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CD169+ Macrophages Restrain Systemic Inflammation Induced by Staphylococcus aureus Enterotoxin A Lung Response

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ABSTRACT

Alveolar macrophages (AMs) are considered the first line of defense in the airways. Exposure to harmful substances and certain infections can lead to dysfunction or depletion of AMs. Importantly, these conditions have been associated with increased risk of sepsis and acute lung injury. Staphylococcus aureus enterotoxins are superantigens that induce oligoclonal activation of T cells and a robust cytokine release, leading to systemic inflammatory response and tissue injury. In this study we investigated the relationship between S. aureus enterotoxins and AMs. Following inhalation, S. aureus enterotoxin was preferentially bound to AMs and MHC class II was not required. Furthermore, the enterotoxin was internalized and its presence in the cells decreased by 24 h after exposure. Ablation of AMs in CD169−diphtheria toxin receptor mice was associated with increased activation of enterotoxin-specific T cells and enhanced cytokine release into circulation. Thus, conditions causing depletion of AMs may increase the risk of S. aureus enterotoxin−induced diseases. ImmunoHorizons, 2017, 1: 213–222.

INTRODUCTION

Alveolar macrophages (AMs) are specialized tissue-resident cells that play a crucial role in lung development, homeostasis, and immune surveillance. Their unique position in the lung alveoli enables them to sample pathogens, inhaled particulates, and other environmental cues. Their reciprocal interactions with neighboring cells through various receptors and the cytokine/chemokine axis can then alarm the immune system and orchestrate responses to remove potential threats and respond to tissue injury, making them the first line of pulmonary immune defense (1). AMs have been shown to play a critical anti-inflammatory role in a number of disease animal models, including influenza, asthma, and acute lung injury (2−4), but they could be also proinflammatory (5).

AMs are a relatively stable population of cells with very slow turnover rate; however, when the injury is severe enough, AMs may be depleted and later replenished from circulating monocytes or from in situ proliferation (1). Exposure to various substances, pathogens, and their products may lead to AM depletion or impaired function; these include influenza (6), bacterial pneumonia (7), cigarette smoke (8), anesthesia (9), diabetes (10), or alcohol consumption (11). Importantly, many of these conditions or exposures have been associated with increased risk of serious infections and development of systemic inflammatory response syndrome/sepsis and acute respiratory distress syndrome (ARDS).
In particular, influenza can be complicated by a secondary bacterial pneumonia, which leads to higher rates of hospitalization and death (12). Increased alcohol consumption and cigarette smoke exposure have been associated with increased risk of systemic inflammatory response syndrome/septic shock and ARDS, respectively (13, 14). Finally, a number of chronic conditions, particularly diabetes mellitus, and immunosuppressive state are well-described comorbidities in sepsis patients (15, 16). Collectively, these studies suggest that depletion or impaired function of AMs may be associated with increased risk of serious infection, sepsis, and ARDS.

*Staphylococcus aureus* enterotoxins are a group of potent bacterial toxins that bypass the classical Ag processing and presentation, directly bind to MHC class II (MHC II) on APCs, and crosslink it with specific Vβ-chains of TCRs. This unique feature of the so-called superantigens induces a massive inflammatory response marked by oligoclonal T cell activation and cytokine storm (17). Superantigens have been established as the mediators of toxic shock syndrome, a type of systemic inflammatory response, which can lead to tissue injury, shock, and even death (18). However, recent evidence suggests that they may be involved in a number of diseases, including pneumonia, endocarditis, and sepsis (19–21). In fact, superantigens and superantigen-expressing *S. aureus* strains have been recovered from septic patients (22). *S. aureus* enterotoxins likely spread systemically from focal sites of infection rather than being directly produced in the bloodstream, as hemoglobin peptides were shown to inhibit superantigen production (19). The most common colonization site of *S. aureus* is the anterior nares (23); therefore, exposure to aerosolized or inhaled *S. aureus* enterotoxin may well mimic the route of dissemination. Accidental exposure to aerosolized *S. aureus* enterotoxin in humans was reported to cause systemic symptoms of fever, chills, headache, myalgia, cough, dyspnea, vomiting, and diarrhea in humans (24). In nonhuman primates, aerosolized lethal doses of *S. aureus* enterotoxin caused severe pulmonary lesions and death in some subjects (25). Finally, *S. aureus* enterotoxin inhalation in mice was shown to cause systemic inflammatory response, involving rapid activation of T cells, cytokine release, recruitment of innate cells, and subsequent lung injury (26).

