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IL-1R signaling in dendritic cells replaces pattern recognition receptors to promote CD8+ T cell responses to influenza A virus

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Abstract

Immune responses to vaccines require direct recognition of pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) on dendritic cells (DCs). Unlike vaccines, infection by a live pathogen often impairs DC function and inflicts additional damage to the host. Here, we found that following live influenza A infection, signaling through the interleukin-1 receptor (IL-1R), but not the PRRs, TLR7 and RIG-I, is required for productive $CD8^+$ T cell priming. DCs activated by IL-1 *in trans* were both required and sufficient for the generation of virus-specific $CD8^+$ T cell immunity. Our data reveal a critical role of a bystander cytokine in $CD8^+$ T cell priming during a live viral infection.

Keywords

inflammasome; respiratory mucosa; migration; viral immunity; monocytes

INTRODUCTION

The initiation of protective immune responses to infectious microorganisms is thought to rely on recognition by innate microbial sensors[1]. Upon infection, influenza A virus (IAV) is detected by three classes of innate sensors, namely, members of the Toll-like receptors (TLRs), RIG-I like receptors (RLRs) and the NOD-like receptors (NLRs)[2]. Viral genomic single-stranded RNA is recognized by TLR7 (Ref [3, 4]) and TLR8 (Ref [5]) in the endosome, and RIG-I in the cytosol[6, 7]. Signaling through both RIG-I and TLR7 leads to the production of type I interferon (IFN), which limit viral replication and increase innate resistance to infection[8]. In contrast to TLRs and RLRs that recognize viral PAMPs, NLRP3 senses cellular damage or distress as a consequence of infection[9]. The infected host cell recognizes IAV through NLRP3 based on the activity of the M2 ion channel[10]. Once activated, NLRP3

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AUTHORS CONTRIBUTION

Experiments were conceived and designed by I.K.P, T.I. and A.I. Experiments were performed by I.K.P. and T.I. Data were analyzed by I.K.P., T.I. and A.I. The paper was written by I.K.P. and A.I.

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engages the inflammasome complex involving ASC and caspase-1, resulting in the activation of caspase-1 and the cleavage of its substrates including the pro-inflammatory cytokines, interleukin 1 (IL-1) β and IL-18 (Ref [11]).

The relevance of host recognition of viral PAMPs versus viral-inflicted damage in linking innate recognition of IAV to adaptive immunity has not been systematically explored. Neither the absence of TLR3 (Ref [12]) nor of the RIG-I signaling adaptor MAVS[8] diminishes adaptive immunity to IAV. In addition, *Tlr7*$^{-/-}$ mice are able to mount an intact CD8$^+$ T cell response, despite having variable defects in CD4$^+$ T cell responses and immunoglobulin production to IAV[8, 13]. In contrast, the importance of NLRP dependent activation of inflammasomes in innate[14, 15] and adaptive[16] immune defense against IAV has been demonstrated. The requirement for the inflammasomes to generate protective adaptive immunity to IAV was attributed to the production of IL-1α and IL-1β, as mice deficient for the IL-1 receptor type I (IL-1R)[16–18] suffer from a similar lack of immune responses as ASC- and caspase-1-deficient mice[16]. IL-1R-deficient mice have defective CD4 T cell and antibody responses to influenza infection[16, 17], and have impaired CD8 T cell responses at seven[17] and fourteen[16] days post infection. Although these data collectively implicate the importance of the inflammasome-IL-1R axis in CD8 T cell priming to IAV, it remains possible that TLR7 and RIG-I induce redundant signaling pathways that compensate for each other in single deficient mice.

With respect to the importance of direct detection of PAMPs by antigen-presenting dendritic cells (DCs) in priming T cell responses, previous studies have revealed a requirement for DC-intrinsic recognition of PAMP through TLR in initiating CD4$^+$ (Ref [19]) and CD8$^+$ (Ref [20]) T cell responses following immunization with a model antigen plus adjuvant (TLR ligand). Under these conditions, activation of bystander DCs by inflammatory cytokines alone is not sufficient to render them capable of stimulating productive T cell immune responses. However, whether similar principles of T cell priming apply during infection with various microbes remains unclear. During live infections, directly infected DCs are often rendered incapable of T cell priming[21]. Under such circumstances, bystander DC activation might be required for efficient T cell priming.

Here, we examined the relative contributions of pattern recognition *versus* inflammasome-dependent damage recognition in the initiation of adaptive CD8 T cell responses after respiratory infection with influenza virus. Our data provide evidence that CD8 T cell priming does not depend on PRRs, but critically depends on NLR-inflammasome-induced release of IL-1α and IL-1β and on IL-1R signaling in bystander DCs. Thus, our data suggest that upon pathogenic virus infection leading to lysis and/or impairment in DC function, IL-1R signaling, like TLR signaling, is capable of providing the innate signal necessary for the initiation of adaptive immune responses.

RESULTS

IL-1R is required for influenza virus-specific CD8$^+$ T cell responses

Because mice deficient in TLR7 or MAVS alone have intact CD8$^+$ T cell immune responses to IAV[8, 13], we tested whether TLR7 and RIG-I share any functional redundancy in

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providing the requisite signals for the activation of adaptive immune responses. Mice lacking both TLR7 and MAVS (*Tlr7*$^{-/-}$ × *Mavs*$^{-/-}$) were challenged intranasally with a sublethal dose (10 PFU per mouse, 0.4 LD_{50}) of mouse-adapted influenza strain A/PR8 (H1N1). This dose of A/PR8 was shown to be optimal in revealing the importance of innate pathways involved in adaptive immunity-dependent clearance of IAV[16] without inducing mortality. Compared to wild-type and *Mavs*$^{-/-}$ mice, *Tlr7*$^{-/-}$ and *Tlr7*$^{-/-}$ *Mavs*$^{-/-}$ mice mounted a substantially reduced inflammatory response, including reduced cellular recruitment into the airway and decreased production of the proinflammatory cytokine IL-6 at day 4 post infection (Supplementary Fig. 1a, b). The inflammatory response was the most diminished in *Tlr7*$^{-/-}$ *Mavs*$^{-/-}$ mice, followed by *Tlr7*$^{-/-}$ mice. These data indicate that the innate immune response against respiratory IAV is largely TLR7-dependent.

To examine the impact of TLR7 and MAVS deficiency on the development of adaptive immunity to IAV, we assessed the frequency and number of virus-specific CD8$^+$ T cells in the lung. We detected no difference in the frequency (Fig. 1a) or the number (Fig. 1b) of MHCI tetramer$^+$ CD8$^+$ T cells in the lung at day 9 post infection. In contrast, *Tlr7*$^{-/-}$ *Mavs*$^{-/-}$ mice had significantly reduced number of IFN-γ$^+$ CD4$^+$ T cells in the lung 9 days after IAV infection (Fig. 1c). These data indicate that while both the TLR7- and RIG-I-dependent recognition pathways contribute to the development of virus-specific IFN-γ-producing CD4 T cells, CD8 T cell responses develop in the absence of both of these PRRs.

Protective immunity against influenza virus is known to require the ASC- and caspase-1-dependent inflammasome activation and IL-1R[16]. Compared to wild-type mice, *Il1r1*$^{-/-}$ mice mounted a substantially diminished response to a sublethal dose of IAV, in terms of frequency (Fig. 1a) and number (Fig. 1b) of virus-specific CD8$^+$ T cell in the lung and in the bronchoalveolar lavage (BAL) fluid (Fig. 1d) 9 days after infection. These results indicated a dominant role of IL-1R over TLR7 and RIG-I in the generation of CD8$^+$ T cell responses, as previously reported for a recombinant influenza A virus[16].

We next examined the proliferative and functional capacities of influenza-specific CD8$^+$ T cells in *Il1r1*$^{-/-}$ mice. Eight days after a sublethal dose of PR8 infection the number of proliferating (Ki67$^+$) virus-specific CD8$^+$ T cells was substantially lower in the lung and the draining mediastinal lymph node (mLN) of *Il1r1*$^{-/-}$ mice (Fig. 1e). Compared to wild-type mice, *Il1r1*$^{-/-}$ mice also showed decreased number of IFN-γ-producing virus-specific CD8 T cells in the lung and the spleen (Fig. 1f). Further, the number of polyfunctional CD8 T cells capable of secreting IFN-γ and TNFα and of undergoing degranulation (CD107a$^+$) was significantly lower in the mLN of *Il1r1*$^{-/-}$ compared to wild-type mice (Fig. 1g). These results indicate that IL-1R signaling is required for CD8 T cell activation, expansion and acquisition of effector functions following influenza virus infection.

IL-1R signaling requirement is intrinsic to hematopoietic lineage

IL-1R is widely expressed by many cell types belonging to the hematopoieitic and non-hematopoieitic compartments[22]. To investigate the cells responsible for supporting the IL-1R-dependent priming of CD8$^+$ T cells during IAV infection, we generated bone-marrow (BM) chimeric mice in which IL-1R expression was confined either to the hematopoietic (wild-type BM into *Il1r1*$^{-/-}$ hosts) or the stromal (*Il1r1*$^{-/-}$ BM into wild-type hosts)

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compartment. Following complete bone-marrow reconstitution, chimeric mice were challenged with a sublethal dose of PR8 influenza virus. Compared to wild-type into wild-type control mice, *Il1r1*$^{-/-}$ into wild-type mice showed reduced frequency and number of virus-specific CD8 T cells in the lung and the spleen 9 days p.i., similar to *Il1r1*$^{-/-}$ into *Il1r1*$^{-/-}$ chimeras (Fig. 2a, b). In addition, IFN-γ secretion from CD8 T cells upon restimulation with influenza NP peptide was impaired in *Il1r1*$^{-/-}$ into wild-type mice (Fig. 2c) to the same extent as in *Il1r1*$^{-/-}$ into *Il1r1*$^{-/-}$ chimeras. In contrast, CD8 T cell responses in the wild-type into *Il1r1*$^{-/-}$ mice were comparable to the wild-type into wild-type controls, both in terms of number of tetramer^{+} cells and IFN-γ secretion, indicating that IL-1 responsiveness within the radio-resistant cells is not required for CD8 T cell priming. Furthermore, *Il1r1*$^{-/-}$ into wild-type, but not wild-type into *Il1r1*$^{-/-}$ mice had a higher viral titer in the BAL fluid at 9 days p.i. (Fig. 2d). These data indicate that IL-1R signaling in the hematopoietic, but not the radio-resistant compartment, is required for the optimal generation of virus-specific CD8 T cells.

