$CD4^+$ T Cells Regulate Surgical and Postinfectious Adhesion Formation

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Abstract

The development of adhesions in the peritoneal and pelvic cavities, which commonly form after surgery or infection, cause significant morbidity and mortality. However, the pathogenesis of adhesion formation is still poorly understood. Because T cells are important in orchestrating fibrinogenic tissue disorders, we hypothesized that they play a critical role in the pathogenesis of peritoneal adhesion formation. Using a cecal abrasion surgical model in rodents, T cell depletion and adoptive transfer experiments demonstrated that this host response is dependent on $CD4^+$ $\alpha\beta$ T cells. These cells were also critical to adhesion formation associated with experimental intraabdominal sepsis. T cell transfer studies with mice deficient in signal transducer and activator of transcription (Stat)4 and Stat6 revealed that adhesion formation was dependent on a T helper 1 response. Activated T cells homed to the peritoneal cavity 6 hours after cecal abrasion surgery and predominated at this site during adhesiogenesis. Increased levels of the T cell–derived proinflammatory cytokine interleukin (IL)-17 and of neutrophil chemoattractant CXC chemokines macrophage inflammatory protein-2/CXCL8 and cytokine-induced neutrophil chemoattractant/CXCL1 were associated with adhesion formation. The production of these chemokines was dependent on T cells. Furthermore, the administration of neutralizing antibodies specific for IL-17 or the receptor that binds these CXC chemokines, CXC chemokine receptor 2, significantly reduced the degree of adhesion formation. These results demonstrate for the first time that the immunopathogenesis of adhesion formation is under the control of T cells and that T cell–derived cytokines and chemokines play important roles in the development of this deleterious host response.

Key words: T cells • adhesions • interleukin-17 • chemokines • peritonitis

Introduction

Adhesion formation is a common and often severe complication of abdominal or pelvic surgery. This under-appreciated surgical problem, associated with 67–93% of all abdominal and pelvic procedures, can cause long-term morbidity, mortality, and female infertility (1–5). Adhesions also arise as a result of bacterial infections such as peritonitis (6). Currently, only a few options are available to prevent adhesion development and these are not well accepted (7, 8). Although the mechanism of adhesion formation is poorly understood, recent studies suggest that the balance between fibrin deposition and fibrin degradation in the early phase of tissue repair dictates the outcome (9–11).

Investigations of the cellular mechanism of postsurgical adhesion formation have centered on the role of macrophages and PMN (12–15). A role for T lymphocytes has not been postulated, although these cells play a critical role in coordinating and regulating chemotactic responses in a variety of inflammatory tissue diseases, including experimental autoimmune encephalomyelitis, inflammatory bowel disease, and acute lung injury (16–21). Investigations in this area have clearly shown that T cell–derived cytokines and chemokines are among the predominant factors governing appropriate inflammatory responses. Recent investigations have shown that IL-17, a proinflammatory cytokine produced almost exclusively by activated $CD4^+$ T cells, in-

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duces the release of neutrophil-specific chemokines such as macrophage inflammatory protein (MIP)*-2 and cytokine-induced neutrophil chemoattractant (KC) and controls PMN trafficking in the peritoneal cavity (22–24). Based on these data, we hypothesized that T cells play a central role in orchestrating the inflammatory process leading to the development of peritoneal adhesions.

In this report, we demonstrate that Th1 $CD4^+$ αβ T cells are critical to the development of postsurgical and postinfectious adhesion formation. Moreover, activated T cells home to the peritoneal cavity shortly after the induction of adhesions and become a dominant cell type at this site during adhesiogenesis. Finally, soluble mediators such as the T cell–derived proinflammatory cytokine IL-17 and chemokines that bind to the CXC chemokine receptor 2 (CXCR2) have a direct role in the pathogenesis of adhesion formation.

Materials and Methods

Animals. Lewis rats and C57BL/6 mice were obtained from Charles River Laboratories. $\alpha\beta TCR^{-/-}$ (B6.129P2-Tcrb), $CD4^{-/-}$ (B6.129S6-Cd4), $CD8^{-/-}$ (B6.129S6-Cd8a), signal transducer and activator of transcription (Stat)$4^{-/-}$ (C.129S2-Stat4), $Stat6^{-/-}$ (C.129S2-Stat6), C57BL/6J, and BALB/cJ mice were obtained from The Jackson Laboratory. All animals were provided with food and water ad libitum and housed under specific pathogen-free conditions. The 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.