The relationship between AMs and *S. aureus* enterotoxins has not been fully explored. In vitro stimulation of human AMs with *S. aureus* enterotoxin A (enterotoxin A) induced IL-8 production in a dose-dependent manner (27). In another in vitro study, addition of macrophages (AMs or peritoneal macrophages) reduced proliferation of enterotoxin A–activated T cells via NO production (28). Although there is only a limited direct link between AMs and *S. aureus* enterotoxins, indirect evidence exists that shows a relationship between superantigens and conditions that deplete AMs. In particular, several reports from the 1980s described development of toxic shock syndrome in patients with influenza and influenza-like illness (29, 30). Furthermore, a recent study showed that the combination of cigarette smoke exposure and *S. aureus* enterotoxin B (enterotoxin B) induced a greater extent of pulmonary inflammation than did cigarette smoke or enterotoxin B alone (31). Based on these findings, we hypothesized that AMs play a critical role in the inflammatory responses triggered by *S. aureus* enterotoxin inhalation.

In this study we show that enterotoxin A was taken up by AMs upon inhalation and the enterotoxin was found intracellularly and not on the cell surface. Perhaps surprisingly, MHC II expression was not required for binding, as enterotoxin A was similarly taken up by AMs of wild-type (WT) and MHC II−/− mice. AMs in the murine lung express sialoadhesin, also known as CD169, on their surface (32). Thus, to study the role of AMs, CD169–diphtheria toxin receptor (DTR) mice were treated with DT and then exposed to enterotoxin A. The absence of AMs was associated with increased CD25 expression on enterotoxin A–specific T cells, but this effect was only observed when lower concentrations of enterotoxin A were administered. Finally, enterotoxin A–exposed CD169-DTR mice also showed a significantly greater cytokine secretion to blood compared with WT mice. Thus, AMs may represent a defense mechanism against *S. aureus* enterotoxins, as their depletion may increase the severity of toxic shock or other *S. aureus* enterotoxin–induced diseases.

**MATERIALS AND METHODS**

**Mice**

C57BL/6J (WT) and MHC II−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and CD169-DTR mice (CD169-DTR−/−) were provided by Dr. K.M. Khanna (University of Connecticut Health Center via Dr. M. Tanaka). Mice were used between 1.5 and 4 mo of age and they were housed in the Central Animal Facility at the University of Connecticut Health Center according to federal guidelines. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Connecticut Health Center.

**Toxin administration**

In most studies, 1 μg of enterotoxin A (Toxin Technology, Sarasota, FL) diluted in 50 μl of balanced salt solution or balanced salt solution alone (vehicle) was pipetted on the nostrils (intranasal route). In enterotoxin A titration experiments, 0.033, 0.1, 0.33, or 1 μg of enterotoxin A was administered intranasally. To ablate CD169+ cells, both WT and CD169-DTR mice received 40 ng/g body weight of DT (Sigma-Aldrich, St. Louis, MO) i.p. 2 d before enterotoxin A or vehicle inhalation.

**Flow cytometry**

Harvest and processing of bronchoalveolar lavage (BAL) fluid, lung, and spleen, and staining for flow cytometry were performed as described previously (26, 33). The single-cell suspensions were labeled with Live/Dead fixable blue dead cell stain (Thermo Fisher Scientific, Waltham, MA) and stained with the following Abs: CD45.2 clone 104 (shown as CD45), anti–Siglec F clone E50-2440, anti-CD11c clone N418, anti-CD169 clone SER-4, anti-CD3 clone 17A2, anti-VB3 clone KJ25, anti-VB14 clone 14-2, anti-CD25 clone PC61, and anti-CD69 clone H1.2F3 (purchased from BD Biosciences).
Biosciences, San Jose, CA or eBioscience, San Diego, CA). Staining for enterotoxin A was performed (after blocking with undiluted goat serum) with polyclonal anti–enterotoxin A Ab produced in rabbit (whole antiserum; Sigma-Aldrich) followed by secondary goat anti-rabbit IgG–Alexa Fluor 488 (Thermo Fisher Scientific). For intracellular staining, cells were first fixed in 2% formaldehyde and then permeabilized with 0.25% saponin (Sigma-Aldrich). Flow cytometry was performed using a FACS LSR II (BD Biosciences) and the data were analyzed with FlowJo software (Tree Star, Ashland, OR).

