Staphylococcus aureus Infection in Humanized Mice: A New Model to Study Pathogenicity Associated With Human Immune Response

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Background. Staphylococcus aureus is a common pathogen among humans worldwide, with an increasing prevalence of multidrug resistance. The understanding of virulence factors inducing pathogenicity is still incomplete, and thus far the transfer of results from animal studies into successful clinical trials has been difficult.

Methods. In this study, we established an S. aureus infection model in mice engrafted with a human immune system, compared it with infected wild-type and nonhumanized mice, and investigated pathogenesis in these models.

Results. Staphylococcus aureus infection was aggravated in humanized mice, compared with wild-type or non-engrafted mice. The humanized mice displayed a significantly reduced survival percentage, increased weight loss, and a more-rapid increase in bacterial burden. In addition, S. aureus infection induced T-cell activation, apoptosis, and Fas receptor expression in humanized but not wild-type mice.

Conclusions. Our findings demonstrate the different pathogenetic mechanisms in wild-type and humanized mice and the possible benefit of including humanized mice in future studies involving S. aureus as a prior step to human clinical trials.

Keywords. humanized mice (HM); Staphylococcus aureus; T-cell apoptosis; fas receptor; caspase-3.

Staphylococcus aureus is a versatile pathogen in humans, causing a variety of different diseases ranging from minor skin abscesses to life-threatening infections [1, 2]. About 20% of individuals are permanent carriers, and 30%–60% are intermittent carriers [3]. The rate of community-acquired or hospital-acquired infections is increasing steadily, and given the growing prevalence of methicillin-resistant S. aureus’ strains [4], new treatment strategies and an appropriate animal model to test these therapeutic approaches under human-like conditions are urgently needed.

Rodent models have been important tools to study the pathogenesis of Staphylococcus-produced toxins [5], enzymes [6], and the host-bacterium relationship [7–9]. However, murine and human immune systems exhibit some substantial differences [10, 11], and the immune responses against pathogens and thereby the outcome of infections differs dramatically between species. For example, sensitivity to endotoxins [12] and, most notably, to lipopolysaccharide is more pronounced in humans than in rodents [13, 14]. In addition, while most rodents are less susceptible to the effects of Panton-Valentine leukocidin (PVL) [15], rabbits show hypersensitivity to α-toxin [16]. These differences might offer some explanation as to why many new therapeutic strategies with promising results in animal studies failed in clinical trials. For instance, many attempts have been made to develop effective antibody therapies against different bacterial targets. These antibodies showed sufficient effects when tested in mice but failed when tested in humans [17–19]. In addition, the development of new treatment options for sepsis, which
had significant beneficial influences in murine models, have failed to demonstrate efficacy in human clinical trials [20–22]. Moreover, as many as 150 clinical studies of potential antiinflammatory drugs failed in clinical settings, although they showed promising results in mice studies [23]. Therefore, new animal models with characteristics of the human immune system are needed to better understand the host-pathogen interplay.

In this study, human CD34+ hematopoietic stem cells were transplanted into the immunodeficient mouse strain NOD scid gamma (NSG) to generate mice with a human immune system [24]. These so-called humanized mice (HM) were used as a new model to study intraperitoneal S. aureus infections and the effect on human immune cells, especially human T cells.

MATERIAL AND METHODS

Animals

NOD.Cg-Prkdcscid Ili2rgtm1Wjl/SzJ (NSG) mice were obtained from Jackson Laboratories (Bar Harbor, Maine). Mice were housed at the animal facility of the University of Regensburg in a specific-pathogen-free environment. For the generation of HM, newborn NSG mice were irradiated sublethally (1 Gy) within 48 hours of birth and underwent transplantation with 2.5 × 107 CD34+ human hematopoietic stem cells as previously described [25].

Ethics Statements

All animal work was approved by the local veterinary authorities of the district government on the basis of European guidelines and the national regulations of the German animal protection act (permission no. 54-2532.1-18/10). Cord blood samples were collected with approval from the Ethics Committee of the University of Regensburg (permission no. 11-101-0231). All patients included in the study provided written informed consent.

