Differential Role of the Interleukin-17 Axis and Neutrophils in Resolution of Inhalational Anthrax

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The roles of interleukin-17 (IL-17) and neutrophils in the lung have been described as those of two intricate but independent players. Here we identify neutrophils as the primary IL-17-secreting subset of cells in a model of inhalation anthrax using A/J and C57BL/6 mice. With IL-17 receptor A knockout (IL-17RA−/−) mice, we confirmed that IL-17A/F signaling is instrumental in the self-recruitment of this population. We also show that the IL-17A/F axis is critical for surviving pulmonary infection, as IL-17RA−/− mice become susceptible to intranasal infection by Bacillus anthracis Sterne spores. Strikingly, infection with a fully virulent strain did not affect IL-17RA−/− mouse survival. Eventually, by depleting neutrophils in wild-type and IL-17RA−/− mice, we demonstrated the crucial role of IL-17-secreting neutrophils in mouse survival of infection by fully virulent B. anthracis. This work demonstrates the important roles of both IL-17 signaling and neutrophils in clearing this pathogen and surviving pulmonary B. anthracis infection.

The lung is a delicate and vital organ which is constantly exposed to pollutants and pathogens through air sampling (22). To achieve gaseous exchange through the thin membranes separating air in the alveoli from the capillaries, the lung has developed a highly efficient and balanced immune system which maintains balance between inflammation and tolerance. Studies on the role of the innate immune system in protecting against inhalational anthrax have focused mainly on alveolar phagocytes such as macrophages and dendritic cells. Anthrax is a disease caused by Bacillus anthracis, a Gram-positive, rod-shaped, spore-forming bacterium whose spores are considered a serious bioterrorism agent (25). B. anthracis spores cause three main clinical forms of the disease, depending on the route of infection: cutaneous, inhalational, and gastrointestinal anthrax (16). Intriguingly, prognoses of B. anthracis mucosal infections (pulmonary and intestinal) are dreadful, whereas cutaneous infection is known to be diagnosed easily and is readily cured. This suggests that the mucosal and skin-associated immune systems differ greatly in their handling of the pathogen.

The pulmonary route of infection is of paramount interest in terms of bioterrorism. The pathophysiology of inhalational anthrax is still a matter of discussion (7, 16, 47). It has been studied in multiple models, including mainly mouse, guinea pig, rabbit, and nonhuman primate (NHP) models, among which rodents are by far the most studied species (16, 50). In mice, recent studies have proven that entry of B. anthracis is not restricted to the lower respiratory tract, as initially thought, but may occur dually at the upper airways through the nasal mucosa-associated lymphoid tissue (NALT) (14, 15). In the lower respiratory tract, we and others have already shown that B. anthracis spores are managed during early infection in a coordinated manner by lung phagocytes (46, 49). Spores are immediately phagocytosed by alveolar macrophages lodged along the alveolar pneumocytes. Lung epithelial cells have also been described to phagocytose spores after inhalation (43). After a slight delay, lung dendritic cells (DCs) capture spores and rapidly transport them to the draining thoracic lymph nodes, playing the role of a Trojan horse (5). There, the spores start to transform into metabolically active, toxin-producing, replicating bacilli. B. anthracis toxins are formed by the association of enzymatically active moieties, lethal factor (LF) and edema factor (EF), with protective antigen (PA) as a cell surface binding component, generating lethal toxin (LT) and edema toxin (ET), respectively (36). Both toxins play a major role in bacterial evasion of the host innate and adaptive immune systems (47, 48).

So far, little is known about the lung innate immune responses that are triggered after spore phagocytosis and processing. The specific role of neutrophils in lung immunity has not been explored thoroughly, although one report suggests it to be minor (6). Indirect arguments based on subcutaneous and intravenous models of infection were recently reported. Using myeloid-specific B. anthracis toxin receptor capillary morphogenesis protein 2 (CMG2) knockout mice, it was shown that toxin impairment of myeloid cells, including neutrophils, is critical for establishing disease (31).

