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Interleukin-1 Is Responsible for Acute Lung Immunopathology but Increases Survival of Respiratory Influenza Virus Infection

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Interleukin-1α (IL-1α) and IL-1β are proinflammatory cytokines, which induce a plethora of genes and activities by binding to the type 1 IL-1 receptor (IL-1R1). We have investigated the role of IL-1 during pulmonary antiviral immune responses in IL-1R1$^{-/-}$ mice infected with influenza virus. IL-1R1$^{-/-}$ mice showed markedly reduced inflammatory pathology in the lung, primarily due to impaired neutrophil recruitment. Activation of CD4$^+$ T cells in secondary lymphoid organs and subsequent migration to the lung were impaired in the absence of IL-1R1. In contrast, activation of virus-specific cytotoxic T lymphocytes and killing of virus-infected cells in the lung were intact. Influenza virus-specific immunoglobulin G (IgG) and IgA antibody responses were intact, while the IgM response was markedly reduced in both serum and mucosal sites in IL-1R1$^{-/-}$ mice. We found significantly increased mortality in the absence of IL-1R1; however, lung viral titers were only moderately increased. Our results demonstrate that IL-1α/β mediate acute pulmonary inflammatory pathology while enhancing survival during influenza virus infection. IL-1α/β appear not to influence killing of virus-infected cells but to enhance IgM antibody responses and recruitment of CD4$^+$ T cells to the site of infection.

Interleukin-1α (IL-1α) and IL-1β are potent proinflammatory cytokines that are produced by a variety of cells and act on virtually every organ system of the body. Their biological activity is mediated by binding to the IL-1 receptor type 1 (IL-1R1), which recruits an accessory protein necessary for intracellular signal transduction. The potentially devastating autotoxic inflammatory response induced by IL-1α/β is balanced by the IL-1R antagonist (IL-1Ra), a naturally occurring inhibitor that binds to the IL-1R1 with a higher affinity than IL-1α/β and fails to recruit the accessory protein. The importance of IL-1Ra as an anti-inflammatory cytokine is shown in IL-1Ra-deficient mice, which spontaneously develop chronic synovial inflammation (18) and lethal arterial inflammation (34).

A body of evidence implicates IL-1 in resistance to infectious agents (10) including the intracellular bacteria *Listeria monocytogenes* and *Mycobacterium tuberculosis* (25, 30, 37). Evidence supporting a role for IL-1 in viral clearance is more indirect. IL-1 production is triggered by most viruses through activation of the extracellular signal-regulated kinase (ERK), double-stranded-RNA-dependent protein kinase (PKR), and NF-κB (7, 23, 31), which are all induced by double-stranded RNA that accumulates during viral replication. Some viruses have evolved strategies to increase virulence by interfering with the IL-1 response, highlighting the importance of this defense pathway (24). For instance, vaccinia virus has acquired a soluble form of the naturally occurring IL-1 decoy receptor (IL-1R type 2) and, additionally, an inhibitor of the interleukin-1β-converting enzyme, which prevents the proteolytic activation of IL-1β (1, 35, 42). Furthermore, vaccinia virus encodes two proteins with homologies to the TIR domain, which inhibits IL-1-mediated activation of the transcription factor NF-κB (5). Prior studies have demonstrated that IL-1 production was decreased in patients with chronic infections of hepatitis C virus (47) and in memory responses against Epstein-Barr virus infection (22). Of note, resistance to human cytomegalovirus (20), Epstein-Barr virus (19), and human immunodeficiency virus (44) is increased in individuals homozygous for allele 2 of the IL-1Ra gene (IL1RN*2), which is associated with a more prolonged and severe proinflammatory response than that in individuals with other IL-1Ra genotypes (19).

As yet, the precise role of IL-1 in the immune response against viral infections remains to be determined. To address this, we have studied, with mice lacking the IL-1R1, the cellular immunological pathways known to be important against influenza virus infection. Influenza virus infection represents a significant health problem, causing high morbidity and mortality worldwide despite vaccines and antiviral drugs. It induces a massive pulmonary inflammatory response during acute infection. Here we show that IL-1R1$^{-/-}$ mice are protected from the acute pathological granulocytic inflammatory response in the lung following infection, while survival was markedly decreased in the absence of IL-1R1.

MATERIALS AND METHODS

Animals, virus, and infection. IL-1R1$^{-/-}$ mice (30) (kindly provided by M. A. Labow) were backcrossed for seven generations onto a C57BL/6 background and maintained in facilities free of specific pathogens at the Basel Institute for Immunology and BioSupport, Zurich. C57BL/6 control mice were purchased from Charles River (France or Germany). Influenza virus strain PR8 (A/Puerto Rico8/34, H1N1) was originally provided by J. Pavlovic, University Zürich. At the age of 8 to 12 weeks, mice were infected intranasally with 100 PFU influenza virus. The mice were briefly anesthetized with isofluran and received for inhalation two times successively 50 μl virus in endotoxin-free phosphate-buffered saline (PBS) (10^3 PFU/ml). For lethality experiments, mice were infected with 2×10^3 PFU influenza virus PR8. For a period of 14 days mice were weighed and monitored for mortality daily.