Bacterial Strain

The S. aureus strain PS80 (a type 8 encapsulated clinical isolate) was kindly provided by Prof J. C. Lee (Channing Laboratory, Brigham and Women’s Hospital, Boston, Massachusetts). PS80 was grown on soybean casein digest (CASO) agar overnight and suspended in phosphate-buffered saline (PBS). For infection, the bacterial suspension was adjusted to an optical density of 0.4, corresponding to 5 × 108 colony-forming units (CFU)/mL in PBS.

Infection Model

Mice were infected intraperitoneally with S. aureus at the age of 3–5 months. To determine sublethal infectious doses, HM were injected with 1 × 107–5 × 108 CFU. To study the effect of acute infection on human T cells, mice were infected with a nonlethal infectious dose of 1 × 105 PS80 intraperitoneally and analyzed after 24 hours or infected with a lethal dose of 5 × 108 PS80 and analyzed after 4 or 24 hours. As controls, nonmanipulated NSG, irradiated NSG without reconstitution, and C57Bl/6 mice were infected with 5 × 108 PS80 and either analyzed after 24 hours or monitored for up to 6 days. Cell suspensions were prepared from peripheral blood specimens, spleen, bone marrow specimens, and the peritoneum (peritoneal exudate cells); stained with specific fluorochrome-conjugated antibodies (Supplementary Materials); and analyzed using a FACS Canto II flow cytometer (BD Bioscience, San Jose, California) as previously described [25].

Bacterial Burden in Various Organs

The homogenates of spleen, liver, lung, kidneys, brain, bone marrow, and peritoneal exudate cells were plated on CASO agar in serial dilutions. After incubation overnight at 37°C, colonies were counted, and the number of CFU per gram of organ or, with regard to bone marrow and peritoneal lavage specimens, the number of CFU per milliliter were calculated.

Apoptosis Assay

The apoptotic fraction in samples was determined by staining cells as described above, washing in FACS buffer (PBS, 1% fetal bovine serum, and 0.01% NaN3), and incubating with 5 µL of annexin V-FTTC (Immuno Tools) in 70 µL of annexin-binding buffer (10 mM HEPES, 0.14 M NaCl, and 2.5 mM CaCl2; pH 7.4) for 20 minutes at 4°C in the dark. One minute before reading samples by flow cytometry, 10 µL of DAPI (5 µg/mL) was added and mixed.

Histological Analysis

Tissue samples of spleen, liver, lung, brain, and kidney were fixed in 4% formalin, embedded in paraffin, and stained as previously described [25]. The following antibodies from the ultraView Universal DAB Detection Kit (Ventana, Roche) were used for the HM: anti-CD4 (SP35), anti-CD8 (SP57), anti-CD45LCA (2B11), anti-CD79a (SCB117), anti-CD68 (PG-MI), anti-Caspase-3 (Asp175), and anti-CD44 (clone DF1485; all from Dako), and anti-CD69 (clone 15B5G2; from Acris). For wild-type mice, the anti-CD44 antibody (clone IM7) and anti-CD69 (H1.2F3; both from eBioscience) were used. All samples were analyzed microscopically (Zeiss Axiovert 200).

RESULTS

Characterization of Pathogenicity of PS80 Isolate Used in This Study

Staphylococcus aureus displays a complex, isolate-specific array of virulence factors. Most of these virulence factors (PVL, TSST-1, SEA, SEE, and ETA/ETB) were not detectable in the PS80 isolate used in this study, with the exception of α-toxin (data not shown). In agreement with previously published data [26], the spa type of the PS80 strain could be verified as t021, which is also seen in methicillin-susceptible S. aureus and methicillin-resistant S. aureus isolates, along with variable patterns of virulence genes.
The antimicrobial susceptibility analysis of the isolate revealed resistance to the β-lactam antibiotics penicillin G, ampicillin, mezlocillin, and piperacillin but displayed susceptibility to oxacillin (data not shown).