Interleukin-17 (IL-17) is the prototype of a large family of six cytokines that play a potent role in regulating neutrophil responses (10, 51). IL-17 (also referred as IL-17A) in association with IL-17F—both of which are capable of signaling through IL-17 receptor A (IL-17RA)—acts as a master coordinator of the response. Neutrophil recruitment is induced indirectly by IL-17 through triggering of epithelial cells to secrete CXC chemokines.
The protective role of IL-17 in host defense against pathogens in the lung has been demonstrated for numerous pathogens, including bacteria, fungi, and viruses (reviewed in reference 26). IL-17 can be either protective or detrimental, as recently exemplified in cases of influenza virus infection, as IL-17RA knockout mice survived significantly longer than wild-type (WT) mice (9). This highlights the delicate balance between inflammation and clearance of infection in the lung, as well as the role played by neutrophils and IL-17.

Several studies have pointed out the variety of IL-17 sources at mucosal sites, emphasizing that IL-17 bridges innate and adaptive immunity (10). In the lung, besides the classical Th17 lineage, innate cells such as γδ T and NKT cells produce IL-17 during infection, sparking a quick start to the immune response. Recent reports suggest that neutrophils may also be a novel player in the game, as shown in noninfectious inflammation models such as acute ischemia-reperfusion in the kidney (30), lung lipopolysaccharide (LPS)-induced neutrophilia (13), and acute vasculitis (23). More recently, neutrophils have been shown to produce IL-17A in the lung after fungal infection by Aspergillus fumigatus (54). Taken together, the extended diversity of IL-17 sources in response to stress or pathogens emphasizes its roles from early to late stages of infection. Given the diversity of IL-17 sources in response to stress or pathogens depending on the strain type.

Materials and Methods

Animals. Six- to 10-week-old A/J mice (Harlan), C57BL/6 WT mice (CERI), IL-17RA−/− mice (obtained from Bernhard Ryffel, CNRS UMR6218, Orléans, France), and CX3CR1gfp/hp mice (Jackson Laboratories) were housed under clean standard conditions. IL-17RA−/− and CX3CR1gfp/hp mice (all on a C57BL/6 background) were bred at the Plateforme de Haute Technologie Animale (La Tronche, France). All experimental protocols were approved by the local animal use committee Agede Haute Technologie Animale (La Tronche, France). All experiments were performed in biosafety level 2 (BSL2) and BSL3 facilities, depending on the strain type.

Bacterial counting. For bacterial enumeration, mice were infected intranasally with 2 × 108 spores of Sterne strain 7702 and killed at 3 days postinfection. Suspensions of lung cells were obtained by mechanical disruption in 1 ml of ice-cold distilled water. Homogenates were then serially diluted (for bacillus counting) or heated at 65°C for 30 min prior to postinfection. Sample dilutions and agar medium were thoroughly mixed by alternating rotation and back-and-forth motion of plates. We let the agar solidify and incubated it promptly for 24 h at 37°C prior to CFU counting.

Neutrophil depletion. Use of a Ly6G-specific monoclonal antibody is an established technique for depleting neutrophils in mice in vivo (11, 38).

In our study, mice were injected intraperitoneally (i.p.) with 100 μl of 1A8 monoclonal antibody (BioXCell) at a concentration of 0.25 mg/ml in PBS. Five hours following neutrophil depletion, mice were infected by the intranasal route with 2 × 108 spores of Sterne strain 7702, and survival was monitored each day until day 15. Injection was repeated on days 1 and 2 and then every 2 days until day 15, the last time point of the survival experiments.

Complement depletion. C57BL/6 mice were injected i.p. with two 5-U doses of cobra venom factor (CVF) 4 h apart to deplete the animals of C5 (19, 44). The mice were injected with 5 U of CVF every fifth day until day 15.

Forty-eight hours following CVF injection, mice were infected by the intranasal route with 2 × 108 spores of Sterne strain 7702, and survival was monitored each day until day 15.

BAL and lung cell analysis. For bronchoalveolar lavage (BAL), 1 ml of ice-cold PBS–2 mM EDTA (Sigma-Aldrich) was injected into the trachea, collected, and kept on ice prior to treatment. Single-cell suspensions of lung cells were obtained after physical dissociation using a GentleMACS dissociator (Miltenyi Biotech) followed by enzymatic digestion (30 min in a humidified incubator at 37°C with 5% ambient CO2) of lung tissue in RPMI 1640 medium (Sigma-Aldrich) containing collagenase type I (1 mg/ml; Worthington Biochemical) and Dnase I (50 U/ml; Sigma-Aldrich). Red blood cells were lysed in NH4Cl (1×) solution. Cells were resuspended in PBS–1% bovine serum albumin (BSA)–0.1% azide before staining.

