Key Role for Respiratory CD103+ Dendritic Cells, IFN-γ, and IL-17 in Protection Against Streptococcus pneumoniae Infection in Response to α-Galactosylceramide

Stoyan Ivanov,1,2,3,4,5 Josette Fontaine,1,2,3,4,5 Christophe Paget,1,2,3,4,5 Elodie Macho Fernandez,1,2,3,4,5 Laury Van Maele,1,2,3,4,5 Joelle Renneson,1,2,3,4,5 Isabelle Maillet,6 Natalia Muñoz Wolf,7 Anaia Rial,8 Hélène Léger,8 Bernard Ryffel,6 Benoit Frisch,7 José A. Chabalgoity,9 Jean Claude Sirard,1,2,3,4,5 Arndt Benecke,8 Christelle Faveeuw,1,2,3,4,5 and François Trottein1,2,3,4,5

1Institut Pasteur de Lille, Centre d’Infection et d’Immunité de Lille, 2Université Lille Nord de France, 3Centre National de la Recherche Scientifique, UMR 8204, 4Institut National de la Santé et de la Recherche Médicale, U1019, and 5Institut Fédératif de Recherche 142, Lille, 6Centre National de la Recherche Scientifique, UMR 6218, Université d’Orléans, Orléans, 7Centre National de la Recherche Scientifique, UMR 7199, Université de Strasbourg, Faculté de Pharmacie, Illkirch, and 8Centre National de la Recherche Scientifique, USR 3078, Institut des Hautes Études Scientifiques, Bures sur Yvette, France; and 9Instituto de Higiene, Facultad de Medicina, Universidad de la República, CP-11600 Montevideo, Uruguay

Background. Exogenous activation of pulmonary invariant natural killer T (iNKT) cells, a population of lipid-reactive αβ T lymphocytes, with use of mucosal α-galactosylceramide (α-GalCer) administration, is a promising approach to control respiratory bacterial infections. We undertook the present study to characterize mechanisms leading to α-GalCer-mediated protection against lethal infection with Streptococcus pneumoniae serotype 1, a major respiratory pathogen in humans.

Methods and Results. α-GalCer was administered by the intranasal route before infection with S. pneumoniae. We showed that respiratory dendritic cells (DCs), most likely the CD103+ subset, play a major role in the activation (IFN-γ and IL-17 release) of pulmonary iNKT cells, whereas alveolar and interstitial macrophages are minor players. After challenge, S. pneumoniae was rapidly (4 hours) eliminated in the alveolar spaces, a phenomenon that depended on respiratory DCs and neutrophils, but not macrophages, and on the early production of both IFN-γ and IL-17. Protection was also associated with the synthesis of various interferon-dependent and IL-17–associated genes as revealed by transcriptomic analysis.

Conclusions. These data imply a new function for pulmonary CD103+ DCs in mucosal activation of iNKT cells and establish a critical role for both IFN-γ and IL-17 signalling pathways in mediating the innate immune response to S. pneumoniae.

The extracellular, gram-positive bacterium Streptococcus pneumoniae (also called pneumococcus) is a serious human pathogen that causes more than 50% of the cases of community-acquired bacterial pneumonia [1, 2]. Innate immunity is crucial in the early phase of natural anti-pneumococcal host defence and alveolar macrophages and neutrophils play key roles in the clearance of bacteria. However, in situations in which the immune system is overwhelmed (for instance, after infection with a high dose of S. pneumoniae) or deficient, bacterial replication is not controlled and hosts die of immune cell-mediated pneumonia and sepsis because of bacterial dissemination. Stimulation of the lung-innate immunity with use of bacterial crude extracts or purified flagellin protects against lethal respiratory pneumococcal infection in mouse...
models [3–5]. In these settings, activation of Toll-like receptors (TLRs) in stromal (epithelial cells) and/or hematopoietic (dendritic cells [DCs], macrophages) cells is a crucial step to promote macrophage and/or neutrophil-mediated bacterial clearance. In this study, we assessed the role of α-galactosylceramide (α-GalCer), a synthetic, non-TLR agonist glycolipid known to activate potent innate responses, in the control of acute respiratory pneumococcal infection.