Rodent Model of Surgical Adhesion Formation. Rats were anesthetized with a single intraperitoneal injection of 0.15 ml pentobarbital sodium (50 mg/ml; Abbott Laboratories). Mice were anesthetized with 0.15 ml 1:5 (vol/vol) diluted pentobarbital sodium solution (10 mg/ml). For adhesion induction, we used a modified rat model of abdominal adhesion formation originally described by Krause et al. (25). An anterior midline incision was made through the abdominal wall and peritoneum. The cecum was isolated and abraded until visibly damaged by scrubbing with a sterile dry 4 × 4 surgical gauze. Apposing areas of the abdominal wall were also abraded. The incision was closed in two layers with silk sutures. Animals were killed and examined for adhesion formation 6 d later by an observer blinded to the identity of the experimental groups. Each animal was evaluated according to the following standard scoring system, which has been widely used in this field (26–29): 0, no adhesion; 1, one thin filmy adhesion; 2, more than one thin adhesion; 3, thick adhesion with focal point; 4, thick adhesion with plantar attachment or more than one thick adhesion with focal point; and 5, very thick vascularized adhesions or more than one plantar adhesion. The adhesion scores for the various groups were compared by the Mann-Whitney U test or the Kruskal-Wallis with Dunn's multiple comparisons test. Differences between experimental groups were considered significant at $P < 0.05$.

Mouse Model of Intraabdominal Sepsis. We used a model of intraabdominal sepsis as previously described (30). C57BL/6 mice were injected intraperitoneally with 0.2 ml 1:5 (vol/vol) diluted cecal contents inoculum containing both aerobic (9×10^5 CFU/ml) and anaerobic bacteria (8×10^7 CFU/ml). This dose was shown to yield a sublethal infection in these animals (unpublished data). Animals were killed and examined for adhesion formation 6 d later.

$CD3^+$ T Cell Depletion in Rats. Lewis rats were randomly assigned to one of three groups and subjected to cecal abrasion surgery. Group 1 was treated with 100 μg of a CD3-specific mAb (G4.18; BD PharMingen) via the intraperitoneal route at the time of surgery, group 2 was treated with 100 μg of an isotype-matched IgG antibody, and group 3 was treated with the same volume of saline via the same route. FACS® analysis showed that treatment with the anti-CD3 mAb depleted >95% of T cells (unpublished data). Animals were killed 6 d later and their adhesions were scored as previously described.

$\alpha\beta TCR^+$ T Cell Depletion in Rats and Mice. For studies with rats depleted of T cells bearing αβTCR, mAb R73 (BD PharMingen) specific for rat αβTCR was used. Lewis rats were treated with 100 μg mAb R73 or an isotype-matched control antibody via the intracardiac route 24 h before surgery. For experiments with mice, C57BL/6 mice were treated with 300 μg TCR β chain–specific mAb H57-597 (BD PharMingen) or an isotype-matched control antibody via the intraperitoneal route 4 d before surgery. All rats and mice underwent cecal abrasion surgery and were assessed for adhesion formation as previously described.

$CD4^+$ or $CD8^+$ T Cell Depletion in Mice. $CD4^+$ and $CD8^+$ T cells were depleted with CD4-specific mAb GK1.5 (BD PharMingen) and CD8-specific mAb 53-6.7 (BD PharMingen), respectively. C57BL/6 mice were treated with 0.2 mg of these mAbs via the intraperitoneal route 48 h before surgery. An additional group was treated with isotype-matched control antibody. All animals underwent cecal abrasion surgery and were killed and assessed for adhesion formation 6 d later.

Adoptive $CD4^+$ T Cell Transfer Experiments. Splenic T cells from $Stat4^{-/-}$, $Stat6^{-/-}$, or wild-type mice were purified on a nylon wool column and then $CD4^+$ T cells were purified on $CD4^+$ T cell enrichment immunocolumns (Cedarlane). $CD4^+$ T cell–enriched populations (>95% $CD4^+$) were transferred to $\alpha\beta TCR^{-/-}$ mice (2.4×10^6 cells per mouse) via the intracardiac route 24 h before surgery. All recipient animals underwent cecal abrasion surgery and were killed and assessed for adhesion formation 6 d later.

Kinetics of Cellular Influx into the Peritoneal Cavity After Cecal Abrasion. C57BL/6 mice underwent cecal abrasion for studies measuring the cellular influx into the peritoneal cavity after this procedure. A control group underwent laparotomy without cecal manipulation. Animals ($n = 5$) underwent peritoneal lavage with 1 ml PBS 6, 24, 48, and 72 h after surgery. Lavage fluid from each animal (25 μl) was smeared on a microscope slide and stained with a modified Giemsa stain. Slides were examined microscopically and monocytes/macrophages, lymphocytes, and PMN (per 200 cells) were enumerated. The remaining specimens were pooled for FACS® analysis and red blood cells were removed via lysis with NH_4Cl. After preincubation with rat anti–mouse CD16/CD32 (BD PharMingen) to block Fc receptors, cells were stained with FITC- or PE-labeled isotype control antibodies or mAbs to CD3, CD19, CD25, and CD69. Stained cells were analyzed on a Coulter EPICS XL™ cytometer (Beckman Coulter), the CELLQuest™ (Becton Dickinson), and WinMDI 2.8 analysis software (http://facs.scripps.edul; Scripps Research Institute). The absolute number of peritoneal cells collected was determined by trypan blue staining and a hemacytometer. The

