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Granzyme A impairs host defense during *Streptococcus pneumoniae* pneumonia

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*Streptococcus (S.) pneumoniae* is the most frequently isolated causative pathogen in community-acquired pneumonia (CAP), responsible for up to 60% of bacterial cases (15, 28). CAP is a common illness worldwide, accounting for considerable morbidity and substantial mortality rates, ranging from 2 up to 30% as it progresses into sepsis (3). Despite the availability of an extensive arsenal of antibiotics, outcome has not improved over the past decades and therefore adjunctive measures are of vital importance to optimize treatment.

Granzymes are a family of serine proteases that have been characterized in man, rat, and mouse. Stored in secretory granules of cytotoxic lymphocytes (CLs), the search for physiologic substrates of granzymes has focused on their ability to induce apoptosis. Of the five human granzymes (A, B, H, K, M), the cytotoxic potential of the trypsin-like granzyme A (GzmA) (24), however, has become controversial, as GzmA-induced cytotoxicity was only observed in vitro at superphysiological concentrations in synergy with the pore-forming protein perforin (8, 20, 21). In addition, GzmA-deficient (GzmA−/−) mice demonstrated normal cytotoxic mechanisms (6). Instead, mounting evidence points towards a role for GzmA in the host inflammatory response. GzmA was shown to cleave pro-IL-1β into its biologically active form (14) and to induce the release of proinflammatory cytokines from monocytes/macrophages, fibroblasts, and epithelial cell lines (21, 25, 26). Furthermore, extracellular matrix proteins are potential substrates for GzmA, which may influence cell migration (5).

GzmA is constitutively expressed by NK, NKT, and CD8+ T cells, while other cells require stimulation to induce expression (10). More recently, GzmA expression was reported in bronchiolar epithelial cells, alveolar macrophages, and type II pneumocytes in human lung tissue with enhanced expression in the latter cell type in chronic obstructive pulmonary disease (31). Plasma GzmA levels were elevated in patients with
various parasitic, viral, and bacterial infectious diseases (5, 13, 19), severe sepsis caused by different bacterial pathogens (35), and endotoxemia induced in healthy humans (19). Moreover, whole blood stimulation with Gram-negative and Gram-positive bacteria resulted in GzmA release by leukocytes (13). In contrast, GzmA did not seem to play an important role during infection with lymphocytic choriomeningitis virus, Listeria monocytogenes (6), or Klebsiella (K.) pneumoniae (9) and was reported to contribute to lethality during influenza (2) and endotoxic shock (21).

Here we set out to investigate the role of GzmA during pneumococcal pneumonia. For this we studied GzmA expression in lung tissue and measured GzmA levels in BALF samples of patients with CAP. To study the involvement of GzmA in host defense, we induced pneumococcal pneumonia in GzmA−/− and wild-type (WT) mice. In addition, we depleted NK cells to evaluate the contribution of NK cell-derived GzmA to the outcome of this experimental model of CAP.

METHODS

Human Studies

Subjects. From healthy subjects and from patients with confirmed pneumonia (7), bronchoalveolar lavage fluid (BALF) was harvested. Written informed consent was obtained from all individuals and the study was approved by the Institutional Medical Ethical Committee of the St. Antonius Hospital, Nieuwegein, The Netherlands.

Stored lung tissue slides of 10 patients who had succumbed to culture proven S. pneumoniae pneumonia and 9 patients who had died from a nonpulmonary cause were used, according to the “Code for Proper Secondary Use of Human Tissue,” Dutch Federation of Medical Scientific Societies.

GzmA enzyme-linked immunosorbent assay. GzmA protein levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (Pelikine Compact; Sanquin, Amsterdam, The Netherlands) with a detection limit of 10 U/ml.