For microscopy analysis, lung preparations were cytocoentrifuged and mounted with Vectashield DAPI medium (4′,6-diamidino-2-phenylindole; Dako) or stained with Giemsa stain (RAL 555 kit). Cells were examined on a Zeiss Axioscope 2 microscope to image Giemsa staining and on an Olympus IX-81 microscope for fluorescence microscopy.

Flow cytometry analysis. Lung cell suspensions were incubated with Fc receptor blocking antibody (anti-mouse CD16/CD32 Ab, clone 2.4G2; BD Biosciences) for 30 min at 4°C. We used the following monoclonal antibodies (Mabs) (all from BD Biosciences, except where stated otherwise): anti-mouse CD3ε (145-2C11), CD4 (L3T4), CD8α (53-6.7), CD11b (M1/70), CD11c (H3L), CD25 (3C7), CD45 (30-F11), CD335 (NKP46, 29A1.4), Gr-1 (RB68C5), T-cell receptor γδ (TCRγδ) (GL3), Ly6G (1A8), TCRβ (H57-597; eBioscience), and CD11d tetramer (a kind gift of Murielle Pichavant, Institut Pasteur de Lille), as well as correspond-
ing isotypes. Cells were stained for 30 min at 4°C. For intracellular staining, cells were permeabilized with saponin and stained for IL-17A MAb (eBio17B7; eBiosciences) at 4°C. Cell acquisition was conducted on a model FC500 (Beckman Coulter) flow cytometer, and data were analyzed with FlowJo software.

Cytokine measurement. Cytokine concentrations in BAL fluid (BALF) were measured by enzyme-linked immunosorbent assay (ELISA), using a DuoSet assay for IL-17A and IL-17F and a Quantikine immunoassay (R&D Systems) for IL-22, IL-6, IL-12p70, and gamma interferon (IFN-γ), according to the manufacturer’s instructions.

Statistical analysis. Statistical analysis was performed using GraphPad Prism software (version 4.0c; GraphPad Software, Inc.). Statistical differences between the groups were determined by a nonparametric test, the Mann-Whitney test, and by the log rank test for survival analysis.

RESULTS
IL-17A and IL-17F are present in BALF after inhalational anthrax. We first aimed to determine whether IL-17 is produced after i.n. infection with B. anthracis. We therefore investigated the
cytokines present in the BALFs of A/J mice (C5 deficient), a largely used model of mouse infection with strain Sterne (Fig. 1A). Mice were infected with B. anthracis Sterne strain 7702 via the i.n. route and were sacrificed serially from day 0 (noninfected) to day 4 to determine the cytokine concentrations in the BALF samples.

We observed a robust production of IL-17A, IL-22, IL-6, IL-12p70, and IFN-γ in the BALF samples starting as soon as 24 h postinfection and reaching a peak at day 3 (Fig. 1A). Beyond this time point, mice started to die, and all mice were dead by day 4. Since IL-17A, IL-17F, and IL-22 were produced in significant amounts on day 3, we focused the rest of our investigations on this time point (Fig. 1B). Using different doses of B. anthracis spores, we observed that IL-17A was produced significantly only with an infective quantum above 2 × 10^8 spores per mouse (Fig. 1C). Furthermore, for A/J mice as well as C57BL/6 and IL-17RA−/− mice (used later in this study), CFU analysis did not show any CFU in pulmonary homogenates 3 days after intranasal infection with B. anthracis Sterne strain spores. A/J (A) and C57BL/6 WT and IL-17RA−/− (B) mice were infected intranasally with 2 × 10^8 spores of Sterne strain 7702 of B. anthracis. (A) CFU of bacilli (open diamonds) and spores (closed diamonds) in lungs of A/J mice at 3 days postinfection. Each symbol represents one mouse. * P < 0.05; ** P < 0.01; *** P < 0.001; ns, not significant.