Abbreviations used in this paper: CXCR2, CXC chemokine receptor 2; KC, cytokine-induced neutrophil chemoattractant; MIP, macrophage inflammatory protein; Stat, signal transducer and activator of transcription.

absolute numbers of macrophages/monocytes, PMN, and lymphocytes were calculated by multiplying the total number of peritoneal cells by the percentage of each cell type identified by microscopic examination and dividing the result by 100. The numbers of T and B lymphocytes were calculated based on the number of lymphocytes and the percentage of $CD3^+$ and $CD19^+$ cells obtained from FACS® analysis. The percentages of $CD69^+$ and $CD25^+$ T cells were determined by FACS® analysis and the absolute cell number was calculated by multiplying these percentages by the total peritoneal cell number.

Kinetics of IL-17 and CXC Chemokines in Peritoneal Fluid of Mice Undergoing Cecal Abrasion. C57BL/6 mice underwent the cecal abrasion procedure for studies measuring IL-17, KC, and MIP-2 in peritoneal fluid after this procedure. A control group underwent laparotomy without cecal manipulation. Animals ($n = 5$ at each time interval) underwent peritoneal lavage with 1 ml PBS 6, 24, 48, and 72 h after surgery. The peritoneal fluid was stored at −80°C until assayed for IL-17, KC, and MIP-2 with ELISA kits (R&D Systems) according to the manufacturer's protocols.

Intracellular FACS® Analysis for IL-17. Intracellular staining and subsequent FACS® analyses were used to determine the cell types in the peritoneal cavity that were responsible for the production of IL-17. Groups of C57BL/6 mice underwent cecal abrasion surgery and were killed 0, 4, or 6 h later. Peritoneal lavage was performed on each animal. Mononuclear cells from each group of animals were pooled and red blood cells were removed using Lymphoprep media (Nycomed Pharma). Cells from each group were stained with an mAb specific for CD3e conjugated to cychrome (BD PharMingen), an mAb specific for CD4 conjugated to FITC, and the appropriate isotype controls. The cells were washed, fixed, and permeabilized using cytofix/cytoperm solution and 13 perm/wash solution (BD PharMingen). Intracellular staining was performed with PE-conjugated mAb specific for IL-17 (BD PharMingen) or a PE-conjugated isotype control. Stained cells were analyzed on a Coulter EPICS XL™ cytometer as previously described. Analyses were performed to determine the proportion of $CD3^+4^+$ T cells that produce IL-17 compared with the proportion of non-$CD3^+4^+$ T cells that produce this cytokine.

Generation of Murine IL-17– and CXCR2-specific Polyclonal Antibodies. Polyclonal antibody to murine IL-17 was produced by the immunization of rabbits at multiple intradermal sites with recombinant mouse IL-17 (R&D Systems) mixed with complete Freund's adjuvant as previously described (31). The IgG fraction was purified by HiTrap protein G affinity chromatography per the manufacturer's instructions (Amersham Pharmacia Biotech). Eluted fractions from the column were concentrated using Centriprep YM-10 (Millipore) and buffer exchanged to PBS using PD-10 columns. Specific antibody was obtained after additional IL-17 affinity chromatography. The concentration of purified antibody was determined using a standard protein assay. Polyclonal antibody to murine CXCR2 was produced by the immunization of goats at multiple intradermal sites with murine CXCR2 peptide mixed with complete Freund's adjuvant as previously described (32, 33). The peptide sequence Met-Gly-Glu-Phe-Lys-Val-Asp-Lys-Phe-Asn-Ile-Glu-Asp-Phe-Phe-Ser-Gly has been shown to contain the ligand-binding portion of CXCR2. This peptide-specific antibody does not deplete PMN (34). Purified antibody was obtained after HiTrap and CXCR2 affinity chromatography of the antiserum as previously described.

Effect of IL-17– and CXCR2-specific Neutralizing Antibody Treatment on Adhesion Formation. For neutralization experiments, C57BL/6 mice were injected with increasing doses of affinity-purified IL-17– or CXCR2-specific antibodies (10, 50, or 100 mg of antibody per animal) via the intraperitoneal route at the time of cecal abrasion surgery and 6 h thereafter. Control groups were given 50 mg affinity-purified rabbit or goat IgG. All groups of mice were killed after 6 d and assessed for adhesion formation.

Results

Postsurgical Adhesion Formation in Rodent Cecal Abrasion Models. Adhesions that developed after cecal abrasion surgery typically involved the cecum, large bowel, and abdominal wall (Fig. 1). These fibrotic structures were dense and difficult to remove from involved organs. In contrast, animals that underwent control laparotomy without cecal manipulation only developed a few thin, filmy, membranous adhesions or no adhesions at all.