Determination of viral titers in lungs of influenza virus-infected mice. Lung virus titers were determined as described previously in detail (2). Briefly, at the indicated days, lungs were isolated and homogenized in 1 ml SF medium, consisting of Iscove's modified Dulbecco's medium (IMDM) (Gibco) supplemented

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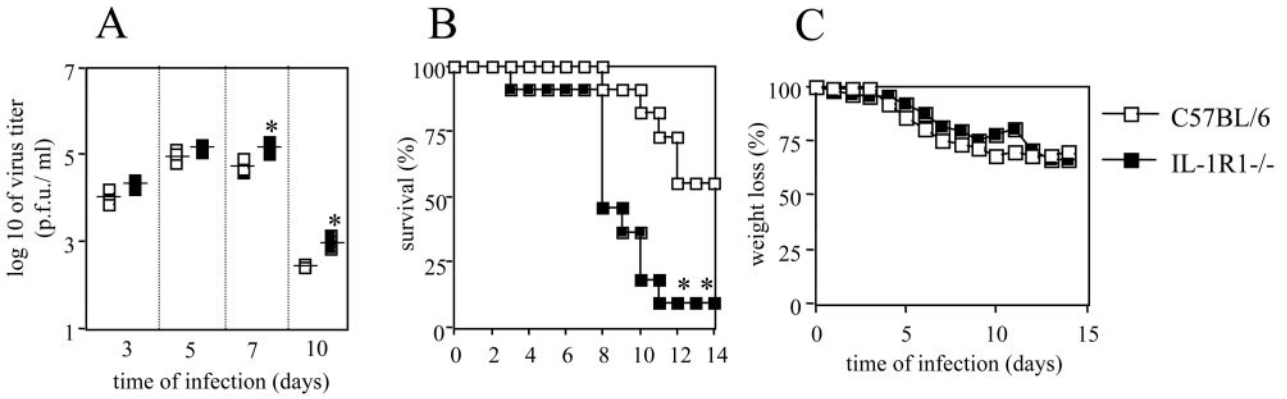

FIG. 1. Delayed viral clearance and enhanced mortality in IL-1R1$^{-/-}$ mice after infection with influenza virus. (A) C57BL/6 wild-type and IL-1R1$^{-/-}$ mice were infected intranasally with influenza virus PR8 (100 PFU). Lungs were removed at the indicated days and kept frozen until determination of viral titers. Values show $\log_{10}$ PFU/ml for individual mice (n = 4/group/day). Averages are indicated with horizontal lines. *, significant differences (Wilcoxon test). Groups of mice (n = 11/group) were infected intranasally with the influenza virus 50% lethal dose (2×10^3 PFU), and (B) survival and (C) weight were monitored during the course of infection. **, significant differences (P = 0.0023 by the Mantel-Haenszel test).

with 0.5% fetal calf serum (FCS), insulin (5 mg/liter), and Primatone RL (Quest International, Bussum, Netherlands), and subsequently centrifuged at 3,000 rpm at 4°C. Supernatants containing virus were diluted serially fivefold starting with 50 μl virus sample in 200 μl medium in 24-well plates in SF medium before addition of MDCK cells (7.5×10^5/ml) in 0.2 ml medium. Mixtures were incubated at 37°C for 6 h to allow cell adherence to the plastic. After an overlay of 300 μl methylcellulose was added, the virus mixture was incubated for 40 to 48 h at 37°C. Viral foci were stained with rat-anti influenza monoclonal antibody (MAb) (kindly provided by A. J. Caton, Wistar Institute) followed by horseradish peroxidase-labeled goat anti-rat immunoglobulin G (IgG) Fc (Chemicon) (diluted 1/1,000 in PBS, 10% FCS).

BAL. Mice were sacrificed with CO_2 at the specified time points after infection. The trachea of individual mice were cannulated with a 22-gauge needle (Microlane 3; Becton Dickinson, New Jersey) surrounded by plastic tubing (polyethylene intramedic tubing; Becton Dickinson). PBS (0.3 ml) was injected and withdrawn repeatedly with a syringe until a final volume of 1.2 ml was collected. Bronchoalveolar lavage (BAL) cells were harvested by centrifugation. Total cell numbers per BAL were determined with a Coulter Counter (IG Instrumenten Gesellschaft AG), and cells were processed for further analysis. Bronchoalveolar lavage fluid was used to measure specific antibody levels.

Characterization of BAL cells. BAL cells (5×10^4) of individual mice were isolated and fixed onto glass slides in PBS with 20% bovine serum albumin (BSA) by centrifugation ($600 \times g$, 10 min). Cells were differentiated by May-Grünwald/Giemsa staining: After fixation with methanol (96%; 2.5 min) cells were stained with undiluted May-Grünwald solution (Fluka) for 3 min. Thereafter the staining was performed in a 50% May-Grünwald solution for an additional 3 min. In a last step, cells were stained in 7% Giemsa solution (Fluka) for 10 to 12 min. Slides were rinsed with tap water and air dried overnight. Dried cells were embedded within Eukit solution under glass coverslips. The percentages of eosinophils, lymphocytes, macrophages, and neutrophils were determined by counting 200 cells, and the total number of each cell type was calculated from the total number of BAL cells (100%). Aliquots of BAL samples were stained with allophycocyanin-labeled anti-CD4 and phycoerythrin (PE)-labeled anti-CD8 MAbs after blocking of Fc receptor with anti-CD32/CD16 MAb (all from BD PharMingen) at 4°C and analyzed by flow cytometry.

