The Journal of Immunology

$CD4^+$ 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 Chtinis, Michael J. Grusby, Mohamed H. Sayegh and Arthur O. Tzianabos

J Immunol 2003; 170:1958-1963; ;
doi: 10.4049/jimmunol.170.4.1958
http://www.jimmunol.org/content/170/4/1958

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

CD4$^+$ T Cells Mediate Abscess Formation in Intra-abdominal Sepsis by an IL-17-Dependent Mechanism[1]

Doo Ryeon Chung,* Dennis L. Kasper,*§ Ronald J. Panzo,* Tanuja Chtinis,† Michael J. Grusby,¶ Mohamed H. Sayegh,‡ and Arthur O. Tzianabos[2]*

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 $\alpha\beta$TCR-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 $\alpha\beta$TCR$^{-/-}$ 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.**

The pathogenesis of abscess formation associated with intra-abdominal sepsis is poorly understood. Abscess formation in this setting ensues subsequent to fecal contamination of the peritoneal cavity (1). The progression of this disease process imposes a significant clinical burden, causing severe morbidity with associated mortality. *Bacteroides fragilis* is the most common anaerobic isolate from clinical cases, despite its being numerically among the least dominant organisms of the normal flora (2, 3). The capsular polysaccharides of *B. fragilis* are the principal virulence determinants and promote the formation of abscesses in rodent models of intra-abdominal sepsis (4). The biologic basis for this activity is dependent on the presence of positively and negatively charged functional groups on the repeating units of these capsular polysaccharides. Structurally distinct zwitterionic polysaccharides (Zps)[3] (3) from other bacteria such as the capsular polysaccharide from *Streptococcus pneumoniae* type 1 (CP1) also promote abscess formation in animal models (4). Recently, we have shown that the type 8 capsule of *Staphylococcus aureus* exhibits this zwitterionic charge motif and induces experimental abscess formation in a similar manner (5).

Relatively little is known regarding the biology of T cell interactions with carbohydrate-based Ags. Recently, we have shown that the zwitterionic charge motif associated with Zps confers the ability of these polymers to activate human and rat CD4$^+$ T cells in vitro (5, 6). T cell activation by Zps is dependent on the presence of free amino and carboxyl groups on these repeating unit structures. Zps that have been chemically modified to remove either of these charged groups do not stimulate T cells. T cell activation by these polymers requires MHC class II on APCs and T cell costimulation via the CD28-CD86 pathway (6, 7). T cell hybridomas specific for one type of Zps respond to other, structurally distinct Zps, but not to polysaccharides that lack this charge motif (unpublished data). Zps-activated T cells are functional, as they induce abscess formation when transferred to the peritonea of rats (5, 7). These data provide a framework for understanding how bacterial pathogens such as *B. fragilis* and *S. aureus* that display Zps on their cell surface predominate in clinical cases of abscess formation and the role of T cells in this process. In the present study, we sought to characterize the specific T cell response that mediates abscess formation in vivo. These data reveal that CD4$^+$ T cells have an essential role in the development of intra-abdominal abscesses. These cells home to and predominate at the site of infection and comprise the wall of these fibrinous structures. This disease process is mediated, at least in part, by the T cell-derived proinflammatory cytokine IL-17.

Materials and Methods

Animals

$\alpha\beta$TCR$^{-/-}$, CD4$^{-/-}$, CD8$^{-/-}$, CD28$^{-/-}$, C57BL/6, and BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). STAT4$^{-/-}$ and STAT6$^{-/-}$ mice were generated by Grusby and colleagues

*Channing Laboratory, Department of Medicine, †Department of Neurology, and ‡Laboratory of Immunogenetics and Transplantation, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; and §Department of Microbiology and Molecular Genetics, and ¶School of Public Health, Department of Immunology and Infectious Disease, Harvard Medical School, Boston, MA 02115

Received for publication September 17, 2002. Accepted for publication December 11, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health/National Institute of Allergy and Infectious Diseases Grants R21 AI45563 (to A.O.T.), R01 AI39576 (to D.L.K.), and R01 AI34965 (to M.H.S.).

