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

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

The initiation of protective immune responses to infectious microorganisms is thought to rely on the recognition of microbes by innate sensors1. After infection, influenza A virus (IAV) is detected by the following three classes of innate sensors: members of the Toll-like receptor (TLR) family; RIG-I like receptors (RLRs); and 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 by RIG-I in the cytosol6,7. Signaling through either RIG-I or TLR7 leads to the production of type I interferons, which limits viral replication and increases innate resistance to infection8. In contrast to TLRs and RLRs, which recognize viral pathogen-associated molecular patterns (PAMPs), NLRP3 senses cellular damage or distress as a consequence of infection7. The infected host cell recognizes IAV through NLRP3 on the basis of activity of the M2 ion channel9. Once activated, NLRP3 engages the inflammasome complex that includes the adaptor ASC and caspase-1, which results in the activation of caspase-1 and the cleavage of its substrates, including the proinflammatory cytokines interleukin 1β (IL-1β) and IL-18 (ref. 11).

The relevance of host recognition of viral PAMPs versus virus-inflicted damage in linking innate recognition of IAV to adaptive immunity has not been systematically explored. Neither the absence of TLR3 (ref. 12) nor the absence of the RIG-I signaling adaptor MAVS8 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 in response to IAV8,13. In contrast, the importance of NLRP-dependent activation of inflammasomes in innate14,15 and adaptive16 immune defense against IAV has been demonstrated. The requirement that the inflammasomes generate protective adaptive immunity to IAV has been attributed to the production of IL-1α and IL-1β, as mice deficient in IL-1 receptor type I (IL-1R)16–18 suffer from a lack of immune responses similar to that of ASC- or caspase-1-deficient mice16. IL-1R-deficient mice have defective CD4+ T cell and antibody responses to infection with IAV16,17 and have impaired CD8+ T cell responses at 7 d after infection17 and 14 d after infection16. Although these data collectively indicate the importance of the inflammasome–IL-1R axis in the priming of CD8+ T cells in response to IAV, it remains possible that TLR7 and RIG-I induce redundant signaling pathways that compensate for each other in mice with single deficiency in either of these PRRs.

As for the importance of direct detection of PAMPs by antigen-presenting dendritic cells (DCs) in the priming of T cell responses, published studies have shown a requirement for DC-intrinsic recognition of PAMPs via TLRs in initiating CD4+ T cell responses19 and CD8+ T cell responses20 after immunization with a model antigen plus a TLR ligand as adjuvant. Under those conditions, activation of bystander DCs by inflammatory cytokines alone is not sufficient to render them able to stimulate productive T cell immune responses. However, whether similar principles of T cell priming apply during infection with various other microbes remains unclear. During infection with a live pathogen, directly infected DCs are often rendered unable to prime T cells21. Under such circumstances, activation of bystander DCs might be required for efficient priming of T cells.

Here we examined the relative contributions of pattern recognition versus inflammasome-dependent recognition of damage in the initiation of adaptive CD8+ T cell responses after respiratory infection with IAV.

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with IAV. Our data provide evidence that priming of CD8+ T cells did not depend on pattern-recognition receptors (PRRs) but critically depended on NLR inflammasome–induced release of IL-1α and IL-1β and on signaling through the IL-1R in bystander DCs. Thus, our data suggest that after infection with a pathogenic virus that leads to the lysis of DCs and/or impairment of DC function, IL-1R signaling, like TLR signaling, is able to provide the innate signal necessary for the initiation of adaptive immune responses.

RESULTS

IAV-specific CD8+ T cell responses require IL-1R

Because mice deficient in TLR7 or MAVS alone have intact CD8+ T cell immune responses to IAV8,13, we investigated whether TLR7 and RIG-I share any functional redundancy in providing the requisite signals for the activation of adaptive immune responses. We challenged mice lacking both TLR7 and MAVS (Tlr7−/−Mavs−/− mice) intranasally with a sublethal dose of mouse-adapted IAV strain A/PR8 (H1N1; 10 plaque-forming units (PFU) per mouse; 0.4 LD50 (dose lethal to 50% of mice tested)). This dose of A/PR8 has been shown to be optimal in demonstrating the importance of innate pathways involved in adaptive immunity–dependent clearance of IAV14 without inducing mortality. Tlr7−/− mice and Tlr7−/−Mavs−/− mice mounted a much lower inflammatory response than did wild-type mice or Mavs−/− mice, including less recruitment of cells into the airway and lower production of the proinflammatory cytokine IL-6 at day 4 after infection (Supplementary Fig. 1a,b). The inflammatory response was the most diminished in Tlr7−/−Mavs−/− mice and less diminished in Tlr7−/− mice. These data indicated that the innate immune response to respiratory IAV was largely TLR7 dependent.