Invariant natural killer T (iNKT) cells are innate-like αβ T lymphocytes expressing markers associated with the NK lineage. These cells express a semi-invariant TCR and rapidly secrete cytokines upon stimulation with certain lipid antigens (Ags), such as α-GalCer, which is presented by the nonpolymorphic CD1d molecule expressed by Ag-presenting cells (APC) [6–10]. In the mouse system, iNKT cells can be subdivided into 2 main populations based on the expression of NK1.1; the NK1.1+ subset is described to preferentially produce IFN-γ and IL-4, and the NK1.1− subset is more prone to release IL-17 [7, 11, 12]. Human iNKT cells can also produce IFN-γ, IL-4, and IL-17 [7]. iNKT cells are critical regulators of the immune response to infectious agents, and therapies to promote iNKT cell activation may represent a promising approach to treat respiratory (antibiotic-resistant) bacterial infections [7, 13]. Here, we first sought to identify the role of pulmonary APC in the primary activation of lung iNKT cells after intranasal (i.n.) administration of α-GalCer. Using a pathogenic strain of S. pneumoniae serotype 1, a major serotype associated with invasive disease in humans [14], we then studied the mechanisms underlying α-GalCer-mediated protection against a lethal S. pneumoniae challenge.

MATERIALS AND METHODS

Reagents and Abs
α-GalCer was from Axxora Life Sciences (Coger). Cyanine (Cy5)-conjugated α-GalCer [15] and clodronate-containing liposomes [16] were produced as described elsewhere. Monoclonal Abs against mouse CD3 (APC-conjugated), NK1.1 (PE- or PerCP-Cy5.5-conjugated), TCR-β (FITC or Pacific Blue–conjugated), CD69 (PerCP-Cy5.5–conjugated), CD11b (PerCP-Cy5.5–conjugated), Ly6G (PE–conjugated), CD103 (PE–conjugated), CD11c (APC or PE-Cy7–conjugated), F4/80 (PerCP-Cy5.5 or PE-Cy7–conjugated), CD45 (FITC or eFluor65NC–conjugated), CD1d (biotin–conjugated), Streptavidin (Alexa Fluor-700–conjugated), IFNγ (Alexa Fluor-488–conjugated), IL-17A (Alexa Fluor-647–conjugated), and isotype controls were purchased from BD Pharmingen. The LIVE/DEAD Fixable Dead Cell Stain Kit was from Invitrogen. PE-conjugated PBS-57 glycolipid-loaded CD1d tetramer was from the National Institute of Allergy and Infectious Diseases Tetramer Facility (Emory University, Atlanta, GA).

Mice
Wild-type (WT) C57BL/6 mice were purchased from Janvier. Ja18−/−, CD11c-DTR, and Il17ra−/− mice are described elsewhere [17–19]. Eight-week-old male mice were used in this study. For S. pneumoniae infection, mice were maintained in a biosafety level 2 facility. All animal work conformed to the Pasteur Institute, Lille, Animal Care and Use Committee guidelines (agreement number N°AF16/20090).

Analysis of iNKT and NK Cell Activation
Lung mononuclear cells (MNCs) were prepared as described elsewhere [20]. MNCs were incubated with appropriate combinations of Abs and/or PBS-57-loaded CD1d tetramer to identify iNKT and NK cells. For intracellular staining, cells were fixed in PBS 1% paraformaldehyde and incubated with appropriate Abs in 0.1% saponin buffer. Cells were acquired and analyzed on a LSR Fortessa (Becton Dickinson) cytometer using the FACSDiva software.