T Cells Are Critical for Surgical Adhesion Formation. To evaluate the role of T cells in surgical adhesion formation, T cell depletion studies were performed in rats with an antibody specific for the pan T cell epitope CD3. Groups of animals were treated with an mAb to CD3 (G4.18) or a control antibody and subjected to cecal abrasion. The median adhesion score was 1 in the CD3-specific mAb-treated group compared with 4.5 in the control antibody–treated group ($P < 0.001$; Fig. 2 A). Animals treated with saline had a median adhesion score of 5. The depletion of $\alpha\beta TCR^+$ T cells in rats after treatment with specific mAb resulted in a significant decrease in adhesion scores (Fig. 2 B). Treatment with the αβTCR-specific mAb R73 resulted in a median score of 2, whereas treatment with an isotype-matched control antibody yielded a median score of 5 (Fig. 2 B).

Similar studies with mice corroborated these data. Treatment with a TCR β chain–specific mAb (H57-597) that depletes αβ T cells reduced the median adhesion score from 5 in the control group to 0 ($P = 0.003$; Fig. 2 C). These data were confirmed in αβTCR-deficient (−/−) mice, which had a median adhesion score of 2 compared with wild-type littermate animals that had a median score of 5 ($P = 0.001$; Fig. 2 D).

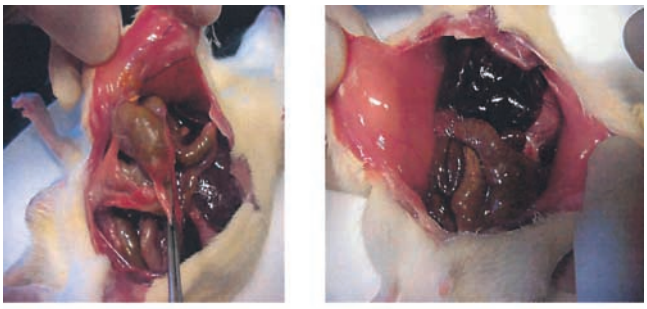

Figure 1. Postsurgical adhesion formation in a rat cecal abrasion model. Lewis rats underwent cecal abrasion surgery as described in Materials and Methods. The control group underwent laparotomy without cecal manipulation. Animals were killed and examined for adhesion formation 6 d later.

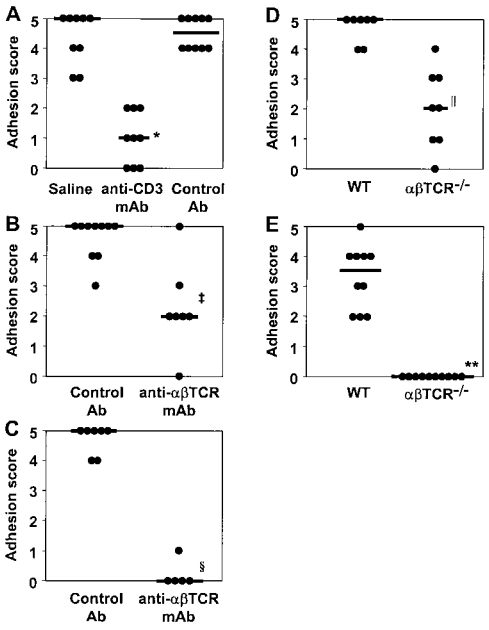

Figure 2. T cells play a critical role in adhesion formation. (A) Effect of $CD3^+$ T cell depletion on adhesion formation in Lewis rats undergoing cecal abrasion. Each point represents an individual adhesion score. Line indicates a median score for the group. *, $P < 0.001$ compared with control antibody group (Mann-Whitney U test). (B) Effect of $\alpha\beta TCR^+$ T cell depletion on adhesion formation in Lewis rats undergoing cecal abrasion. ‡, $P = 0.004$. (C) Effect of $\alpha\beta TCR^+$ T cell depletion on adhesion formation in C57BL/6 mice undergoing cecal abrasion. §, $P = 0.003$. (D) Comparison of adhesion scores for $\alpha\beta TCR^{-/-}$ and wild-type mice after cecal abrasion surgery. ‖, $P = 0.001$. (E) Adhesion formation in $\alpha\beta TCR^{-/-}$ challenged with cecal contents. **, $P < 0.001$.

Role of T Cells in Adhesions Induced by Intraabdominal Sepsis. To address the role of T cells in adhesions induced by an infectious process, we investigated whether adhesions induced during intraabdominal sepsis would develop in $\alpha\beta TCR^{-/-}$ mice. Wild-type and $\alpha\beta TCR^{-/-}$ mice were challenged intraperitoneally with a sublethal cecal contents inoculum. Wild-type mice developed adhesions after challenge (median score = 3.5; Fig. 2 E), whereas $\alpha\beta TCR^{-/-}$ mice did not (median score = 0, $P < 0.001$; Fig. 2 E).