To examine the effect of deficiency in TLR7 and MAVS on the development of adaptive immunity to IAV, we assessed the frequency and number of virus-specific CD8+ T cells in the lungs. We detected no difference in the frequency (Fig. 1a) or number (Fig. 1b) of IAV-specific CD8+ T cells in the lungs at day 9 after infection. In contrast, Tlr7−/−Mavs−/− mice had significantly fewer IFN-γ+ CD4+ T cells in the lungs 9 d after infection with IAV (Fig. 1c). These data indicated that whereas both the TLR7- and RIG-I-dependent recognition pathways contributed to the development of virus-specific IFN-γ-producing CD4+ T cells, CD8+ T cell responses developed in the absence of both of these PRRs.

Protective immunity to IAV is known to require ASC- and caspase-1-dependent activation of the inflammasome and IL-1R16. Il1r1−/− mice mounted a much lower response to a sublethal dose of IAV than did wild-type mice, in terms of the frequency (Fig. 1a) and number (Fig. 1b) of virus-specific CD8+ T cell in the lungs and in the bronchoalveolar lavage (BAL) fluid (Fig. 1d) 9 d after infection. These results indicated a dominant role of IL-1R over TLR7 and RIG-I in the generation of CD8+ T cell responses.

We next examined the proliferative and functional abilities of IAV-specific CD8+ T cells in Il1r1−/− mice. At 8 d after infection with a sublethal dose of IAV strain A/PR8, the number of proliferating (Ki67+) virus-specific CD8+ T cells was much lower in the lungs and draining mediastinal lymph nodes (mLN) of Il1r1−/− mice than in those of wild-type mice (Fig. 1e). Il1r1−/− mice also had fewer IFN-γ-producing virus-specific CD8+ T cells in the lungs and spleen than did wild-type mice (Fig. 1f). Furthermore, there were significantly fewer polyfunctional CD8+ T cells able to secrete IFN-γ and tumor-necrosis factor and to undergo degranulation (CD107a+) in Il1r1−/− mLN than in wild-type mLN (Fig. 1g). These results indicated that IL-1R signaling was required for the activation and population expansion of and acquisition of effector functions by CD8+ T cells after infection with IAV.

Hematopoietic lineage–intrinsic requirement for IL-1R signaling

IL-1R is widely expressed by many cell types that belong to the hematopoietic and nonhematopoietic compartments22. To identify the cells responsible for supporting the IL-1R-dependent priming of CD8+ T cells during infection with IAV, we generated chimeras in which IL-1R expression was confined to the hematopoietic compartment (lethally irradiated Il1r1−/− host mice given wild-type bone marrow).