Measurement of Cy5-α-GalCer Incorporation by Respiratory APC
Lung cells were prepared 45 minutes after i.n. inoculation of Cy5-conjugated or unconjugated α-GalCer (2 μg). Then, 2 × 106 cells/well were resuspended in the appropriate combination of Abs to allow DC and macrophage subset identification, and incorporation of Cy5 was analyzed on the LSR Fortessa.

Respiratory APC and iNKT Co-cultures
Cell-sorted respiratory DC and macrophages were pulsed with graded doses of α-GalCer for 2 hours, washed, and co-cultured for 48 hours with hepatic CD5+ NK1.1+ cells, which were purified as described elsewhere [21] or with the iNKT cell hybridoma DN32.D3 [22]. Cytokine production was measured in the culture supernatants by enzyme-linked immunosorbent assay.

Inoculation of α-GalCer, Infection With S. pneumoniae, and Assessment of Bacterial Counts
Mice were inoculated by the i.n. route with α-GalCer (2 μg). S. pneumoniae serotype 1 clinical isolate E1586 sequence type ST304 is described elsewhere [4, 23, 24]. Mice were anesthetized and administered i.n. with 5 × 108 bacteria. Mice were monitored daily for illness and mortality for 14 days. A morphology-based differential cell count was conducted on cytospin preparations from the bronchoalveolar lavage (BAL) fluid samples and stained with Diff-Quik solution (Sigma). Bacterial burden in the lungs and BAL samples was measured by plating lung homogenates or BAL samples onto blood agar plates. Colony-forming units were enumerated 24 hours later.

Micro-array Analysis
Total RNA (2 μg) from whole lungs was processed on the Mouse Whole Genome Arrays, version 2.0 (Applied
RESULTS

Depletion of DCs, Neutrophils, and Alveolar Macrophages

For depletion of DCs, mice heterozygous for the CD11c-DTR transgene were injected by the intra-peritoneal route (i.p.) with 100 ng diphtheria toxin (DT; Sigma) as described elsewhere [18]. Neutrophil depletion was achieved with a single i.p. injection of 250 µg of anti-Ly6G (clone NIMPR14) or isotype control (LTF-2) mAb 1 day before S. pneumoniae challenge. To deplete alveolar macrophages, mice were administered with the i.n. route with 20 µg of clodronate-containing liposomes.

Neutralization of IFN-γ

Recipient mice were injected (i.p.) with 100 µg anti-IFN-γ (AN18) or with the isotype control mAb (HRPN) 1 hour before α-GalCer treatment.

Statistical Analyses

Results are expressed as the mean ± standard deviation or standard error of the mean. The statistical significance of differences between experimental groups was calculated using analysis of variance with a Bonferroni post-test or an unpaired Student t test (GraphPad Prism 4 Software). Survival of mice was compared using Kaplan–Meier analysis and log-rank test.

RESULTS

Respiratory DCs are Essential to Activate Pulmonary iNKT Cells In Vivo

We first analyzed the kinetics of the pulmonary iNKT cell response after i.n. inoculation of α-GalCer. As shown in Figure 1A, α-GalCer decreased the frequency of lung iNKT cells 12 hours and 24 hours, but not 6 hours, after treatment, a phenomenon probably attributable to TCR internalization [28, 29] and/or to activation-induced cell death [30, 31]. This effect was associated with an enhanced expression of intracellular IFN-γ and IL-17A in iNKT cells (Figure 1B and 1C). Of note, although the NK1.1⁺ iNKT cell subset only produced IFN-γ, NK1.1⁻ iNKT cells produced both IFN-γ and IL-17A (Figure 1C).