[2] Address correspondence and reprint requests to Dr. Arthur O. Tzianabos, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115. E-mail address: atzianabos@channing.harvard.edu

[3] Abbreviations used in this paper: Zps, zwitterionic polysaccharide; CP1, *S. pneumoniae* type 1 capsule; PMN, polymorphonuclear neutrophil; SCC, sterile cecal contents.

0022-1767/03/$02.00

(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 $\alpha\beta$TCR$^+$ T cell depletion, C57BL/6 mice were treated with 300 μg of the TCR β-chain-specific mAb (H57-597; BD PharMingen, 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 PharMingen) or CD8-specific mAb (53-6.7; BD PharMingen) 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 CTLA4Ig and control L6Ig were obtained from Bristol-Myers Squibb (Princeton, NJ). CTLA4Ig binds specifically to CD80 and CD86. L6Ig 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 $\alpha\beta$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 abscess 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 dH_2O and incubated with 10% goat serum in PBS to block nonspecific binding sites. Intracytoplasmic IL-17 was stained with rabbit anti-mouse IL-17 IgG (Santa Cruz Biotechnology, Santa Cruz, CA) and then stained with goat anti-rabbit IgG conjugated to Alexa Fluor 568 (Molecular Probes, Eugene, OR). Staining for CD4 was performed with hamster anti-mouse CD4 IgG (BD PharMingen) and goat anti-hamster IgG conjugated to Alexa Fluor 488 (Molecular Probes). These sections were compared with those stained with irrelevant isotype control primary Abs and the respective Alexa Fluor conjugates. Confocal microscopy images were collected with a Bio-Rad (Hercules, CA) scanning confocal microscope equipped with a krypton-argon laser. Images were analyzed with Bio-Rad Confocal Assistant and Adobe Photoshop 5.5 (Adobe Systems, San Jose, CA). For colocalization studies, red channel fluorescence was plotted against red channel fluorescence to correlate the number of cells that stain for both CD4 and IL-17.

Measurement of IL-17 in the peritoneal cavity

IL-17 levels in the peritoneal cavities of STAT4$^{-/-}$, STAT6$^{-/-}$, and wild-type mice were assessed after *B. fragilis* infection. Four mice in each group were exsanguinated, and peritoneal lavage was performed with 1 ml of PBS. Supernatant fluids were centrifuged at $12{,}000 \times g$, and assayed for IL-17 using specific ELISA kit (R&D Systems, Minneapolis, MN), according to the manufacturer's protocols.

Kinetics of cellular influx into the peritoneal cavity

Mice were challenged with *B. fragilis* (1×10^8 CFU), and the cellular influx into the peritoneal cavity was assessed. Animals ($n = 5$) underwent peritoneal lavage with 1 ml of PBS at 6, 24, and 48 h following challenge. Lavage fluid from each animal (25 μl) was treated with NH_4Cl to lyse RBCs, and a total cell count was performed with a hemocytometer. Each sample was then analyzed by flow cytometry. After preincubation with rat anti-mouse CD16/CD32 (BD PharMingen) to block Fc receptors, cells were stained with FITC- or PE-labeled isotype control Abs or mAbs to CD4, CD16 (polymorphonuclear neutrophils (PMNs)), Mac-1 (macrophages), CD25 (IL-2R), and CD69 (T cell activation marker). Stained cells were analyzed on a Coulter EPICS XL cytometer (Beckman Coulter, Fullerton, CA), the CellQuest (BD Biosciences, San Jose, CA), and WinMDI 2.8 analysis software (Scripps Research Institute, La Jolla, CA; http://facs.scripps.edu). The absolute number of peritoneal cells collected was determined by trypan blue staining and counted with a hemocytometer. The absolute number of each respective cell type present was calculated by taking the proportion of each cell type (as determined by FACS analysis) and multiplying it by the total number of cells obtained from each mouse.