$CD4^+$ T Cells Are the Major T Cell Subset Mediating Adhesion Formation. To further characterize the T cell phenotype that mediates surgical adhesion formation, mice were depleted of $CD4^+$ or $CD8^+$ T cells with a CD4-specific (GK1.5) or a CD8-specific mAb (53-6.7). Treatment with CD4-specific mAb resulted in a significant reduction in adhesion formation compared with control antibody–treated mice, whereas treatment with CD8-specific mAb had no such effect (Fig. 3 A). Mice deficient in $CD4^+$ T cells had lower median adhesion scores than wild-type animals ($P = 0.002$), whereas $CD8^{-/-}$ mice had scores comparable with those of wild-type animals (Fig. 3 B). Similarly, $CD4^{-/-}$ mice challenged intraperitoneally with a sublethal cecal contents inoculum failed to develop adhesions (median score = 0), whereas wild-type animals developed adhesions (median score = 2, $P = 0.018$). These results clearly indicate that the $CD4^+$ $\alpha\beta$ T cell is the main

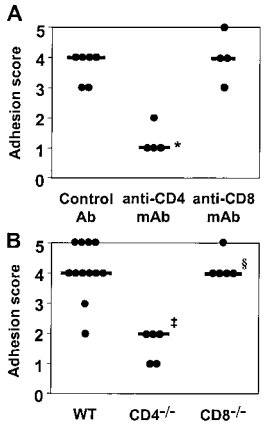

Figure 3. The $CD4^+$ T cell is the major T cell subset mediating surgical adhesion formation. (A) Effect of $CD4^+$ or $CD8^+$ T cell depletion on surgical adhesion formation. *, $P = 0.007$ compared with control-antibody (Kruskal-Wallis test). (B) Comparison of adhesion scores for $CD4^{-/-}$ or $CD8^{-/-}$ mice and wild-type mice after adhesion induction. ‡, $P = 0.002$; §, not significant compared with wild type.

phenotype mediating surgical and postinfectious adhesion formation in rodents.

Adhesion Formation Is Mediated by a Th1 Response. Previous studies have shown that different $CD4^+$ T cell subsets control host responses in different infectious, autoimmune, and inflammatory tissue diseases (35). To study the role of Th1 and Th2 responses in the development of peritoneal surgical adhesions, Stat4 and Stat6 knockout mice were used. $Stat4^{-/-}$ and $Stat6^{-/-}$ mice are genetically impaired in their ability to generate Th1 and Th2 responses, respectively, and have been used with success to demonstrate the type of Th response responsible for inflammatory host disorders (36–38). Cecal abrasion surgery in $Stat4^{-/-}$ mice resulted in significantly lower adhesion scores compared with control wild-type mice, whereas $Stat6^{-/-}$ mice had scores comparable with those from wild-type animals (Fig. 4 A). The role of Th1 cells was confirmed in T cell transfer experiments. Splenic $CD4^+$ T cells from $Stat4^{-/-}$, $Stat6^{-/-}$, or wild-type mice were purified and transferred to $\alpha\beta TCR^{-/-}$ mice previously shown to be genetically deficient in their ability to form adhesions, which were then subjected to cecal abrasion surgery. Transfer of $CD4^+$ T cells from wild-type or $Stat6^{-/-}$ mice enabled recipient $\alpha\beta TCR^{-/-}$ mice to develop adhesions in a manner comparable with wild-type mice ($P = 0.012$ and 0.013, respectively, compared with $\alpha\beta TCR^{-/-}$ mice receiving no T cell transfer, Fig. 4 B). In contrast, transfer of $CD4^+$ T cells from $Stat4^{-/-}$ mice did not yield a significant change in adhesion scores compared with $\alpha\beta TCR^{-/-}$ mice receiving no T cell transfer. These data strongly support a critical role for $CD4^+$ Th1 cells in the development of surgical adhesions in mice.

T Cells Are Activated during Adhesiogenesis. It is well documented that the role of T cells in different inflammatory tissue responses is dependent upon T cell activation and the release of soluble mediators, such as chemokines, to coordinate these responses (16, 39, 40). We hypothesized that upon activation, T cells home to the peritoneal cavity and coordinate the release of cytokines and chemokines during adhesiogenesis.