Determination of virus-specific CD8$^+$ T cells. BAL cells (1×10^5) were incubated at 37°C for 15 min together with soluble phycoerythrin-conjugated tetramers (provided by A. Gallimore, University of Oxford, United Kingdom) composed of biotinylated murine class I monomers (H2-D^b), human β2 M, and influenza virus peptide NP68 (ASNENMDAM). Allophycocyanin-conjugated anti-CD8 monoclonal antibodies (BD PharMingen) were subsequently added and incubated on ice for another 15 min. Cells were washed and analyzed by flow cytometry (FACSCalibur; Becton Dickinson, Mountain View, California).

Measurement of cytotoxicity. Mediastinal lymph node (MLN) cells and BAL cells from influenza virus-infected mice were isolated at day 10 after infection. MLN cells (2×10^6) were restimulated with peptide NP68 (20 ng/ml) in 1 ml IMDM supplemented with 10% FCS and antibiotics for 5 days. Both 2×10^6 restimulated effector cells and 2×10^6 total BAL cells (pooled from four mice per group) were collected, washed, and resuspended in 0.3 ml medium. Duplicates of effector cells were serially diluted threefold in microtiter plates (starting with 1×10^6/well) and mixed with ^{51}Cr-labeled, NP68 peptide-pulsed EL-4 target cells (1×10^4 per well) before 5 h incubation. ^{51}Cr-labeled but non-peptide-pulsed EL-4 target cells were used as a control. ^{51}Cr release was measured in 70-μl supernatants of each 96 well.

Proliferation of spleen CD4$^+$ T cells. CD4$^+$ T cells were positively purified from spleens of individual mice by magnetic cell sorting (MACS) (purity, >95%) according to the instructions of the supplier (Miltenyi Biotec). A total of 2×10^5 CD4$^+$ T cells were restimulated in IMDM–10% FCS with 2×10^5 irradiated syngeneic antigen-presenting cells (APCs) from spleen. APCs were pulsed with UV light-inactivated influenza virus PR8 (starting concentration, 10^7 PFU/ml) in threefold serial dilution steps. At the third day of restimulation, cells were pulsed with [^{3}H]thymidine (1 μCi per well) for 12 h before [^{3}H]thymidine incorporation (in cpm) was measured.

Detection of virus-specific Igs. At the indicated time points, BAL fluids and sera of mice were analyzed for virus-specific IgM, IgG, and IgA antibodies. Ninety-six-well plates (Maxisorp; Nunc) were coated with UV light-inactivated influenza virus (PR8, 10^7 PFU/ml) in PBS overnight at 4°C. Between all of the following steps, plates were washed five times with PBS. Coated plates were blocked with PBS–1% BSA for 2 h at room temperature. BAL fluids and sera from individual mice were serially diluted in PBS–0.1% BSA starting with a 1:1 dilution for BAL fluids and a 1:100 dilution for sera, followed by incubation overnight at 4°C. Thereafter, alkaline phosphatase-labeled goat anti-mouse antibodies to IgG, IgM, and IgA (Southern Biotechnology Associates, Inc.) were added at room temperature for 2 h, followed by addition of the substrate *p*-nitrophenyl phosphate (Sigma-Aldrich). Optical densities were measured on an enzyme-linked immunosorbent assay (ELISA) reader at 405 nm.

RESULTS

IL-1 promotes clearance of influenza virus and protects from virus-induced mortality. To examine the role of IL-1 in the control of virus infection, C57BL/6 wild-type and IL-1R1$^{-/-}$ mice were infected intranasally with a sublethal dose (100 PFU) of influenza virus (PR8/A) and lungs were collected at the indicated days for determination of virus titers. IL-1R1$^{-/-}$ mice tended to have slightly increased lung viral titers throughout the course of infection; however, differences were statistically significant only on days 7 and 10 postinfection (Fig. 1A), and the virus was cleared by day 14 postinfection (data not shown). Infection with a high virus dose (2,000 PFU) led to markedly increased mortality in IL-1R1$^{-/-}$ mice (Fig. 1B), which was not associated with increased weight loss (Fig. 1C).