IL-1R signaling in CD8^{+} T cells is not required for their activation

We next examined the requirement for IL-1R signaling in CD8 T cells to support their development and expansion during flu infection. To measure epitope-specific CD8 T cell responses following infection, we used a recombinant A/PR8 influenza virus that expresses the LCMV GP-33–41 epitope (A/PR8/GP-33) and P14 T cell receptor-transgenic (P14 TCR Tg) CD8 T cells, which recognize the GP-33–41 epitope[23]. IL-1R-deficient antigen-specific CD8 T cells were produced by crossing P14 TCR transgenic mice with *Il-1r1*$^{-/-}$ mice. Equal numbers of allelically marked wild-type (Thy1.1^{+} CD45.2^{+}) and *Il1r1*$^{-/-}$ (Thy1.1^{-} CD45.2^{+}) P14 CD8^{+} T cells were co-transferred into congenic wild-type CD45.2^{-} C57BL/6 mice (Supplementary Fig. 2a) that were infected intranasally with A/PR8/GP-33 influenza virus. Ten days after infection, wild-type and *Il1r1*$^{-/-}$ lung P14 Tg CD8^{+} T cells produced comparable levels of IFN-γ upon re-stimulation with the LCMV GP-33–41 peptide (Supplementary Fig. 2b). In addition, similar numbers of wild-type and *Il1r1*$^{-/-}$ P14 Tg CD8^{+} T cells were found in the lung, mLN and the spleen of the infected hosts (Supplementary Fig. 2b, c). These data indicate that the development of epitope-specific CTL response following influenza virus infection occurs independently of IL-1R expressed by CD8 T cells, and implicate a non-CD8 T cell hematopoietic population in the IL-1R-dependent CTL priming.

Impaired CD8^{+} T cells priming by *Il1r1*$^{-/-}$ DCs during influenza infection

We next investigated the importance of IL-1 in supporting the ability of DCs to prime CD8 T cells following influenza virus infection. The priming and expansion of influenza virus-specific CD8 T cells depend on the CCR7-mediated migration of tissue DCs from the lung to the draining mLN and on antigen presentation via peptide-MHCI on DCs[2425, 26]. To maximize the antigen load, we challenged wild-type and *Il1r1*$^{-/-}$ mice with a higher dose of A/PR8-GP33 (1,000 PFU). Three days p.i., CD11c^{+} DCs isolated from the mLN were used to stimulate naïve P14 Tg CD8 T cells *ex vivo*. Compared to DCs isolated from A/PR8-GP33-infected wild-type mice, DCs isolated from the mLN of infected *Il1r1*$^{-/-}$ mice had a reduced capacity to stimulate naïve P14 Tg CD8 T cells to secrete IFN-γ (Fig. 3a). This was not due to a general defect in antigen presentation by the *Il1r1*$^{-/-}$ DCs, because DCs isolated

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from infected wild-type or *Il1r1*$^{-/-}$ mice were able to activate naïve P14 CD8 T cells when exogenous GP33–41 peptide was added to the culture (Fig. 3b). These results indicate that in the absence of IL-1R, fewer DCs are present in the draining lymph nodes to activate naïve CD8 T cell following influenza virus infection.

IL-1R controls CCR7 expression and migration of DCs to the lymph node

Next, we examined whether IL-1R signaling is critical for pulmonary DC homeostasis. We found that lung CD11c^{+} MHCII^{+} DCs from *Il1r1*$^{-/-}$ mice had a less mature phenotype at steady-state compared to wild-type mice, with reduced surface expression of the co-stimulatory molecule CD86 and the chemokine receptor CCR7 (Supplementary Fig. 3a). Although the total number of DCs in the lung and mLN of wild-type and *Il1r1*$^{-/-}$ mice remained similar, we observed reduced numbers of CD103^{+} CD11b^{lo}, but not CD11b^{+} CD103^{-} DCs in the lung and the mLN of *Il1r1*$^{-/-}$ mice at steady state (Supplementary Fig. 3b). These data indicate that signaling via IL-1R is particularly important in maintaining the CD103^{+} DCs numbers in the respiratory tract.

During influenza virus infection, trafficking of respiratory-tract DCs to the draining mLN, a process that is essential for the generation of T cell immunity, occurs in a CCR7-dependent manner[24]. CD103^{+} CD11b^{lo} DCs originating from the lung are critical in presenting influenza virus antigens to naïve CD8 T cells in the mLN, with the peak of this process occurring 48–72 h post infection[26, 27]. In addition, CD11b^{hi} CD103^{-} DCs are continuously recruited to the mLN (with peak migration on day 5 p.i.) and cross-present to CD8 T cells in a CD70-dependent manner following influenza virus infection[28]. Because IL-1R-deficient mice have reduced expression of CCR7 and CD86 at steady-state and contain fewer antigen presenting DCs in the mLN following IAV infection, we investigated the importance of IL-1R in respiratory DC migration and activation. CD103^{+} CD11b^{lo} lung DCs in *Il1r1*$^{-/-}$ mice failed to upregulate the expression of CCR7 at 18 hours p.i. (Fig. 4a), leading to reduced frequency and number of CCR7^{+} CD103^{+} CD11b^{lo} DCs in the lung and mLN (Supplementary Fig 4). These data suggest that antigen-bearing CD103^{+} CD11b^{lo} DCs are less capable of migrating to the draining mLN during the first few days of infection. CD103^{-} CD11b^{hi} DCs displayed comparable induction of CCR7 expression in wild-type and *Il1r1*$^{-/-}$ mice at 18 hours p.i. However, these cells do not reach the mLN in significant numbers until later time points[28].

Three to 5 days p.i., the lungs of both influenza-infected wild-type and *Il1r1*$^{-/-}$ mice were dominated by the inflammatory monocyte-derived CD11b^{hi} CD103^{-} DC population[29], with minimal presence of CD103^{+} CD11b^{lo} DCs, consistent with previous reports[28] (Fig. 4a and Supplementary Fig 4a). However, compared to wild-type mice, the newly recruited DCs in the lung of *Il1r1*$^{-/-}$ mice showed reduced viability (Supplementary Fig. 3c) and substantially lower surface expressions of CCR7 and CD86 (Supplementary Fig. 3a) 3 days after infection. Thus, these data indicate that IL-1R signaling is required to promote survival of lung DCs after infection, upregulate the expression of CD86 on DCs, and induce the expression of CCR7 on lung-resident CD103^{+} CD11b^{lo} DCs but not CD11b^{hi} CD103^{-} DC, and recruited monocyte-derived CD103^{-} CD11b^{hi} DCs following influenza infection.

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To directly examine the importance of IL-1R in lung DC migration to the mLN, mice were inoculated intranasally with fluorescent-latex beads together with live influenza virus. Beads mixed with LPS were used as a positive control[30]. Reduced frequency of latex bead-bearing (Lx^+) lung DCs was detected in the draining mLN of flu-infected *Il1r1*$^{-/-}$ mice compared to wild-type mice (Fig. 4b). Decreased frequency of Lx^+ DCs in *Il1r1*$^{-/-}$ mice was not due to impaired uptake of latex beads, as the intrinsic ability of *Il1r1*$^{-/-}$ BMDCs to take up fluorescent latex beads *in vitro* was comparable to that of wild-type BMDCs (Supplementary Fig. 3d), and the frequencies of Lx^+ DCs and Lx^+ alveolar macrophages in the BAL remained similar between wild-type and *Il1r1*$^{-/-}$ mice after intranasal bead administration (Supplementary Fig. 3e). Of note, *Il1r1*$^{-/-}$ mice also had reduced frequency of migratory DCs reaching the mLN upon LPS^+ and poly(I:C)$^+$, but not CpG^+ bead injections (Fig. 4b and Supplementary Fig. 5a), indicating that the importance of IL-1R in DC migration extends to other stimuli including TLR3 and MDA5 (poly(I:C)) and TLR4 (LPS), but not TLR9 (CpG) activation. To test whether the requirement for IL-1R in CD8 T cell priming is restricted to influenza virus infections, we infected wild-type and *Il1r1*$^{-/-}$ mice intranasally with herpes simplex virus (TK^- HSV2). DCs isolated from the mLN of HSV2-infected *Il1r1*$^{-/-}$ mice had a reduced capacity to induce IFN-γ secretion from naïve gBT-I $CD8^+$ T cells, which are specific for the HSV glycoprotein B (Supplementary Fig. 5b). These data demonstrate that IL-1R promotes respiratory DC migration and the activation of antigen-specific T cells beyond influenza virus infection.

We next asked whether IL-1β is sufficient to promote DC migration from the lung to the mLN. Intranasal administration of recombinant mouse IL-1β significantly induced the surface expression of CD86 and CCR7 and mobilized $MHCII^{hi}$ $CD11c^+$ lung migratory DCs[28] to the mLN of wild-type, but not *Il1r1*$^{-/-}$ mice (Fig. 4c). In addition, intranasal injection of recombinant IL-1α was sufficient to upregulate surface expressions of CD86 and CCR7 on migratory $CD103^+$ $CD11b^{lo}$ DCs in the lung and mLN (Supplementary Fig. 6b). Unlike lung $CD11b^{hi}$ DCs, the migratory $CD103^+$ $CD11b^{lo}$ DCs were particularly responsive to both IL-1β– and IL-1α–induced activation (Supplementary Fig. 6). Collectively, these results indicate that signaling through IL-1R is both required and sufficient for the migration and activation of $CD103^+$ DCs in the lung.