We then focused on neutrophils recruited to the lungs of C57BL/6 mice. In parallel, we performed the same analysis on C57BL/6 mouse lungs (Fig. 4A), because A/J mice are deficient in C5 and this factor is critical for IL-17 production by T cells (20). Using knock-in heterozygous CX3CR1^+/−/Gr-1^high mice, we could differentiate monocytes (CX3CR1^+/−) and neutrophils (CX3CR1^−) that both express Gr-1 (29). We confirmed that the IL-17^+^ population corresponded to CD11b^+^ Gr-1^high^ CX3CR1^+^ cells (Fig. 4B). Accordingly, IL-17A-positive cells exhibited multilobulated ring-shaped nuclei characteristic of neutrophils (Fig. 4C).

The neutrophil population was recruited to the lung upon infection in A/J and C57BL/6 mice (Fig. 5A). Interestingly, IL-17^+^ cells represented a significant subset of about 40% in A/J mice and 30% in C57BL/6 mice among the total neutrophils (Fig. 5B).

To determine a putative role of Th17 cells in resolving infection in A/J mice, we performed experiments on PA-immunized mice allowing mature T cell differentiation. In contrast to the case for naïve animals, IL-17A and IL-17F levels in BALFs of vaccinated mice were not different from those for noninfected animals at day 3 postinfection (Fig. 6A). Even 15 days following infection, we did not observe any IL-17 production in immunized mice (data not shown). Since C57BL/6 mice survive Sterne strain infection, we analyzed IL-17-producing cells in lungs for up to 6 days after infection to rule out the role of Th17 cells. At day 6, we found no significant increase of the Th17^+^ cell phenotype compared to that in uninfected animals (Fig. 6B). Taken together, these results show that mature T cells did not participate in IL-17 production in the A/J and C57BL/6 models of inhalational anthrax by a Sterne strain.

Impaired recruitment of neutrophils in IL-17RA−/− mice leads to susceptibility to intranasal Sterne strain infection but not to infection with a fully virulent strain. We next examined the role of IL-17A/F axis signaling during recruitment of the neutrophil population by using IL-17RA−/− mice. Although we did not observe a decrease of the IL-17A^+^ cell percentage in the lungs of IL-17RA−/− mice compared with WT animals when we gated on total cells, it was significantly diminished when the gating strategy was restricted to the neutrophils (Fig. 7A). One can infer that in the absence of an IL-17A/F retrofit, some other cell populations compensate for the lower recruitment of IL-17-secreting neutro-
phils. We observed an increased percentage of CD11b^+ Gr-1^int neutrophils, which may be monocites (Fig. 7A).

In contrast, neutrophil recruitment was significantly impaired in IL-17RA^−/− mice compared with that in WT animals (Fig. 7B). We also observed a lower level of neutrophils in IL-17RA^−/− naive mice compared to WT noninfected mice, in accordance with a previous study (57) and other literature suggesting a role of IL-17 during granulopoiesis and migration of neutrophils in tissues (3, 33).

Next, we investigated the effects of IL-17A/F signaling knock-out on mouse survival by using the acapsulated Sterne and fully virulent 17JB strains. IL-17RA^−/− mouse survival was impaired

FIG 3 IL-17-positive cells are CD11b^+ Gr-1^high Ly6G^+ neutrophils. (A to D) A/J mice were infected intranasally with 2 × 10^8 spores of Sterne strain 7702 of B. anthracis. (A) Percentage of IL-17^+ cells gated among total pulmonary cells of A/J mice at 3 days postinfection. These plots are representative of three independent experiments with 10 mice in each experiment. (B) Mean fluorescence intensities of IL-17^+ cells from noninfected (open triangles) and infected (closed triangles) A/J mice. Each triangle represents one mouse. (C) Phenotypic analysis of the R1 population from panel A. These plots are representative of three independent experiments with 10 mice in each experiment. (D) Mean fluorescence intensities of IL-17^+ cells among Th17 cells (i.e., TCRβ^+ CD3^+ cells; open circles) and neutrophils (i.e., CD11b^+ Gr-1^high cells; closed circles). Each circle represents one mouse. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant.
FIG 4  IL-17-positive cells are mostly CD11b⁺ Gr-1⁺ CX3CR1⁺ multilobed neutrophils. (A to C) Each group of C57BL/6 and CX3CR1⁺/gfp mice was infected intranasally with 2 × 10⁸ spores of Sterne strain 7702 of *B. anthracis*. (A) Lung cell analysis of C57BL/6 mice. These plots are representative of two independent experiments with 10 mice in each experiment. (B) Lung cell analysis of CX3CR1⁺/gfp mice. These plots are representative of two independent experiments with five mice in each experiment. (C) Cytospin analysis of C57BL/6 lung cells double stained with DAPI (blue) and with Alexa Fluor 647 for intracellular IL-17A (red) (magnification, ×60).
significantly compared with that of WT mice, which are resistant to i.n. *B. anthracis* Sterne infection (52). This result highlights the critical role played by the IL-17 axis against *B. anthracis* at the lung mucosa in an acapsulated strain infection model (Fig. 7C). In sharp contrast, we did not observe any differences when we used a capsulated fully virulent strain, suggesting that protection against a capsulated strain does not depend on the IL-17 axis (Fig. 7D).