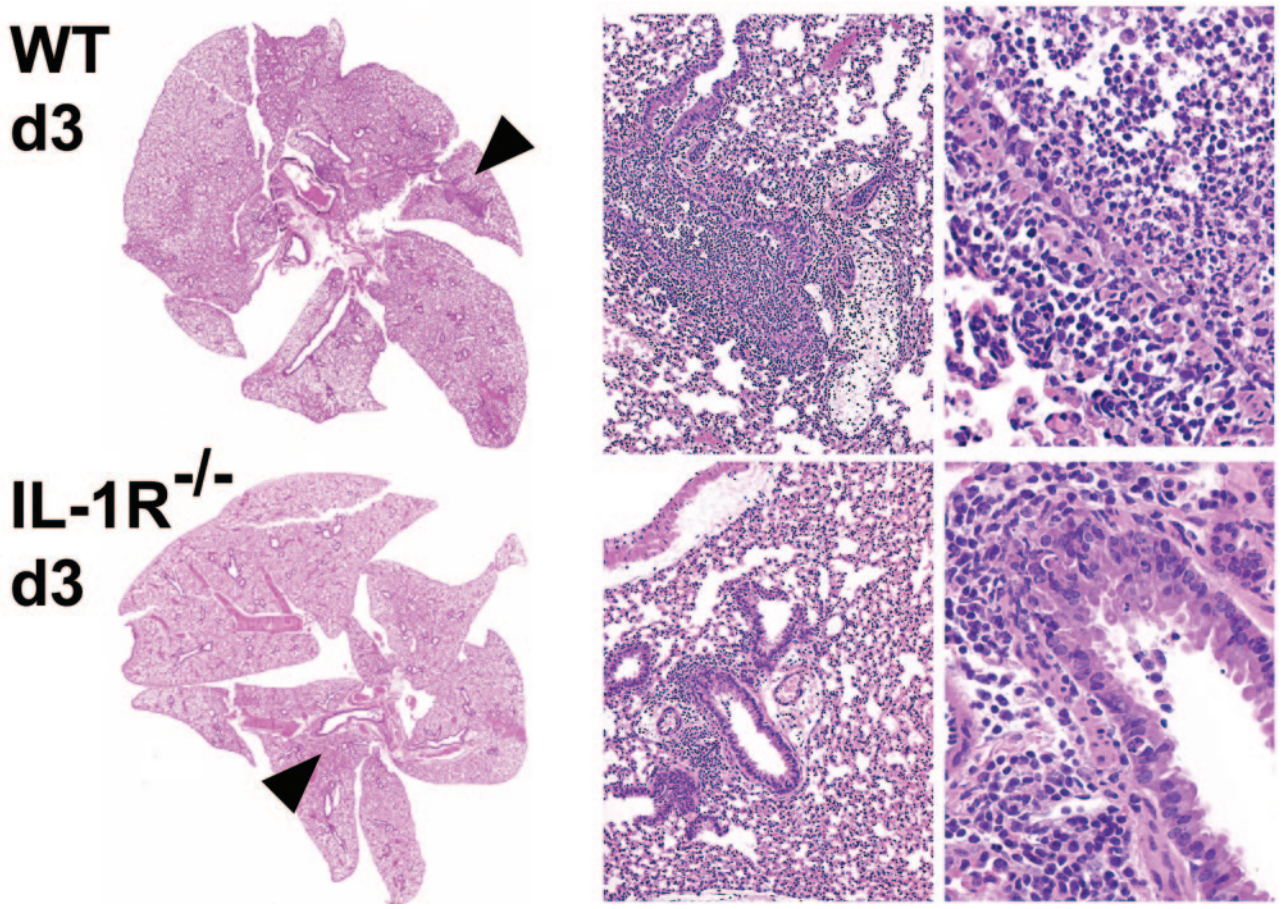

FIG. 2. Reduced pulmonary inflammation in IL-1R1$^{-/-}$ mice. C57BL/6 wild-type (WT) mice (top row) and IL-1R1$^{-/-}$ mice (bottom row) were infected with influenza virus PR8 (100 PFU), and lungs were taken for histology on day 3. C57BL/6 mice showed areas of severe pathology, with massive peribronchial lymphocytic inflammation and the lumen filled with exudates of neutrophils and mucus. Affected areas of the lungs from IL-1R1$^{-/-}$ mice showed mild pathology with few intraepithelial neutrophils and peribronchial lymphocytes (hematoxylin-eosin staining was used; magnifications [left to right], ×5, ×50, and ×200).

Increased mortality was also found after infection with an intermediate virus dose (500 PFU), which killed 33% of the IL-1R1$^{-/-}$ mice but none of the wild type. These results demonstrate that IL-1α/β–IL-1R1 interactions promote survival after influenza virus infection, although it plays a moderate role in control of viral replication.

IL-1 is responsible for neutrophil recruitment and inflammatory lung pathology. Lungs were evaluated by histology on days 3 and 5 postinfection. On day 3 postinfection, C57BL/6 wild-type mice showed a spectrum of lesions (Fig. 2). It ranged from single bronchial epithelial cell apoptosis with intraepithelial neutrophil granulocytes to extended bronchial epithelial cell erosions and lymphocytic peribronchial infiltrates. In IL-1R1$^{-/-}$ mice the histological lesions at day 3 were largely limited to single bronchial epithelial cell apoptosis with few intraepithelial neutrophils and peribronchial lymphocytes. On day 5 postinfection both C57BL/6 wild-type mice and IL-1R1$^{-/-}$ mice showed more extensive lung pathology with peribronchial and perivascular inflammation, as well as interstitial inflammation and hemorrhage involving the lung parenchyma in a patchy distribution. Differences between C57BL/6 wild-type mice and IL-1R1$^{-/-}$ mice were not as striking as on day 3 (not shown).

To assess the role of IL-1 in recruitment of cells into the lungs of virus-infected mice, we isolated BAL fluid on the indicated days after infection, counted total cell numbers, and determined cell types by morphological differentiation. Compared to those from wild-type mice, BAL samples from IL-1R1$^{-/-}$ mice showed a strikingly reduced total cell number on days 3, 5, and 7, primarily due to decreased numbers of neutrophils and lymphocytes (Fig. 3A). In contrast, BAL cell numbers were comparable on day 10 after infection (Fig. 3A).

