12* expression in BM is attributable to neutrophils (Fig. 1C). A recent study suggested that *Nlrp12* expression decreased during differentiation of BM progenitors towards mature BMDM (Vladimer et al., 2012). Our data showing weak expression of *Nlrp12* in neutrophil-depleted BM instead suggested that *Nlrp12* expression declined in differentiating BM cultures due to the gradual loss of neutrophils as they die by apoptosis. To test this hypothesis, we CSF-1-differentiated neutrophil-depleted and neutrophil-intact (whole) BM cultures over a time course, and quantified mRNA expression of *Nlrp12* and the neutrophil marker *Gcsfr*. Indeed, *Nlrp12* expression in the whole BM declined over time (Fig. 1D), concurrent with the