The role of respiratory APC in the activation of pulmonary iNKT cells is ill-defined. To investigate the potential role of DCs in lipid Ag presentation in vivo, transgenic CD11c.DTR mice were used [18]. As shown in Figure 2A, DT treatment depleted by approximately 80% lung DCs (CD11c⁺ F4/80⁺). However, the number of alveolar (CD11cpos F4/80hi) and interstitial (CD11cneg F4/80int) macrophages remained unchanged. Because 2 predominant populations of respiratory DCs have been described [32, 33], respiratory DCs were discriminated on the basis of CD11b and CD103 expression (Figure 2B). Of interest, DT treatment resulted in an almost complete disappearance of CD11b⁺ CD103⁺ (here, referred to as CD103⁺) DCs, whereas it reduced by approximately 58% the number of CD11bhiCD103neg (referred to as CD11bhi) DCs. This can be explained by the lower level of CD11c expression by the remaining CD11bhi DCs (not shown). Of note, administration of DT completely blunted IFN-γ and IL-17 production by pulmonary iNKT cells (Figure 2C). Depletion of DCs also resulted in a complete ablation of IFN-γ production by NK cells (Figure 2D). Thus, respiratory DCs (most likely, the CD103⁺ subset), but not macrophages, are the major players of pulmonary iNKT cell activation in response to α-GalCer administered intranasally.

Respiratory DCs Display a High Capacity to Activate Primary iNKT Cells In Vitro

We then investigated whether these data could be explained by differential CD1d expression, in vivo incorporation rate of α-GalCer, or intrinsic ability to directly promote cytokine release by iNKT cells. As shown in Figure 3A, CD103⁺ and CD11b⁺ DCs expressed equal levels of CD1d, and this expression was higher in alveolar macrophages and lower in interstitial macrophages. As indicated in Figure 3B, 45 minutes after Cy5-α-GalCer i.n. administration, both CD103⁺ and CD11b⁺ DCs labeled positively and to a similar extent. Of note, Cy5-conjugated α-GalCer was strongly incorporated by alveolar macrophages, whereas interstitial only poorly took-up α-GalCer (Figure 3B). The ex vivo iNKT cell-activating properties of respiratory APCs were then compared. DC and macrophage subsets were sorted from the lung tissue, loaded with α-GalCer, and then exposed to primary iNKT cells. CD103⁺ DC and CD11b⁺ DC activated the release of IFN-γ by iNKT cells to a similar extent, whereas alveolar macrophages, particularly interstitial macrophages, had a much reduced capacity to do so (Figure 3C). In contrast, DC and macrophage subsets activated the iNKT cell hybridoma DN32.D3 to the same extent (Figure 3D).

α-GalCer Protects Mice Against Lethal S. pneumoniae Infection

We next evaluated the impact of α-GalCer treatment on S. pneumoniae serotype 1 lethal infection. As shown in Figure 4A, at the dose used (5 LD50), all vehicle-treated mice died 5 days after infection. In marked contrast, mice
previously treated (24 hours) with α-GalCer did not present any visible signs of clinical disease and were fully protected from mortality. Of note, α-GalCer did not confer protection in mice deficient in iNKT cells (Ja18−/− mice). The protective effect of α-GalCer was long-lasting, because 60%–75% of mice treated 3 and 7 days, but not 14 days, earlier were protected against S. pneumoniae challenge (Figure 4B). The numbers of live bacteria in the whole lungs 36 hours after infection correlated inversely with protection against lethal pneumonia (Figure 4C). Furthermore, there was significantly less bacterial dissemination to the spleen in mice treated with α-GalCer.

We next investigated whether elimination of bacteria occurs...
rapidly after challenge in the alveolar space or at later time points in the lung tissue after the bacteria have crossed the lung epithelium. As shown in Figure 4D, α-GalCer induced a microbicidal or bacteriostatic environment in the alveolar spaces that rapidly, within the first 4 hours of inoculation, led to the control of bacterial replication in the airways. This effect was associated with an enhanced recruitment of neutrophils, but not macrophages, in the BAL samples (Figure 4E).