To further assess T-cell subsets, BAL cells were stained with antibodies against CD4 and CD8 and analyzed by flow cytometry. On day 7, average frequencies of 9.4% CD4$^+$ and 50.4% CD8$^+$ T cells in the lymphocyte population were found in BAL fluid of wild-type mice, indicating a predominant CD8$^+$ T-cell infiltration (Fig. 3B). IL-1R1$^{-/-}$ mice contained 5.1% CD4$^+$ and 33.5% CD8$^+$ T cells, a reduction of 43% and 30%, respectively, compared to the wild type. On day 10, when virus was almost cleared from the lung and T-cell infiltration peaks, BAL fluid of wild-type mice contained 20.9% CD4$^+$ and 70.2% CD8$^+$ T cells. CD4$^+$ T-cell numbers in lungs of IL-1R1$^{-/-}$ mice remained twofold reduced, whereas CD8$^+$ T-cell numbers were comparable to those in wild-type mice. In keeping with these results, staining of BAL cells with major histocom-

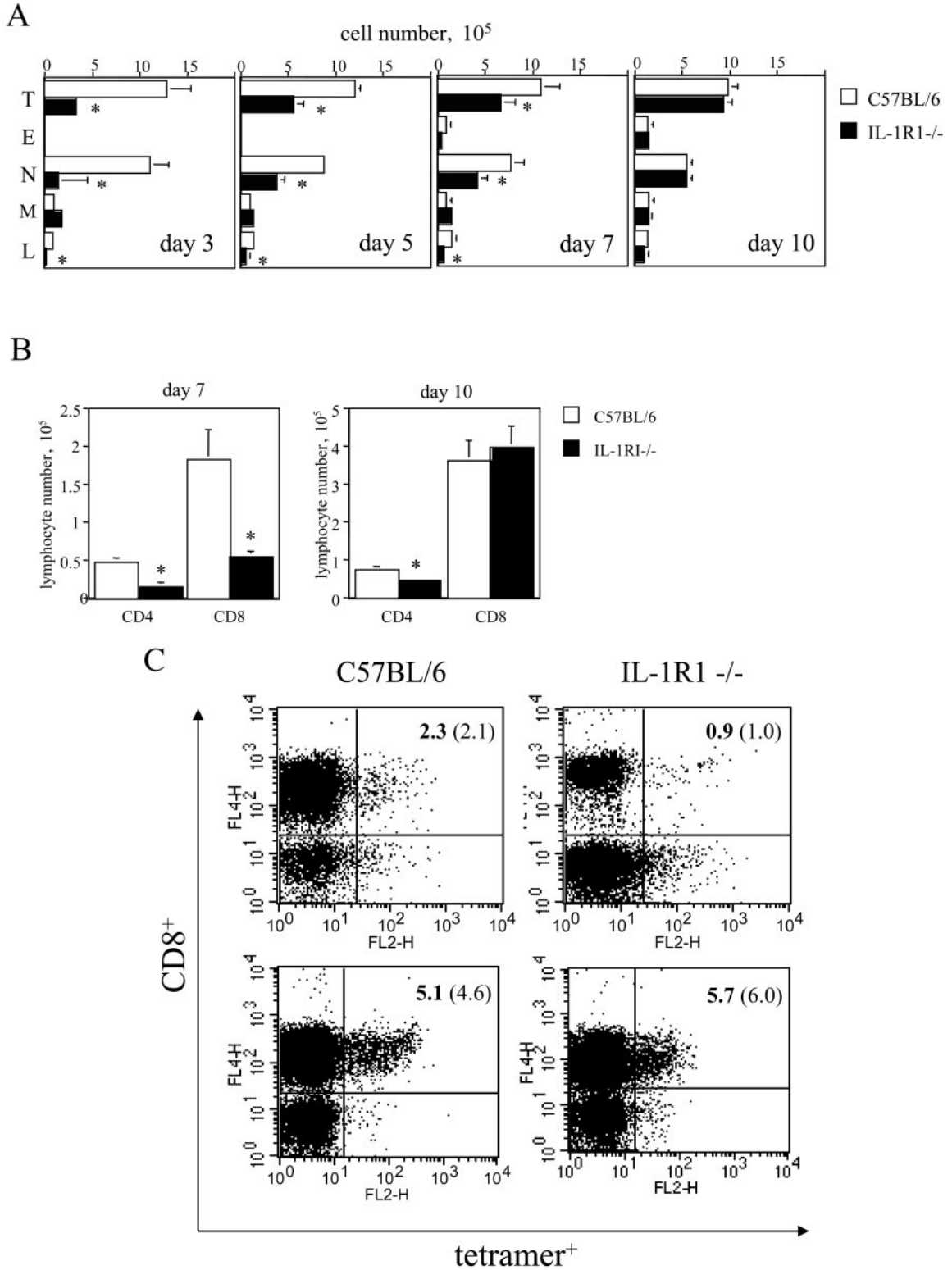

FIG. 3. Impaired recruitment of neutrophils and $CD4^+$ T cells to the lung in the absence of IL-1R1. Groups of mice were infected intranasally with 100 PFU influenza virus PR8. Lung-infiltrating cells were isolated by BAL. (A) Total BAL cell number (T) was measured using a Coulter Counter (Instrumenten Gesellschaft IG, Basel, Switzerland), and eosinophils (E), neutrophils (N), monocytes/macrophages (M), and lymphocytes (L) were determined by differential counts according to morphological criteria as described in Materials and Methods. Numbers represent average values for four mice ± standard deviations. (B) On days 7 and 10, BAL cells were incubated with anti-CD32/CD16 MAb to block unspecific binding and subsequently stained with allophycocyanin-labeled anti-CD4 and PE-labeled anti-CD8 MAbs. Cells were acquired using a FACSCalibur (Becton Dickinson) and data analyzed with Cell Quest software. $CD4^+$ and $CD8^+$ T-cell numbers were calculated from percentages of BAL total cell numbers excluding dead cells. Values show averages for four mice per group. *, significant differences (Student test). (C) On days 7 (upper panels) and 10 (lower panels), aliquots of BAL cells were stained with PE-conjugated class I tetramers loaded with influenza virus peptide NP68 and allophycocyanin-conjugated anti-CD8 MAb before analysis by flow cytometry. Values show percentages of virus-specific $CD8^+$ T cells gated on lymphocytes of an individual mouse representative of the group. Average percentages from groups of mice ($n = 4$) are shown in parentheses.

