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CD4⁺ T Cells Mediate Abscess Formation in Intra-abdominal Sepsis by an IL-17-Dependent Mechanism

Doo Ryeon Chung,* Dennis L. Kasper,*§ Ronald J. Panzo,† Tanuja Chthinis,‡ Michael J. Grusby,¶ Mohamed H. Sayegh,‡ and Arthur O. Tzianabos2*

Abscess formation associated with intra-abdominal sepsis causes severe morbidity and can be fatal. Previous studies have implicated T cells in the pathogenesis of abscess formation, and we have recently shown that CD4⁺ T cells activated in vitro by zwitterionic capsular polysaccharides from abscess-inducing bacteria such as Staphylococcus aureus and Bacteroides fragilis initiate this host response when transferred to naive rats. In this study, we show that mice deficient in αβTCR-bearing T cells or CD4⁺ T cells fail to develop abscesses following challenge with B. fragilis or abscess-inducing zwitterionic polysaccharides, compared with CD8⁻/⁻ or wild-type animals. Transfer of CD4⁺ T cells from wild-type mice to αβTCR⁻/⁻ animals reconstituted this ability. The induction of abscesses required T cell costimulation via the CD28-B7 pathway, and T cell transfer experiments with STAT4⁻/⁻ and STAT6⁻/⁻ mice demonstrated that this host response is dependent on STAT4 signaling. Significantly higher levels of IL-17, a proinflammatory cytokine produced almost exclusively by activated CD4⁺ T cells, were associated with abscess formation in Th2-impaired (STAT6⁻/⁻) mice, while STAT4⁻/⁻ mice had significantly lower levels of this cytokine than control animals. The formation of abscesses was preceded by an increase in the number of activated CD4⁺ T cells in the peritoneal cavity 24 h following bacterial challenge. Confocal laser-scanning microscopy analysis revealed that CD4⁺ T cells comprise the abscess wall in these animals and produce IL-17 at this site. Administration of a neutralizing Ab specific for IL-17 prevented abscess formation following bacterial challenge in mice. These data delineate the specific T cell response necessary for the development of intra-abdominal abscesses and underscore the role of IL-17 in this disease process. The Journal of Immunology, 2003, 170: 1958–1963.

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(8, 9), as described previously, and backcrossed into a C57BL/6 background for at least 10 generations. Animals were maintained according to the Harvard Medical School animal management program, which is accredited by the American Association for the Accreditation of Laboratory Animal Care.

Bacterial strains and polysaccharide preparation

*B. fragilis* NCTC 9343 was obtained from the Channing Laboratory stock culture collection. CP1 was obtained from the American Type Culture Collection (Manassas, VA) and purified, as described previously (7).

Animal model for intra-abdominal abscess formation

An intra-abdominal sepsis model was used for these studies (10, 11). In brief, mice were injected i.p. with *B. fragilis* (1 × 10^8 CFU/animal) or CP1 (50 μg/animal) mixed with sterile cecal contents (SCC; 1:1 v/v, 0.2 ml total volume). SCC is a required adjuvant for the development of abscess formation by intact bacteria or purified polysaccharide in this model and is administered to reflect the spillage of colonic contents that occurs during the onset of intra-abdominal sepsis in humans (5, 11). Administration of SCC alone does not induce abscess formation in animals. Six days later, animals were examined at necropsy and for the presence of one or more abscesses within the peritoneal cavity.

*T cell depletion*

For αβ T cell depletion, C57BL/6 mice were treated with 300 μg of the TCR β-chain-specific mAb (H57-597; BD PhарMingen, San Diego, CA) or isotype-matched control Abs via the i.p. route 4 days before *B. fragilis* challenge. For depletion of CD4 or CD8 T cells, C57BL/6 mice were treated with 0.2 mg of CD4-specific mAb (GK1.5; BD PhарMingen) or CD8-specific mAb (53-6.7; BD PhарMingen) via the i.p. route 48 h before challenge. Depletion of the targeted cell type was confirmed by subsequent FACS analysis, which showed >93% depletion of the respective cell type.

Blockade of the CD28-B7 pathway

Murine CTLA4g and control L6dg were obtained from Bristol-Myers Squibb (Princeton, NJ). CTLA4g binds specifically to CD80 and CD86. L6dg was used as a control Ig fusion protein. This molecule has the same Ig H chain fused to an irrelevant protein. Fusion proteins (500 μg) were administered to animals via the intracardiac route at the time of challenge.