IL-1R-MyD88 signaling is sufficient to promote respiratory DC migration

Next, we examined whether IL-1R-MyD88 signaling in DCs is sufficient to drive their activation and migration to the mLN during influenza virus infection. We used transgenic mice expressing MyD88 under the control of the CD11c promoter crossed to *Myd88*$^{-/-}$ mice (hereafter called CD11c–MyD88-transgenic)[31]. In these mice, MyD88 is only expressed in $CD11c^+$ DCs and some macrophages (including $CD11c^+$ alveolar macrophages), but not in B cells or T cells (Pasare, C., personal communications). Lung DCs in CD11c–MyD88-transgenic mice migrated equally efficiently from the lung to the draining mLN and displayed similar levels of CCR7 expression as wild-type lung DCs, despite slightly lower expression of CD86 at 2.5 days after infection (Fig. 5a and Supplementary Fig. 5d). Furthermore, unlike *MyD88*$^{-/-}$ mice, CD11c–MyD88-transgenic mice generated an effective virus-specific CD8 T cell response in the lung 7 days after sublethal PR8 infection (Fig. 5b, c). These results indicate that MyD88-dependent signaling in DCs alone is

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sufficient to induce DC migration from the lung to the lymph node to promote CD8 T cell responses after flu infection.

Next, we tested whether intranasal delivery of wild-type DCs could restore the defective CD8 T cell response following influenza virus infection in IL-1R-deficient mice. We intranasally injected wild-type or IL-1R-deficient BMDCs together with live PR8-GP-33 influenza virus into wild-type or *Il1r1*$^{-/-}$ mice previously adoptively transferred with naïve P14 Tg CD8^{+} T cells. By day 7 p.i., P14 Tg CD8^{+} T cells in wild-type but not *Il1r1*$^{-/-}$ mice were readily detectable in the draining mLN. Notably, intranasal injection of wild-type, but not *Il1r1*$^{-/-}$ BMDCs fully restored P14 expansion in *Il1r1*$^{-/-}$ mice (Fig. 6). These data show that wild-type DCs can restore CD8^{+} T cell priming in the mLN of IL-1R-deficient hosts, suggesting that IL-1 responsiveness by the migrant DCs is sufficient for CD8^{+} T cell priming following influenza infection.

Direct DC activation by caspase-1 is not required for CD8^{+} T cell priming

Studies using protein plus adjuvant have shown that bystander DCs activated by inflammatory signals cannot substitute for PAMP-exposed DCs directly activated by PRR signaling for successful priming of CD4 (Ref [19]) and CD8 T cells[20]. Thus far, our data indicated that priming of CD8 T cells does not depend on PRR signaling, but rely on the inflammasome-IL-1R axis. We next tested whether direct DC activation via NLR-inflammasomes is required to couple damage recognition to the activation of adaptive CD8 T cell response. To this end, we generated mixed bone marrow chimeric mouse model based on a previously established strategy[20] in which half the APCs cannot directly activate caspase-1, but can present to CD8 T cells, whereas the other half can activate caspase-1 for the processing and secretion of inflammasome-dependent cytokines but cannot present to CD8 T cells following IAV infection (Supplementary Fig. 7a). In these chimeric mice (*Casp1*$^{+/+}$ *H-2D*$^{b-/-}$ + *Casp1*$^{-/-}$ *H-2D*$^{b+/+}$ → wild-type B6 mice; "*Casp1*$^{-/-}$"), H2Db-restricted virus-specific CD8 T cells are exclusively primed by caspase-deficient DCs (*Casp1*$^{-/-}$ *H-2D*$^{b+/+}$). Nevertheless, these antigen-presenting DCs are exposed *in trans* to IL-1α and IL-1β secreted by the caspase-1-expressing, non-presenting DCs (*Casp1*$^{+/+}$ *H-2D*$^{b-/-}$). As controls, mixed chimeras in which all cells can activate caspase-1 were generated (*Casp1*$^{+/+}$ *H-2D*$^{b-/-}$ + *Casp1*$^{+/+}$ *H-2D*$^{b+/+}$ → wild-type B6; "wild-type"). Eight days following intranasal A/PR8 influenza infection (10 PFU per mouse), the numbers of H-2D^{b}-restricted NP (366–374) and PA (244–233) CD8 T cells were comparable between wild-type and *Casp1*$^{-/-}$ chimeras (Fig. 7a, b). These data indicated that direct recognition of IAV via NLR inflammasome by DCs is not required for viral antigen processing and presentation on MHCI.

To test the possibility that IL-1 responsiveness in the antigen-presenting DCs is required for the priming of H-2D^{b}-restricted virus-specific CD8 T cells, we generated chimeric mice in which half the APCs can signal through IL-1R but cannot present to H-2D^{b}-restricted CD8 T cells, whereas the other half lack IL-1R but can present to CD8 T cells (*Il1r1*$^{+/+}$ *H-2D*$^{b-/-}$ + *Il1r1*$^{-/-}$ *H-2D*$^{b+/+}$ → wild-type B6 mice; "*Il1r1*$^{-/-}$"). H-2D^{b}-restricted IAV-specific CD8 T cell responses in this these mice eight days post A/PR8 infection were significantly reduced compared to the wild-type chimeras. Of note, despite the diminished H-2D^{b}-

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restricted virus-specific CD8 T cell responses in the lung of *Il1r1*$^{-/-}$ chimeras, we observed similar viral titers in the airway of wild-type, *Casp1*$^{-/-}$ and *Il1r1*$^{-/-}$ chimeras (Supplementary Fig. 7b), indicating that non-CD8-T cell dependent antiviral mechanism compensates for the impaired CD8 T cell responses in these mice. Together, these data demonstrated that cell-intrinsic IL-1 responsiveness by DCs, but not innate recognition of IAV through NLR inflammasomes, is required for activating virus-specific CD8 T cell responses after IAV infection.

DISCUSSION

Influenza virus infection is recognized by two classes of innate recognition receptors, by those that rely on detection of viral PAMPs (TLR7, TLR8 and RIG-I), and those that monitor virus-inflicted damage (NLRPs). In this study, we examined the relative contributions of these classes of innate viral sensors in the development of adaptive CD8 T cell response following IAV infection. After a physiological dose of infection with IAV *in vivo*, mice that lack both TLR7 and MAVS were able to mount a robust virus-specific CD8 T cell response against IAV. In contrast, ASC-dependent inflammasomes[16] and IL-1R were required for the optimum virus-specific CD8 T cell response to IAV. Expression of IL-1R in hematopoietic cells, but not in CD8 T cells themselves, was found to be essential for CTL activation. IL-1R signaling was critical in regulating both constitutive and infection-induced activation of lung-resident DCs. Furthermore, MyD88 signaling in DCs alone was sufficient for DC migration from the lung to the mLN and CD8 T cell priming following influenza infection. Likewise, intranasal administration of wild-type DCs was sufficient to restore CD8 T cell priming in IL-1R-deficient mice after influenza virus infection. Moreover, our data indicated that direct stimulation of APCs through IL-1R, but not caspase-1 activation, was required for antigen presentation to CD8 T cells following IAV infection. These data indicate that an inflammatory cytokine, but not PAMP recognition, is both required and sufficient for conveying the signals necessary for the generation of virus-specific CD8 T cells after IAV infection.

These findings reveal that following a live viral infection, the rules that govern DC activation for CD8 T cell priming differ dramatically from those that apply following immunization with a non-live vaccine. Elegant work has demonstrated that direct recognition of PAMPs by antigen presenting DCs is required for productive activation of CD4 (Ref [19]) and CD8 (Ref [20]) T cells following vaccination with protein antigen plus adjuvant. The requirement for direct PAMP recognition even extends to the phagosome within which the microbial antigens are processed for MHCII presentation[32]. In contrast, following IAV infection, our data indicated that activation of DCs *in trans* through IL-1R is both required and sufficient to promote the expansion of virus-specific CD8 T cells. These seemingly conflicting results raise several questions. First, why is PAMP recognition not sufficient to induce DC activation and CD8 T cell priming following influenza infection? One possible explanation is that DCs that recognize influenza virus through cytosolic sensors are necessarily infected by the virus, rendering them incapable of performing their antigen presenting functions in the mLN[33, 34]. This may also be the case for HSV-2 infection, as we saw fewer antigen presenting DCs in the lymph nodes of IL-1R–deficient mice. The other possibility is that the DC subset responsible for priming CD8 T cell

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responses does not possess the appropriate PRRs. In this regard, the $CD103^+$ tissue-resident DCs in the lung do not express TLR7 (Ref. [35]), a key PAMP associated with IAV. Second, why aren't other inflammatory cytokines able to replace the function of IL-1? It is possible that since IL-1R, like TLRs, contains a TIR domain and signals through the adaptor protein MyD88, it induces sets of gene expression that overlap with those induced by TLR signaling. Third, why isn't IL-1R signaling sufficient to activate DCs upon immunization with protein plus adjuvant? This may relate to the extent of IL-1 secretion induced by a typical immunization compared to live viruses[10]. Whether the NLR-inflammasomes and the cytokines secreted through this pathway represent a common strategy used by the host to stimulate adaptive immune responses to pathogens that evade innate sensors remains to be determined.

Pulmonary DCs are critical for the priming of naïve CD8 T cells in the lung and efficient viral clearance following respiratory influenza virus infection[26]. In the present study, IL-1R was found to be important in maintaining the homeostasis and maturation of pulmonary DCs in the lung at steady state and in activating and mobilizing tissue DC migration in the respiratory tract following influenza virus infection. In addition, DCs in IL-1R-deficient mice had impaired ability to survive in the lung following influenza virus infection. The overall reduction in CD8 T cell priming in IL-1R-deficient mice is likely due to a combined effect of reduced frequency and number of $CD103^+$ DCs at steady state, and impaired CCR7 expression upon IAV infection, resulting in fewer antigen presenting DCs in the lymph node. The importance of IL-1R signaling in promoting respiratory DC migration and activation is not restricted to influenza virus infections and extends to other stimuli including TLR activation and HSV-2 infections. Moreover, in other experimental systems, IL-1R signaling activates DCs for the induction of $CD4^+$ T cell-mediated autoimmune heart disease[36]. In the skin, IL-1β is essential for the activation and migration of Langerhans cells to the lymph nodes during contact hypersensitivity response[37]. Thus, in addition to PAMPs, IL-1R, in certain situations, can trigger DC migration by upregulating CCR7 expression and promote DC maturation and survival leading to optimal CD8 T cell priming.