Immunohistochemistry. Tissue blocks were taken from human lungs at autopsy, fixed without inflation in 10% phosphate-buffered formalin, and embedded in paraffin wax. Sections (4 μm) were cut and processed for immunohistochemical analysis of GzmA. For this, deparaffinized sections were boiled in 10 mM citrate buffer for antigen retrieval and treated with 5% bovine serum albumin in Tris-buffered saline (TBS) to reduce background staining. Mouse monoclonal antibodies against GzmA (GA6, M1791; Sanquin) were used followed by biotin-conjugated rabbit anti-mouse IgG antibody (E-0413; Dako Cytomation, Glostrup, Denmark). After application of alkaline-phosphatase-labeled avidin-biotin complex (ABC-AP, K-0376; Dako Cytomation), enzymatic reactivity was visualized using Vector Blue (Vector Laboratories, Burlingame, CA) and sections were counterstained with Nuclear Fast Red (Vector Laboratories) and mounted. Spleen tissue was used as a positive control. Negative controls for nonspecific binding by omitting the primary detecting antibodies or applying normal mouse IgG instead of the primary antibodies revealed no signal.

Mouse Studies

Animals. Granzyyme A knockout (GzmA−/−) mice on a C57Bl/6 background were originally obtained from Dr. M. M. Simon (Max Planck Institute, Freiburg, Germany) (6) and bred at the animal care facility of the Academic Medical Center. C57Bl/6 mice were purchased from Charles River (Maastricht, The Netherlands) and all experiments were conducted with 10- to 12-wk-old age-and gender-matched mice. The Institutional Animal Care and Use Committee of the Academic Medical Center approved all experiments.

Experimental study design. S. pneumoniae serotype 3 (American Type Culture Collection, ATCC 6303, Rockville, MD) were grown as described previously (18, 27) and ~5 × 10⁵ colony-forming units (CFU) in 50 μl were inoculated intranasally to induce pneumococcal pneumonia. Mice were observed in a survival study or killed at 6, 24, or 48 h after induction of pneumococcal pneumonia. NK cell depletion was performed by intraperitoneal administration of 500 μg NK1.1 cell depleting antibody (clone PK136) 2 and 1 days before induction of pneumonia. Lung, spleen, blood, and BALF cells were isolated using methods described previously (18, 27). Total cell numbers in BALF were determined by an automated cell counter (Coulter Counter; Coulter Electronics, Hialeah, FL). Differential cell counts were performed on cytopsin preparations stained with a modified Giemsa stain (Diff-Quick; Dade Behring, Düdingen, Switzerland).

Bacterial quantification. To assess bacterial loads undiluted whole blood and serial 10-fold dilutions of whole blood, organ homogenates and BALF were made in sterile isotonic saline and plated onto sheep-blood agar plates. Following 16 h of incubation at 37°C CFU were counted.

Flow cytometry. Lung cell suspensions were obtained by crushing lungs through a 40-μm-cell strainer (Becton Dickinson, Franklin Lakes, NJ) as described previously (33). Erythrocytes were lysed by ammonium chloride and the cells were washed twice and resuspended in FACS staining buffer (PBS with 0.5% BSA, 0.01% NaN₃ and 0.02% potassium-EDTA). Leukocytes were incubated for 30 min at 4°C with the following primary antibodies: fluorescein isothiocyanate (FITC)-labeled anti-CD122, peridinin-chlorophyll proteins (PerCP)-Cy5-labeled anti-NK1.1, allophycocyanin (APC)-labeled anti-CD49b, and phycoerythrin (PE)-labeled anti-CD3 (all anti-mouse from eBioscience, San Diego, CA). To determine intracellular GzmA expression, cells were fixed and permeabilized with fixation/permeabilization solution (BD Biosciences, Franklin Lakes, NJ) and stained with FITC-labeled mouse anti-mouse GzmA mAb (Santa Cruz Biotechnology, Heidelberg, Germany) in the presence of anti-CD16/CD32 block (2.4G2; Bio- cers, Utrecht, The Netherlands) to prevent nonspecific antibody binding. GzmA expression in NK1.1 cells was determined by flow cytometry; NK cells were identified based on forward and side scatter (lymphocyte gate) and as CD3-/NK1.1+ cells. The FAC- SCalibur flow cytometer (BD Biosciences) and FlowJo software (Tree Star, Ashland, OR) were used for analysis.

Assays. Levels of myeloperoxidase (PMO; Hycompound, Uden, The Netherlands), macrophage-inflammatory protein (MIP)-2, keratinocyte-derived cytokine (KC), interleukin (IL)-1β, IL-6, IL-12, tumor necrosis factor-α (TNF-α), and interferon-gamma (IFN-γ) were determined using commercially available assays (R&D Systems, Abingdon, UK and BD Biosciences, San Jose, CA).