Confocal microscopy
Confocal microscopy on frozen tissue sections was performed as described before (26). The sections were stained with polyclonal anti–enterotoxin A Ab (Sigma-Aldrich) for 1 h at room temperature, which was followed by Alexa Fluor 488–conjugated anti-rabbit IgG (Thermo Fisher Scientific) and fluorophore-conjugated Abs for 1 h at room temperature. The following Abs were used: anti-CD11c clone SER-4, MHC II clone M5/114.15.2, and anti-CD169 clone N418 or HL3 (eBioscience and BD Biosciences). The images were acquired with a Zeiss LSM780 confocal microscope (Zeiss, Oberkochen, Germany) as confocal z-stack projections, using ×20/0.8 numerical aperture objective. Image analysis, including adjustment for brightness and contrast, quantification of cells, and colocalization studies (threshold value set to 7), was performed with Imaris software (Bitplane, Zurich, Switzerland).

Multiplex assay
Serum was collected using serum separator tubes (BD Biosciences). The concentrations of cytokines in the serum were measured with customized Luminex-based multiplex assays (EMD Millipore, Billerica, MA).

Statistical analysis
Statistical analyses in all experiments were performed using Prism (GraphPad Software, La Jolla, CA) and Microsoft Excel. A p value < 0.05 was considered significant (*p < 0.05, **p < 0.01, ***p < 0.001).

RESULTS

MHC II in lung is not required for binding of enterotoxin A to AMs
One of the crucial properties of S. aureus enterotoxins is their ability to rapidly induce oligoclonal activation of T cells, which is accompanied by a massive inflammatory response and cytokine storm (17). However, how enterotoxin A and other enterotoxins spread systemically, especially from the airways, as anterior nares are a common site of S. aureus colonies (23), is not fully understood. In our previous studies, we showed that enterotoxin A permeates into blood immediately after inhalation and is found in the serum rather than bound to cells (26). The ability to access the circulatory system from airway mucosa is likely an important feature that enables the toxin to spread systemically and to activate T cells in lymphoid tissues within minutes of inhalation (26, 34). Furthermore, using a titration curve, the concentration of enterotoxin A in serum after inhalation was estimated to be only a small fraction of the total amount inhaled by a mouse (~<1/35th; J. Svedova, unpublished observations). Intriguingly, in another report using mass spectrometry, we showed that enterotoxin A could be recovered from BAL fluid at 16 h after inhalation and the toxin preserved its biological activity (35). These findings suggested that when enterotoxin A is inhaled, only a small portion enters the circulation, whereas the remainder may be retained in the lung mucosa or perhaps neutralized by various defense mechanisms, such as defensins or phagocytosis.

Therefore, we sought to determine whether enterotoxin A binds to a certain cell population within the pulmonary tissue. Lung was obtained from mice 8 h after enterotoxin A or vehicle inhalation and examined by confocal microscopy for enterotoxin A using polyclonal anti–enterotoxin A Ab. Only mice exposed to enterotoxin A displayed cells that were enterotoxin A+ (Fig. 1A, 1B, Supplemental Fig. 1A), confirming the specificity of the Ab. Surprisingly, we found that enterotoxin A was preferentially bound to CD11c+CD169+ AMs (Fig. 1A). In fact, a total of 87% of enterotoxin A+ cells were also positive for CD11c and 64% were both CD11c+ and CD169+ (Fig. 1C). To further define enterotoxin A binding to AMs, BAL fluid was obtained from mice 1, 4, or 24 h after enterotoxin A inhalation and BAL AMs defined as CD45+ CD11c+Siglec F+CD169+ (Fig. 1D) were stained for enterotoxin A. Importantly, no enterotoxin A was detected on the surface of AMs; however, there was a significant increase in enterotoxin A presence intracellularly, peaking at 4 h (Fig. 1E, 1F, Supplemental Fig. 1B). Interestingly, there was no significant difference detected at 24 h after enterotoxin A (Fig. 1F). Similar results were obtained when AMs from lung tissue were analyzed (Fig. 2A). In contrast, lung dendritic cells showed increased enterotoxin A expression only at 24 h both on the surface and intracellularly (Fig. 2).

