CD4+ T cells and CXC chemokines modulate the pathogenesis of Staphylococcus aureus wound infections


Channing Laboratory, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115

Edited by John J. Mekalanos, Harvard Medical School, Boston, MA, and approved May 11, 2006 (received for review October 13, 2005)

T cells are critical for the formation of intraabdominal abscesses by Staphylococcus aureus. We hypothesized that T cells modulate the development of experimental staphylococcal infections by controlling polymorphonuclear leukocyte (PMN) trafficking, in models of staphylococcal s.c. abscess formation, hindpaw infection, and surgical wound infection. S. aureus multiplied in the tissues of WT C57BL/6J mice and elicited a marked inflammatory response. CD4+ αβ T cells homed to the surgical wound infection site of WT animals. In contrast, significantly fewer S. aureus were recovered from the tissues of mice deficient in αβ T cells, and the inflammatory response was considerably diminished compared with that of WT animals. αβ T cell receptor (−/−) mice had significantly lower concentrations of PMN-specific CXC chemokines in wound tissue than did WT mice. The severity of the wound infection was enhanced by administration of a CXC chemokine and abrogated by antibodies that blocked the CXC receptor. An acapsular mutant was less virulent than the parental S. aureus strain in both the s.c. abscess and the surgical wound infection models in WT mice. These data reveal an important and underappreciated role for CD4+ αβ T cells in S. aureus infections in controlling local CXC chemokine production, neutrophil recruitment to the site of infection, and subsequent bacterial replication.

Staphylococcus aureus is the causative agent of a range of serious human infections, including pneumonia, endocarditis, and sepsis (1). In addition, this organism is a major cause of localized infections, such as s.c. abscesses, impetigo, and surgical wound infections. The increasing prevalence of nosocomial and community-acquired S. aureus infections has prompted studies to better understand the host–pathogen interactions.

S. aureus produces a myriad of virulence factors that contribute to its ability to cause disease, allowing the organism to gain entry into tissues, evade the host immune system, attach to host cells, and secrete exoproteins and toxins. Cell wall components such as peptidoglycan, teichoic acids, and capsule, as well as adhesins, proteases, exoproteins, and exotoxins, all promote the virulence of this pathogen (2–4).

In contrast, less is known about the host response to S. aureus and how host factors influence the pathogenesis of staphylococcal infections. Animal models of staphylococcal infections have been used to investigate the interactions between S. aureus and the host. However, few recapitulate the major hallmarks of the disease as it occurs in humans, because a large S. aureus inoculum (>106 colony-forming units (cfu)) is necessary to initiate infection, and little bacterial replication occurs in vivo. The amount of inoculum needed to provoke disease can be reduced with the use of foreign bodies, such as Cytodex I beads (Sigma), to potentiate infection (5).

Polymorphonuclear leukocytes (PMNs) represent the host’s first line of defense against invasion by S. aureus and are a critical determinant in the outcome of staphylococcal infections (6). Depletion of PMNs in vivo before bacterial challenge results in overwhelming disease (7–9), and patients who are neutropenic or who have congenital or acquired defects in PMN function are highly susceptible to S. aureus infection (6). However, Gresham et al. (10) demonstrated that survival of S. aureus inside PMNs contributes to the pathogenesis of staphylococcal infection in a murine peritonitis model.

We have shown that T cells control the development of abscesses in an animal model of S. aureus intraabdominal abscess formation and that the S. aureus capsular polysaccharide (CP) activates CD4+ T cells in vitro (11). Because abscess formation depends on the recruitment of PMNs to the site of infection, we hypothesized that T cells control the pathogenesis of S. aureus infection by modulating the PMN response in vivo. In this study, we investigated the influence of T cells on the pathogenesis of S. aureus infections in three distinct animal models. Experiments in a clinically relevant surgical wound infection model showed that T cells modulate the development of staphylococcal wound infections by controlling local CXC chemokine production, and ultimately PMN migration to sites of infection. This work demonstrates that, although PMNs may be beneficial to the host at the outset of S. aureus infection, they may also play a deleterious role during the course of disease.