Histopathology and immunohistochemistry. Paraffin-embedded 4-μm-thick sections of the left lung lobe were stained with hematoxylin and eosin (H&E). All slides were coded and scored by a pathologist, who was blinded for group identity for the following parameters: interstitial inflammation, endothelialitis, bronchitis, edema, and pleuritis. Confluent (diffuse) inflammatory infiltrate was quantified separately and expressed as percentage of the lung surface. The remaining parameters were rated separately on a scale from 0 (condition absent) to 4 (severe condition). Neutrophil stainings were performed using an anti-mouse Ly-6G monoclonal antibody (BD Pharmingen, San Diego, CA) and analyzed as described previously (16).

Statistical analysis

Human data are presented as means ± SE. Experimental data are expressed as box-and-whisker diagrams. Differences between groups were analyzed by (un)paired t-tests, Mann-Whitney U-tests, or
Kruskal-Wallis test when appropriate. Survival curves were compared using the log-rank test. All analyses were done using GraphPad Prism (GraphPad Software, San Diego, CA). $P < 0.05$ was considered statistically significant.

RESULTS

GzmA in BALF and Lung Tissue of CAP Patients

To obtain insight into the local release of GzmA during pneumonia, we measured GzmA in BALF derived from the infected and the uninfected lungs of six patients with unilateral CAP and from eight healthy subjects. CAP patients were 52 ± 6 yr of age (mean ± SE, 3 females, 3 males) and all recovered; causative organisms were identified as $S$. pneumoniae in four, $Mycoplasma pneumoniae$ in one, and $Haemophilus influenzae$ in one patient. GzmA levels were higher in BALF harvested from the infected lung compared with the uninfected lung from the same individual (Fig. 1A).

To gain insight into which cell types express GzmA in human lung tissue during CAP, we stained lung tissue slides from 10 patients who had died from pneumonia caused by $S$. pneumoniae (69 ± 6 yr) and from 9 patients who died without known pulmonary disease (62 ± 9 yr) with an anti-GzmA antibody. Figure 1B shows representative photomicrographs of these stainings. Positive GzmA staining was found in a variety of pulmonary cells of some, but not all, samples. Positive immunostaining for GzmA was found in pneumocytes, endothelial cells (Fig. 1B) and bronchiolar epithelium (not shown). In addition, few alveolar macrophages stained positive for GzmA. Moreover, in CAP patients, GzmA expression was found in intra-alveolar neutrophilic infiltrates (Fig. 1B). Besides these GzmA-positive neutrophilic infiltrates in CAP patients, there were no differences in GzmA staining between lung tissue slides from patients and control subjects.

GzmA Reduces Survival in Murine S. Pneumoniae Pneumonia and Has a Detrimental Effect on Bacterial Dissemination During S. Pneumoniae Pneumonia

To study the potential influence of GzmA on mortality from pneumococcal pneumonia, GzmA$^{-/-}$ and WT mice were observed in a survival study for 7 days following intranasal infection with viable $S$. pneumoniae. GzmA$^{-/-}$ mice showed a strong protection from mortality (25%) compared with WT mice (67%, $P = 0.007$) (Fig. 2A). We compared bacterial loads in samples from WT and GzmA$^{-/-}$ mice at several time points (6, 24, and 48 h) after induction of pneumonia to gain insight into the role of GzmA in the host resistance to $S$. pneumoniae (Fig. 2B). In the early phase of infection GzmA$^{-/-}$ mice had lower bacterial loads in BALF (6 h, $P < 0.05$ vs. WT mice), whereas in the more advanced stage of pneumonia GzmA$^{-/-}$ mice showed less systemic bacterial dissemination, reflected by lower bacterial counts in blood and spleen (24 h, both $P < 0.05$ vs. WT mice).