Neutrophils are critical for resolution of infection by a fully virulent capsulated strain of *B. anthracis*. Finally, we sought to assess the role of neutrophils recruited to the lung in protection against Sterne strain and fully virulent models of infection. In the C5-deficient A/J mouse model depleted of neutrophils (Fig. 8A and B) and infected with the Sterne strain, we observed only a slight significant decrease in time to death among depleted mice (Fig. 8C). We then determined the effects of neutrophil depletion in complement-sufficient WT and IL-17RA−/−/− mice (Fig. 8D). After infection by the Sterne strain, we observed only a slight, nonsignificant difference between the depleted and nondepleted groups (Fig. 8E). These results suggested that protection against an acapsulated strain depends strongly on the IL-17 axis but only partially upon neutrophil recruitment. Since we observed an increase of time to death only for A/J C5-deficient mice compared to the survival among C5-sufficient C57BL/6 mice, we tried to address the role of C5 in survival. To that end, we assessed the survival of C57BL/6 mice after depletion of C5 by CVF. We did not observe any differences between WT and C5-depleted mice (data not shown), suggesting that C5 deficiency did not account for the differences between the A/J and C57BL/6 backgrounds. We eventually infected depleted and nondepleted WT mice with the fully virulent capsulated 17JB strain and observed a significant decrease in survival time, thereby demonstrating that neutrophils play a critical role in protection against lung *B. anthracis* infection (Fig. 8F).

**DISCUSSION**

Our study identified IL-17 as one of the first innate immune alarms after *B. anthracis* infection of the lungs and demonstrated that neutrophils are the primary source of IL-17. We also demonstrated that IL-17+ neutrophils are recruited upon infection and that the IL-17 axis is necessary for amplifying its own recruitment. To our knowledge, this is one of the first reports that IL-17-secreting neutrophils are a central producer of IL-17 in an infected mucosa, playing a critical role in survival.

Neutrophils are a vital component of the acute cellular inflammatory system and defense against intruding microorganisms (3, 37). At homeostasis, neutrophils are recruited through their CXCR4 chemokine receptor for rapid clearance of bacteria. However, they can cause lung injury when their recruitment is dysregulated (28). IL-17 is instrumental in lung neutrophil recruitment via its effect on epithelial cells, which in turn produce granulopoietic factors such as granulocyte colony-stimulating factor (G-CSF) and CCL20 (10). The role of IL-17 in neutrophil recruitment has been demonstrated largely with large panels of extracellular