Figure 1 Adaptive immune responses to respiratory IAV that are dependent on and independent of TLR7-MAVS. (a) IAV-specific CD8+ T lymphocytes in the lung and mLN of wild-type (WT), Tlr7−/−Mavs−/− and Il1r1−/− mice 9 d after intranasal infection with a sublethal dose (10 PFU) of IAV strain A/PR8, as detected by staining with MHC class I tetramer and for CD44 (a surface marker associated with virus-specific CD8+ T cells). Numbers adjacent to outlined areas indicate percent tetramer-positive (Tet+) CD4+ (gated) cells. (b, c) Quantification of virus-specific CD8+ T cells (b) and IFN-γ+CD4+ T cells (c) in the lungs of wild-type, Mavs−/−, Tlr7−/−, Tlr7−/−Mavs−/− and Il1r1−/− mice 9 d after infection as in a. detected by staining with MHC class I tetramer (b) or by staining for intracellular cytokines after restimulation with influenza virus peptide in vitro (c). (d) Quantification of virus-specific Tet+CD44+ CD8+ T cells in the BAL fluid of wild-type and Il1r1−/− mice 9 d after infection as in a, detected as in b. Numbers adjacent to outlined areas (left) indicate percent Tet+CD44+ cells among CD8+ T cells. (e–g) Quantification of Tet+Ki67+ CD8+ T cells (e), IFN-γ+ CD8+ T cells (f) and CD107a+IFN-γ+ tumor-necrosis factor–positive (TNF+) CD8+ T cells (g) in the mLN (e,g), lungs (e,f) and spleen (f) of wild-type and Il1r1−/− mice 8 d after infection with IAV strain A/PR8, as detected by staining with IAV-specific MHC class I tetramer and Ki67 (e) or by intracellular cytokine staining (f,g). NS, not significant; *P < 0.05 and **P < 0.01 (Student’s t-test). Data are representative of three independent experiments with three to four mice per group (mean and s.e.m. in b–g).
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 population expansion during infection with IAV. To measure epitope-specific CD8+ T cell responses after infection, we used a recombinant IAV strain A/PR8 that expresses the epitope of amino acids 33–41 of lymphocytic choriomeningitis virus glycoprotein (GP33–41; IAV strain A/PR8-GP33), as well as CD8+ T cells from P14 mice, which transgenically express a T cell antigen receptor that recognizes the GP33–41 epitope 23. We obtained IL-1R-deficient antigen-specific CD8+ T cells from the lungs produced similar amounts of IFN-γ after restimulation with LCMV GP33–41 (Supplementary Fig. 2b). In addition, similar numbers of wild-type and Il1r1−/− P14 CD8+ T cells were present in the lungs, mLNs, and spleen of the infected hosts (Supplementary Fig. 2b,c). These data indicated that the development of an epitope-specific cytotoxic T lymphocyte response after infection with IAV occurred independently of IL-1R expressed by CD8+ T cells and indicated the involvement of a hematopoietic non-CD8+ T cell population in the IL-1R-dependent priming of cytotoxic T lymphocytes.

Impaired CD8+ T cell priming by Il1r1−/− DCs

We next investigated the importance of IL-1 in supporting the ability of DCs to prime CD8+ T cells after infection with IAV. The priming and population expansion of IAV-specific CD8+ T cells depend on the migration of tissue DCs from the lungs to the draining mLNs mediated by the chemokine receptor CCR7 and also depend on antigen presentation via peptide–MHC class I on DCs. To maximize the antigen load, we challenged wild-type and Il1r1−/− mice with a high dose (1,000 PFU) of IAV strain A/PR8-GP33. Then, 3 d after infecting the mice, we isolated CD11c+ DCs from the mLNs, and used those cells to stimulate naive P14 CD8+ T cells ex vivo. DCs isolated from the mLNs of infected Il1r1−/− mice had less ability to stimulate naive P14 CD8+ T cells to secrete IFN-γ than did DCs isolated from their wild-type counterparts (Fig. 3a). This was due to a general defect in antigen presentation by the Il1r1−/− DCs, because DCs isolated from infected wild-type or Il1r1−/− mice were able to activate naive P14 CD8+ T cells when exogenous GP33–41 peptide was added to the culture (Fig. 3b). These results indicated that in the absence of IL-1R, fewer antigen-presenting DCs were present in the draining lymph nodes to activate naive CD8+ T cell after infection with IAV.

IL-1R controls CCR7 expression and migration of lung DCs

Next we investigated whether IL-1R signaling is critical for the homeostasis of pulmonary DCs. We found that lung CD11c+ MHC class II–positive Il1r1−/− DCs had a less mature phenotype at steady state than that of their wild-type counterparts, with lower surface expression of the costimulatory molecule CD86 and CCR7 (Supplementary Fig. 3a). Although the total number of DCs in the lungs and mLNs of wild-type and Il1r1−/− mice remained similar,
we observed fewer CD103⁺CD11bhi DCs, but not fewer CD11bhiCD103⁺ DCs, in the lungs and mLNs of Il1r1−/− mice than in that of wild-type mice at steady state (Supplementary Fig. 3b). These data indicated that signaling via IL-1R was particularly important in maintaining the number of CD103⁺ DCs in the respiratory tract.