GzmA Contributes to the Early Inflammatory Response in the Lung

Invasion of the lower airways by $S$. pneumoniae results in an early inflammatory response that is essential for protective innate immunity (28, 30). To obtain information about the early host response in the lungs, we analyzed BALF and lung tissue harvested from GzmA$^{-/-}$ and WT mice 6 h after infection. Considering the importance of neutrophils in host defense, we examined the extent of neutrophil recruitment by determining neutrophil counts in BALF and the number...
of Ly-6G-positive cells in lung tissue. BALF neutrophil numbers were lower in GzmA−/− mice relative to WT mice 6 h after intranasal inoculation with S. pneumoniae \((P < 0.01, \text{Fig. 3A})\); at this early time point, neutrophils were barely detectable in lung tissue and not different between mouse strains (data not shown). GzmA has been shown to induce proinflammatory mediators in vitro \((7, 9 –11)\). In accordance with an attenuated early inflammatory response, GzmA−/− mice had lower levels of neutrophil attracting chemokines (KC and MIP-2) and cytokines (TNF-α and IL-1β) in whole lung homogenates, whole blood, and spleen homogenates as indicated 6, 24, or 48 h after intranasal infection with S. pneumoniae \((B)\). Data are expressed as box-and-whisker diagrams \((n = 8 \text{ per group})\). n.d. None detected. *\(P < 0.05\) compared with WT mice.

Role of NK Cells in Pneumococcal Pneumonia

Since NK cells are a major source of GzmA \((2, 10)\), we next determined the contribution of NK cells to GzmA positivity in lungs of mice before and 24 h after infection with S. pneumoniae via the airways (Fig. 6). GzmA positivity was almost exclusively found in NK cells in lungs and the hematopoietic compartment (Fig. 6A). Whereas the percentage of NK cells did not change after infection in lungs (Fig. 6B) or spleens (Fig. 6C) as quantified in WT mice, the percentage GzmA-positive NK cells and the GzmA MFI increased in lungs of infected WT mice; as expected GzmA staining was negative in GzmA−/− mice.

We next set out to deplete WT and GzmA−/− mice from NK cells using a NK1.1 depleting antibody. This treatment resulted in a 97% reduction of the NK1.1 \((\text{CD122}^+/\text{CD3}^-)\) lymphocyte
population in lungs of WT mice (vs. 94% in GzmA−/− mice) and 92% reduction in blood (vs. 91% in GzmA−/− mice) at the time of infection (Table 1). In line with the initial 24-h time point experiment (Fig. 2), GzmA−/− mice showed lower bacterial loads in spleen (P = 0.006) and blood (P < 0.001) compared with WT mice (Fig. 7). However, NK cell depletion did not influence bacterial loads in either WT or GzmA−/− mice (Fig. 7). In this experiment, GzmA−/− mice (relative to WT mice) showed lower concentrations of cytokine and chemokine levels in whole lung homogenates, significantly so for KC

Fig. 3. GzmA enhances the early inflammatory response in the lung during pneumococcal pneumonia. Number of neutrophils (PMN) per milliliter bronchoalveolar lavage fluid (BALF) and levels of keratinocyte-derived cytokine (KC), macrophage-inflammatory protein (MIP)-2, interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) in lung homogenates of wild-type (WT; grey boxes) and GzmA knockout (GzmA−/−, open boxes) mice, 6 h after intranasal infection with Streptococcus pneumoniae. Data are expressed as box-and-whisker diagrams (n = 8 per group). *P < 0.05, **P < 0.01, and ***P < 0.001, compared with WT mice.

Fig. 4. GzmA has little effect on lung injury in the early phase of pneumococcal pneumonia. Total lung histopathology scores (A) with representative microphotographs of hematoxylin and eosin-stained lung sections (B) of wild-type (WT) and GzmA knockout (GzmA−/−) mice 6 h after induction of pneumococcal pneumonia. Data are expressed as box-and-whisker diagrams (n = 8 per group). Scale bar = 200 μm.
NK cell depletion did not impact on lung cytokine or chemokine levels in either WT or GzmA−/− mice.

DISCUSSION

Research on the function of granzymes has mainly focused on their ability to cause target cell death. However, the role of GzmA as inducer of target cell death is controversial (21) and recent studies have pointed to a role of GzmA in the regulation of innate immunity during infectious and inflammatory conditions (2, 21). Here we report GzmA expression in BALF and lungs of CAP patients and show that GzmA deficiency during experimental CAP caused by S. pneumoniae is associated with reduced bacterial growth and improved survival.