Production of IL-17-specific Ab

Polyclonal Ab to murine IL-17 was produced by immunizing rabbits at multiple intradermal sites with mouse rIL-17 (R&D Systems) mixed with CFA, as previously described (10). The IgG fraction was purified by HiTrap protein G affinity chromatography, according to the manufacturer's instructions (Amersham Pharmacia Biotech, Piscataway, NJ). Fractions eluted from the column were concentrated with the Centriprep YM-10 (Millipore, Bedford, MA) and buffer exchanged to PBS on PD-10 columns. Specific Ab was obtained after additional IL-17 affinity chromatography. Briefly, purified rIL-17 was coupled to Sepharose beads (Amersham Pharmacia Biotech) and used for affinity column chromatography. IL-17-specific IgG that bound to the column was eluted with 0.1 M glycine, neutralized, dialyzed, and concentrated for use. The concentration of purified Ab was determined by a standard protein assay. This Ab was specific for IL-17, as determined by ELISA, but did not cross-react IL-2, IFN-γ, IL-4, IL-10, IL-5, or IL-13. The control Ab used in these experiments was the IgG fraction from normal rabbit serum purified by HiTrap protein G affinity chromatography, as described above.

Effect of IL-17-specific neutralizing Ab on abscess formation

For neutralization experiments, C57BL/6 mice were injected with affinity-purified IL-17 Abs (100 μg of Ab/animal) via the i.p. route at the time of challenge and 6 h thereafter. Control groups were given 100 μg 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 χ^2 analysis (InStat, GraphPad Software, San Diego, CA). The results shown are a compilation of at least two separate experiments. Comparison of means from 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 $\alpha\beta$TCR$^+$, CD4$^+$, or CD8$^+$ T cells, respectively. When challenged with *B. fragilis*, animals depleted of $\alpha\beta$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. $\alpha\beta$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).

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

Group	No. of Animals with Abscess/Total (%)[a]	*p* Value
$\alpha\beta$TCR$^+$ T cell depleted	2/8 (25)	0.01[b]
CD4$^+$ T cell depleted	2/10 (20)	0.02[b]
CD8$^+$ T cell depleted	8/10 (80)	NS
Sham depleted	9/10 (90)	
Wild type$^{+/+}$	15/18 (83)	
$\alpha\beta$TCR$^{-/-}$	3/10 (30)	0.01[c]
CD4$^{-/-}$	4/20 (20)	0.0002[c]
CD8$^{-/-}$	15/20 (75)	NS

[a] Challenged via the i.p. route with 1×10^8 CFU of *B. fragilis* and SCC.
[b] Compared with sham-depleted control.
[c] Compared with wild-type littermate control.

Role of CD28-B7 pathway in T cell-mediated intra-abdominal abscess formation

We investigated the role of T cell costimulation via the CD28-B7 pathway in T cell-mediated abscess formation induced by *B. fragilis*. Mice deficient in CD28 showed a significantly lower abscess rate (20%) as compared with wild-type mice (90%) (Table II, $p = 0.006$). Furthermore, treatment with the fusion protein CTLA4Ig, which inhibits CD28-mediated signaling, reduced the incidence of abscess formation to 30% as compared with treatment with a fusion protein control (L6Ig), which resulted in a 90% abscess rate ($p = 0.02$ compared with CTLA4Ig-treated animals).

Intra-abdominal abscess formation is mediated by STAT4 signaling

The role of Th1 and Th2 responses in the development of intra-abdominal abscesses was studied in STAT4$^{-/-}$ and STAT6$^{-/-}$ mice (Table III). Challenge with *B. fragilis* resulted in a significantly lower abscess rate in STAT4$^{-/-}$ (15%) than in wild-type littermate control mice (81%), whereas the abscess rate in STAT6$^{-/-}$ mice (94%) was comparable to that in control animals. Challenge of these mouse strains with a known abscess-inducing Zps, CP1, yielded similar results (Table III).

To further delineate this response, we performed T cell transfer experiments in which $\alpha\beta$TCR$^{-/-}$ mice, previously shown to be genetically impaired in their ability to develop abscesses (Table I), were used as recipients. In these studies, the transfer of purified CD4$^+$ T cells from STAT6$^{-/-}$ or wild-type mice reconstituted abscess formation in $\alpha\beta$TCR$^{-/-}$ mice, whereas transfer of these cells from STAT4$^{-/-}$ mice did not have this effect (Table IV). The transfer of CD4$^+$ T cells from STAT6$^{-/-}$ to $\alpha\beta$TCR$^{-/-}$ mice resulted in abscess formation in recipient animals (90% abscess rate), a rate comparable to that in $\alpha\beta$TCR$^{-/-}$ mice that received CD4$^+$ T cells from wild-type littermate controls (78%, Table IV).