Our study reveals a molecular signature of innate signals critical for the development of robust antiviral CD8 T cell immunity following a live virus infection. Our findings highlight a contingency system for priming CD8 T cell responses when a virus infection renders the host cells, particularly DCs, incapable of performing their antigen-presenting functions. Our results imply a potential use of IL-1 as an adjuvant in vaccine settings. Conversely, IL-1 may play a detrimental role by priming autoreactive T cells against self-antigens, even in the absence of PAMPs. Developing novel approaches for utilizing the immunogenicity of IL-1 signaling in DCs directed at desired antigens may make this cytokine an ideal adjuvant in the future.

METHODS

Mice

Age- and sex-matched C57BL/6 (WT) and B6.SJL-$Ptprc^a$ $Pep3^b$/BoyJ (congenic CD45.1 mice on B6 background) mice from National Cancer Institute (Frederick, MD) were used as wild-type controls. *Il1r1*$^{-/-}$ mice were obtained from The Jackson Laboratory (Bar Harbor,

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ME). The generations of *Tlr7*$^{-/-}$[3], *Mavs*$^{-/-}$[38], CD11c–MyD88-TG[31], *Myd88*$^{-/-}$[39] and gBT-I T-cell receptor (TCR) transgenic mice[40] specific for the immunodominant HSV glycoprotein B (gB) peptide $gB_{498-505}$ have been described. *Tlr7*$^{-/-}$ *Mavs*$^{-/-}$ double-deficient mice were bred in the animal facility at Yale. P14 TCR transgenic mice expressing the TCR specific for the lymphocytic choriomeningitis virus glycoprotein (gp_{33-41}) peptide were a gift from S. Kaech (Yale University, CT). H-2D^{b} deficient mice[41] in the C57BL/6 background were purchased from Taconic Farms (Germantown, NY). All procedures used in this study complied with federal and institutional policies of the Yale Animal Care and Use committee.

Bone marrow chimera

Generation of bone marrow chimera was carried out as previously described[16]. Briefly, mice were γ-irradiated with 950 Rad and subsequently reconstituted with $4 - 6 \times 10^{6}$ bone-marrow cells of the indicated genotype and allowed to recover for six to eight weeks before influenza infection. For mixed bone marrow chimera, mice were tested for chimerism 6–8 weeks after reconstitution. Chimeras were used for subsequent experiments only if analysis of blood leukocytes showed the presence of $K^{b+/+}$ $D^{b-/-}$ vs. $K^{b+/+}$ $D^{b+/+}$ cells at a ratio close to 1:1. All procedures used in this study complied with federal guidelines and were approved by the Yale Animal Care and Use Committee.

Virus infections *in vivo*

A/PR8 virus (H1N1) and recombinant PR8 virus expressing the LCMV glycoprotein epitope GP_{33-41} (Ref.[42]) used for all experiments were grown in allantoic cavities from 10–11-day-old fertile chicken eggs for 2 d at 35°C. Viral titer was quantified by a standard plaque assay using Madin-Darby canine kidney cells and viral stock was stored at −80°C. The thymidine kinase mutant HSV2 strain 186TK Kpn[43], which is incapable of viral reactivation, was propagated in Vero cells. All stocks were titered on the Vero cell line before use. For intranasal infection, mice were fully anesthetized by i.p. injection of ketamine and xylazine and then infected by intranasal application of 20 μl of virus suspension (10 – 1,000 PFU of influenza in PBS or 2×10^{6} PFU of TK^{-} HSV2 as indicated). This procedure leads to the upper and lower respiratory tract infection.

Measurement of virus titer

Bronchoalveolar lavage (BAL) was collected for measurement of virus titer at the indicated time point post infection. BAL was collected by washing the trachea and the lungs three times with 1 ml of PBS containing 0.1% BSA. The virus titer was determined by inoculating Madin-Darby canine kidney cells in 6-well plate with 200 μl of serial 10-fold dilutions of the BAL wash at 37°C. After 1 hr of incubation, each well was overlaid with 2ml of agar medium. Forty-eight hrs after inoculation, the cell monolayers were stained with 0.1% crystal violet in 20% ethanol and the number of plaques in each well was counted.

Cell preparation and flow cytometry

Single-cell suspensions of lung samples were prepared as previously described[16]. For staining, fluorochrome-labeled anti-CD8α (53-6.7), anti-CD4 (RM4–5), anti-CD44 (IM7),

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anti-CD11b (M1/70), anti-CD103 (2E7), anti-CCR7 (4B12), anti-CD11c (N418), anti-TNF-α (MP6-XT22) and anti-IFN-γ (XMG1.2) antibodies were from BioLegend, anti-Ly6C (AL-21) and anti-CD107a (1D4B) antibodies were from BD Biosciences, anti-Ki67 (SolA15), anti-CD86 (GL1) and anti-MHC-II (M5/114.15.2) antibodies were from eBioscience. Staining for Ki-67 was done according to the manufacturer's instruction using paraformaldehyde fixation and permeabilization buffer (eBioscience). For the detection of intracellular cytokines and degranulation by T cells, cells were restimulated with influenza CD8-specific peptide for 5 – 6 hrs in 96-well round-bottom plates in the presence of brefeldin A (Sigma) and CD107a antibody. Afterwards, cells were stained with anti-mouse CD8α antibody for 30 min on ice, fixed and permeabilized using paraformaldehyde fixation and Perm/Wash buffer (BD Biosciences), and stained with anti-IFN-γ and anti-TNF-α antibodies. Acquisition of samples was performed on a cytometer (LSR II, BD). Leukocytes were gated based on forward and side scatter properties, and live cells were gated based on 7-aminoactinomycin D (BioLegend) or Live/Dead fixable aqua stain (Invitrogen) exclusion. In the lung, alveolar macrophages were excluded from FACS gating by autofluorescence, high expression of CD11c and side scatter[44]. For MHCI tetramer staining of CD8 T cells, cells were incubated with APC-labeled tetramers (NIH Tetramer Core Facility) specific for H-$2D^b$ complexed with peptides from the viral acid polymerase PA(224–233) (SSLENFRAYV) or from the viral nucleoprotein NP(366–374) (ASNENMETM) in 0.1 ml of 1% FBS PBS for 30 min on ice. After washing, samples were resuspended in 1% paraformaldehyde in PBS. The frequency of tetramer-positive $CD8^+$ T cells was analyzed by FACS within the $CD44^+$ cells. The final analysis and graphical output were performed using FlowJo software (Tree Star, Inc.).

Purification and adoptive transfer of cell populations

For isolation of mLN DCs for the priming of naïve P14 $CD8^+$ T cells or gBT-I $CD8^+$ T cells ex vivo, mice were infected intranasally with 1,000 pfu of PR8-GP33 influenza virus or 2×10^6 pfu TK^- HSV2. Three days later, $CD11c^+$ DCs were isolated from the mLN or spleen of the virus infected mice using anti-CD11c-microbeads (Miltenyi Biotec). Naïve P14 or gBT-I $CD8^+$ T cells were isolated from splenic single-cell suspension of the P14 or gBT-I TCR transgenic mice using $CD8^+$ T cell isolation kit (Miltenyi Biotec) according to the manufacturer's instructions. 1×10^5 naïve p14 $CD8^+$ T cells were restimulated with 2.5×10^4, 5×10^4 or 1×10^5 DCs for 72 hours at 37°C. P14 $CD8^+$ T cells were co-cultured with 5×10^4 DCs in the presence of 1 μg of LCMV $GP_{33–41}$ peptide or HSV $gB_{498–505}$ peptide. IFN-γ production in the culture supernatants was measured by ELISA in triplicates. For measuring $CD8^+$ T cell response as described in Figure 2c, $CD8^+$ T cells were isolated from the spleen of mice infected with 10 PFU A/PR8 at 9 d p.i. using anti-CD8 microbeads (Miltenyi Biotec). 10^5 $CD8^+$ T cells were restimulated with 5×10^5 NP peptide–pulsed APCs for 72 h as previously described[16]. IFN-γ production in the culture supernatants was measured by ELISA. For adoptive transfer of BMDCs, BMDCs were prepared as described[16] and intranasally transferred (1×10^6 cells / mouse) at the time of infection with live PR8-GP33 influenza virus. For adoptive transfer of P14 $CD8^+$ T cells, $CD8^+$ T cells were isolated from the spleen of P14 TCR Tg mice and labeled for 10 min at 37°C with 1 μM CFSE (Molecular Probes). 2×10^5 cells were transferred i.v. into recipients the day before PR8-GP33 infection.

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In vivo labeling of pulmonary DCs with fluorescent latex beads

Labeling of pulmonary DCs was described previously[44]. Briefly, fluorescent 0.5 μm latex particles (Polysciences, cat#17152) were diluted 1:25 in PBS containing live PR8 virus, 1 μg LPS from *Escherichia coli* (InvivoGen), 10 μg Poly(I:C) (Sigma Aldrich) or 10 μg CpG 2216 (Tri-Link Biotech) and 30 μl of the inoculum were delivered intranasally into each mouse after anesthesia.