S. aureus enterotoxins are known for their ability to directly bind to MHC II molecules and crosslink them with specific Vβ-chains of TCRs, leading to a systemic inflammatory response (17). However, AMs were reported to only express low levels of MHC II under homeostatic conditions (1) with MHC II expression increasing during an inflammatory response, particularly in the presence of IFN-γ (36). Furthermore, AMs tend to be poor Ag presenters and may, in fact, inactivate T cells (37). These findings suggested that enterotoxin A binding to AMs can occur independently of MHC II expression. To assess whether enterotoxin A requires MHC II for binding to AMs, lung tissue was harvested from WT and MHC II−/− mice 8 h after enterotoxin A inhalation and analyzed by confocal microscopy. There was no difference in the number of enterotoxin A+ cells between WT and MHC II−/− mice (Fig. 3A, 3B). Additionally, enterotoxin A+ cells colocalized with CD11c marker with a similar frequency (Fig. 3C). Finally, only a small fraction of enterotoxin A+ cells colocalized with MHC II (Fig. 3D) and, in fact, we observed CD11c+ MHC II+ dendritic cells with no significant enterotoxin A binding (Fig. 3A, white arrows). Collectively, these results show that enterotoxin A binding to AMs does not require MHC II expression.
Depletion of AMs is associated with increased T cell activation and cytokine secretion

Next, we wanted to determine whether enterotoxin A binding to AMs impacts T cell activation and cytokine production. We hypothesized that AMs sequester a large portion of the inhaled toxin and neutralize it. Thus, AMs could play a protective role in enterotoxin A–induced inflammation. To investigate the role of AMs in enterotoxin A–induced inflammatory response, we used CD169-DTR mice to selectively deplete CD169+ macrophages after DT administration. Because AMs express CD169 (Fig. 1), this technique can be used to study the role of AMs in pulmonary responses (32). Thus, CD169-DTR mice and WT control were treated with DT 2 d prior to enterotoxin A or vehicle inhalation. To avoid oversaturation of enterotoxin A dosage given to mice (which could lead to masking of differences between WT and CD69-DTR mice), the enterotoxin A dose was titrated (0.033, 0.1, 0.33, and 1 μg). Blood, lung, and spleen were harvested 4 h after enterotoxin A or vehicle (0 μg) inhalation. Depletion of CD169+ AMs was
confirmed in the lung by gating on CD45⁺CD11c⁺Siglec F⁻CD169⁺ cells (Fig. 3A). The overall depletion of AMs was ~87% (Fig. 3B). We first investigated the effect of CD169⁺ macrophage ablation on CD69 expression on enterotoxin A–specific Vβ3⁺ T cells in the spleen. Interestingly, even with a low dose of enterotoxin A (0.033 μg), the percentage of CD69⁺Vβ3⁺ T cells was 88.5% in WT mice and 89.8% in CD169-DTR mice, suggesting that a very small amount of enterotoxin A is sufficient to induce CD69 expression on T cells (Fig. 4C, 4D). Thus, we concluded that CD69 expression might not be a sensitive marker to detect differences in T cell activation. In contrast, CD25 expression on Vβ3⁺ T cells increased in a dose-dependent manner (Fig. 4F). Importantly, CD169-DTR mice showed significantly greater percentage of CD25⁺Vβ3⁺ T cells when administered 0.033 and 0.1 μg of enterotoxin A (Fig. 4E, 4F). We also investigated the effect of AM ablation on the bystander Vβ14⁺ T cells. There was no difference in the percentage of CD69⁺ and CD25⁺ of Vβ14⁺ T cells with the exception of a significant increase in CD25⁺Vβ14⁺ T cells in CD169-DTR mice after a dose of 0.033 μg of enterotoxin A (Fig. 4D, 4F, right panels).

A hallmark of toxic shock syndrome as well as sepsis is a robust release of cytokines into the bloodstream (18). Therefore, we next investigated whether the depletion of CD169⁺ macrophages affected cytokine secretion following enterotoxin A inhalation. Serum cytokine concentrations were measured by a multiplex assay in DT-pretreated WT and CD169-DTR mice 4 h after vehicle or enterotoxin A (0.033 or 0.1 μg) inhalation. There was no significant difference in the levels of serum cytokine when vehicle control was administered. However, following enterotoxin A inhalation, CD169-DTR mice had greater concentrations of serum G-CSF, IFN-γ, TNF, IL-2, IL-6, and also IL-10 compared with WT mice (Fig. 5). These findings show that ablation of AMs is associated with increased expression of CD25 on enterotoxin A–specific Vβ3⁺ T cells and also increased secretion of cytokines into the circulation.