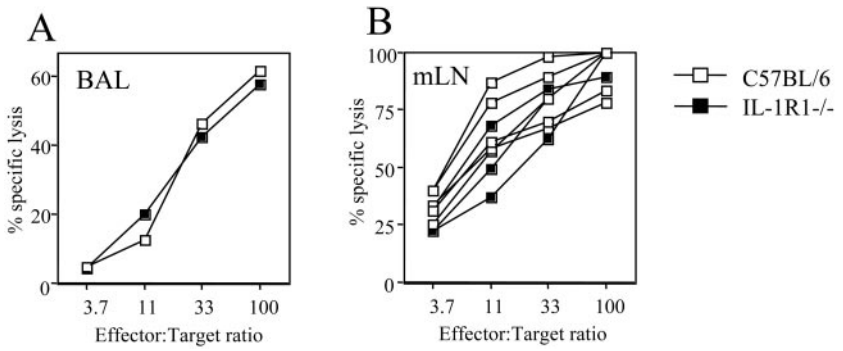

FIG. 4. IL-1R1$^{-/-}$ mice exhibit normal cytotoxic T-cell effector responses. Mice were infected with 100 PFU influenza virus PR8 and analyzed on day 10 postinfection. (A) To determine primary CTL responses at the site of virus infection, pools of BAL cells from groups of mice (n = 4/group) were serially diluted threefold in microtiter plates starting with 1×10^6 cells before addition of ^{51}Cr-labeled NP68 peptide-pulsed EL-4 target cells (1×10^4 per well) and incubated for 5 h before measurement of ^{51}Cr release. (B) Mediastinal lymph node cells were restimulated in vitro with specific peptide for 5 days. Effector cells were mixed with EL-4 target cells at the ratios indicated. Values show percent lysis. Nonspecific lysis of target cells not pulsed with peptide was below 2% (not shown).

patibility complex class I tetramers loaded with influenza virus peptide showed that the frequency of virus-specific CD8$^+$ T cells in IL-1R1$^{-/-}$ mice was reduced by 50% at day 7 but comparable to controls at day 10 after infection (Fig. 3C). Therefore, we conclude that IL-1 is critical for efficient pulmonary recruitment of CD4$^+$ T cells but less important for CD8$^+$ T-cell migration.

IL-1 does not affect the CTL response to influenza virus infection. BAL cells were used to measure killing of virus-infected cells directly ex vivo in a primary ^{51}Cr release assay on days 7 and 10 postinfection. In addition, MLN cells were restimulated with specific peptide in vitro for 5 days before measurement of lytic activity. On day 10 postinfection, cells isolated directly from the site of infection (Fig. 4A) or expanded from the draining lymph node (Fig. 4B) showed comparable specific cytotoxicities, irrespective of the absence of the IL-1R. On day 7 postinfection, the lytic activity of cells derived from both the MLN and BAL was reduced compared to that on day 10, reflective of the lower number of virus-specific cells, and no significant differences were detected between groups (not shown). These data demonstrate that IL-1 is dispensable for priming and effector function of cytotoxic T lymphocytes (CTLs) after influenza virus infection.

IL-1 enhances priming of CD4$^+$ T-helper cells during influenza virus infection. Since we found a reduced number of CD4$^+$ T cells in BAL fluid of IL-1R1$^{-/-}$ mice, we analyzed whether IL-1 affects T-helper cell priming in peripheral lymphoid organs. We therefore isolated spleen CD4$^+$ T cells and performed a proliferation assay on day 7 after infection. As expected, we found a strong antigen-specific proliferation of CD4$^+$ T cells from wild-type mice (Fig. 5). In contrast, IL-1R1$^{-/-}$ mice showed a dramatic decrease in CD4$^+$ T-cell proliferation, suggesting that efficient T-helper cell priming in peripheral organs is dependent on a functional IL-1 signal.

IL-1 promotes the production of antiviral IgM antibodies by B cells. Both IgM and IgG antibody responses have been shown to be important for virus clearance and protection against infection (3, 28, 36). To measure systemic and mucosal antiviral antibody responses, we isolated sera and BAL fluid from mice on days 7 and 10 after infection and performed an ELISA. Virus-specific IgM antibody levels were strongly reduced in BAL fluid (Fig. 6A and B) and sera (Fig. 6A and C) of IL-1R1$^{-/-}$ mice on days 7 (Fig. 6A) and 10 (Fig. 6B and C) postinfection. Mucosal IgA responses in the lung were slightly, but not significantly, impaired in the absence of IL-1R1. In contrast, antiviral IgG antibody responses appeared to be intact in IL-1R1$^{-/-}$ mice (Fig. 6B and C). Taken together, these results demonstrate that IL-1 promotes IgM production but is dispensable for IgG and IgA antibody responses during influenza virus infection.