**PS80 Infection Increased Apoptosis of Human T Cells In Vitro**

Coincubation of *S. aureus* with human mononuclear cells (MNCs) isolated from cord blood slightly increased the cell number after 4 hours but significantly reduced the MNC count after 24 hours (Supplementary Figure 1A; *P* < .001). Total cell counts of the different immune cell subsets revealed that levels of human T cells (Supplementary Figure 1B; *P* = .0296) and human B cells (Supplementary Figure 1C; *P* = .0072) were significantly reduced after 24 hours of incubation but not after 4 hours or 48 hours. To evaluate the effect on human T-cell subsets, CD4+ and CD8+ T-cell subsets were further characterized using annexin and DAPI staining. There were no differences at 4 hours, but a trend toward increased apoptosis, especially of CD4+ T cells, was observed after 24 hours. After 48 hours, there was a significantly enhanced apoptosis of CD4+ T cells (*P* < .05) and a tendency toward increased apoptosis of CD8+ T cells (Supplementary Figure 1D).

To analyze possible pathways of apoptosis induction, we investigated the apoptosis-associated Fas receptor (CD95) expression on both subsets. Interestingly, 5 of 6 CD4+ and 4 of 6 CD8+ T-cell samples from cord blood MNCs had high basal expression levels of Fas receptor (mean percentage of CD4+ T cells expressing CD95 [±standard error of the mean {SEM}], 93% ±3.6%; mean percentage of CD8+ T cells expressing CD95 [±SEM], 89.8%±4.8%), and therefore the percentage of CD95+ cells was not significantly enhanced by cocultivation with PS80 (Supplementary Figure 1E). However, the overall expression intensity (mean fluorescence intensity) on PS80-cocultured MNCs was significantly influenced by the infection (Supplementary Figure 1F and 1G), and in 2 of 6 experiments, we observed an approximately 100% increase in the frequency of CD95 expression on both subsets of T cells.

We did not observe a significant change in the levels of Fas ligand (data not shown), interleukin 2 (IL-2), interleukin 6 (IL-6), tumor necrosis factor (TNF), interleukin 10 (IL-10), and interferon γ (IFN-γ) in PS80-infected MNC cultures (Supplementary Figure 2A). However, the highest concentration of cytokines (eg, for IL-6, up to 12 340 pg/mL; and for TNF, up to 11 100 pg/mL) were detected in PS80-cocultured MNC samples.

![Figure 1](http://jid.oxfordjournals.org/)

**Figure 1.** PS80 infection had the most-serious effects in mice with a human immune system. *A*, Dose-dependent survival of PS80-infected humanized mice (HM). *B*, Survival curves of 5 × 10^8^ PS80-infected HM, C57BL/6 wild-type (WT) mice, conditioned NOD scid gamma (NSG) mice (irradiated but not reconstituted with human CD34+ cells; irrad. NSG), and unmanipulated NSG mice (NSG). Statistical differences were calculated using the Mantel–Cox (log-rank) test (***P* < .001, **P* < .01, and *P* < .05). *C*, Weight loss of infected HM and control mice was monitored during 24 hours of 1 × 10^8^ PS80 infection, and the significance of differences was calculated with the Bonferroni posttest (***P* < .01). *D*, The colony-forming unit (CFU) count for each organ was determined 4 hours after infection with 5 × 10^8^ PS80 or 24 hours after infection with 1 × 10^8^ PS80. The number of animals is indicated in brackets. Abbreviations: BM, bone marrow; PEC, peritoneal exudate cells.
HM Were More Severely Affected by *S. aureus* Infection Than Nonreconstituted or Wild-Type Mice

Next we investigated the effect of *S. aureus* on human T cells in vivo. To determine the dose of *S. aureus* capable of causing severe disease in HM without leading to the death of the animals, mice were infected with different CFU concentrations. A total of 1 × 10^8 CFU led to moderate signs of infection (such as hunched posture and slightly reduced movement) and 91% survival, whereas infection with 5 × 10^8 CFU caused significantly reduced survival (*P* = .0007) within the first 24 hours after infection (Figure 1A).