Statistical Analysis

Statistical significance was tested by Student's t test using GraphPad PRISM software (Version 5; GraphPad software). Data are presented as mean ± SEM. $p < 0.05$ was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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Abbreviations used

BAL	bronchoalveolar lavage
IL-1R	IL-1 receptor
IAV	Influenza A virus
Lx	latex
mLN	mediastinal lymph node
NLR	NOD-like receptor

REFERENCES

1. Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol. 1989; 54 Pt 1:1–13. [PubMed: 2700931]
2. Pang IK, Iwasaki A. Inflammasomes as mediators of immunity against influenza virus. Trends Immunol. 2011; 32:34–41. [PubMed: 21147034]
3. Lund JM, et al. Recognition of single-stranded RNA viruses by Toll-like receptor 7. Proc Natl Acad Sci U S A. 2004; 101:5598–5603. [PubMed: 15034168]
4. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 2004; 303:1529–1531. [PubMed: 14976261]
5. Heil F, et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 2004; 303:1526–1529. [PubMed: 14976262]
6. Pichlmair A, et al. RIG-I-Mediated Antiviral Responses to Single-Stranded RNA Bearing 5'-Phosphates. Science. 2006; 314:997–1001. [PubMed: 17038589]

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7. Hornung V, et al. 5'-Triphosphate RNA is the ligand for RIG-I. Science. 2006; 314:994–997. [PubMed: 17038590]
8. Koyama S, et al. Differential role of TLR- and RLR-signaling in the immune responses to influenza A virus infection and vaccination. Journal of immunology. 2007; 179:4711–4720.
9. Franchi L, Munoz-Planillo R, Nunez G. Sensing and reacting to microbes through the inflammasomes. Nature immunology. 2012; 13:325–332. [PubMed: 22430785]
10. Ichinohe T, Pang IK, Iwasaki A. Influenza virus activates inflammasomes via its intracellular M2 ion channel. Nature immunology. 2010; 11:404–410. [PubMed: 20383149]
11. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. Annual review of immunology. 2009; 27:229–265.
12. Le Goffic R, et al. Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. PLoS Pathogens. 2006; 2 0526.
13. Heer AK, et al. TLR signaling fine-tunes anti-influenza B cell responses without regulating effector T cell responses. J Immunol. 2007; 178:2182–2191. [PubMed: 17277123]
14. Allen IC, et al. The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. Immunity. 2009; 30:556–565. [PubMed: 19362020]
15. Thomas PG, et al. The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. Immunity. 2009; 30:566–575. [PubMed: 19362023]
16. Ichinohe T, Lee HK, Ogura Y, Flavell R, Iwasaki A. Inflammasome recognition of influenza virus is essential for adaptive immune responses. The Journal of Experimental Medicine. 2009; 206:79–87. [PubMed: 19139171]
17. Schmitz N, Kurrer M, Bachmann MF, Kopf M. Interleukin-1 is responsible for acute lung immunopathology but increases survival of respiratory influenza virus infection. Journal of virology. 2005; 79:6441–6448. [PubMed: 15858027]
18. Szretter KJ, et al. Role of host cytokine responses in the pathogenesis of avian H5N1 influenza viruses in mice. Journal of virology. 2007; 81:2736–2744. [PubMed: 17182684]
19. Sporri R, Reis e Sousa C. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4+ T cell populations lacking helper function. Nature immunology. 2005; 6:163–170. [PubMed: 15654341]
20. Kratky W, Reis e Sousa C, Oxenius A, Sporri R. Direct activation of antigen-presenting cells is required for CD8+ T-cell priming and tumor vaccination. Proc Natl Acad Sci U S A. 2011; 108:17414–17419. [PubMed: 21987815]
21. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. Science. 2010; 327:291–295. [PubMed: 20075244]
22. Dinarello CA. Immunological and Inflammatory Functions of the Interleukin-1 Family. Annual Review of Immunology. 2009; 27:519–550.
23. Mueller SN, et al. Qualitatively different memory CD8+ T cells are generated after lymphocytic choriomeningitis virus and influenza virus infections. Journal of immunology. 2010; 185:2182–2190.
24. Heer AK, Harris NL, Kopf M, Marsland BJ. CD4+ and CD8+ T Cells Exhibit Differential Requirements for CCR7-Mediated Antigen Transport during Influenza Infection. The Journal of Immunology. 2008; 181:6984–6994. [PubMed: 18981118]
25. Belz GT, et al. Distinct migrating and nonmigrating dendritic cell populations are involved in MHC class I-restricted antigen presentation after lung infection with virus. Proc Natl Acad Sci U S A. 2004; 101:8670–8675. [PubMed: 15163797]
26. GeurtsvanKessel CH, et al. Clearance of influenza virus from the lung depends on migratory langerin+CD11b− but not plasmacytoid dendritic cells. J Exp Med. 2008; 205:1621–1634. [PubMed: 18591406]
27. Kim TS, Braciale TJ. Respiratory dendritic cell subsets differ in their capacity to support the induction of virus-specific cytotoxic CD8+ T cell responses. PLoS One. 2009; 4:e4204. [PubMed: 19145246]

28. Ballesteros-Tato A, Leon B, Lund FE, Randall TD. Temporal changes in dendritic cell subsets, cross-priming and costimulation via CD70 control CD8(+) T cell responses to influenza. Nature immunology. 2010; 11:216–224. [PubMed: 20098442]
29. Aldridge JR Jr, et al. TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. Proc Natl Acad Sci U S A. 2009; 106:5306–5311. [PubMed: 19279209]
30. Jakubzick C, Tacke F, Llodra J, van Rooijen N, Randolph GJ. Modulation of Dendritic Cell Trafficking to and from the Airways. Journal of immunology. 2006; 176:3578–3584.
31. Pasare C, Medzhitov R. Control of B-cell responses by Toll-like receptors. Nature. 2005; 438:364–368. [PubMed: 16292312]
32. Blander JM. Phagocytosis and antigen presentation: a partnership initiated by Toll-like receptors. Ann Rheum Dis. 2008; 67(Suppl 3):iii44–iii49. [PubMed: 19022813]
33. Fernandez-Sesma A, et al. Influenza virus evades innate and adaptive immunity via the NS1 protein. Journal of virology. 2006; 80:6295–6304. [PubMed: 16775317]
34. Smed-Sorensen A, et al. Influenza A virus infection of human primary dendritic cells impairs their ability to cross-present antigen to CD8 T cells. PLoS pathogens. 2012; 8 e1002572.
35. del Rio ML, et al. CX3CR1+ c-kit+ bone marrow cells give rise to CD103+ and CD103− dendritic cells with distinct functional properties. Journal of immunology. 2008; 181:6178–6188.
36. Eriksson U, et al. Activation of dendritic cells through the interleukin 1 receptor 1 is critical for the induction of autoimmune myocarditis. The Journal of experimental medicine. 2003; 197:323–331. [PubMed: 12566416]
37. Shornick LP, Bisarya AK, Chaplin DD. IL-beta is essential for langerhans cell activation and antigen delivery to the lymph nodes during contact sensitization: evidence for a dermal source of IL-1beta. Cell Immunol. 2001; 211:105–112. [PubMed: 11591114]
38. Sun Q, et al. The Specific and Essential Role of MAVS in Antiviral Innate Immune Responses. Immunity. 2006; 24:633. [PubMed: 16713980]
39. Adachi O, et al. Targeted Disruption of the MyD88 Gene Results in Loss of IL-1- and IL-18-Mediated Function. Immunity. 1998; 9:143–150. [PubMed: 9697844]
40. Mueller SN, Heath W, McLain JD, Carbone FR, Jones CM. Characterization of two TCR transgenic mouse lines specific for herpes simplex virus. Immunology and cell biology. 2002; 80:156–163. [PubMed: 11940116]
41. Perarnau B, et al. Single H2Kb, H2Db and double H2KbDb knockout mice: peripheral CD8+ T cell repertoire and anti-lymphocytic choriomeningitis virus cytolytic responses. European journal of immunology. 1999; 29:1243–1252. [PubMed: 10229092]
42. Ichinohe T, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proceedings of the National Academy of Sciences. 2011
43. Jones CA, Taylor TJ, Knipe DM. Biological properties of herpes simplex virus 2 replication-defective mutant strains in a murine nasal infection model. Virology. 2000; 278:137–150. [PubMed: 11112490]
44. Jakubzick C, Randolph GJ. Methods to study pulmonary dendritic cell migration. Methods Mol Biol. 2010; 595:371–382. [PubMed: 19941125]

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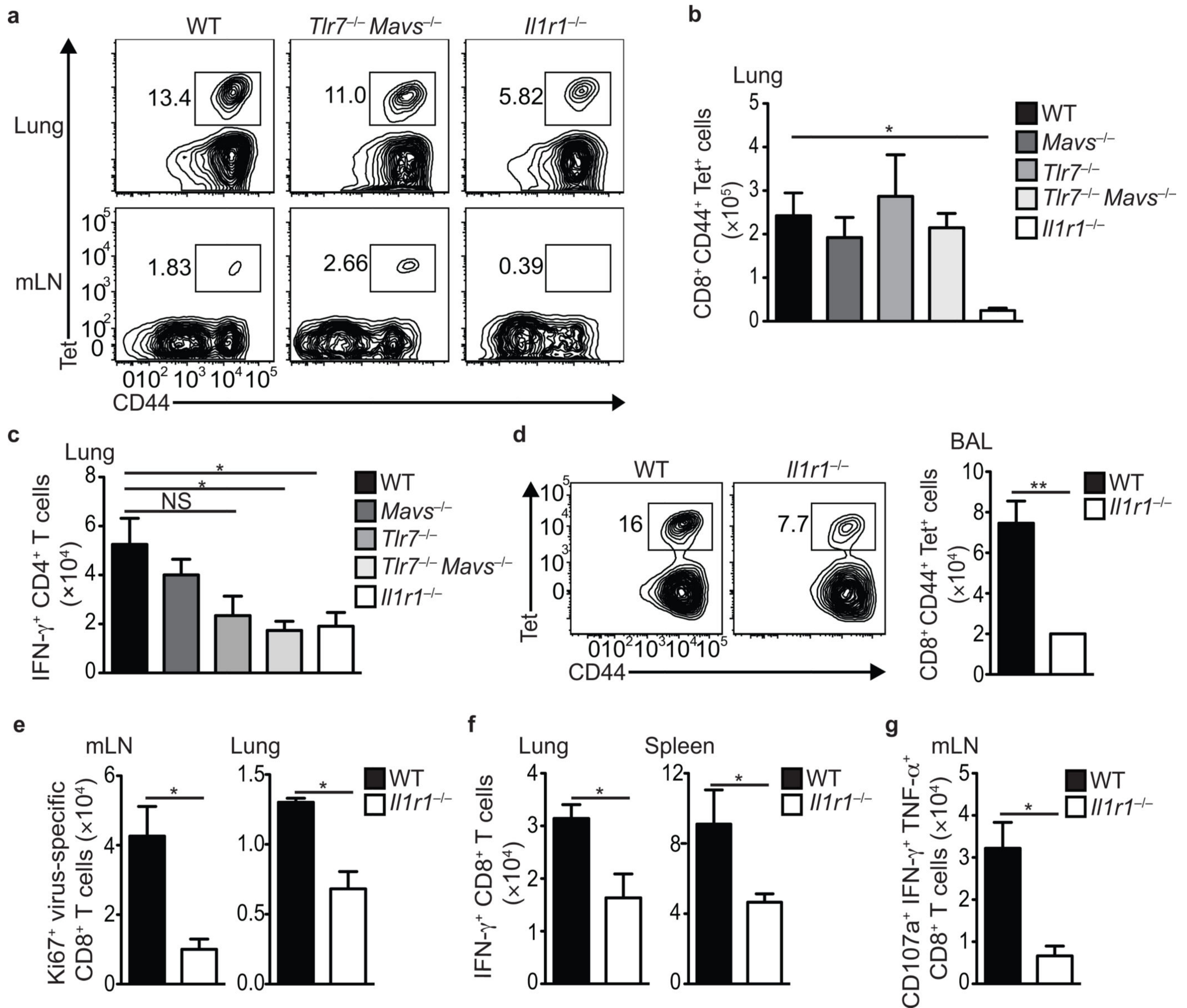