During infection with IAV, trafficking of respiratory-tract DCs to the draining mLNs, a process that is essential for the generation of T cell immunity, occurs in a CCR7-dependent manner. CD103⁺ CD11bhi DCs that originate in the lungs are critical in presenting IAV antigens to naive CD8⁺ T cells in the mLNs, with the peak of this process occurring 48–72 h after infection. In addition, CD11bhiCD103⁺ DCs are continuously recruited to the mLNs (with peak migration on day 5 after infection) and cross-present to CD8⁺ T cells in a CD70-dependent manner after infection with IAV. Because IL-1R-deficient mice had lower expression of CCR7 and CD86 at steady state and had fewer antigen-presenting DCs in the mLNs after infection with IAV, we investigated the importance of IL-1R in the migration and activation of respiratory DCs after infection with IAV. CD103⁺ CD11bhi DCs in Il1r1−/− lungs did not upregulate CCR7 expression at 18 h after infection with IAV strain A/PR8 (Fig. 4a), which led to a

Figure 3 DCs from the mLNs of Il1r1−/− mice are less able to prime naive P14 CD8⁺ T cells after infection with IAV. Enzyme-linked immunosorbent assay of IFN-γ in supernatants of P14 CD8⁺ T cells cultured with various numbers of lung-draining mLNs of wild-type or Il1r1−/− mice 3 d after intranasal infection with 1,000 PFU of recombinant IAV strain A/PR8-GP33, cultured without exogenous IAV (a) or with the addition of exogenous GP(33–41) (b). Spleen (far right, a), splenic DCs from infected wild-type mice (negative control). ND, not detected; * P < 0.01 and ** P < 0.001 (Student’s t-test). Data are representative of three independent experiments with four to five mice per group, with similar results (mean and s.e.m.).

Figure 4 IL-1R signaling promotes the activation of respiratory DCs and their CCR7-dependent migration to the lymph nodes after infection with IAV. (a) CCR7 expression on CD11c⁺ MHC class II–positive DCs in the lungs of wild-type and Il1r1−/− mice after mock infection (Mock) or at 18 h and 3 d after intranasal infection with 1,000 PFU of IAV strain A/PR8, assessed by flow cytometry. Numbers in quadrants indicate percent cells in each; red outlined areas indicate lung-migratory DCs. (b) Frequency of latex bead–bearing (Lx⁺) CD11c⁺ MHC class II–positive DCs in the mLNs of wild-type and Il1r1−/− mice 2 d after intranasal inoculation of fluorescent latex beads alone (Mock) or in the presence of 1,000 PFU of IAV strain A/PR8 (IAV) or 1 µg LPS (LPS), assessed by flow cytometry. (c) Frequency of MHC class II (MHCII)–high CD11c⁺ DCs (numbers adjacent to outlined areas (gates) at left) and their expression of CD86 and CCR7 (right) in the mLNs of wild-type and Il1r1−/− mice 2 d after no treatment (Untreated (left) or shaded histograms (right)) or intranasal inoculation of recombinant IL-1β on day 0 and day 1 (IL-1β (left) or open histograms (right)). * P < 0.05 (Student’s t-test). Data are representative of three separate experiments with three mice per group (mean and s.e.m. in b).
lower frequency and number of CCR7+CD103+CD11b− DCs in the lungs and mLN s (Supplementary Fig. 4). These data suggested that antigen-bearing CD103+CD11b+ DCs in IIr1−/− mice were less capable of migrating to the draining mLN s than were their wild-type counterparts during the first few days of infection. CD11b+CD103− DCs had similar induction of CCR7 expression in wild-type and IIr1−/− mice after infection with IAV. Thus, these data demonstrated that IL-1R signaling was capable of promoting the migration of respiratory DCs and the activation of antigen-specific T cells beyond infection with IAV.

We next sought to determine whether IL-1β was sufficient to promote the migration of DCs from the lungs to the mLN s. Intranasal administration of recombinant mouse IL-1β induced substantial surface expression of CD86 and CCR7 and mobilized MHC class II-high CD11c+ migratory lung DCs28 to the mLN s of wild-type mice but not those of IIr1−/− mice (Fig. 4c). In addition, intranasal injection of recombinant IL-1α was sufficient to upregulate surface expression of CD86 and CCR7 on migratory CD103+CD11b+ DCs in the lungs and mLN s (Supplementary Fig. 6b). Unlike CD11b+ lung DCs, the migratory CD103+CD11b+ DCs were particularly responsive to activation induced by either IL-1β or IL-1α (Supplementary Fig. 6). Collectively, these results indicated that intranasal injection of wild-type DCs restores the priming of CD8+ T cells in IIr1−/− mice. Total adoptively transferred P14 CD8+ T cells in the mLN s of wild-type and IIr1−/− host mice (Host) 7 d after intranasal infection with 100 PFU of recombinant IAV strain A/PR8-GP33, alone or together with intranasal injection of 1 × 10^6 wild-type or IIr1−/− bone marrow DCs at the time of infection (below graph). NS, not significant; *P < 0.05 and **P < 0.001 (Student's t-test). Data are representative of three separate experiments with three to four mice per group (mean and s.e.m.).
Indirectly activated DCs drive the CD8+ T cell response