To evaluate the influence of the human immune system on *S. aureus* infection, nonmanipulated NSG mice, irradiated but not reconstituted NSG mice, and C57BL/6 wild-type mice were infected with the high dose of PS80 (5 × 10^8 CFU). Surprisingly, the mortality rate of HM was significantly enhanced, compared with wild-type mice (*P* = .0009), nonmanipulated NSG mice (*P* = .0027), and irradiated NSG mice (*P* = .0075; Figure 1B). Similarly, mice reconstituted with human immune cells had significantly higher weight loss (*P* < .01; Figure 1C).

The highest bacterial burden was detected at 24 hours in infected HM, with the highest concentrations observed in the lung (4.87 × 10^8 CFU/g), spleen (8.33 × 10^7 CFU/g), liver (2.18 × 10^7 CFU/g), and kidney (6.63 × 10^7 CFU/g) specimens (Figure 1D). The highest concentration in the peritoneum (3.4 × 10^7 CFU/g) was observed 4 hours after infection in HM infected with a high dose. Systemic spreading of *S. aureus* was also detectable in all other organs at this early time point (Figure 1D).

**Figure 2.** Human reconstitution and invasion in different organs after infection. A, Humanized mice (HM) were infected with moderate doses of PS80 (1 × 10^8 colony-forming units) for 24 hours, and human engraftment (determined as the percentage of human CD45+ immune cells) was measured by flow cytometry. B, Percentages of human B cells (CD19^+^), human myeloid cells (CD33^+^), and human T cells (CD3^+^) were determined in the different organs, and the changes in peritoneal exudate cells (PEC) subpopulations in uninfected (PEC ctrl) and infected HM (PEC) were calculated using the Bonferroni posttest. The number of animals is indicated in brackets (** **P < .001). C, Immunohistological staining of liver and lung detected immune cell extravasation in infected organs. Bars indicate 100 µm. Abbreviations: BM, bone marrow; contr, HM without infection; PB, peripheral blood; *S. aureus*, *Staphylococcus aureus*.
S. aureus Infection Increases Invasion of Human Immune Cells Into Infected Organs and Induces T-Cell Activation In Vivo

There was no significant increase in the total human immune cells in organs from infected HM (Figure 2A; exemplarily shown for peritoneal exudate cells), compared with uninfected HM. Nevertheless, 24 hours after infection with $1 \times 10^8$ CFU of PS80, the composition of the leukocyte fraction in the peritoneal cavity (the site of inoculation) changed significantly, with an enhanced infiltration of T cells ($P < .001$) and a decreased presence of myeloid cells ($P < .01$; Figure 2B). The distribution of human immune cells did not change significantly in all other organs tested (peripheral blood, bone marrow, and spleen; data not shown for uninfected HM) after infection. Despite the constant total number of human immune cells in spleen, peripheral blood, and the peritoneum, extravasation of human effector cells into the liver and lung of infected HM was traceable by immunohistochemical staining within 24 hours after infection (Figure 2C).

To evaluate the possible effects of S. aureus infection on human T cells, spleen sections were stained for the surface markers CD44 and CD69, which are known to be upregulated on activated T cells. CD69 expression in uninfected control HM showed a small area of positive cells at the margin of the spleen, the size of which increased in mice 4 hours after high-dose infection and 24 hours after moderate-dose infection. The highest concentration of CD69-expressing T cells was detected 24 hours after high-dose infection, with additional migration of positive cells to the center of the spleen (Figure 3A). CD44 expression was present on single cells in uninfected HM and increased in frequency following S. aureus infection, with maximum expression, including strong cluster formation all over the spleen, 24 hours after high-dose infection (Figure 3B). CD44 expression in wild-type mice was detectable in uninfected control mice at the margin of the spleen (Figure 3C). Its expression was not increased 4 hours after infection (data not shown), but scattered CD44-positive cells were also present in the central area of the spleen 24 hours after high-dose infection. CD69 expression was present on single cells in uninfected wild-type mice, and the frequency of expression increased slightly 4 hours (data not shown) and 24 hours (Figure 3D) after infection.