α-GalCer–Mediated Protection is Fully Mediated by Neutrophils
Because macrophages and neutrophils represent the most predominant cell populations in the alveolar spaces, we investigated their respective role in α-GalCer–mediated protection against S. pneumoniae. As shown in Figure 5A, administration of anti-Ly6G mAb (NIMPR14) strongly depleted neutrophils in BAL samples and in the lung. In agreement with another study [34], depletion of neutrophils accelerated mortality in PBS–treated mice. Remarkably, in α-GalCer–injected animals, neutrophil depletion resulted in a fatal outcome and higher bacterial loads in BAL (Figure 5A).

To study the involvement of alveolar macrophages, known in some contexts to participate in neutrophil recruitment and activation [35, 36], mice were administered intranasally with clodronate-loaded liposomes just before α-GalCer treatment. This induced a strong depletion of alveolar macrophages...
Alveolar macrophage depletion had no effect on the survival rate or on the bacterial load of α-GalCer–treated animals (Figure 5B). Thus, after α-GalCer stimulation, neutrophils play a key role in bacterial killing, independently from macrophages.

Protection Is Associated With Increased Expression of IFN- and IL-17–related Defence Genes

To gain insight into the mechanism of protection conferred by α-GalCer, we analyzed the gene expression signature that characterizes the lung after α-GalCer administration. At 4 hours after treatment, a transient regulation of gene expression (477 induced, 211 repressed; P < .01) was detected (Figure 6A). This initial gene expression program then evolved toward a more balanced profile at 18 hours, characterized by an enhanced number of regulated genes (1023 induced, 1078 repressed). Direct comparisons of the transcriptomic profiles at 4 hours and 18 hours with the baseline (0 hour) measurement identified genes with significant changes in expression (Figure 6B and 6C). These 2 gene sets (4 hours and 18 hours) showed significant enrichment of different gene ontology sets, such as immunity and defence and cytokine and chemokine (Figure 6D and 6E). Of interest, the neutrophil-recruiting chemokines Cxcl1 and Cxcl2 were strongly up-regulated at 4 hours (Log Q >3) (Figure 6F). Numerous IFN-dependent genes, such as chemokines (Cxcl5/9/10/11) and members of the so-called IFN-stimulated gene (ISG) family (eg, Irg1 and Igtp) were found to be strongly (Log Q >3) up-regulated.
18 hours after treatment. In parallel, genes associated with IL-17 (Cxcl1/2/9/10/11, Il6, Csf3, Ccl2/7, Saa3) were induced in response to α-GalCer. Thus, α-GalCer induced IFN- and IL-17–mediated gene expression in the lungs, a signature that may account for its protective effect against S. pneumoniae lethal challenge.

IFN-γ, IL-17, and Respiratory DCs Are Required for α-GalCer–mediated Protection Against S. pneumoniae

Inoculation of α-GalCer resulted in IFN-γ and IL-17 production by iNKT cells, a phenomenon that depended on respiratory DCs. We next addressed the role of these cytokines in the α-GalCer–mediated protection against S. pneumoniae lethal challenge. Compared with the isotype control, the neutralizing anti–IFN-γ Ab (AN18) partially reduced the protective effect conferred by α-GalCer, because approximately 50% of mice died 5 days after infection (Figure 7A). Similarly, the lack of IL-17Ra reduced the protective effect of α-GalCer by half. When these 2 deficiencies were combined, α-GalCer failed to confer its protective effects; the totality of Il17ra−/− mice treated with the anti–IFN-γ Ab died. Thus, IFN-γ and IL-17 function cooperatively to clear S. pneumoniae in our setting. To our knowledge, this is the first time that IL-17 has been shown to exert a protective anti-infectious effect after α-GalCer treatment.