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 in the successful priming of CD4+ T cells9 and CD8+ T cells20. Thus far, our data indicated that priming of CD8+ T cells after infection with live IAV did not depend on PRR signaling but relied on the inflammasome–IL-1R axis. We next determined whether direct activation of DCs by NLR inflammasomes was needed to couple damage recognition to the activation of adaptive CD8+ T cell responses. For this, we generated a mixed–bone marrow chimera model based on a published, established strategy20 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 after infection with IAV (Supplementary Fig. 7a). In these chimeric mice (lethally irradiated wild-type C57BL/6 host mice given Casp1+/H2-D1−/− and Casp1+/H2-D1+/+ bone marrow (Casp1−/− or Il1r1−/−H2-D1−/− and Il1r1−/−H2-D1+/+ bone marrow (Il1r1−/−)), assessed 9 d after intranasal infection of recipient mice with a sublethal dose (10 PFU) of IAV strain A/PR8, detected by staining with H-2Db-specific MHC class I tetramers that recognize IAV nucleoprotein (amino acids 366–374: NP(366–374); a) or acidic polymerase (amino acids 224–233: PA(224–233)); b). *P < 0.05 and **P < 0.001 (Student’s t-test). Data are representative of two separate experiments with five mice per group (mean and s.e.m.).

signaling through IL-1R was both required and sufficient for the migration and activation of CD103+ DCs in the lungs.

IL-1R–adaptor MyD88 signaling in DCs drives their migration

Next we investigated whether signaling via IL-1R and the adaptor MyD88 in DCs was sufficient to drive their activation and migration to the mLN during infection with IAV. We used the progeny of transgenic mice that express MyD88 under the control of the Cdl1c promoter crossed with Myd88−/− mice31. In these mice, MyD88 is expressed only in Cdl1c+ DCs and some macrophages (including Cdl1c+ alveolar macrophages) but not in B cells or T cells (C. Pasare, personal communications). DCs in these mice migrated as efficiently from the lungs to the draining mLN as did those in wild-type mice and had expression of CCR7 similar to that of wild-type lung DCs, despite having slightly lower expression of CD86 at 2.5 d after infection (Fig. 5a and Supplementary Fig. 5d). Furthermore, unlike Myd88−/− mice, these transgenic mice generated an effective virus-specific CD8+ T cell response in the lung 7 d after sublethal infection with IAV strain A/PR8 (Fig. 5b,c). These results indicated that MyD88-dependent signaling in DCs alone was sufficient to induce the migration of DCs from the lungs to the lymph nodes to promote CD8+ T cell responses after infection with IAV.

Next we determined whether intranasal delivery of wild-type DCs could restore the defective CD8+ T cell response in IL-1R-deficient mice after infection with IAV. We injected wild-type or IL-1R-deficient bone marrow DCs intranasally together with live recombinant IAV strain A/PR8-GP33 into wild-type or Il1r1−/− mice previously given adoptive transfer of naive P14 CD8+ T cells. By day 7 after infection, P14 CD8+ T cells were readily detectable in the draining mLN of wild-type mice but not in those of Il1r1−/− mice. Notably, intranasal injection of wild-type bone marrow DCs fully restored the population expansion of P14 CD8+ T cells in Il1r1−/− mice, but intranasal injection of Il1r1−/− bone marrow DCs did not (Fig. 6). These data showed that wild-type DCs were able to restore the priming of CD8+ T cells in the mLN of Il1r1−/− hosts, which suggested that the responsiveness of the migrant DCs to IL-1 was sufficient for the priming of CD8+ T cells after infection with IAV.