Figure 1. TLR7-Mavs–dependent and independent adaptive immune responses against respiratory influenza virus

(**a**) Influenza-specific $CD8^+$ T lymphocytes in the lung, mLN and BAL of wild-type, *Il1r1*$^{-/-}$ and *Tlr7*$^{-/-}$ *Mavs*$^{-/-}$ mice 9 days post-intranasal infection with a sublethal dose (10 pfu) of A/PR8 influenza virus as detected by staining with MHCI tetramer. (**b–d**) Numbers of virus-specific $CD8^+$ (b,d) and IFN-γ^+ $CD4^+$ (c) T cells in the lungs (**b,c**) or BAL (**d**) of wild-type, *Il1r1*$^{-/-}$ and *Tlr7*$^{-/-}$ *Mavs*$^{-/-}$ mice 9 days p.i. with A/PR8 influenza virus as in **a**, detected by MHCI tetramer staining (**b,d**) or by intracellular cytokine staining following restimulation with CD4-specific influenza peptide (**c**). (**e–g**) Numbers of tetramer$^+$ Ki67$^+$ (**e**), IFN-γ^+ (**f**), and CD107a$^+$ IFN-γ^+ TNF$^+$ (**g**) $CD8^+$ T cells in the mLN (**e, g**), lung (**e, f**) and spleen (f) of wild-type and *Il1r1*$^{-/-}$ mice 8 days p.i. with A/PR8 influenza virus as detected by staining with influenzaspecific MHCI tetramer and Ki67 (**e**) or intracellular cytokine staining following restimulation with influenza PA_{224} peptide (**f, g**) were

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enumerated. Data represent the mean ± S.E.M. These figures are representative of three independent experiments with three to four mice per group. *, $p < 0.05$, **, $p < 0.01$.

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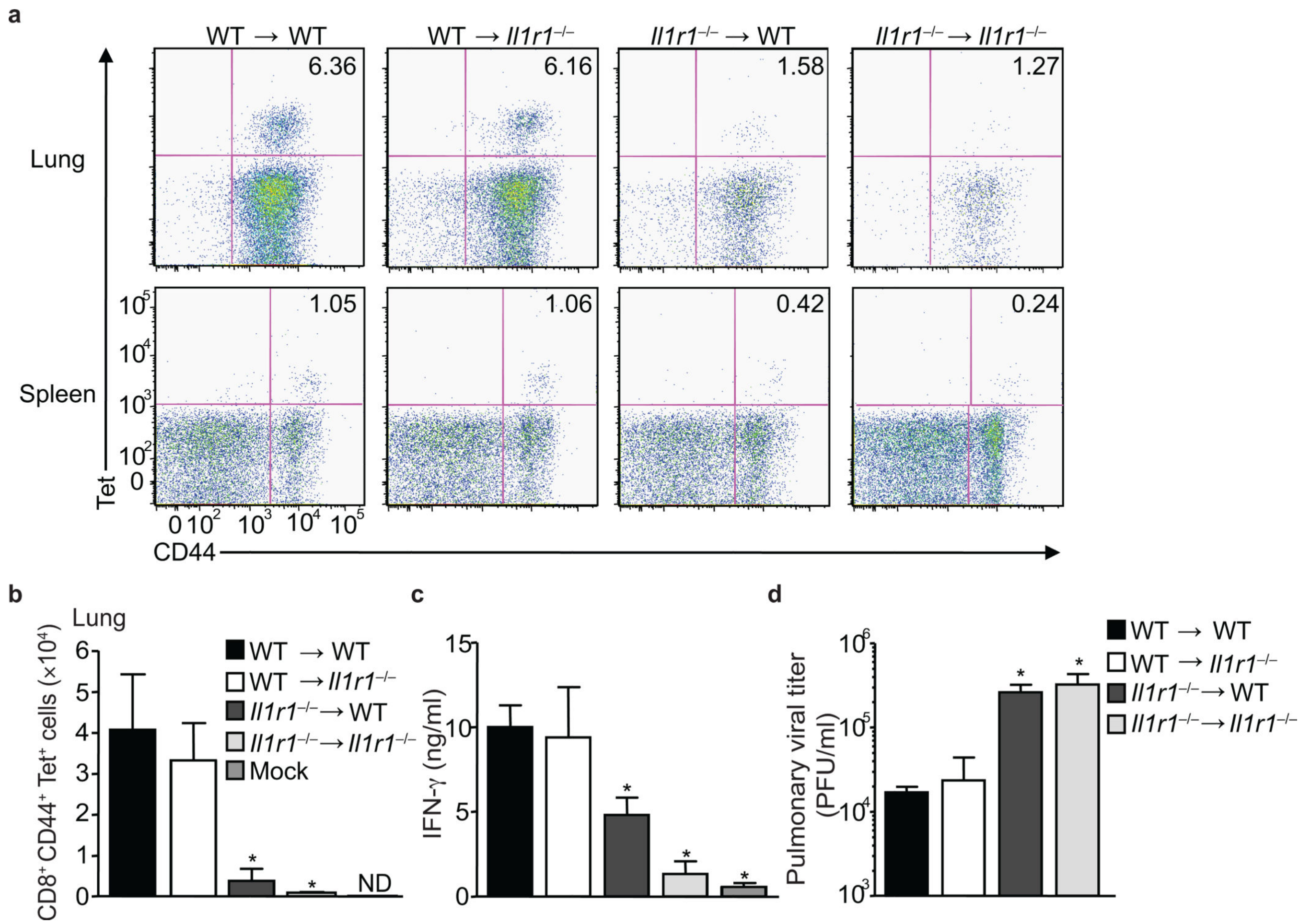

Figure 2. IL-1R on hematopoietic cells but not stromal cells is required for the activation of CD8 T cells after influenza virus infection

(a) Influenza-specific CD8+ T lymphocytes in the lung and spleen of WT→WT, WT→*Il1r1*−/−, *Il1r1*−/−→WT and *Il1r1*−/−→*Il1r1*−/− BM chimeric mice 9 days post-intranasal infection with a sublethal dose (10 pfu) of A/PR8 influenza virus as detected by staining with MHCI tetramer. **(b)** Number of virus-specific CD8+ T cells in the lung 9 days p.i. as in **a**, detected by MHCI tetramer staining was enumerated. **(c)** IFN-γ secretion by splenic CD8 T cells from BM chimeric mice 9 days p.i. as detected by ELISA following co-culture with APCs pulsed with CD8 peptide. **(d)** Pulmonary viral titer from the BAL of BM chimeric mice 9 days p.i. as determined by plaque assay. Data represent the mean ± S.E.M. These figures are representative of three independent experiments with four mice per group. *, $p < 0.05$.

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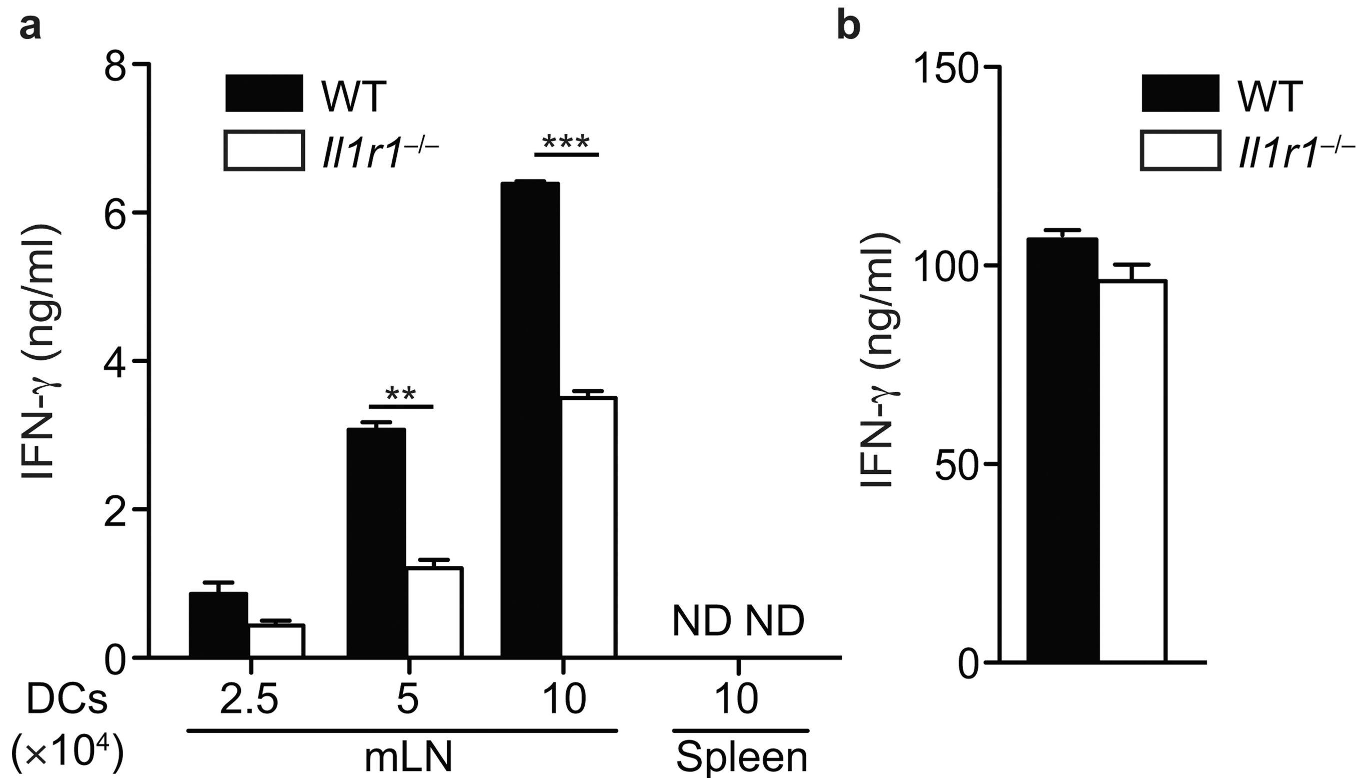