We finally assessed the role of respiratory DCs in α-GalCer–mediated protection. DT treatment completely abrogated the protective effect of α-GalCer in transgenic CD11c−/− DTR mice (Figure 7B).
DISCUSSION

In this study, we assessed the role of α-GalCer, a non-TLR agonist that specifically activates iNKT cells, in the control of acute respiratory pneumococcal infection. Alpha-GalCer has been reported to enhance host resistance to extracellular capsulated bacteria, such as pneumococcus, but the underlying mechanisms are still elusive. Kawakami et al reported that i.p. delivery of α-GalCer at the time of S. pneumoniae serotype 3 infection led to a partial, but significant, reduction in the number of live bacteria in the lungs 3 days after infection [37]. It was next suggested that iNKT cells, through IFN-γ production, may act by enhancing neutrophil-mediated host defence, although the mechanism of the protection was not fully described [38]. In the present study, we re-evaluated this question, using the lung as the delivery site of α-GalCer administration and S. pneumoniae serotype 1, a major serotype in humans. Before assessing the potential role of α-GalCer in protective immunity in this system, we first studied the features of the pulmonary iNKT cell response and the role that respiratory APCs play in this system.

Whereas activation of splenic/hepatic iNKT cells in response to systemic delivery of α-GalCer is rapid (1–2 hours), its i.n. administration led to a delayed response in the lungs.
Figure 6. Analysis of gene expression in the lungs of mock and α-GalCer–treated mice. Mice were either treated or not with α-GalCer for 4 hours or 18 hours after total RNA from the lungs was extracted and analyzed. Transcripts isolated from the lungs were analyzed by whole-genome microarray. (A) Kinetic analysis of transcriptomic profiles recorded at 0 hour, 4 hours, and 18 hours. A heatmap of logarithmic base 2 fold-changes (log Q) at the indicated time-points is shown for all genes that are significantly (fold-change z-test with positive False Discovery Rate correction (pFDR) corrected post hoc) differentially expressed from baseline at least at one time point. Differences in relative levels of gene expression are indicated in colors, where red indicates up-regulation and blue indicates down-regulation. (B and C) Heatmap of the subtraction profile 4 hours vs 0 (B) and 18 hours vs 0 hour (C) of the biological triplicate recordings showing the statistically significantly regulated genes (P < .01). (D and E) Gene ontology enrichment analysis 4 hours or 18 hours vs 0 hour (Binomial distribution, Bonferroni corrected). Diagrams are drawn to size of the number of statistically significantly regulated genes. One representative experiment of 2 performed (n = 6) is shown. (F) Genes are ordered based on fold induction relative to mice inoculated with vehicle alone at 4 hours and 18 hours (P < .001). Genes whose expression are up-regulated at least 8 times are indicated (Log Q > 3). IFN-dependent genes are in bold and IL-17-related genes are indicated with an asterisk.
This cannot be explained by a delayed uptake of α-GalCer by APCs, because as is the case for splenic DCs after intravenous delivery [15], α-GalCer is rapidly picked up by respiratory APC subsets. It is more likely that the preferential location of iNKT cells in the lung microvasculature, but not in the interstitial spaces where APCs preferentially reside [39, 40], is responsible for this delayed response. In the lung, it is not clear how iNKT cells become activated in response to mucosal delivery of α-GalCer. Two recent reports showed that respiratory DCs have the potential to activate iNKT cells in vitro, although CD103+ DC and CD11b+ DC were not discriminated in these studies [39, 41]. Using CD11c-DTR mice, we showed that DCs, but not macrophages, mediated the activation of pulmonary iNKT cells in vivo. Lung macrophages, particularly alveolar macrophages, express CD1d and take-up α-GalCer in vivo. However, they display a weak capacity to activate primary iNKT cells, but not the iNKT cell hybridoma DN32.D3. Activation of primary iNKT cells, unlike iNKT hybridomas, depends on not only CD1d/Ag-mediated TCR triggering, but also cofactors produced by APC. Thus, the reduced role of macrophages in iNKT cell activation in vivo can be explained, at least in part, by their inability to deliver appropriate signals to iNKT cells. Despite a remaining population of CD11b+ DCs in DT-treated CD11c-DTR mice, α-GalCer failed to activate lung iNKT cells, suggesting that CD103+ DCs are the major players in iNKT cell stimulation. Functional differences between these DC subsets have clearly been described [33, 42, 43]. However, our data indicated no difference between the two DC subsets to express CD1d, to incorporate α-GalCer in vivo, or to activate iNKT cells in vitro. It is possible that the anatomical location of CD103+ DCs, close to the airway epithelium and in the luminal and parenchymal sides of the vascular wall [33], and their potent migratory properties [42, 43] play an important role in iNKT cell activation in vivo.