Table II. *Role of CD28-B7 costimulatory pathway in abscess formation*

Group	No. of Animals with Abscess/Total (%)[a]	*p* Value
Wild type$^{+/+}$	9/10 (90)	
CD28$^{-/-}$	2/10 (20)	0.006[b]
CTLA4Ig[c]	3/10 (30)	0.02[d]
L6Ig[c]	9/10 (90)	NS[d]
Saline	9/10 (90)	

[a] Challenged via the i.p. route with 1×10^8 CFU of *B. fragilis* and SCC.
[b] Compared with wild-type control.
[c] The fusion protein (500 μg/ml) was administered to mice via the intracardiac route.
[d] Compared with sham-depleted control.

Table III. *Abscess formation in STAT$^{-/-}$ mice*

Group	Challenge[a]	No. of Animals with Abscess/Total (%)	*p* Value[b]
Wild type	*B. fragilis*	13/16 (81)	
STAT4$^{-/-}$	*B. fragilis*	3/20 (15)	0.001
STAT6$^{-/-}$	*B. fragilis*	15/16 (94)	NS
Wild type	CP1	7/8 (88)	
STAT4$^{-/-}$	CP1	1/9 (11)	0.003
STAT6$^{-/-}$	CP1	8/9 (89)	NS

[a] Inocula given via the i.p. route with SCC.
[b] Compared with respective wild-type control.

However, the transfer of CD4$^+$ T cells from STAT4$^{-/-}$ mice to $\alpha\beta$TCR$^{-/-}$ mice resulted in a significantly lower rate of abscess formation (17%, $p = 0.009$ compared with control animals). These data clearly show that the development of abscesses is dependent on STAT4 signaling, and indicate that Th1 cells are responsible for this host response.

Kinetics of the cellular response in the peritoneal cavity

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 1.5×10^5 cell/ml to $\sim 4 \times 10^6$ 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 STAT4$^{-/-}$ 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 STAT6$^{-/-}$ mice was significantly higher than wild-type animals

Table IV. *CD4$^+$ T cells from STAT6$^{-/-}$ mice transfer abscess induction*

Donor	Recipient[a]	No. of Animals with Abscess/Total (%)[b]	*p* Value[c]
Wild type	$\alpha\beta$TCR$^{-/-}$	7/9 (78)	
STAT4$^{-/-}$	$\alpha\beta$TCR$^{-/-}$	2/12 (17)	0.009
STAT6$^{-/-}$	$\alpha\beta$TCR$^{-/-}$	9/10 (90)	NS

[a] Recipient animals received 3×10^6 CD4$^+$ T cells from donor animals via the intracardiac route 24 h prior to challenge.
[b] Animals challenged via the i.p. route with 1×10^8 CFU of *B. fragilis* and SCC.
[c] Compared with animals that received T cells from wild-type animals.

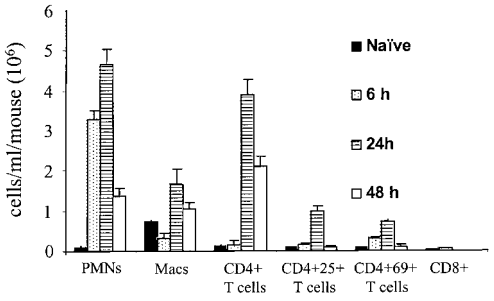

FIGURE 1. Kinetics of cellular infiltration and T cell activation in the peritoneal cavity following challenge with *B. fragilis*. Peritoneal lavage fluids from five C57BL/6 mice were analyzed individually 6, 24, and 48 h postchallenge. PMNs accumulated very rapidly at this site (within 6 h) following bacterial challenge. The level of these cells peaked at 24 h and then declined. The number of macrophages initially dropped at the 6-h time interval, but increased by 24 h postchallenge. The number of $CD4^+$ T cells rapidly increased in the peritoneal cavity at the 24-h time interval rising from 1.5×10^5 cells/ml to $\sim 4 \times 10^6$ 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.