Figure 3. DCs from the mLN of *Il1r1*$^{-/-}$ mice had reduced capacity to prime naïve P14 CD8 T cells following influenza infection

(**a,b**) IFN-γ production in culture supernanants by P14 Tg $CD8^+$ T cells co-cultured with indicated numbers of wild-type and *Il1r1*$^{-/-}$ $CD11c^+$ DCs isolated at 3 days post-intranasal infection with 1000 pfu of PR8-GP33 virus together with (**b**) or without (**a**) GP33 peptide as detected by ELISA. Splenic DCs from the infected wild-type mice were used as negative controls (**a**). Data represent the mean ± S.E.M. Similar results were obtained from three independent experiments with four to five mice per group. **, $p < 0.01$. ***, $p < 0.001$.

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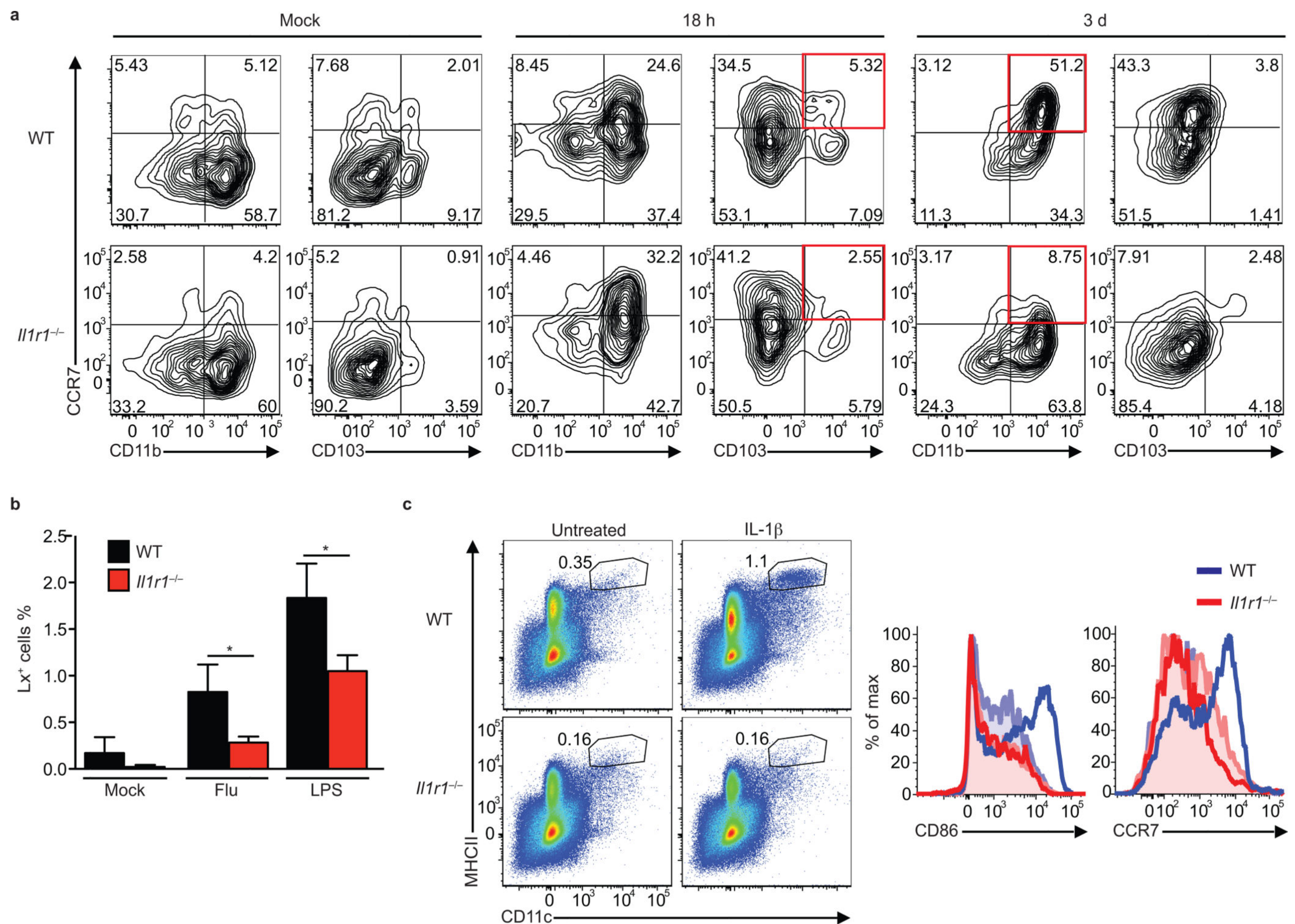

Figure 4. IL-1R signaling promotes respiratory DC activation and CCR7-dependent migration to the lymph node after influenza infection

(a) CCR7 expression on CD11c+ MHCII+ DCs in the lung of wild-type and *Il1r1*−/− mice at 18 hours and 3 days after intranasal infection with 1,000 pfu of PR8 influenza virus as detected by flow cytometry. **(b)** Frequency of Lx+ CD11c+ MHCII+ DCs in the mLN of wild-type and *Il1r1*−/− mice 2 days post-intranasal inoculation with fluorescent latex beads in the presence of 1,000 pfu of PR8 influenza virus or 1μg LPS as detected by flow cytometry. **(c)** Frequency of CD11c+ MHCII^{hi} DCs and their expressions of CD86 and CCR7 in the mLN of wild-type and *Il1r1*−/− mice 2 days after intranasal inoculation with recombinant IL-1β on day 0 and day 1. Shaded histograms indicate untreated controls, and open histograms indicate rIL-1β treated groups. These results were representative of three separate experiments with three mice per group. *, $p < 0.05$.

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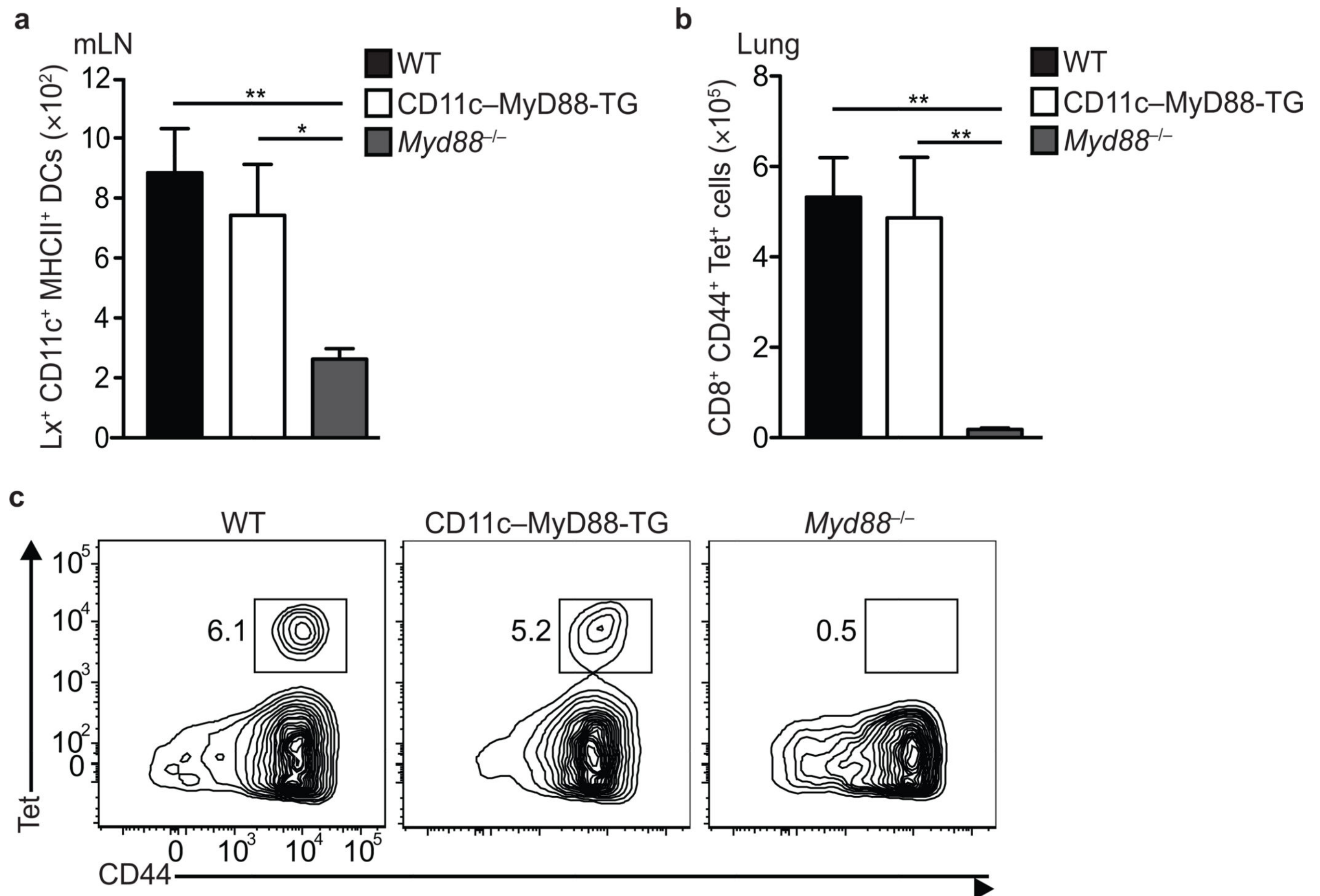