**Figure 7** Direct activation of caspase-1 in the antigen-presenting DCs is not required for the population expansion of virus-specific CD8+ T cells. Quantification of IAV-specific CD8+ T lymphocytes in the lungs of wild-type C57BL/6 mice given Casp1+/−H2-D1−/− and Casp1+/−H2-D1+/+ bone marrow (WT), Casp1+/−H2-D1−/− and Casp1+/−H2-D1+/+ bone marrow (Casp1−/−) or Il1r1−/−H2-D1−/− and Il1r1−/−H2-D1+/+ bone marrow (Il1r1−/−), assessed 9 d after intranasal infection of recipient mice with a sublethal dose (10 PFU) of IAV strain A/PR8, detected by staining with H-2Db-specific MHC class I tetramers that recognize IAV nucleoprotein (amino acids 366–374: NP(366–374); a) or acidic polymerase (amino acids 224–233: PA(224–233)); b). *P < 0.05 and **P < 0.001 (Student’s t-test). Data are representative of two separate experiments with five mice per group (mean and s.e.m.).

**DISCUSSION**

IAV is recognized by two classes of innate recognition receptors: 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 an adaptive CD8+ T cell response after infection with IAV. After infection with a physiological dose of IAV in vivo, mice that lacked both TLR7 and MAVS were able to mount a robust virus-specific CD8+ T cell response to IAV. In contrast, ASC-dependent inflammasomes and IL-1R were required for the optimum virus-specific CD8+ T cell response to IAV. Expression of IL-1R in hematopoietic cells was essential for the activation of cytosolic T lymphocytes, but expression of IL-1R in CD8+ T cells themselves was not. 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 the migration of DCs from the lungs to the mLNs and for the priming of CD8+ T cells after infection with IAV. Likewise, intranasal administration of wild-type DCs was sufficient to restore the priming of CD8+ T cells in IL-1R-deficient mice after infection with IAV. Moreover, our data indicated that direct stimulation of APCs through IL-1R was required for antigen presentation to CD8+ T cells after infection with IAV, but activation of caspase-1 was not. These data indicated that an inflammatory cytokine, but not recognition of PAMPs, was both required and sufficient for conveying the signals necessary for the generation of virus-specific CD8+ T cells after infection with IAV.

Our findings have shown that after infection with a live virus, the rules that govern DC activation for CD8+ T cell priming differ considerably from those that apply after immunization with a non-live vaccine. Published work has demonstrated that direct recognition of PAMPs by antigen-presenting DCs is required for productive activation of CD4+ T cells and CD8+ T cells after vaccination with protein antigen plus adjuvant. The requirement for direct PAMP recognition extends even to the phagosome in which the microbial antigens are processed for MHC class II presentation. In contrast, our data indicated that after infection with IAV, activation of DCs in trans through IL-1R was both required and sufficient to promote the population 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 priming of CD8+ T cells after infection with IAV? One possible explanation is that DCs that recognize IAV through cytosolic sensors are necessarily infected by the virus, which renders them unable to serve their antigen-presenting functions in the mLNs. This may also be the case for infection with HSV-2, as we noted 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 responses does not have the appropriate PRRs. In this context, the CD103+ tissue-resident DCs in the lung do not express TLR7 (ref. 35), a key PRR associated with recognition of IAV. Second, why are other inflammatory cytokines unable to serve the function of IL-1 in its absence? It is possible that as IL-1R, like TLRs, contains a Toll–IL-1R domain and signals 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 naive CD8+ T cells in the lungs and for efficient viral clearance after respiratory infection with IAV. In our study here, we found that IL-1R was important in maintaining the homeostasis and maturation of pulmonary DCs in the lungs at steady state and in activating and mobilizing the migration of tissue DCs in the respiratory tract after infection with IAV. In addition, DCs in IL-1R-deficient mice had an impaired ability to survive in the lungs after infection with IAV. The overall diminished priming of CD8+ T cells in IL-1R-deficient mice was probably due to a combined effect of a lower frequency and number of CD103+ DCs at steady state and impaired CCR7 expression after infection with IAV, which resulted in fewer antigen-presenting DCs in the lymph node. The importance of IL-1R signaling in promoting the migration and activation of respiratory DCs was not restricted to infection with IAV and extended to other stimuli, including activation of TLRs and infection with HSV-2. Moreover, in other experimental systems, IL-1R signaling activates DCs for the induction of CD4+ T cell–mediated autoimmune heart disease. In the skin, IL-1R is essential for the activation and migration of Langerhans cells to the lymph nodes during contact-hypersensitivity responses. Thus, in addition to PAMPs, IL-1R can (in certain situations) trigger the migration of DCs by upregulating CCR7 expression and can promote the maturation and survival of DCs that leads to optimal priming of CD8+ T cells.