Results

Influence of T Cells in Subcutaneous Abscess Formation. Previous studies (11, 12) have indicated that CD4+ T cells play a role in the development of S. aureus intraabdominal abscesses. To determine whether T cells might also influence other types of staphylococcal infection, WT C57BL/6J and αβ T cell receptor (TCR) (−/−) mice were challenged s.c. with 105 to 106 cfu of S. aureus PS80 [a serotype 8 CP (CP8) strain]. As shown in Fig. 1, WT mice exhibited a clear dose response at low inocula (<3 × 103 cfu per mouse). The frequency of abscess formation reached 100% at inocula ≥3 × 103 cfu, and ≤103 cfu per abscess was recovered from these animals. Mice that were culture-negative developed smaller foreign-body responses that contained Cytodex beads only. Gross pathologic inspection of the abscesses in WT mice revealed large vascularized structures with a thick fibrin wall (Fig. 1B). Histologic analysis revealed that abscesses contained PMNs, bacteria, and Cytodex beads (Fig. 1C). Confocal microscopic examination of abscesses from WT mice demonstrated that CD3+ T cells had infiltrated the fibrin wall of these structures (data not shown). This observation is consistent with previous studies demonstrating that T cells home to and comprise the wall of intraabdominal abscesses (13).

At inocula ranging from 3 × 102 to 3 × 103 cfu, αβ TCR (−/−) mice had fewer culture-positive abscesses and significantly fewer cfu...
per abscess than WT animals (Fig. 1A). Most of the αβ TCR (−/−) mice challenged with <10^4 cfu were culture-negative. αβ TCR (−/−) mice that were culture-positive developed a tissue response that differed from that of a classic abscess. These structures were smaller and lacked a thick fibrin wall (Fig. 1B). Histologic analysis revealed that the structures were composed of hyperproliferative fibroblasts characteristic of granulation tissue found in a foreign-body response seen in the abscess tissue. Histologic analysis showed fibroblasts characteristic of granulation tissue (gt).

Fig. 1. Role of T cells in s.c. abscesses induced by S. aureus PS80. (A) Quantitative cultures of abscesses from WT or αβ TCR (−/−) mice were significantly different at inocula of 3 × 10^4 (P = 0.026) and 3 × 10^5 (P = 0.038) cfu per mouse. The number of animals that were culture-positive in each group is indicated. (B) WT mice challenged with S. aureus formed well-defined abscesses with a thick fibrin wall (fw). In contrast, αβ TCR (−/−) mice developed small foreign-body responses (fb). Histological analysis showed fibroblasts (inf) within the abscesses in WT mice and significant leukocyte infiltration (alt). Cytokex beads (cb) are visible in the abscess tissue. (D) The foreign-body response seen in the αβ TCR (−/−) mice consisted primarily of granulation tissue (gt).

Influence of T Cells in the Hindpaw Infection Model. Rich and Lee (14) described an S. aureus infection model in the hindpaw of nondiabetic and diabetic mice. We inoculated a hindpaw of WT and αβ TCR (−/−) mice with 10^6 cfu of PS80 and euthanized the mice after 5 days. Quantitative culture results revealed that WT mice had significantly (P = 0.0178) more S. aureus recovered from the hindpaws than did αβ TCR (−/−) animals: 5.24 ± 0.43 vs. 2.19 ± 0.44 cfu/g of tissue, respectively (mean log10 cfu ± SEM).

Development of a Surgical Wound Infection Model. Our initial experiments were aimed at optimizing the inoculum needed to provoke a staphylococcal surgical wound infection in mice. Inoculation of surgical wounds with ≥10^3 cfu of S. aureus PS80 resulted in a localized purulent infection on day 3 that showed marked inflammation on gross pathological inspection. Challenge with inocula of ≥10^4 cfu resulted in a severe and purulent infection. Tissues from mice inoculated with 10^2 to 10^5 cfu of S. aureus strains PS80, Reynolds (a CP5 strain), or COL (a methicillin-resistant CP5 strain) (Fig. 2A). The tissue bacterial load was not significantly different between 3 and 6 days after inoculation. Animals infected with 2 × 10^6 cfu of S. aureus PS80 in the absence of a sutured wound yielded ~1/100th as many S. aureus as animals with sutured wounds (data not shown). A time-course experiment in mice challenged with 10^2 cfu of S. aureus PS80 revealed that the bacterial burden in the wound tissue achieved maximum levels (10^5 to 10^7 cfu/g of tissue) between 3 and 12 days after inoculation (Fig. 2B). The infection declined by day 18, when two of four mice had cleared the infection.