To address this question, we performed microscopic cell counts and FACS® analyses to determine the temporal kinetics of peritoneal cellular infiltration in mice after cecal

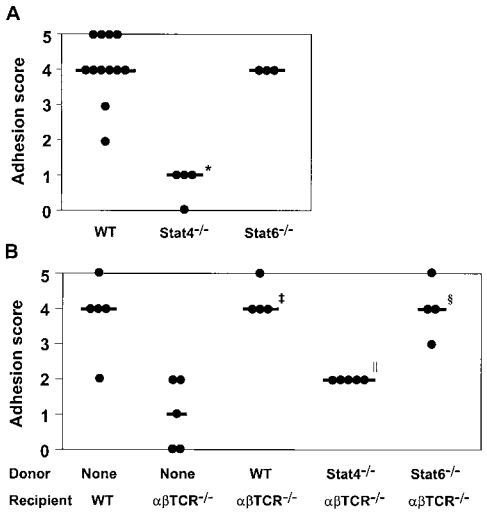

Figure 4. Th1 CD4$^+$ T cells mediate postsurgical adhesion formation. (A) Comparison of adhesion scores for Stat4$^{-/-}$ or Stat6$^{-/-}$ mice and wild-type mice after cecal abrasion surgery. *, $P = 0.003$ compared with wild type (Kruskal-Wallis test). (B) Adoptive CD4$^+$ T cell transfer experiment. CD4$^+$ T cells purified from spleens of indicated mouse group were transferred to recipients and cecal abrasion surgery was performed. ‡, $P = 0.012$; §, $P = 0.013$; ‖, not significant compared with αβTCR$^{-/-}$ mice receiving no T cell transfer.

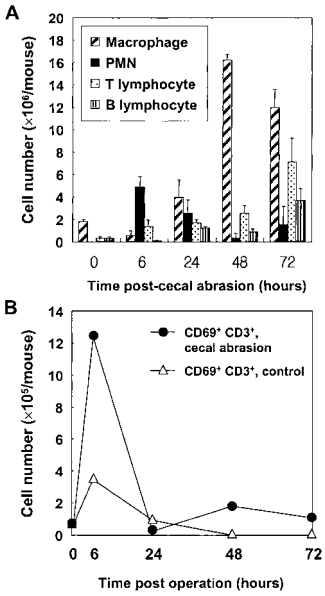

Figure 5. Kinetics of cellular infiltration and T cell activation in the peritoneal cavity after cecal abrasion surgery. (A) Kinetics of inflammatory cell influx into the peritoneal cavity in C57BL/6 mice that underwent cecal abrasion. Bar represents a mean number of each cell type in peritoneal fluid collected by lavage from five animals at each time point. Error bar indicates a standard deviation. (B) Kinetics of activated T cell number in the peritoneal cavity after cecal abrasion.

abrasion surgery. After peritoneal injury, PMN accumulated within 6 h in the peritoneal cavity, becoming a dominant cell type at this site (Fig. 5 A), whereas there was approximately a fourfold increase in the number of T cells at this time interval. Macrophages entered the peritoneal cavity at 24 h and predominated at 48–72 h, whereas the number of T cells continued to rise. By 72 h after surgery, macrophages and T cells were the predominant cell types in the peritoneal cavity. FACS® analysis of the cellular infiltrate showed that T cells entering the peritoneal cavity at 6 h expressed the activation marker CD69 compared with control animals that underwent laparotomy without cecal abrasion (Fig. 5 B). The finding that T cells are rapidly activated after peritoneal injury suggests a role for these cells in producing soluble mediators that coordinate cellular trafficking.

Cecal Abrasion Is Associated with Increased Levels of IL-17 and CXC Chemokines in the Peritoneal Cavity. IL-17 is a proinflammatory cytokine that is reportedly produced by activated T lymphocytes (22, 41, 42). IL-17 can selectively recruit neutrophils into the peritoneal cavity via the release of neutrophil-specific chemokines, such as KC, from the peritoneal mesothelium (23). Because PMN quickly enter the peritoneal cavity after cecal abrasion and T cells play a critical role in adhesion formation, we hypothesized that IL-17 could be one of the soluble factors that mediate this activity. In addition to testing this hypothesis, we sought to determine whether members of the CXC chemokine family were associated with the development of this tissue response. Cytokine- and chemokine-specific ELISAs were performed on peritoneal lavage fluids from mice subjected to cecal abrasion. The results showed a rapid (i.e., within 6 h) increase in IL-17 after surgery (Fig. 6 A) compared with levels in animals that underwent laparotomy with no cecal abrasion. The elevated level in animals with cecal abrasion dropped to baseline by 24 h. Intracellular FACS® analysis of the cellular infiltrate at the time of cecal abrasion surgery as well as 4 or 6 h after surgery demonstrated that T cells were the only cell type present that produced IL-17 (Table I). This is in agreement with previous studies investigating the cellular source of this cytokine (22, 23).