GzmA is constitutively expressed by NK, NKT, and γδT cells, by approximately half of circulating CD8+ cells and few CD4+ cells (10, 11). Additionally, GzmA expression can be induced in mast cells, macrophages, T-regulatory cells, and human B cells (12); however, the possible function of GzmA within nonlymphoid cells thus far has not been established. We aimed to look at GzmA expression in human lung tissue in the setting of CAP caused by S. pneumoniae. In line with a previous report (31), we demonstrated GzmA expression by bronchial epithelial cells, pneumocytes, tissue macrophages, and a small number of alveolar macrophages. Notably, endothelial cells also demonstrated GzmA expression. Although enhanced GzmA expression by type II pneumocytes in patients with severe chronic obstructive pulmonary disease (31) points towards a role for GzmA in chronic lung inflammation, we did not observe any difference in GzmA expression between CAP patients and patients who died from a nonpulmonary cause apart from GzmA-positive neutrophilic infiltrates in the former group. We did detect elevated GzmA levels in BALF obtained from the infected lung of patients with unilateral CAP. Together these data indicate that GzmA is constitutively expressed by multiple cell types in human lungs and that infection results in local release of GzmA.

The generation of GzmA-deficient mice has provided the opportunity to gain more insight into the functional role of GzmA in infectious diseases. Previous studies have indicated that the involvement of GzmA in the host response to infection varies dependent on the causative organism. GzmA−/− mice were more susceptible to ectromelia infection (1, 22) and the parasite Trypanosoma cruzi (12, 23) than WT mice. However, GzmA did not seem to play an important role during infection with either lymphocytic choriomeningitis virus or Listeria monocytogenes (6) or K. pneumoniae (9) and GzmA−/− mice were protected from death during influenza infection (2). GzmA−/− mice were resistant to endotoxic shock, illustrating the proinflammatory properties of this protease (21). In the present study we infected GzmA−/− and WT mice with viable S. pneumoniae and found reduced bacterial numbers in BALF in the early phase and in the systemic compartment at 24 h postinfection with protection from mortality in an observational study. Of note, the observed difference in systemic bacterial counts 24 h postinfection was no longer present at 48 h, which may reflect maximal systemic bacterial loads in both study groups. These data suggest that the presence of GzmA facilitates early pneumococcal multiplication in the alveolar compartment, causing the bacterial load to exceed a certain threshold for systemic dissemina-
tion over the lung epithelial-endothelial barrier, ultimately leading to increased mortality.

A proinflammatory role for GzmA was first suggested when GzmA was shown to cleave pro-IL-1β into its biologically active form (14). Subsequent studies have shown that GzmA induces the release of proinflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α from monocytes/macrophages, fibroblasts, and epithelial cell lines (21, 25, 26). In addition, GzmA−/− mice showed a reduced inflammatory response during parasitic infection (12). In line with these studies, GzmA−/− mice demonstrated less neutrophil influx in BALF 6 h postinfection, together with lower levels of IL-1β, TNF-α, KC, and MIP-2 in lung homogenates. In the late stage of pneumonia (48 h postinfection), apart from elevated levels of

Table 1. NK cell depletion rates in lung and blood of wild-type and granzyme A knockout mice

<table>
<thead>
<tr>
<th></th>
<th>WT Control</th>
<th>WT NK Depl</th>
<th>GzmA−/− Control</th>
<th>GzmA−/−: NK Depl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung CD122+/CD3−</td>
<td>10.0 ± 0.5</td>
<td>0.3 ± 0.1*</td>
<td>8.2 ± 1.0</td>
<td>0.4 ± 0.1†</td>
</tr>
<tr>
<td>%Depletion</td>
<td>97%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood CD122+/CD3−</td>
<td>3.4 ± 0.4</td>
<td>0.3 ± 0.0 (92%)*</td>
<td>4.2 ± 0.5</td>
<td>0.4 ± 0.1 (91%)+</td>
</tr>
<tr>
<td>%depletion</td>
<td>92%</td>
<td></td>
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</tbody>
</table>