Influence of T Cells on Surgical Wound Infections. To determine whether T cells influenced the pathogenesis of S. aureus-induced surgical wound infections, initial experiments were performed with an inoculum of 40 cfu of strain PS80. Similar to our findings with the abscess and hindpaw infection models, WT mice had significantly (P = 0.0445) more S. aureus recovered from the infected wound tissue than did αβ TCR (−/−) animals on day 3: 5.05 ± 0.60 vs. 2.59 ± 0.76 cfu/g of tissue, respectively (mean log cfu ± SEM). Mice deficient in different T cell subsets were then used to determine the phenotype of T cells that contribute to the pathogenesis of S. aureus wound infections. CD8-deficient mice challenged with 10^2 cfu had 6.88 ± 0.67 log cfu/g of tissue recovered from wounds at day 3, similar to the bacterial burden recovered from WT mice (6.81 ± 0.23). In contrast, mice deficient in αβ TCR or CD4 or having T cells lacking the TCRα chain had significantly fewer bacteria at the wound site 3 days after inoculation with 10^2 cfu of S. aureus than WT mice (Table 1). Likewise, the T cell-deficient mice had fewer S. aureus recovered from the wounds than did WT animals on day 6, and this difference was significant (P = 0.035) for the CD4 (−/−) mice. WT and T cell-deficient animals challenged with ≥10^3 cfu of S. aureus had similar numbers of bacteria recovered from the wounds on both days.

Fig. 2. S. aureus wound infection model dose–response curve (A) and time course (B). (A) WT mice were challenged with increasing inocula of strain PS80 (circles), Reynolds (squares), or COL (triangles) and euthanized at either 3 days (open symbols) or 6 days (filled symbols). (B) WT mice were challenged with 10^3 cfu of S. aureus PS80 and euthanized at the designated time points.
Histopathology of Surgical Wound Tissues. Examination of the wound tissues from WT mice challenged with PBS revealed aspects of the normal host response to a foreign body. PMNs were detected at the suture site by 24 h after surgery. By day 3 the PMN infiltration was accompanied by a densely staining infiltrate of hyperproliferative fibroblasts associated with granulation tissue (Fig. 3). This observation is consistent with the normal process of wound repair that follows tissue trauma. More granulation tissue and fewer PMNs were observed at the wound site by day 6 (data not shown), indicating that the normal wound healing process was under way.

Challenge of WT animals with $10^2$ cfu of *S. aureus* resulted in numerous PMNs infiltrating the wound site 24 h after surgery. Additional PMNs entered the wound site by day 2 and were found around the suture and in the surrounding muscle tissue. Mononuclear cells were observed infiltrating the wound site on day 2. By day 3, wounds exhibited an intense, purulent infection with numerous PMNs and mononuclear cells visible near the suture and in the surrounding tissue (Fig. 3B). The cellular infiltrate in infected tissues was greater than that observed in PBS-challenged mice at each time point. *S. aureus*-infected wounds on day 6 were histologically similar to those from day 3, an indication that the host response had not abated. Mice challenged with $10^3$ cfu of *S. aureus* PS80 exhibited a more severe infection than mice given $10^2$ cfu, but the time course of histopathologic changes was similar (data not shown).

Histologic examination of surgical wound tissues from αβ TCR (−/−) mice on day 3 revealed less inflammation in response to challenge with $10^2$ (Fig. 3C) or $10^3$ cfu of *S. aureus* compared with WT animals. Tissues from αβ TCR (−/−) mice showed reduced PMN infiltration and fewer mononuclear cells around the suture site in the surrounding muscle tissue compared with that seen in WT animals. Granulation tissue associated with normal wound repair was noted in tissues from αβ TCR (−/−) mice.

Histologic examination of surgical wound tissues from αβ TCR (−/−) mice on day 3 revealed less inflammation in response to challenge with $10^2$ (Fig. 3C) or $10^3$ cfu of *S. aureus* compared with WT animals. Tissues from αβ TCR (−/−) mice showed reduced PMN infiltration and fewer mononuclear cells around the suture site and in the surrounding muscle tissue compared with that seen in WT animals. Granulation tissue associated with normal wound repair was noted in tissues from αβ TCR (−/−) mice.

T Cells Traffic to *S. aureus*-Infected Wounds. Confocal microscopy was used to investigate the types of cells that infiltrated the wound site during *S. aureus* infection. WT mice were challenged with PBS or $10^2$ cfu of *S. aureus* PS80, and the wounds were harvested daily until day 6 after surgery. Mice challenged with PBS showed some PMN infiltration at the wound site, but no αβ T cells were observed on day 3 (Fig. 3D). Infiltration of PMNs occurred within the first 24 h of infection in WT mice challenged with *S. aureus*, whereas T cells could be detected by day 2 after inoculation. Cellular infiltration by T cells and PMNs achieved maximal levels on day 3 (Fig. 3E). PMNs, T cells, and bacteria were present around the sutures and in the surrounding muscle tissue. The αβ T cells in the wound tissues (Fig. 3E) stained positive for CD4 but not for CD8 (data not shown).