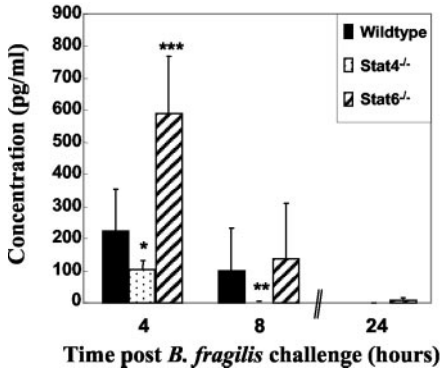

FIGURE 2. IL-17 levels in peritoneal cavities of C57BL/6 wild-type littermate control and $STAT^{-/-}$ mice challenged with *B. fragilis*. All mice were challenged via the i.p. route with *B. fragilis* (10^8 CFU/animal) and SCC. Groups of four mice underwent peritoneal lavage 4, 8, and 24 h postchallenge. 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. 3*A*). Abscesses had a defined fibrinous wall surrounding a dark-staining purulent focus of PMNs and bacteria. Confocal microscopic analysis of these sections after staining with a CD4-specfic Ab showed the presence of $CD4^+$ T cells within the fibrinous wall of the abscess (Fig. 3*B*, 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. 3*C*, 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. 3*D*), 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 fibrinous 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 $\alpha\beta TCR^{-/-}$ mice, which do not develop abscesses, supports this result. Based