Figure 5. MyD88 signaling in CD11c$^+$ cells is sufficient for DC migration and CD8 T cell activation following influenza infection

(**a**) Number of Lx$^+$ CD11c$^+$ MHCII$^+$ DCs in the mLN of wild-type, CD11c–MyD88-transgenic mice on *Myd88*$^{-/-}$ background (CD11c–MyD88-TG) and *Myd88*$^{-/-}$ mice 2.5 days post-intranasal infection with 1,000 pfu of A/PR8 influenza virus in the presence of fluorescent latex beads (as in Fig. 4**b**) as detected by flow cytometry was enumerated. **(b, c)** Number **(b)** and frequency **(c)** of influenza-specific CD8$^+$ T lymphocytes in the lung of wild-type, CD11c–MyD88-TG and *Myd88*$^{-/-}$ mice 7 days post-intranasal infection with 10 pfu of A/PR8 influenza virus as detected by staining with MHCI tetramer. Data represent the mean ± S.E.M. These figures are representative of two to three independent experiments with three mice per group. *, $p < 0.05$. **, $p < 0.01$.

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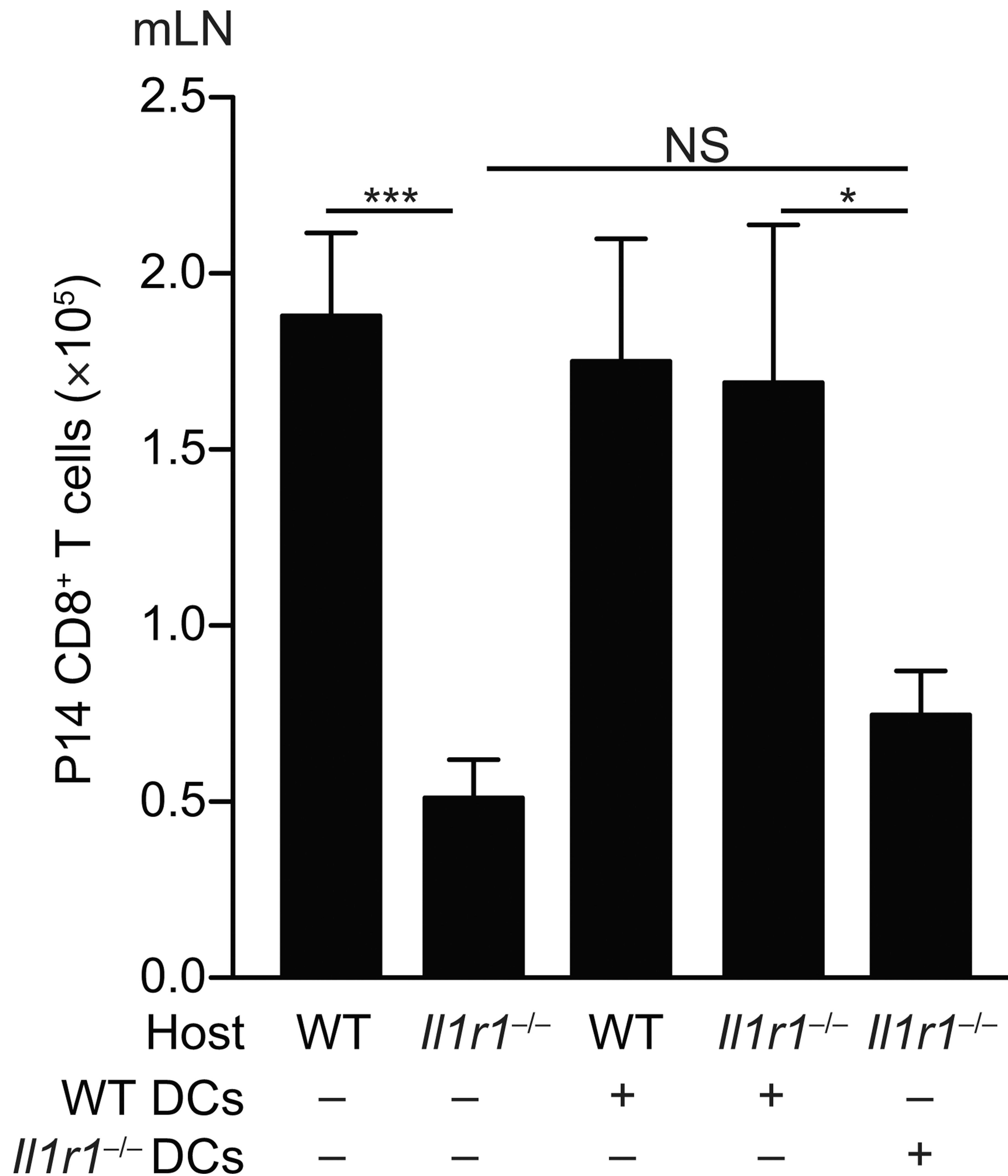

Figure 6. Intranasal injection of WT DCs restores CD8 T cell priming in *Il-1r1–/–* mice

Total number of adoptively transferred P14 $CD8^+$ T cells in the mLN of wild-type and *Il1r1*$^{-/-}$ mice 7 days post-intranasal infection with 100 pfu PR8-GP33 virus. Some *Il1r1*$^{-/-}$ mice intranasally received 1×10^6 wild-type or *Il1r1*$^{-/-}$ BMDCs pulsed with PR8-GP33 virus. Data represent the mean ± S.E.M. These results were representative of three separate experiments with three to four mice per group. *, $p < 0.05$. ***, $p < 0.001$.

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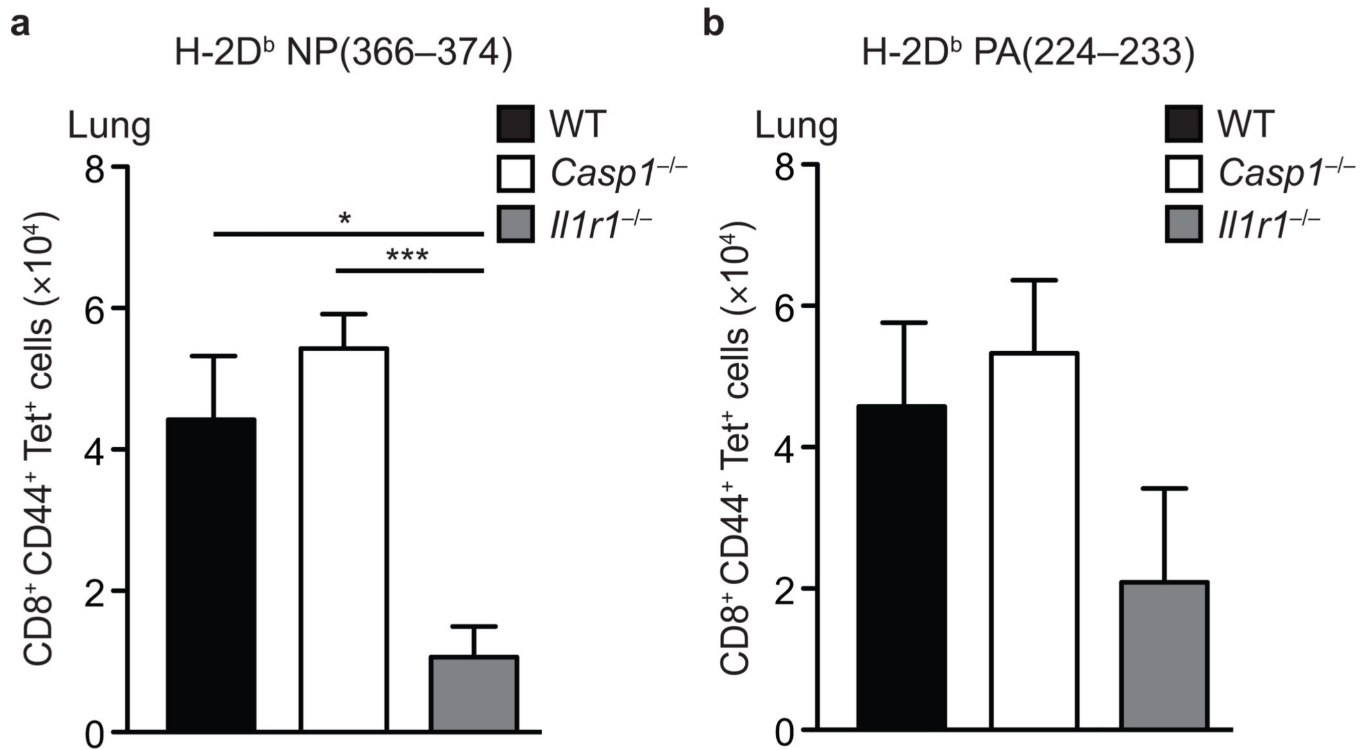

Figure 7. Direct activation of caspase-1 in the antigen-presenting DCs is not required for the expansion of virus-specific CD8 T cells

(a,b) Numbers of influenza-specific $CD8^+$ T lymphocytes in the lung of wild-type, *Casp1*$^{-/-}$ and *Il1r1*$^{-/-}$ mixed bone marrow chimeric mice 9 days post-intranasal infection with a sublethal dose (10 pfu) of A/PR8 influenza virus as detected by staining with H-2D^b-specific MHCI tetramers recognizing NP(366–374) **(a)** or PA(224–233) **(b)** were enumerated. Data represent the mean ± S.E.M. These results were representative of two separate experiments with five mice per group. *, $p < 0.05$. ***, $p < 0.001$.