Confocal microscopic analysis of tissues from αβ TCR (−/−) mice challenged with $10^2$ or $10^3$ cfu of *S. aureus* PS80 confirmed our
histologic findings that T cell deficiency alters the cellular host response. Compared with WT mice, αβ TCR (−/−) mice showed a mitigated inflammatory response in the wounds on day 3, with notably fewer PMNs and mononuclear cells present (Fig. 3F).

Table 2. The S. aureus capsule promotes virulence in mouse models of S. aureus s.c. abscess formation and surgical wound infection

<table>
<thead>
<tr>
<th>Inoculum, log_{10} cfu/mouse</th>
<th>Subcutaneous abscess</th>
<th>Wound infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log_{10} cfu of S. aureus per abscess</td>
<td>Log_{10} cfu of S. aureus per g of tissue</td>
</tr>
<tr>
<td></td>
<td>PS80</td>
<td>RMS-1 (acapsular)</td>
</tr>
<tr>
<td>3</td>
<td>6.29 ± 0.4</td>
<td>4.92 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>2</td>
<td>3.7 ± 0.63</td>
<td>2.13 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>(n = 12)</td>
<td>(n = 14)</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SEM.
acapsular mutant RMS-1 were not significantly different from those in PS80-infected mice (data not shown).

**Discussion**

An important aspect of the host innate immune response to *S. aureus* is opsonophagocytic killing by PMNs. The essential role of phagocytic killing in host clearance of *S. aureus* is apparent because patients who are neutropenic or have defects in PMN function suffer from recurrent staphylococcal infections. In contrast, little information is available on how various T cell populations influence the outcome of staphylococcal infections. We reported that the *S. aureus* CP activates CD4+ T cells in vitro and that activated T cells induce intraabdominal abscesses when transferred to naïve animals (11). These results led us to consider the T cell as an important mediator of the host response to this organism.

We evaluated the role of T cells in modulation of host innate immunity in three different animal models of *S. aureus* infection. Whereas WT mice formed well-defined abscesses at inocula as low as 10^3 cfu, αβ TCR (−/−) mice were impaired in their ability to develop defined abscesses. Few PMNs were noted in tissue sections from αβ TCR (−/−) mice, and significantly fewer bacteria were recovered from the injection site of the mutant animals compared with WT animals. Similarly, in the hindpaw infection model, challenge of αβ TCR (−/−) mice resulted in a significantly lower bacterial burden in the infected tissue than in WT animals. These data are concordant with the results of our earlier studies indicating that T cells control the development of *S. aureus* intraabdominal abscesses (11, 12).

These experimental findings were investigated in greater depth in a wound infection model that mimics *S. aureus* infection in a surgical setting. As few as 10 cfu initiated infection in this model, and bacterial numbers increased *in vivo* by 3 to 4 orders of magnitude. The infection remained localized; the mice did not become bacteremic even when >10^6 cfu were delivered to the wound.

Few studies have critically assessed the contribution of the host response to the pathogenesis of staphylococcal wound infections. Confocal microscopic analyses of *S. aureus*-infected wounds from WT mice confirmed histologic findings that PMNs infiltrated the wound tissue by 24 h after bacterial inoculation. αβ CD4+ T cells were observed at the wound site on day 2 and were abundant in the tissues by day 3. Compared with WT or CD8-deficient mice, fewer bacteria were cultured from the wound site of αβ TCR−/−, CD4−/−, and TCRα−/− chain-deficient mice at 3 and 6 days after challenge with 10^2 cfu of *S. aureus*. A markedly reduced PMN infiltrate was observed at the suture site of αβ TCR-deficient mice compared with WT mice, suggesting that CD4+ αβ T cells contribute to the pathogenesis of staphylococcal wound infections by controlling PMN infiltration.

Because CXC chemokine levels were significantly lower in αβ TCR (−/−) mice than in WT animals, it is likely that T cells control PMN recruitment to the infected site through modulation of CXC chemokine levels and subsequent PMN migration to surgical wounds. Antibody blockade of the CXCXR2, which binds both MIP-2 and KC in vivo, significantly reduced the bacterial burden at the infection site. Conversely, administration of MIP-2 directly into the wound at the time of challenge resulted in significantly greater bacterial growth in the pleural space than WT animals. Mice infected with *S. aureus* in our surgical wound model responded with early (6 h) KC production, whereas maximal MIP-2 production occurred at 48 h. These data are consistent with a previous study that showed a distinct temporal pattern of expression for these two functionally similar chemokines in a surgical injury model (27). The authors postulate that other inflammatory mediators at the wound site may differentially regulate the expression of KC and MIP-2. KC may be predominantly involved in directing initial PMN recruitment to the infection site, whereas MIP-2 may function in later aspects of the immune response to injury, e.g., CXC chemokines have been implicated in wound healing (28).