![FIG 5](http://iai.asm.org)
pathogens, mainly as an early requirement, within 4 to 8 h after exposure (10). In our model, IL-17 was detected from day 2 onwards, and its level increased until death. Interestingly, the mean time to death was 8 to 9 days for IL-17RA−/−/− mice, which is much later than that for acute infection with a susceptible strain. This suggests that neutrophil-related IL-17 sustains the recruitment of new neutrophils to the infected site at the later stages of infection. Although neutrophil-related IL-17 is classified as an innate response, its role is delayed until the late phase of lung scavenging. IL-17 is known to induce CXC chemokines (57), some of which possess antimicrobial properties against B. anthracis spores and bacilli (8). This could be another IL-17 effector mechanism of action, independent of neutrophils. Here we demonstrated that lung neutrophils produce IL-17. A previous study with a model of lung neutrophilia induced by LPS instillation reported the detection of IL-17 mRNA in neutrophils (13). Subsequently, it was shown that IL-17 can be produced by non-T cells during “sterile” inflammation of the kidneys (30) and in a model of acute vasculitis (23). Intriguingly, in a model of Helicobacter hepaticus-induced colitis, Gr-1+ CD11b+ cells produced a significant amount of IL-17 in the gut lamina propria (24). More recent data favor a role of neutrophils in IL-17 production in the lung after fungal infection by C. neoformans (55) or A. fumigatus (54). Our study provides a new, nonfungal pathogen on the list of neutrophil IL-17 inducers. According to our data, IL-17 is produced by a subset of neutrophils which represent about 30 to 40% of the total CD11b+ Gr-1+ cell population in the infected lungs. This subpopulation is crucial for sustained recruitment of neutrophils during infection with an acapsulated strain, as illustrated with IL-17RA knockout mice. Neutrophil-related IL-17 induces a stimulating loop of recruitment for inflammatory effectors such as neutrophils and monocytes. It should be noted that production of IL-17 in A/J mice infected with an acapsulated strain depends on the level of infection. This could mean that below a certain quantum, neutrophils are not activated to produce IL-17. Interestingly, the role of the IL-17 axis might differ during infections by acapsulated versus capsulated B. anthracis, as shown by mouse survival. This is not surprising given that mice are sensitive to capsulated B. anthracis devoid of toxins (21, 53), demonstrating the unique role of capsule in virulence (12). This may suggest that IL-17 axis-dependent effectors are ineffective against capsulated strains. Conversely, major capsule effects upon infection dissemination may mask subtle differences observed with the Sterne strain in mice. Interestingly, the capsulated virulent strain 17JB did not trigger a detectable level of IL-17 in BALF (data not shown). Since the capsule allows dissemination to distal organs (12), it could mean that the pathogen spreads before a certain level of activation is reached in the lung. In our study, we focused on the lung, although IL-17 could participate in controlling infection at alternate sites, such as the NALT or the lymph nodes. Alternatively, the infective dose
used was much lower for infection with the 17JB capsulated strain, far below the threshold for activating IL-17 production. Finally, the roles of IL-17 and neutrophils in resisting infection are not always strictly correlated. It has been exemplified in a systemic model of infection by *Acinetobacter baumannii* that neutrophils may have a prominent role in infections with virulent strains, independent of IL-17 signaling (4).

The role of neutrophils in anthrax disease was first recognized by the seminal work of Elie Metchnikoff on phagocytosis in the late 19th century (34). Over time, the role of neutrophils in pul-

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**FIG 7** Recruitment of CD11b⁺ Gr-1⁺ neutrophils is impaired in IL-17RA⁻/⁻ mice, leading to susceptibility to Sterne strain infection. (A to C) C57BL/6 WT and IL-17RA⁻/⁻ mice were infected intranasally with 2 × 10⁶ spores of the Sterne strain of *B. anthracis*. (A) Percentages of IL-17⁺ cells (left dot plots) and neutrophils (right dot plots) in infected C57BL/6 WT (upper dot plots) and infected IL-17RA⁻/⁻ (lower dot plots) mice. These data are representative of two independent experiments with 10 mice in each group. The lower left graph represents the percentages of IL-17⁺ cells from infected C57BL/6 WT (closed circles) and infected IL-17RA⁻/⁻ (closed squares) mice. The lower right graph represents the percentages of neutrophils gated on IL-17⁺ cells from infected C57BL/6 WT (closed circles) and infected IL-17RA⁻/⁻ (closed squares) mice. Each symbol represents one mouse. (B) Recruitment of total neutrophils in C57BL/6 WT (upper panels) and IL-17RA⁻/⁻ (lower panels) mice, either noninfected (left) or infected (right). These data are representative of two independent experiments with 10 mice in each group. The lower graph represents the recruitment of neutrophils in noninfected (open symbols) and infected (closed symbols) C57BL/6 WT (circles) and IL-17RA⁻/⁻ (squares) mice. Each symbol represents one mouse. (C) Survival curves for wild-type (circles, full line) and IL-17RA⁻/⁻ (squares, dashed line) mice infected with 7.5 × 10⁵ spores of the capsulated virulent strain 17JB. These data are representative of three independent experiments with 10 mice in each group. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant.
monary anthrax has been masked progressively by studies focusing on the first cell responder that is directly on-site: the alveolar macrophages (6). Human neutrophils can kill spores and fully virulent capsulated bacilli (32), suggesting that they scavenge infected tissues. It has been known for a long time that B. anthracis toxins dampen the recruitment (39) and priming (56) of neutrophils. LT impairs production of IL-8, which is a critical chemokine for neutrophil recruitment, acting on epithelial (40) and endothe-