Inoculation of α-GalCer induced protection against a lethal S. pneumoniae challenge. Protection against mortality was
attributable to control of bacterial growth in the alveolar spaces, and this was associated with a strong influx of neutrophils into the BALs. Depletion experiments indicated that neutrophils are crucial in bacterial clearance. iNKT cells are known to drive macrophage activation [44, 45], and macrophages are known to cooperate with neutrophils to promote protective responses in some circumstances. However, after α-GalCer administration, macrophages were dispensable in neutrophil-mediated clearance of S. pneumoniae. The mechanism by which iNKT cells promote anti-streptococcal effects through neutrophils is unknown. To address this issue, we asked how α-GalCer influenced overall transcriptional responses in the lungs. We showed for the first time the mucosal gene signature of α-GalCer. A significant enrichment of ISGs and, to a lesser extent, genes regulated by IL-17 was revealed. Among ISGs, numerous chemokine (Cxcl5, Cxcl9, Cxcl10, Cxcl11) and IFN-γ-inducible guanosine triphosphatase (GTPase) transcripts were up-regulated. Although the majority of ISGs do not yet have any assigned function, some clearly play a critical role in host defence against bacteria through a variety of complementary mechanisms, including cell recruitment, bactericidal activity, oxidative burst, and autophagy [46, 47]. Among the GTPases induced by α-GalCer, it is possible that members of the guanylate-binding protein (Gbp1, Gbp2, Gbp4) or immunity-related GTPase family (Tgtp, Irgm) play a role in S. pneumoniae clearance. Nakamatsu et al. have reported that α-GalCer triggers innate, IFN-γ-dependent mechanisms leading to accelerated clearance of S. pneumoniae serotype 3 [38]. Our data, using S. pneumoniae serotype 1, confirm this finding, because the neutralizing anti-IFN-γ Ab reduced the efficacy of α-GalCer by half. Moreover, the lack of IL-17 signalling decreased the beneficial effect of α-GalCer treatment to a similar extent. Of note, when both signalling pathways were abrogated, α-GalCer failed to protect against mortality. This indicates that IFN-γ and IL-17 function cooperatively but in an independent manner to control S. pneumoniae infection in response to α-GalCer. IL-17 has recently been shown to play a part in protection against S. pneumoniae [48]. Our current study shows for the first time that the dual activation of the IFN-γ and IL-17 pathways confers protection against S. pneumoniae. Although it is likely that the protective effect of IFN-γ is attributable to ISGs, the mechanisms by which IL-17 participates in protection are still unknown. After α-GalCer stimulation, IL-17 strongly participates in neutrophil recruitment in the lungs through its receptor IL-17Ra (not shown). This is in line with previous studies showing that IL-17 production by iNKT cells mediates the neutrophil influx into the lungs [11, 49]. Because no IL-17 targeted effector genes (ie, antimicrobial peptides) were detected in our transcriptomic analysis, with the exception of defensin 8 (3 > logQ > 2), it is likely that the main action of IL-17 in protection is through neutrophil mobilization and activation, such as enhancement of phagocytic activity, a process leading to S. pneumoniae killing.

To conclude, our results show for the first time, that after i.n α-GalCer delivery, respiratory DCs, probably CD103⁺, are critical in the primary activation of pulmonary iNKT cells. The understanding of the mechanisms leading to α-GalCer-mediated neutrophil effector functions and the role played by ISGs in the control of S. pneumoniae should be of interest in the design of new immuno-activators able to control a lethal S. pneumoniae infection.

Notes

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