*T cell transfer studies*

Splenic CD4+ T cells from STAT4−/−, STAT6−/−, or wild-type mice were purified on a nylon wool column and negative selection with magnetic beads, as previously described (7). T cells were transferred to αβ TCR−/− mice at 3 × 10^6 cells per mouse via the intracardiac route 24 h before challenge. All recipient animals were challenged via the i.p. route with 1 × 10^8 CFU of *B. fragilis* and SCC.

Immunohistochemistry and confocal laser-scanning microscopic imaging

Paraffin-embedded tissue sections of intra-abdominal abscesses from *B. fragilis*-challenged C57BL/6 mice were stained with H&E or dewaxed with EZ-DeWax deparaffinization solution (InnoGenex, San Ramon, CA), according to the manufacturer’s protocol. The sections were permeabilized with 0.05% saponin in dH2O and incubated with 10% goat serum in PBS before challenge. Depletion of the targeted cell type was confirmed after 6 days and 6 h thereafter. Control groups were given 100 μl of affinity-purified rabbit IgG. All groups of mice were sacrificed after 6 days and assessed for abscess formation.

Statistical analyses

Evaluation of differences between groups in abscess induction studies was performed by χ2 analysis (InStat, GraphPad Software, San Diego, CA). The results shown are a compilation of at least two separate experiments. Comparison of means from an IL-17 ELISA experiments was made by the unpaired t test.

Results

*T cell phenotype responsible for intra-abdominal abscess formation in animals*

Specific mAbs were used to deplete mice of αβ TCR+, CD4+, or CD8+ T cells, respectively. When challenged with *B. fragilis*, animals depleted of αβ TCR+ T cells or CD4+ T cells showed significantly lower abscess rates as compared with sham-depleted animals (Table I), whereas depletion of CD8+ T cells did not impair abscess formation. This result was confirmed with the use of genetic knockout mice. αβ TCR−/− and CD4−/− mice showed significantly lower abscess rates as compared with wild-type control littermates, whereas the abscess rate in CD8−/− mice was comparable to that in control animals (Table I).
The cellular response subsequent to challenge with _B. fragilis_ was studied to determine the types of cells that home to the peritoneal cavity as a prelude to the induction of abscesses. After bacterial challenge, PMNs accumulated very rapidly (within 6 h) in the peritoneal cavity, becoming a dominant cell type at this site within 24 h (Fig. 1). Macrophages are the primary resident host cell in the peritoneal cavity of naive mice. In animals challenged with _B. fragilis_, the number of these cells declined at the 6-h time interval, but increased by nearly 2-fold 24 h after challenge. A striking increase in the number of T cells in the peritoneal cavity was seen at the 24-h time interval, in which the mean number of these cells per mouse increased from 1.5 × 10^6 cell/ml to ~4 × 10^6 cells/ml. This corresponded to an increase in the number of T cells that expressed the activation markers CD25 and CD69 at this time point. The number of T cells decreased by 48 h, with the number of activated T cells falling to baseline levels. These data indicate that the initial influx of PMNs to the challenge site is soon followed by a large increase in the number of activated T cells.

### IL-17 levels in wild-type and STAT^−/−^ mice

IL-17 is a proinflammatory cytokine produced almost exclusively by activated CD4^+^ T cells (12, 13). To determine whether this cytokine plays a role in the development of abscesses and whether its release is associated with a particular type of Th response, we compared the IL-17 levels following challenge with _B. fragilis_ in STAT^−/−^ and littermate control mice. The level of IL-17 in the peritoneal fluid of STAT^−/−^ mice was significantly lower than in control animals 4 and 8 h after the challenge (Fig. 2, p < 0.0001 and p < 0.001, respectively). In contrast, the level of IL-17 in STAT^−/−^ mice was significantly higher than wild-type animals.