S. aureus Infection Increased the Frequency of Apoptosis Among HM but Not C57BL/6 Wild-Type Mice

To further characterize the effect of S. aureus on T cells, induction of apoptosis among CD4+ (Figure 4A) and CD8+ (Figure 4B)
Figure 4. *Staphylococcus aureus* infection increased apoptosis in humanized mice (HM) but not in C57BL/6 wild-type mice (WT). HM and WT were infected for 4 hours or 24 hours with 1 × 10⁸ colony-forming units (CFU) (for HM) and 5 × 10⁸ CFU (for WT). The percentages of apoptotic (annexin+/annexin+/DAPI+) CD4⁺ (A) and CD8⁺ (B) T cells were analyzed in peripheral blood (PB) and peritoneal exudate cells (PEC) and compared with values for uninfected mice. C, Three HM were analyzed for apoptotic T cells in the PB before and after infection (1 × 10⁸ CFU PS80; 24 hours). The significance of differences between groups was analyzed with 2-way analysis of variance (A****P < .0001 and A***P < .001) and the Bonferroni posttest (*P < .05, **P < .01, and ***P < .001). D, Immunohistochemical caspase-3 staining in the spleen of HM (top row) and WT (bottom row). Bars indicate 100 µm. E, Caspase-3-positive cells in 1000 spleen cells were counted in HM and WT 4 hours and 24 hours after infection with 5 × 10⁸ PS80. The significance of differences between groups was analyzed with Bonferroni posttest (***P < .001).
T cells from the peripheral blood and peritoneal exudates of wild-type mice and HM was analyzed according to the gating strategy indicated in Supplementary Figure 3. Overall, in HM, the percentage of CD4+ (Figure 4A) and CD8+ (Figure 4B) T cells undergoing apoptosis was significantly affected by *S. aureus* infection. The highest apoptosis levels were observed among T cells from peritoneal exudates (the site of infection) 4 hours after following inoculation of 5 × 10⁸ bacteria (*P* < .01), whereas T cells isolated from peripheral blood showed the highest percentage of apoptotic cells 24 hours after inoculation with a low-dose inoculum (1 × 10⁸ CFU; *P* < .001). To confirm the results obtained from peripheral blood samples from HM, which were collected from multiple mice pooled in the control and infected group (Figure 4A and 4B), we analyzed apoptosis induction in 3 individual mice before and 24 hours after infection (Figure 4C). These results verified the significant increase of apoptosis among CD4⁺ (*P* < .001) and CD8⁺ (*P* < .01) T cells from HM following *S. aureus* infection (Figure 4C).

Interestingly, uninfected wild-type mice showed a higher rate of baseline apoptosis among T cells from peripheral blood specimens, which was not significantly influenced by *S. aureus* infection. The lowest percentage of apoptotic T cells in peripheral blood specimens was observed in all animals after 24 hours. The percentage of apoptotic T cells among peritoneal exudate cells was low in all groups and was not affected by *S. aureus* infection (Figure 4A and 4B). Necrosis (DAPI+annexin−) was not detected in any animal model (data not shown).

To further verify apoptosis induction and the related pathway, all organs from infected mice were stained for caspase-3 by an immunohistochemical technique. Caspase-3–positive cells were rarely detectable in the liver, kidney, and lung from all infected mice, including HM, wild-type, nonmanipulated NSG, and irradiated NSG (data not shown). Staining of spleen samples revealed a significant induction of caspase-3 expression 24 hours after infection in HM (Figure 4D and 4E; *P* < .001), whereas wild-type mice displayed high baseline caspase-3 expression without infection, which was not influenced by *S. aureus* (Figure 4D and 4E).