DISCUSSION

Infection of mice with influenza virus leads to a dose-dependent decrease in locomotor activity, body weight, body temperature, and survival (9, 45). While several cytokines, including IL-1, are capable of inducing fever, anorexia, and weight loss (10), the endogenous mechanisms and mediators of these responses and their effects on immune responses to influenza virus infection are not well defined. Studies with IL-1β-knock-

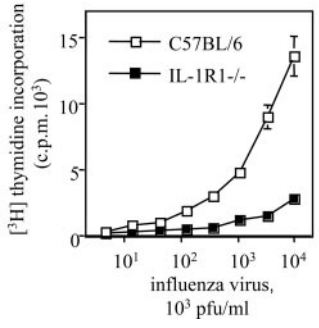

FIG. 5. Strongly reduced priming of spleen CD4$^+$ T cells from influenza virus-infected IL-1R1$^{-/-}$ mice. To determine proliferation of virus-specific Th cells, groups of mice were infected (n = 4/group) with 100 PFU influenza virus, and 7 days later CD4$^+$ T cells were purified from the spleens of individual mice via positive selection by MACS (purity, >95). Isolated cells were stimulated with irradiated splenocytes from uninfected C57BL/6 mice together with graded doses of UV light-inactivated influenza virus for 72 h. [^{3}H]thymidine was added for the final 12 h of the culture. Numbers represent the average [^{3}H]thymidine incorporation (cpm) of wild-type and knockout cells.

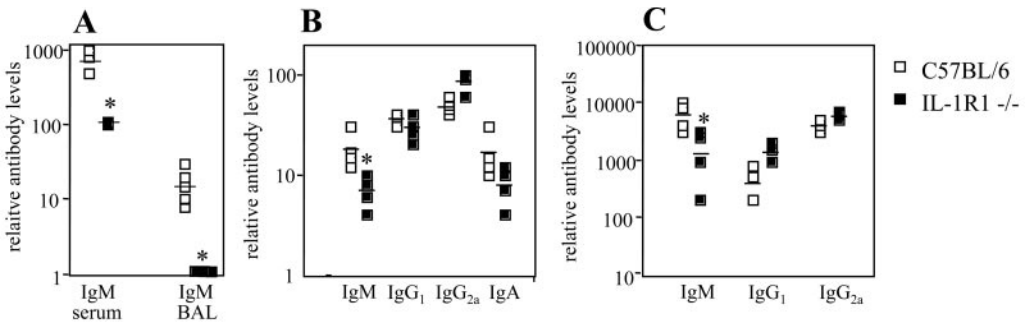

FIG. 6. Reduced IgM and IgA antibody response in influenza virus-infected IL–1R1$^{-/-}$ mice. Groups of mice were infected with 100 PFU influenza virus. To examine antibody responses, mice were bled and BAL fluid was isolated on days 7 (A) and 10 (B and C). Virus-specific antibody levels of the indicated isotypes were measured by ELISA. Values represent the BAL fluid (A and B) and serum (A and C) dilutions at half-maximum absorption (optical density at 405 nm). Lines indicate averages. *, significant differences (Student test).

out mice showed that the drop in body temperature, but not weight, was partially dependent on IL-1β (29) In line with these data, we found that weight loss was comparable in IL-1R1$^{-/-}$ and wild-type mice infected with influenza virus. Previous reports have arrived at the opposite conclusion regarding the importance of IL-1 for survival during influenza virus infection, showing slightly reduced survival in IL-1β-deficient mice and increased survival in mice treated with IL-1Ra (29, 43). Our results clearly demonstrate that absence of IL-1R1 increases mortality from influenza virus infection. Interestingly, lung virus titers were only moderately increased in IL-1R1$^{-/-}$ mice, probably reflective of reduced CD4$^+$ T-cell priming and/or IgM responses. In support of this, mice deficient in the production of IgM, but not other antibody isotypes, show a moderate increase in viral titer but not in mortality (reference 28 and unpublished data). Thus, the moderately increased virus titer detected in IL-1R1$^{-/-}$ mice may be one reason, but it is unlikely the only one, for the highly increased mortality. IL-1β-induced hypothermia in influenza virus-infected mice may be an adaptive response protecting from mortality (29).

In contrast to the beneficial effect IL-1 has on survival after influenza virus infection, we found that IL-1 is responsible for acute inflammatory lung pathology, as the number of neutrophils recruited to the lung on day 7 postinfection was dramatically reduced in the absence of IL-1R1. However, on day 10 the number of neutrophils was comparable in IL-1R1$^{-/-}$ mice and wild-type mice, suggesting that additional factors also regulate neutrophil migration in wild-type mice or that a compensatory mechanism in IL-1R1$^{-/-}$ mice allows neutrophil recruitment late after infection. A role of IL-1 in the mobilization of neutrophils was recently described with murine models of renal and hepatic ischemia reperfusion injury (17, 26). In those studies, IL-1R1$^{-/-}$ mice showed less neutrophils in postischemic renal and hepatic tissue, probably due to reduced expression of the chemokine macrophage inflammatory protein-2 (MIP-2) (26). On the other hand, IL-1R1$^{-/-}$ mice infected with *M. tuberculosis* showed abundant neutrophil infiltration in granulomatous lesions in the lung, comparable to the case for wild-type mice (25). The kinetics of neutrophil accumulation in lungs of influenza virus-infected IL-1R1$^{-/-}$ mice may give some clues to understanding the differences in the importance of IL-1 for neutrophil migration in those reports. IL-1 is essential for the immediate neutrophil accumulation in diseased organs after ischemic injury or after acute viral infection, whereas it plays less of a role in neutrophilia at later stages of infection and in more chronic infections such as tuberculosis.