### Table IV. CD4^+^ T cells from STAT6^−/−^ mice transfer abscess induction

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient[^a]</th>
<th>No. of Animals with Abscess/Total (%)[^b]</th>
<th>p Value[^c]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>αβTCR^−/−^</td>
<td>7/9 (78)</td>
<td>0.009</td>
</tr>
<tr>
<td>STAT4^−/−^</td>
<td>αβTCR^−/−^</td>
<td>2/12 (17)</td>
<td>0.009</td>
</tr>
<tr>
<td>STAT6^−/−^</td>
<td>αβTCR^−/−^</td>
<td>9/10 (90)</td>
<td>NS</td>
</tr>
</tbody>
</table>

[^a]: Recipient animals received 3 × 10^6 CD4^+^ T cells from donor animals via the intracardiac route 24 h prior to challenge.

[^b]: Compared with wild-type littermate control.

[^c]: Composed with animals that received T cells from wild-type animals.
Ab showed the presence of CD4+ cells. Confocal microscopy analysis of these sections after staining with a CD4-dark-staining purulent focus of PMNs and bacteria. Confocal microscopy revealed the cellular organization typical of this host response. B. fragilis abscesses harvested from mice previously challenged with B. fragilis. All mice were challenged via the i.p. route with B. fragilis (10^8 CFU/animal) and SCC. Groups of four mice underwent peritoneal lavage 4, 8, and 24 h postchallenge. IL-17 levels in the lavage fluid of individual mice were assessed by ELISA. Data are representative of three separate experiments. The levels of IL-17 in the peritoneal fluid of STAT4−/− mice were significantly lower than those in wild-type control animals 4 and 8 h after the challenge (*, p < 0.0001, and **, p < 0.001, respectively). The level of IL-17 in STAT6−/− mice was significantly higher than that in wild-type animals 4 h after challenge (***, p = 0.02).

4 h after the challenge (p = 0.02). In both wild-type and STAT−/− mice, IL-17 was barely detectable 24 h postchallenge.

**IL-17-producing CD4+ T cells home to intra-abdominal abscesses**

Histologic analysis of H&E-stained sections of intra-abdominal abscesses harvested from mice previously challenged with B. fragilis revealed the cellular organization typical of this host response (Fig. 3A). Abscesses had a defined fibrous wall surrounding a dark-staining purulent focus of PMNs and bacteria. Confocal microscopic analysis of these sections after staining with a CD4-specific Ab showed the presence of CD4+ T cells within the fibrous wall of the abscess (Fig. 3B, green-stained cells). However, very few of these cells were found within abscesses (not shown). Staining with an IL-17-specific Ab revealed that many of the cells found in the abscess wall produce this cytokine (Fig. 3C, red-stained cells). Two-color colocalization analysis revealed that the CD4+ T cells found within the walls of abscesses produce IL-17 (red + green = yellow-stained cells). A plot of cells fluorescing green (T cells) vs the number of cells fluorescing red (IL-17) revealed a linear relationship (Fig. 3D), suggesting that the majority of CD4+ T cells present in the abscess wall express this cytokine.

**Role of IL-17 in abscess formation**

To demonstrate the role of IL-17 in the development of abscesses, we performed in vivo neutralization experiments. Animals were administered 100 μg of an affinity-purified Ab specific for IL-17 via the i.p. route at t = 0 and 6 h relative to challenge with B. fragilis. Control animals were similarly treated with a control Ab. Animals treated with the IL-17-specific Ab had a significantly lower abscess rate (10%) than animals treated with the control Ab (90%, p = 0.001, Table V).

**Discussion**

The present study clearly demonstrates that CD4+ cells play an essential role in the development of intra-abdominal abscesses. Abscess formation was prevented via blockade of the B7-CD28 pathway, indicating that T cell costimulation is required. In addition, the development of abscesses was preceded by an increase in the number of activated CD4+ T cells in the peritoneal cavity 24 h following bacterial challenge. Surprisingly, a significant number of CD4+ T cells were found comprising the walls of these abscesses, suggesting that these cells home to an infected nidus within the peritoneal cavity and take part in the organization of this fibrous structure. Finally, the proinflammatory cytokine IL-17 plays an important role in the pathogenesis of this host response.