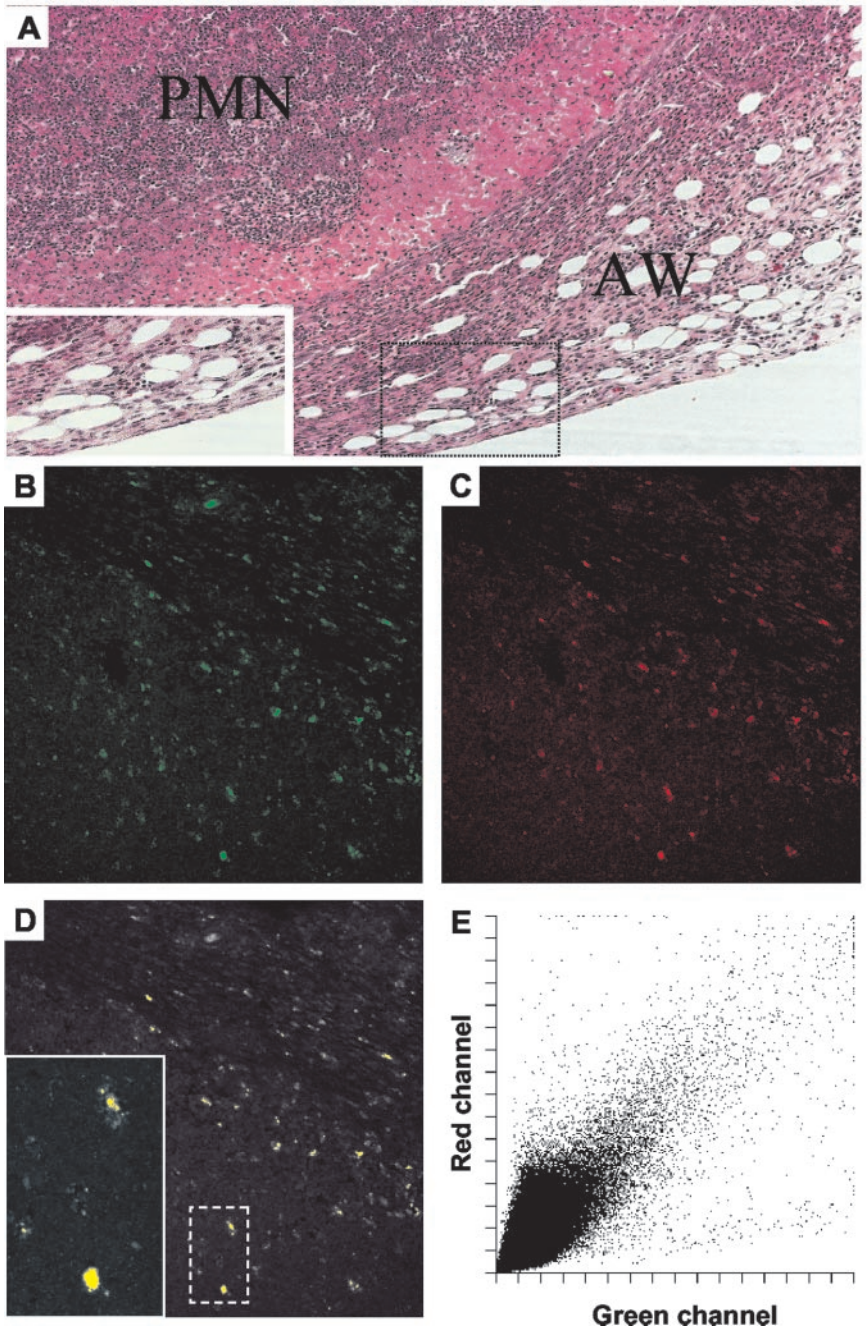

FIGURE 3. *A*, H&E-stained section from intra-abdominal abscesses induced by challenge with *B. fragilis* (original magnification ×100). Abscesses typically comprised a fibrin wall that surrounded a focus of PMNs and bacteria. AW, abscess wall; PMN, neutrophils in center of abscess. Dotted square, higher magnification of the wall of the abscess shown in *inset* demonstrating dense fibrin strands, fibroblasts, and the infiltration of mononuclear cells (original magnification ×250). *B*, Confocal microscopy images of CD4$^+$ T cells found within the wall of the abscess. The section was stained with a CD4$^+$ T cell-specific Ab, and these cells fluoresce green (original magnification, ×400). *C*, Red channel signals show sections stained with an IL-17-specific Ab. Cells with intracellular IL-17 fluoresce red (original magnification, ×400). *D*, Colocalization analysis. Colocalization of CD4 (green) and IL-17 (red) appears as bright yellow (original magnification, ×400). *Inset*, Shows magnified view of cells with colocalized CD4 and IL-17 (original magnification, ×1000). *E*, Histogram showing cell frequency based on the fluorescence intensity of both green and red channel signals. The horizontal and the vertical axes represent the intensity scale of the two images stained with anti-CD4 (green) and anti-IL-17 (red), respectively.

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).

Table V. *Effect of IL-17 neutralization on abscess formation in vivo*

Treatment[a]	No. of Animals with Abscess/Total (%)[b]	*p* Value[c]
Control IgG	9/10 (90)	
Anti-IL-17 Ab	1/10 (10)	0.001

[a] Animals received 100 μg of affinity-purified rabbit Ab via the i.p. route at the time of challenge.
[b] Animals challenged via the i.p. route with 1 × 10^8 CFU of *B. fragilis* and SCC.
[c] Compared with animals that received control IgG Ab.

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 abscesses 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