We showed previously (11) that *S. aureus* CP8 activates CD4+ T cells *in vitro*. Reports of other biological properties of CP8 are scant. The CP5 produced by *S. aureus* promotes virulence in numerous models of staphylococcal infection, including bacteremia, abscess formation, and septic arthritis (11, 17, 18, 29, 30), and protects the bacterium from opsonophagocytosis *in vitro* (17, 18, 29). We genetically modified the serotype 5 strain Reynolds to express CP8. Reynolds (CP5) was more virulent in a mouse bacteremia model and in phagocytic killing assays than an acapsular mutant but less virulent than Reynolds (CP5) (18). In the present study, the impact of *S. aureus* CP8 production was assessed in the s.c. abscesses and the surgical wound infection models. The acapsular mutant RMS-1 was significantly less virulent than strain PS80 in each model. In contrast, the two strains showed equivalent virulence in αβ TCR (−/−) mice, which are unresponsive to *S. aureus* CP. Clearly, bacterial factors other than CP8 influence the outcome of staphylococcal infections because even the acapsular mutant caused infection at inocula >10^6 cfu (data not shown). *S. aureus* wall teichoic acid (produced by both strains) was as potent as CP8 in inducing intraabdominal abscesses in rats (11). Chemokine levels were equivalent in WT mice challenged with either RMS-1 or PS80. Other Gram-positive cell wall components common to both strains, such as peptidoglycan and lipoteichoic acid, have been shown to elicit a host inflammatory response in WT mice (31).

In summary, T cells modulate the pathogenesis of *S. aureus* infection in three different animal models. A low-inoculum, clinically relevant model of surgical wound infection was developed to
study the role of T cells in disease progression and to characterize the host response to infection. The data demonstrate that T cells orchestrate the outcome of this infection through regulation of PMN infiltration and CXC chemokine production at the site. Our study underscores the importance of T cells in the control of certain staphylococcal infections, possibly through modulation of PMN function during the host response.

Materials and Methods

Bacterial Strains. *S. aureus* strains PS80, Reynolds, and COL are described in ref. 11. Mutant RMS-1 is an isogenic acapsular mutant of strain PS80 that was derived by allelic replacement mutagenesis with the temperature-sensitive vector pAP1.2 (18). Staphylococci were cultivated for 24 h at 37°C on Columbia agar (Difco) with 2% NaCl.

Mouse Models of *S. aureus* Infection. Male WT C57BL/6J and congenic gene-deficient mice were obtained at 6–8 weeks of age from The Jackson Laboratory. Animal experiments were performed in accordance with the guidelines of the Harvard Medical School Standing Committee on Animals.

Subcutaneous abscess model. This model is described in ref. 30. Mice were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine, and the hair on their flanks was removed. The mice were injected s.c. in each flank with 0.2 ml of an *S. aureus*/Cytodex bead (Sigma) mixture containing 10^7 to 10^1 cfu. On day 4, the mice were euthanized and the muscle tissue was excised, weighed, and homogenized, and quantitatively.

**Hindpaw infection model.** This model of *S. aureus* infection is described in ref. 14. A 10-μl suspension of *S. aureus* containing 10^6 cfu was injected into the plantar proximal aspect of the hindpaw. The mice were euthanized on day 5, and their hindpaw tissue was homogenized and cultured quantitatively.

**Wound infection model.** In this model, mice were anesthetized and their right thighs were shaved and disinfected. The thigh muscle was inoculated with 10^6 cfu of *S. aureus* PS80 and harvested on day 3 for quantitative culture. Wound tissues were excised and homogenized, and 5 μl of an *S. aureus* suspension or PBS was introduced into the wound site of WT mice 1 h before and 6 h after surgery. Wounds were inoculated with 10^6 cfu of *S. aureus* PS80 and harvested on day 3 for quantitative culture. For chemokine administration, 400 ng of MIP-2 or MIP-1α (R & D Systems) was injected directly into the wound site of αβ TCR (−/−) mice 4 h and 24 h after inoculation with 30 cfu of *S. aureus* PS80. Tissues were harvested on day 3 for quantitative culture.

**Statistical Analyses.** Quantitative culture results and tissue chemokine levels were compared by the Welch modification of the unpaired Student *t* test.

We thank Dr. Vincent Carey for advice regarding statistical analyses. This work was supported by National Institutes of Health Grants AI52397 (to A.O.T.) and AI29040 (to J.C.L.).