We previously have shown that the capsular polysaccharides of B. fragilis and other abscess-inducing bacteria such as S. aureus activate human and rat CD4+ T cells in vitro. The transfer of these activated T cells along with SCC to naive rats promotes abscess formation (5, 7, 14, 15). In the present study, we used a mouse model of intra-abdominal sepsis to take advantage of the availability of different knockout mice to characterize the T cell response that governs this host response in vivo. These studies demonstrate that CD4+ T cells have a definitive role in this disease process and support the concept that T cells can be pathogenic in certain inflammatory tissue disorders, such as experimental autoimmune encephalomyelitis, idiopathic pulmonary fibrosis, progressive systemic sclerosis, experimental colitis, and granuloma formation (16–20). More recently, we have shown that CD4+ T cells promote the development of surgical adhesions, another type of fibrotic tissue response that ensues following surgical trauma (10).

CD4−/− animals had a significantly lower abscess rate than wild-type control animals. However, it should be pointed out that the few abscesses found in CD4−/− mice were abnormal, as determined by histologic examination (data not shown). CD4+ T cells can be classified into Th1 and Th2 subsets, according to the types of cytokines they produce. To determine the Th subset responsible for mediating abscess formation, we used mice deficient in the transcriptional activators STAT4 or STAT6. STAT4−/− and STAT6−/− mice are genetically impaired in the ability to generate Th1 and Th2 responses, respectively, and have been widely used in investigations of the role of Th1 and Th2 subsets in different immune responses. Experiments with STAT4 and STAT6 knockout mice show that Th1 cells are most likely responsible for the development of abscesses induced by B. fragilis or an abscess-inducing Zps, CP1. The finding that the transfer of CD4+ T cells from STAT6−/− mice reconstitutes this ability in αβ TCR−/− mice, which do not develop abscesses, supports this result. Based on the data presented, IL-17 appears to play a crucial role in the pathogenesis of intra-abdominal abscesses.
on these data, we hypothesize that the hallmark Th1 cytokine, IFN-γ, may have a predominant role in this inflammatory response. The impact of this proinflammatory cytokine in this host response is currently under investigation.

Characterization of the cellular response that leads to abscess formation revealed, in addition to the expected increase in the number of PMNs that infiltrate the peritoneal cavity, an increase in the number of T cells expressing activation markers. These data correlate with our finding that T cell costimulation via the CD28-B7 pathway is required for abscess formation in mice, and support our previous studies showing that activation of human and rat T cells by abscess-inducing Zps in vitro is mediated by the CD28-B7-2 pathway (7).

Perhaps the most striking result is the characterization of the singular role that IL-17 plays in abscess formation. This recently described cytokine is a product of activated CD4+ T cells and has been implicated as a mediator of tissue inflammation (14, 15). IL-17 can selectively recruit neutrophils into the peritoneal cavity through the release of neutrophil-specific chemokines, such as KC and macrophage-inflammatory protein-2, from the peritoneal mesothelium (12). Because PMNs are the major cellular components of absesses and T cells play a critical role in abscess formation,
we hypothesized that IL-17 could be one of the soluble factors that mediate this host response. The finding of low levels of IL-17 in the peritoneal fluid of STAT4−/− mice and high levels in STAT6−/− mice suggests a close relationship between IL-17 production and Th1 cells. However, this point is controversial, as there have been reports of a role for IL-17 in inflammatory processes controlled by both Th1 and Th2 CD4+ T cells (21–24).

The importance of IL-17 was corroborated by the demonstration that CD4+ T cells localize in the walls of intra-abdominal abscesses and produce IL-17 at this site. This finding, considered together with the FACS analysis of the cellular response elicited by B. fragilis challenge, suggests that activated CD4+ T cells play a critical role in the development of intra-abdominal abscesses. We hypothesize that following activation of these cells, IL-17 and other soluble mediators are produced and regulate the recruitment of inflammatory host cells to the peritoneal cavity. This series of events ultimately lead to the development of abscesses at this site. The ability of an IL-17-specific Ab to abrogate abscess formation in this setting supports this concept.

The development of abscesses associated with intra-abdominal sepsis is a common infectious complication that can have severe clinical outcomes. In this study, we show that CD4+ T cells mediate abscess formation associated with intra-abdominal sepsis by a mechanism that is dependent on the T cell-derived cytokine IL-17. These results delineate a major component of the cellular and subcellular host response that leads to this inflammatory tissue disorder.

References
CORRECTION


In the original article, the name of the fourth author was misspelled. The correct spelling is Tanuja Chitnis.