Neutrophils are thought to be a first line of defense against invading pathogens. Scientific evidence is based mainly on studies of mice infected with the intracellular bacterium *Listeria monocytogenes*, which become more susceptible after depletion of neutrophils by using anti-Gr1 antibody (8, 38). Our results show that impaired accumulation of neutrophils in the lung did not result in a significantly increased viral titer in IL-1R1$^{-/-}$ mice at day 5 of infection and that IL-1R1$^{-/-}$ mice succumbed to influenza virus infection at a later stage, when accumulation of neutrophils was comparable in IL-1R1$^{-/-}$ mice and wild-type mice. These results suggest that the immunopathology of acute granulocytic lung inflammation mediated by IL-1 plays little if any role in clearance of influenza virus.

We also observed a striking reduction in the number of CD4$^+$ T cells that accumulated in the lungs of virus-infected IL-1R1$^{-/-}$ mice, whereas the recruitment of CD8$^+$ T cells was slightly delayed but total CD8$^+$ T cells numbers in the BAL were not reduced at the peak of the response. Similarly, we reported previously that CD4$^+$ T-cell but not CD8$^+$ T-cell recruitment to the lung was impaired in aeroallergen-immunized IL-1R1$^{-/-}$ mice, which were protected from asthma-like responses, including pulmonary eosinophilia (33, 40). In this model, impaired eosinophil migration in IL-1R1$^{-/-}$ mice could be explained by the reduced number of IL-5-producing Th2 cells which were recruited to the lung.

Migration of neutrophils is critically mediated by the two C-X-C chemokines, MIP-2 and cytokine-induced neutrophil-chemoattractant-1 (41), which are both regulated by IL-1 (6, 27). Thus, reduced influx of neutrophils to the lung after influenza virus infection may result from reduced MIP-2 and cytokine-induced neutrophil-chemoattractant-1 production.

Control of influenza virus infection is mediated by a concerted activity of CTLs, CD4$^+$ T cells, and B cells (11, 12, 16). IL-1 has been suggested to control both T-cell activation and antibody responses. Our data demonstrate that primary and secondary cytotoxic responses against influenza virus-infected cells by CD8$^+$ T cells in the BAL and lung draining lymph nodes, respectively, remained unaffected, consistent with normal numbers of influenza virus-specific CTLs in the BAL fluid of IL-1R1$^{-/-}$ mice at the peak of the response. In contrast, we

found that proliferation of virus-specific $CD4^+$ T cells was impaired in IL-$1R1^{-/-}$ mice, which may explain the reduction in number of $CD4^+$ T cells in the BAL fluid of virus-infected mice. Reduced $CD4^+$ T-cell priming in IL-$1R1^{-/-}$ mice may result from a defect in activation and maturation of APCs rather than a direct requirement for IL-1 in T-cell activation. Nakae et al. showed that APCs from IL-1α/β-deficient mice failed to properly activate wild-type $CD4^+$ T cells in vitro (32), and in support of this we succeeded in restoring development of autoimmune myocarditis by adoptive transfer of wild-type dendritic cells (DCs) into IL-$1R1^{-/-}$ mice, which were otherwise protected from disease (13).

Several papers reported apparently contradictory findings on antibody responses in mice lacking IL-1R1 or IL-1α/β. Using sheep red blood cells as antigen, Nakae et al. found strongly reduced antibody responses of all isotypes in IL-$1\alpha/\beta^{-/-}$ mice (32), whereas others reported normal antibody responses in IL-$1R1^{-/-}$ mice immunized with soluble protein (i.e., TNP-keyhole limpet hemocyanin) in adjuvant (i.e., complete Freund adjuvant) (15). These discrepancies may be explained by usage of different antigens with or without adjuvant. In fact, we found that IL-$1R1^{-/-}$ mice mounted reduced antibody responses after repeated low-dose immunization with OVA in the absence of adjuvant, whereas IgG and IgE responses were normal when mice were immunized with OVA in the presence of alum (40). In the current study, we showed that influenza virus triggered normal IgG1 and IgG2a antibody responses in IL-$1R1^{-/-}$ mice both in serum and in the BAL fluid, suggesting that $CD4^+$ T-cell help for B-cell antibody responses and immunoglobulin class switching to IgG isotypes were largely intact in vivo despite strongly reduced proliferation of virus-specific $CD4^+$ T cells in vitro. Experiments by Sangster et al. using bone marrow chimeric mice that lack expression of major histocompatibility complex class II (Ia^b) molecules only on B cells recently demonstrated that antibody responses of all isotypes, including IgM and IgA, to influenza virus are dependent on help by $CD4^+$ T cells (39). Moreover, influenza virus-induced IgM and IgG antibodies are produced by B2 cells upon infection, whereas innate natural IgM antibodies specific for influenza virus are secreted by B1 cells and do not expand upon infection (3). Our results may suggest that IL-1 is required for efficient IgM responses to influenza virus by B1 cells. We and others have reported that IgM contributes to efficient control of acute influenza virus infection (4, 28). Thus, increased virus titers in IL-$1R1^{-/-}$ mice could be explained by reduced levels of virus-specific IgM antibodies.

Influenza virus causes a highly inflammatory pneumonia, and a treatment that reduces this inflammatory response without inhibiting virus clearance may prevent airway occlusion and increase respiratory functions. Depletion of the inflammatory cytokine tumor necrosis factor alpha reduces recruitment of inflammatory cells to the lung and the severity of disease without affecting virus clearance upon secondary infection with respiratory syncytial virus or influenza virus (21). We show here that IL-1 signaling underlies the acute inflammatory lung pathology evident during influenza virus infection and protects against lethality after high-dose infection, but only partially by limiting viral replication in the lung.

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