FIG 8 Depletion of neutrophil population induces a decrease in survival of mice infected with a virulent strain. (A to E) A/J, C57BL/6, and IL-17RA−/− mice were infected intranasally with 2 × 10⁸ spores of the Sterne strain of B. anthracis. (A) Neutrophil depletion in A/J mice by i.p. injection of 1A8 MAb at 3 days postinfection. These plots are representative of one experiment with five mice in each group. (B) Cytospin analysis of lung cells of A/J mice stained with Giemsa stain (magnification, ×100). These pictures are representative of one experiment with five mice in each group. (C) Survival curves for nondepleted-infected (closed circles, full line) and depleted-infected (closed squares, dashed line) A/J mice. These data are representative of three independent experiments with 10 mice in each group. (D) Neutrophil depletion in C57BL/6 WT and IL-17RA−/− mice by i.p. injection of monoclonal antibody 1A8 at 3 days postinfection. These plots are representative of one experiment with five mice in each group. (E) Survival curve comparison between nondepleted-infected (closed circles) and depleted-infected (open circles) C57BL/6 WT mice and nondepleted-infected (closed squares) and depleted-infected (open squares) IL-17RA−/− mice. These data are representative of two independent experiments with 10 mice in each group. (F) Mice were infected intranasally with 7.5 × 10⁵ spores of the 17JB strain of B. anthracis. The graph shows a survival curve comparison between nondepleted-infected (closed circles, full line) and depleted-infected (open circles, dashed line) C57BL/6 WT mice. Experiments were performed with 10 mice in each group. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant.
lial (2) cells through at least two molecular mechanisms. The role of neutrophils in anthrax was recently brought back into the limelight in a study using myeloid lineage CMG2-deficient mice (31). According to this study, toxin receptor inactivation renders mice resistant to s.c. or intravenous (i.v.) models of B. anthracis infection. The toxin effects were mediated mainly by neutrophil impairment. In another study, mice bearing Nlrp1b-sensitive alleles for macrophage lysis were resistant to infection, through caspase 1 activation, IL-1β signaling, and neutrophil recruitment following s.c. challenge (35).

Beyond our mouse models, one interesting question is the relevance of such findings to neutrophils in other animal models and, ultimately, humans. The main documents on humans are from necropsy analyses of the Sverdlosk incident in 1979 and the U.S. outbreak in 2001 in the Sverdlosk accident cohort, a neutrophilic inflammation was noticed for 37% of microscopic lung analyses (17). In contrast, during the 2001 outbreak, various degrees of monocytic infiltration were noticed on lung necropsy of deceased patients (18). Both studies report major inflammatory lesions with alveolar edema and hyaline membrane formation. Since these reports are based on terminal state examination, they may be biased toward patients whose immune systems did not handle the pathogen properly and thus may miss neutrophil involvement. Research of the most relevant animal model of inhalational anthrax has been compared to a quest for the Holy Grail (16). NHPs have been used widely over the past 60 years (50). Interestingly, neutrophilic inflammation has been reported in most studies using more distant species (1, 45). Neutrophils were found to various degrees on lung tissue necropsy of rabbits (50), as well as in historical studies of guinea pigs (41, 42, 50). Taken together, these data indicate that neutrophils are recruited into the lung in most animal models of B. anthracis infection. The role of neutrophils has largely been undetermined so far, mainly because their recruitment is faint compared to that in other major lung histological disorders.

Our data confirm the critical role of the IL-17 axis in survival with an acapsulated-toxigenic Sterne strain and of neutrophils in survival with a fully virulent strain in a mouse model of pulmonary anthrax. These data draw a more complicated picture of the host innate immune response to B. anthracis infection, including the roles of IL-17 and neutrophils in clearing of the lungs.

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