### Fas Receptor Expression on CD4⁺ and CD8⁺ T Cells Is Triggered by *S. aureus* in HM but Not in Wild-Type Mice
Fas-induced apoptosis in human T cells was detectable in some of the MNC coculture experiments (Supplementary Figure 1E–G) and was therefore also investigated in the in vivo experiments. As observed in MNCs, the percentage of CD95-expressing CD4⁺ and CD8⁺ T cells at baseline was high in the organs from most uninfected HM (Figure 5A and 5B), and therefore no significant

Figure 5. Fas receptor expression on CD4⁺ and CD8⁺ T cells is triggered by *Staphylococcus aureus* in humanized mice (HM) but not in C57BL/6 wild-type mice (WT). HM and WT were infected with 1 × 10⁸ colony-forming units (CFU) or 5 × 10⁸ CFU of PS80 for 4 hours or 24 hours. The percentages of CD4⁺ (A) and CD8⁺ T cells expressing CD95 (Fas) were analyzed by flow cytometry, and the significance of differences between groups was analyzed by the Bonferroni posttest (**P** < .01 and ***P** < .001). The number of experiments is indicated in brackets. The mean fluorescence intensity (MFI) of CD95 expression was determined in peritoneal exudate cells (PEC) on CD4⁺ (C) and CD8⁺ (D) T cells and compared to findings for uninfected animals (control). The significance of differences between groups was analyzed with the Tukey multiple comparisons test (***P** < .01 and ***P** < .001). Abbreviation: PB, peripheral blood.
changes could be observed following infection. However, as seen in T cells ex vivo, mean fluorescence measurements revealed an upregulation in the frequency of CD95 expression on CD4 \(^+\) (\(P < .01\)) and CD8 \(^+\) (\(P < .001\)) T cells among peritoneal exudate cells after *S. aureus* infection (Figure 5C and 5D) but not among CD4 \(^+\) and CD8 \(^+\) T cells among peripheral blood or spleen cells (data not shown). Notably, the highest percentage of CD95 expression was detected in the bone marrow (mean fluorescence \([\pm \text{SEM}]\), 15 068 ± 1445 among CD4 \(^+\) T cells and 10 447 ± 3 among CD8 \(^+\) T cells; \(n = 2\); data not shown), and the lowest level was observed in the lymph nodes (4080 ± 438 and 3023 ± 141, respectively; \(n = 3\); data not shown) measured 4 hours after infection with 5 \(\times\) 10\(^8\) CFU.

As seen for annexin V–indicated apoptosis and caspase-3 expression, wild-type and HM mice also differ significantly with respect to expression of the Fas receptor (ie, CD95; Figure 5). Uninfected wild-type mice had no detectable CD95 expression on peripheral blood or spleen cells, which was also not influenced by infection. However, peritoneal exudate cells contained a small proportion of CD95 \(^+\) T cells but without significance differences in overall expression or mean fluorescence intensity after infection (Figure 5A–D).

### Cytokine Response in Infected HM Differs but Peaks 24 Hours After Infection

As seen in the in vitro experiments, the levels of the cytokines IL-2, IL-6, TNF, IL-10, and IFN-\(\gamma\) varied widely throughout the HM, and no overall significant increases were observed. The highest concentrations were measured in serum specimens of mice 24 hours after infection with 1 \(\times\) 10\(^8\) CFU (Supplementary Figure 2B). Forty-eight hours after infection, cytokine levels declined nearly to the levels observed for uninfected HM.

### DISCUSSION

*Staphylococcus aureus* is a virulent pathogen causing multiple diseases in humans worldwide, with an increasing prevalence of multidrug resistance. New treatment options are urgently needed and require testing in more translational models to overcome the difficulties of transferring results from animal studies into successful clinical trials. In addition, a deeper understanding of the processes leading to severe *S. aureus* infection, including those resulting in sepsis and death, is necessary before effective therapeutic interventions can be successfully applied.