1. Gorbach, S. L., H. Thadepalli, and J. Norsen. 1974. *Anaerobic Microorganisms in Intraabdominal Infections*. Charles C. Thomas, Springfield.
2. Gorbach, S. L., and J. G. Bartlett. 1974. Anaerobic infections. *N. Engl. J. Med. 290:1237.*
3. Polk, B. J., and D. L. Kasper. 1977. *Bacteriodes fragilis* subspecies in clinical isolates. *Ann. Intern. Med. 86:567.*
4. Tzianabos, A. O., A. B. Onderdonk, B. Rosner, R. L. Cisneros, and D. L. Kasper. 1993. Structural features of polysaccharides that induce intra-abdominal abscesses. *Science 262:416.*
5. Tzianabos, A. O., J. Y. Wang, and J. C. Lee. 2001. Structural rationale for the modulation of abscess formation by *Staphylococcus aureus* capsular polysaccharides. *Proc. Natl. Acad. Sci. USA 98:9365.*
6. Tzianabos, A. O., R. W. Finberg, Y. Wang, M. Chan, A. B. Onderdonk, H. J. Jennings, and D. L. Kasper. 2000. T cells activated by zwitterionic molecules prevent abscesses induced by pathogenic bacteria. *J. Biol. Chem. 275:6733.*
7. Tzianabos, A. O., A. Chandraker, W. Kalka-Moll, F. Stingele, V. M. Dong, R. W. Finberg, R. Peach, and M. H. Sayegh. 2000. Bacterial pathogens induce abscess formation by CD4$^+$ T-cell activation via the CD28–B7-2 costimulatory pathway. *Infect. Immun. 68:6650.*
8. Kaplan, M. H., U. Schindler, S. T. Smiley, and M. J. Grusby. 1996. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity 4:313.*
9. Kaplan, M. H., Y. L. Sun, T. Hoey, and M. J. Grusby. 1996. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature 382:174.*
10. Chung, D. R., T. Chitnis, R. J. Panzo, D. L. Kasper, M. H. Sayegh, and A. O. Tzianabos. 2002. CD4$^+$ T cells regulate surgical and postinfectious adhesion formation. *J. Exp. Med. 195:1471.*
11. Tzianabos, A. O., D. L. Kasper, R. L. Cisneros, R. S. Smith, and A. B. Onderdonk. 1995. Polysaccharide-mediated protection against abscess formation in experimental intra-abdominal sepsis. *J. Clin. Invest. 96:2727.*
12. Witowski, J., K. Pawlaczyk, A. Breborowicz, A. Scheuren, M. Kuzlan-Pawlaczyk, J. Wisniewska, A. Polubinska, H. Friess, G. M. Gahl, U. Frei, and A. Jorres. 2000. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GROα chemokine from mesothelial cells. *J. Immunol. 165:5814.*
13. Aarvak, T., M. Chabaud, P. Miossec, and J. B. Natvig. 1999. IL-17 is produced by some proinflammatory Th1/Th0 cells but not by Th2 cells. *J. Immunol. 162: 1246.*
14. Yao, Z., S. L. Painter, W. C. Fanslow, D. Ulrich, B. M. Macduff, M. K. Spriggs, and R. J. Armitage. 1995. Human IL-17: a novel cytokine derived from T cells. *J. Immunol. 155:5483.*
15. Laan, M., Z. H. Cui, H. Hoshino, J. Lotvall, M. Sjostrand, D. C. Gruenert, B. E. Skoogh, and A. Linden. 1999. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J. Immunol. 162:2347.*
16. Kennedy, K. J., and W. J. Karpus. 1999. Role of chemokines in the regulation of Th1/Th2 and autoimmune encephalomyelitis. *J. Clin. Immunol. 19:273.*
17. Gerard, C., and B. J. Rollins. 2001. Chemokines and disease. *Nat. Immun. 2:108.*
18. Lukacs, N. W., and K. K. Tekkanat. 2000. Role of chemokines in asthmatic airway inflammation. *Immunol. Rev. 177:21.*
19. Atamas, S. P., and B. White. 1999. Interleukin 4 in systemic sclerosis: not just an increase. *Clin. Diagn. Lab. Immunol. 6:658.*
20. Chiaramonte, M. G., D. D. Donaldson, A. W. Cheever, and T. A. Wynn. 1999. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J. Clin. Invest. 104:777.*
21. Aarvak, T., M. Chabaud, P. Miossec, and J. B. Natvig. 1999. IL-17 is produced by some proinflammatory Th1/Th0 cells but not by Th2 cells. *J. Immunol. 162: 1246.*
22. Albanesi, C., C. Scarponi, A. Cavani, M. Federici, F. Nasorri, and G. Girolomoni. 2000. Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon-γ- and interleukin-4-induced activation of human keratinocytes. *J. Invest. Dermatol. 115:81.*
23. Infante-Duarte, C., H. F. Horton, M. C. Byrne, and T. Kamradt. 2000. Microbial lipopeptides induce the production of IL-17 in Th cells. *J. Immunol. 165:6107.*
24. Chabaud, M., T. Aarvak, P. Garnero, J. B. Natvig, and P. Miossec. 2001. Potential contribution of IL-17-producing Th(1) cells to defective repair activity in joint inflammation: partial correction with Th(2)-promoting conditions. *Cytokine 13:113.*

CORRECTION

Doo Ryeon Chung, Dennis L. Kasper, Ronald J. Panzo, Tanuja Chtinis, Michael J. Grusby, Mohamed H. Sayegh, and Arthur O. Tzianabos. $CD4^+$ T Cells Mediate Abscess Formation in Intra-abdominal Sepsis by an IL-17-Dependent Mechanism. *The Journal of Immunology* 2003;170:1958–63.

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

0022-1767/03/$02.00