**DISCUSSION**

Superantigens such as *S. aureus* enterotoxins are potent bacterial toxins that can induce serious inflammatory responses and lethal shock (17). It has been estimated that most strains of *S. aureus* are capable of producing superantigens (19). However, *S. aureus* is also an extremely common commensal that permanently colonizes ~20% of all individuals (23). Thus, there is a disparity in understanding why *S. aureus* is so common in asymptomatic individuals and, simultaneously, it can trigger a life-threatening inflammatory response. Although the mechanisms of how a commensal colonization can become pathogenic are not clear (38), it has been well established that patients who have colonization of anterior nares with *S. aureus* and particularly methicillin-resistant *S. aureus* are more susceptible to hospital-acquired *S. aureus* infections (39). This suggests that various defense mechanisms must be in place to prevent the spread of the bacteria and limit the effects of the virulence factors; however, these mechanisms may be impaired in immunocompromised individuals in hospital settings.

Indeed, the immune system possesses several strategies to counteract the harmful effects of superantigens. First, it has been hypothesized that the production of Abs against superantigens is protective and the lack of Abs may a predisposing factor for the development of *S. aureus* infections and toxic shock syndrome (40). In particular, women with toxic shock syndrome had lower Ab titers to TSST-1 compared with healthy women with no prior history of toxic shock syndrome (41). Similarly, immunotherapy using Abs specific to superantigens was found to be protective in animal models (42). In addition to Abs, it has been shown that lactoferrin, an iron-binding glycoprotein located predominantly in mucosal secretions (43), can attenuate the inflammatory responses triggered by *S. aureus* enterotoxins (35). In this study we show that AMs may represent a key cellular defense mechanism that can reduce the effects of *S. aureus* enterotoxin–induced inflammation.

When staining for enterotoxin A in the lung tissue, we found that AMs preferentially bound enterotoxin A after inhalation and

**FIGURE 2. Enterotoxin A presence in lung AMs and dendritic cells.**

Lung tissue was harvested 1, 4, or 24 h after enterotoxin A inhalation or from vehicle control (1 or 4 h). AMs were identified as live CD45⁺CD11c⁺Siglec F⁻CD169⁺ cells and dendritic cells as live CD45⁺CD11c⁺Siglec F⁺MHC II⁺. (A) Relative median fluorescence intensity (MFI) of enterotoxin A on surface and intracellularly plus surface in lung AMs 1, 4, or 24 h after enterotoxin A inhalation. (B) Relative MFI of enterotoxin A on surface and intracellularly plus surface in lung dendritic cells 1, 4, or 24 h after enterotoxin A inhalation. MFI values are relative to the vehicle control (set as 1). Two independent experiments with a total of *n* = 4 per group were performed. Data are represented as mean ± SEM. One-way ANOVA with a Dunnett test was used: *p* < 0.05, **p** < 0.01, ***p*** < 0.001.
internalized it (Fig. 1). Interestingly, the intracellular presence of enterotoxin A in AMs increased in the first several hours but was not significantly different at 24 h (Figs. 1F, 2A). Additionally, dendritic cells, which were hypothesized to bind a significant amount of enterotoxin A due to their high MHC II expression, showed upregulation of enterotoxin A expression on surface and intracellularly only at 24 h after inhalation (Supplemental Fig. 1B). Based on these findings, we postulate that the numerous and highly phagocytic AMs ingest enterotoxin A and thus reduce the ability of dendritic cells either in the lung mucosa or in other tissues to take up the Ag and present it. In fact, AMs have been shown to maintain lung homeostasis by suppressing dendritic cell function (44). The later presence of enterotoxin A on pulmonary dendritic cells could perhaps be due to Ag transfer from AMs to dendritic cells (45).

Superantigens, such as *S. aureus* enterotoxins, are known for their ability to bind to MHC II directly without Ag processing, which results in rapid oligoclonal activation of T cells and a cytokine storm (17). Additionally, compared with enterotoxin B, enterotoxin A was found to have a greater affinity for MHC II due to its ability to not only bind the α-chain but also the β-chain of MHC II with a zinc-dependent binding site, which enabled enterotoxin A to stay on the cell surface for at least 40 h (46). Interestingly, we found that the binding of enterotoxin A to AMs did not require MHC II expression (Fig. 3). Binding of *S. aureus* enterotoxins to molecules other than MHC II have been previously reported. In particular, the costimulatory molecule CD28 and its ligand CD86 are crucial binding sites that enhance the severity of the inflammatory response triggered by *S. aureus* enterotoxin (47). Other binding sites for *S. aureus* enterotoxins have been reported, including MHC class I (48), digalactosylceramide on kidney proximal tubular cells (49), and Gp130 receptor on adipocytes (50). These studies show that *S. aureus* enterotoxins may bind to several different molecules. Thus, it will be important