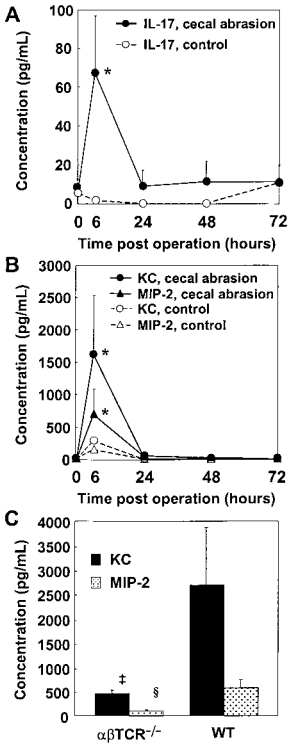

Figure 6. Role of T cells and kinetics of IL-17 and CXC chemokine production after cecal abrasion surgery. Peritoneal fluid was pooled after collection by lavage from five animals at each time point. (A) Kinetics of IL-17 production. (B) Kinetics of MIP-2 and KC production. Data represent mean concentrations from two separate experiments. Error bars indicate SD. *, $P < 0.05$ compared with control group undergoing laparotomy without cecal manipulation (Student's t test). (C) Role of T cells in CXC chemokine production after cecal abrasion. Bars represent mean concentrations and error bars indicate SD for groups of five mice. ‡, $P = 0.013$; §, $P = 0.001$ (Student's t test).

Table I. *Intracellular FACS® Analysis of Cellular Infiltrate after Cecal Abrasion Surgery*

	Percentage of cells expressing marker	
Time relative to surgery	IL-17$^+$/CD3$^+$4$^+$	IL-17$^+$/CD3$^-$
h		
0	0.4	0
4	6.3	0
6	5.3	0

CXC chemokines were found in the peritoneal cavity of animals 6 h after cecal abrasion surgery. KC and MIP-2 levels were significantly higher in animals undergoing abrasion than in control animals at the 6-h time point (Fig. 6 B). Like IL-17, these chemokines returned to baseline levels by 24 h.

T Cells Play an Important Role in CXC Chemokine Production during Adhesiogenesis. To demonstrate the role of T cells in the production of CXC chemokines induced by cecal abrasion, the levels of KC and MIP-2 in the peritoneal fluid of $\alpha\beta$TCR$^{-/-}$ mice and littermate wild-type animals that underwent cecal abrasion surgery were compared. 6 h after surgery, $\alpha\beta$TCR$^{-/-}$ mice showed significantly lower levels of KC and MIP-2 than wild-type animals ($P = 0.013$ and 0.001; respectively, Fig. 6 C).

Neutralization of IL-17 Reduces Adhesion Formation after Cecal Abrasion. To demonstrate the role of IL-17 produced by activated CD4$^+$ T cells in the development of postsurgical adhesions, we tested the effect of neutralization of IL-17 with specific antibody on adhesion formation after cecal abrasion. Although the administration of increasing concentrations of IL-17–specific IgG resulted in the dose-dependent blockade of adhesiogenesis, it did not have this effect in animals treated with normal rabbit IgG (Fig. 7 A).

Blockade of CXCR2 reduces adhesion formation after cecal abrasion. To demonstrate the role of CXC chemokines in the development of surgical adhesion formation, we tested the effect of CXCR2 blockade with a specific antibody on adhesiogenesis. The administration of increasing doses of a purified CXCR2-blocking IgG antibody resulted in the dose-dependent reduction of adhesion formation compared with the administration of normal goat IgG (Fig. 7 B).

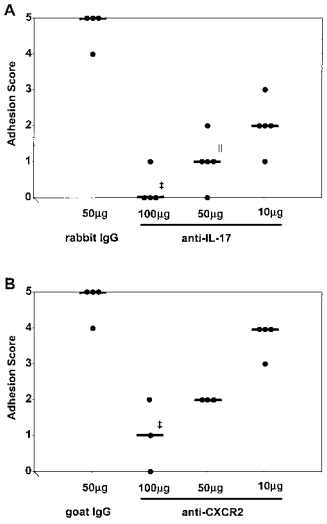

Figure 7. Effect of IL-17 neutralization and CXCR2 blockade on postsurgical adhesion formation. (A) Neutralization of IL-17 reduces postsurgical adhesion formation. ‡, $P < 0.01$; §, $P < 0.05$ compared with control antibody–treated mice (Kruskal-Wallis test). (B) Blockade of CXCR2 with specific antibody reduces adhesion formation in mice undergoing cecal abrasion surgery. ‡, $P < 0.05$ compared with control group.

Discussion

These results demonstrate that CD4$^+$ $\alpha\beta$ T cells play a central role in the development of surgical and postinfectious adhesions in experimental rodent models. The host response is primarily mediated by Th1 cells and is associated with the release of the T cell–derived proinflammatory cytokine IL-17 and CXC chemokines MIP-2 and KC.

Postoperative adhesion formation in the peritoneal and pelvic cavities is a fibrotic tissue disorder manifested by excessive deposition of fibrin during normal wound healing. It is currently believed that a breakdown in the balance between fibrin deposition (fibrinogenesis) and fibrin degradation (fibrinolysis) during wound healing leads to this surgical complication (9–11). Despite a long-term research effort in this area, there is still little understanding of the cellular host response governing this process.