Our study has identified a molecular signature of innate signals critical for the development of robust antiviral CD8+ T cell immunity after infection with a live virus. Our findings highlight a contingency system for priming CD8+ T cell responses when a viral infection renders the host cells, particularly DCs, unable to serve their antigen-presenting functions. Our results suggest a potential use of IL-1 as an adjuvant in vaccine settings. Conversely, IL-1 may serve a detrimental role by priming autoreactive T cells against self antigens, even in the absence of PAMPs. The development of new approaches for using the immunogenicity of IL-1 signaling in DCs directed at desired antigens may make this cytokine an ideal adjuvant in the future.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

I.K.P., T.I. and A.I. conceived of and designed the experiments, and analyzed data; I.K.P. and I.I. did the experiments; and I.K.P. and A.I. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Bone marrow chimera. Bone marrow chimeras were generated as described16. Host mice were γ-irradiated with 950 rads, then were reconstituted with 4 × 10^6 to 6 × 10^6 bone marrow cells of the appropriate genotype and allowed to recover for 6–8 weeks before infection with IAV. For mixed–bone marrow chimeras, chimera was assessed 6–8 weeks after reconstitution. Chimeras were used for subsequent experiments only if analysis of bone leukocytes showed the presence of H-2K^b/+I-D^d/+ cells and H-2K^b−/I-D^d−/+ at a ratio close to 1:1. All procedures used in this study complied with federal and institutional policies of the Yale Animal Care and Use committee.

Viral infection in vivo. The IAV strain A/PR8 (H1N1) and recombinant IAV strain PR8 expressing GP33 (34–31) (A/PR8-GP33) 42 used for all experiments were grown for 2 d at 35 °C in the allantoic cavities of 10- to 11-day-old fertile chicken eggs. Viral titers were quantitated by standard plaque assay with Madin-Darby canine kidney cells, and viral stocks were stored at –80 °C. The thymidine kinase–mutant HSV-2 strain 186TKAp41, which is incapable of viral reactivation, was propagated in Vero (African green monkey kidney epithelial) cells. All stocks were titered on the Vero cell line before use. For intranasal infection, mice were fully anesthetized by intraperitoneal injection of a solution of 20 mg/kg body weight ketamine and 10 mg/kg xylazine. Mice were infected intranasally with 10 PFU of IAV strain A/PR8, with anti-CD8 microbeads (Miltenyi Biotech). CD8+ T cells (1 × 10^5) were stimulated for 72 h at 37 °C with DCs (2.5 × 10^4, 5 × 10^4, or 1 × 10^5). Some P14 CD8+ T cells were cultured with 5 × 10^6 DCs in the presence of 1 μg GP33–41 or HSV peptide (amino acids 498–505 of glycoprotein B). IFN-γ in the culture supernatants was measured by enzyme-linked immunosorbent assay. For measurement of CD8+ T cell response (as described in Fig. 2c), CD8+ T cells were isolated from the spleens of mice 9 d after infection with 10 PFU of IAV strain A/PR8, with anti-CD8 microbeads (Miltenyi Biotech). CD8+ T cells (1 × 10^5) were restimulated for 72 h with nucleoprotein peptide–pulsed APCs (5 × 10^5) as described16. IFN-γ in the culture supernatants was measured by enzyme-linked immunosorbent assay. For adoptive transfer of bone marrow DCs, bone marrow DCs were prepared as described16 and transferred intranasally (1 × 10^6 cells per mouse) at the time of infection with live recombinant IAV strain A/PR8-GP33. For adoptive transfer of P14 CD8+ T cells, CD8+ T cells were isolated from the spleen of P14 mice and then labeled for 10 min at 37 °C with 1 μM CFSE (carboxyfluorescein diacetate succinimidyl ester); Molecular Probes). A total of 2 × 10^5 cells were transferred intravenously into recipients the day before infection with recombinant IAV strain A/PR8-GP33.

In vivo labeling of pulmonary DCs with fluorescent latex beads. Labeling of pulmonary DCs has been described44. Fluorescent 0.5-μm latex particles (17152; Polysciences) were diluted 1:25 in PBS containing live influenza virus strain PR8, 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 was delivered intranasally into each anesthetized mouse.

Statistical analysis. Statistical significance was tested by Student’s-t-test with PRISM software (Version 5; GraphPad software). P values of less than 0.05 were considered statistically significant.