![FIGURE 3. MHC II is not required for binding of enterotoxin A to AMs.](https://doi.org/10.4049/immunohorizons.1700033)
to determine the identity of the binding site of enterotoxin A on AMs as well as the processing of the enterotoxin by these phagocytes. Previous studies showed that following intranasal *S. aureus* enterotoxin exposure, mice experience a systemic oligoclonal T cell activation and cytokine release to blood (26, 33, 51). To examine the role of AMs in the enterotoxin A–induced inflammatory response, WT and CD169-DTR mice were treated with DT and 2 d later, they were exposed to vehicle or enterotoxin A. There was no difference in the expression of activation markers CD25 or CD69 on enterotoxin A–specific Vβ3+ T cells as well as no significant change in serum cytokines when mice were administered vehicle control (Figs. 4, 5). However, CD169-DTR exposed to enterotoxin A showed a significantly greater CD25 expression compared with WT mice (Fig. 4F). This was only observed when lower amounts of enterotoxin A were given, suggesting that the higher doses of enterotoxin A saturate the level of T cell activation. Indeed, even 0.033 μg of enterotoxin A activated almost 90% of enterotoxin A–specific Vβ3+ T cells in the spleen (Fig. 4F).
findings emphasize the potency of enterotoxin A, which even in a small amount is sufficient to trigger a significant response. Furthermore, we examined the concentrations of serum cytokines commonly associated with toxic shock syndrome and sepsis, in particular, TNF, IFN-γ, IL-2, and IL-6 (18, 52). IL-10 and G-CSF were also analyzed. G-CSF, a growth factor that mobilizes neutrophils into circulation during acute inflammation (53), was previously found elevated in patients with bacterial infection (54) whereas the anti-inflammatory cytokine IL-10 can be released in septic patients in an attempt to counteract the hyperinflammatory response (53), was previously found elevated in patients with bacterial infection (54) whereas the anti-inflammatory cytokine IL-10 can be released in septic patients in an attempt to counteract the hyperinflammatory response (53)

Importantly, enterotoxin A–exposed CD169-DTR mice had increased secretion of proinflammatory cytokines TNF, IFN-γ, IL-2, and IL-6, as well as G-CSF, demonstrating that AM ablation is associated with enhanced inflammatory response following enterotoxin A inhalation (Fig. 5). Interestingly, the concentration of anti-inflammatory IL-10 was also elevated in CD169-DTR mice (Fig. 5). This is consistent with our previous study showing that enterotoxin A simultaneously induced the expression of both proinflammatory cytokines IL-2, TNF, and IFN-γ, and anti-inflammatory IL-10 in enterotoxin A–specific VB3+ T cells (26). Thus, depletion of AMs was correlated with an overall enhancement of the enterotoxin A–induced immune response. Finally, CD169 is expressed not only on AMs but also other macrophages, including marginal zone macrophages in the spleen and subcapsular sinus macrophages in the lymph nodes (55). In another study, such non-AM CD169+ macrophages have been shown to capture exosomes and CD169 knockout mice had an enhanced response to Ag-pulsed exosomes (56). Because enterotoxin A has been shown to permeate systemically after intranasal injection (26), depletion of macrophage subsets other than AMs could also contribute to the enhanced inflammatory response observed in CD169-DTR mice. Similarly, it is also possible that DT injection and the subsequent cell apoptosis could play a role in the increased inflammatory response in CD169-DTR mice. Interestingly, the absence of CD169+ macrophages during viral infection in mice reduces antiviral type I IFN activity and also limits PD-L1 expression, leading to overwhelming viral replication in the absence of CD8+ T cell exhaustion (57), a mechanism that could be considered in our model. Because of the broad expression of CD169 in secondary lymphoid tissue and the emerging role of CD169+ macrophages in inflammation and immunoregulation (58), further studies will be necessary to confirm the causal relationship between AMs and S. aureus enterotoxin–induced systemic inflammatory response.

In conclusion, in this study we show that AMs bind S. aureus enterotoxin irrespective of MHC II expression and internalize it. Furthermore, AM ablation in CD169-DTR mice was associated with increased activation of enterotoxin A–specific VB3+ T cells and enhanced secretion of cytokines into circulation. These findings could explain the development of toxic shock syndrome in patients with influenza infection (29, 30) as well as the increased risk of sepsis and ARDS in chronic alcohol users and smokers, respectively (13, 14). Thus, dysfunction or depletion of AMs may be a critical risk factor for the development of S. aureus enterotoxin–induced inflammatory diseases.

**DISCLOSURES**

The authors have no financial conflicts of interest.

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