Values are means ± SE. Percentage of natural killer (NK) cells, defined as CD122+/CD3− lymphocytes, in wild-type (WT) and granzyme A knockout (GzmA−/−) control and natural killer cell depleted (NK depl) WT and GzmA−/− mice and percentage of NK cell depletion at time of infection. *P < 0.001 vs. WT; †P < 0.001 vs. GzmA−/−.
NK cells are considered the main source of GzmA (2, 10) and in naïve mice NK cells were mainly GzmA positive. Of note, some neutrophils in human lung tissue stained positive for GzmA; we did not study GzmA expression in mouse neutrophils either before or after infection. During pneumonia the percentage of NK cells did not increase locally or systemically; however, the percentage of GzmA-positive NK cells, GzmA mRNA expression in lung tissue (data not shown), and GzmA expression per NK cell increased. Remarkably, depletion of NK cells did not alter bacterial loads in either GzmA−/− or WT mice. One explanation of this finding could be that in our model of severe pneumonia NK cells may play a protective role, which is counterbalanced by their GzmA content. However, NK cell depletion in GzmA−/− mice did not have an unfavorable effect on bacterial loads. Thus NK cells in this model may require GzmA to exert a protective role. NK cells indeed were important for protective immunity during Klebsiella pneumonia (34). On the contrary, a previous study reported a detrimental role for NK cells in pneumonia caused by Streptococcus pneumoniae (35). Indeed, NK cells during Streptococcus pneumoniae pneumonia.

Table 2. Levels of cytokines and chemokines in lung homogenates of wild-type and granzyme A knockout mice with/without NK cells during Streptococcus pneumoniae pneumonia

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>WT control</th>
<th>WT NK depl</th>
<th>GzmA−/− control</th>
<th>GzmA−/− NK depl</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP-2, pg/ml</td>
<td>3.87 ± 0.71</td>
<td>7.39 ± 2.049</td>
<td>2.42 ± 0.437</td>
<td>2.00 ± 0.261</td>
</tr>
<tr>
<td>KC, pg/ml</td>
<td>10.387 ± 1.569</td>
<td>16.884 ± 3.155</td>
<td>3.200 ± 1.350*</td>
<td>3.263 ± 1.169†</td>
</tr>
<tr>
<td>IL-1β, pg/ml</td>
<td>525 ± 134</td>
<td>922 ± 225</td>
<td>324 ± 163</td>
<td>134 ± 42‡</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>4.410 ± 1.035</td>
<td>7.788 ± 1.900</td>
<td>1.985 ± 1.044</td>
<td>1.203 ± 0.734†</td>
</tr>
<tr>
<td>TNFα, pg/ml</td>
<td>443 ± 74</td>
<td>775 ± 143</td>
<td>284 ± 92</td>
<td>158 ± 25</td>
</tr>
<tr>
<td>IFN-γ, pg/ml</td>
<td>18.7 ± 5.6</td>
<td>6.1 ± 1.3</td>
<td>5.9 ± 2.9</td>
<td>1.9 ± 0.7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE of n = 8 per group. Levels of cytokines and chemokines in lung homogenates of wild-type (WT) and granzyme A knockout (GzmA−/−) mice and Natural Killer cell depleted (NK depl) WT and GzmA−/− mice 24 h after induction of pneumococcal pneumonia. IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; KC, keratinocyte-derived cytokine; MIP-2, macrophage-inflammatory protein-2. *P < 0.05 vs. WT control; †P < 0.01 vs. WT NK depleted.
by \textit{S. pneumoniae}. Of note, however, these investigations were performed with a less virulent serotype 2 bacterial strain (D39), administered at much higher doses as used here (10^6 vs. 5 \times 10^4 CFU) to a different mouse strain (BALBc) (17). An alternative explanation for the findings in NK cell depleted mice may be that NK cells do not play an important role in host defense and that another cellular source of GzmA may account for the adverse outcome of WT mice relative to GzmA\textsuperscript{+/–} mice. In this regard CD8\textsuperscript{+} T cells are an unlikely GzmA source, considering that antibody induced CD8\textsuperscript{+} T-cell depletion increased, rather than reduced bacterial loads (32; and unpublished results, A. F. de Vos).

This study is the first to investigate the role of GzmA in host defense against CAP. We here link observational data in humans showing pulmonary GzmA expression with functional studies in experimental pneumococcal pneumonia using GzmA\textsuperscript{+/–} mice. Our results point to a detrimental role for GzmA in respiratory tract infection caused by \textit{S. pneumoniae} that is independent of NK cells.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