HM have already been used in different infectious disease models and have also defined treatment effects in the context of a human immune response [25–29]. In this study, we introduced the HM as a feasible animal model that allows further differentiation of *S. aureus*–induced pathogenesis under human-like conditions. Most interestingly, *S. aureus* infection was aggravated in mice engrafted with a human immune system, with a significantly reduced survival percentage, increased weight loss, and higher concentrations of pathogens in different organs, compared with nonhumanized NSG or wild-type mice. In addition, activation of T cells in HM was more pronounced than that in wild-type mice. Uncontrolled inflammation is one important mechanism of inducing organ injury in patients [30]. Polyclonal activation of T cells can be triggered by *S. aureus* toxins binding to particular V beta subgroups of the T-cell receptor, resulting in apoptosis of T cells [31]. In this regard, CD44 is suggested as an important regulator of activation-induced cell death among T cells [32]. The induction of apoptosis is one mechanism to control the overreaction of the immune system and to eliminate activated lymphocytes, such as after infection [33, 34]. This process [35, 36], as well as the loss of lymphocytes [37], has been described in severely infected patients and correlates with an adverse outcome [34, 36, 38].

As published in a previous study of a HM model of sepsis [39], we observed apoptosis as a result of *S. aureus* infection in human T cells in vitro, as well as in vivo, but not in murine T cells. Apoptosis induction might therefore be the response to ongoing hyperactivation of T cells, which should protect the organism but fails when bacterial concentrations are too high.

A common negative regulator of activated T cells is activation-induced cell death, which is initiated by the activation of the Fas receptor (CD95), inducing apoptosis [40]. In addition to the Fas receptor–mediated pathway, the mitochondrial-mediated (Bcl-2–regulated) pathway can induce apoptotic cell death. Both pathways result in an activation of caspase-3 and, in turn, cell death. Sepsis is reported to trigger cell death by both pathways [41], which might be initiated differently, depending on the pathogen involved.

Increased Fas receptor expression, as well as increased caspase-3 expression, was observed in HM but not in wild-type mice and has been described before to be associated with severity and survival in multiorgan dysfunction syndrome in patients with sepsis [35, 37]. Nevertheless, Fas receptor activation might be just one component of a multifactorial mechanism that induces apoptosis during infection [41].

The activation of T cells has been described not only for superantigen stimulation [42, 43] but also in the context of \(\alpha\)-toxin [44]. In addition, Nygaard et al, as well as Bantel et al, have shown that \(\alpha\)-toxin induces human T-cell apoptosis during *S. aureus* infection [45, 46]. Therefore, \(\alpha\)-toxin could be involved in the induction of activation and apoptosis of human T cells in HM.

In conclusion, T-cell activation was more dominant in HM and was accompanied by increased apoptosis rate, increased Fas and caspase-3 expression, and more-severe disease progression. Of note, if apoptosis induction is a protective mechanism that emerges after hyperactivation, its inhibition might not be a convenient approach for new drug development.

With regard to cytokine release, we observed strong variability in HM, as well as in the in vitro MNC experiments, which
was also described in patients with sepsis [47]. HM therefore reflect this heterogeneity (likely caused by different hematopoietic stem cell donors), which mimics the situation in patients. Differences between hematopoietic stem cell donors might be due to allelic variations in major histocompatibility complex class II genes (HLA polymorphisms [48]) or to a specific Vβ repertoire in humans [49], both of which have been described as influencing T-cell activation especially after superantigen stimulation. Of note, in addition to cytokine release, activated T cells have been found to produce reactive oxygen species [50], which could also trigger severe organ damage during infection.

HM will not be able to replace conventional mice as the principle murine research population, but considering the different results from wild-type and HM in this study, it might be beneficial to test new therapeutic strategies in HM before the strategies enter clinical trials.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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