Recent studies have clearly shown that T cells play a critical role in orchestrating early events that lead to different inflammatory host tissue responses, such as experimental autoimmune encephalomyelitis, idiopathic pulmonary fibrosis, progressive systemic sclerosis, myocarditis, hepatic fibrosis, experimental colitis, and granuloma formation (17–19, 43–47). Intraabdominal abscess formation is another example of a T cell–mediated host defense mechanism. CD4$^+$ T cell activation by the capsular polysaccharides of *Bacteroides fragilis* and *Staphylococcus aureus* mediates intraabdominal abscess formation in a rodent model (48). Data from these collective studies led us to hypothesize that T cells and T cell–derived cytokines and chemokines mediate surgical adhesion formation. We addressed this question using T cell depletion and T cell transfer experiments in rats and similar experiments with knockout mice genetically deficient in specific T cell subsets. The significant reduction in adhesion formation after cecal abrasion in T cell–depleted animals or $\alpha\beta$TCR$^{-/-}$ mice indicated that T cells play a definite role in the development of adhesions.

A standard surgical scoring system was used to assess the development of adhesions in rodents. This scoring system is widely used because clinical and experimental experience shows that adhesion formation is difficult to assess by quantitative assays that measure fibrin deposition, which do not accurately reflect the organization and number of defined fibrinous structures in people or animals. Therefore, it is generally accepted that visual determination of the degree of disease is the superior method to assess adhesion formation. This method has been adopted by the American Soci-

ety for Reproductive Medicine in the evaluation of adhesion formation in clinical trials.

Intraabdominal sepsis is a known cause of peritoneal adhesion formation. However, the mechanism by which infection induces adhesions in this scenario is not clear (9). To address this question, we developed an adhesion model that mimicked a bacterial contamination arising from a colonic source. The significant reduction of adhesion formation in $\alpha\beta TCR^{-/-}$ and $CD4^{-/-}$ mice indicated that in addition to surgical adhesion formation, $CD4^+$ T cells play a critical role in postinfectious adhesion formation.

Additional studies using depleting mAbs and knockout mice demonstrated that $CD4^+$ T cells contribute to postsurgical adhesion formation. This finding was confirmed by adoptive transfer experiments documenting the ability of T cell populations enriched for $CD4^+$ T cells to transfer the capacity for adhesion formation to $\alpha\beta TCR^{-/-}$ mice. $CD4^+$ T cells can be assigned to two subsets, Th1 and Th2, according to the cytokines they produce. Experiments with Stat4 and Stat6 knockout mice showed that Th1 $CD4^+$ cells are responsible for the development of postsurgical adhesions.

Surgical trauma to the peritoneum elicits a rapid but transient influx of PMN into the peritoneal cavity with the subsequent accumulation of macrophages (12–14). Our studies of the kinetics of peritoneal inflammatory cell infiltration after cecal abrasion revealed a striking influx of activated T cells homing to this site after trauma. The number of T cells continued to increase in the peritoneum of mice until the experiment was terminated 72 h after surgery. We also observed early PMN infiltration at 6 h and associated macrophage influx at 24 h that has been reported in previous investigations of the cellular response associated with adhesion formation (12, 13). The finding that T cells are also a major component of the local inflammatory response has not been reported and led us to investigate the role of the T cell–derived proinflammatory cytokine IL-17 in this process.

IL-17 is produced by activated T cells and has been shown to induce the release of MIP-2 and KC and regulate PMN infiltration in T cell–mediated inflammatory processes (22, 23). We found that a significant number of T cells entering the peritoneal cavity shortly after cecal abrasion expressed activation marker CD69. Increased levels of IL-17 were found in the peritoneal cavity after cecal abrasion surgery. Intracellular cytokine staining and FACS® analysis showed that T cells were the only cell type present in the peritoneal cavity after cecal abrasion surgery that produces IL-17. The administration of a neutralizing antibody specific for IL-17 significantly reduced adhesion formation. These data demonstrate the critical role of this T cell–derived cytokine in adhesion formation.

Because IL-17 has been shown to elicit the CXC chemokines MIP-2 and KC from peritoneal mesothelial cells during inflammation, we investigated whether these chemokines play a role in adhesion formation. We found increased levels of both MIP-2 and KC after cecal abrasion. Furthermore, significantly lower levels of these chemokines in $\alpha\beta$ T cell–deficient animals confirmed the role of T cells in the production of these chemokines associated with adhesion formation. The administration of an antibody specific for the receptor that binds these chemokines (CXCR2) significantly reduced adhesion formation.

Adhesion formation is a serious yet common host response that is difficult to prevent or treat. On the basis of our findings, we propose that activated $CD4^+$ $\alpha\beta$ T cells with a Th1 phenotype home to the peritoneal cavity after peritoneal injury and orchestrate this host response, which leads to the development of adhesions. These data demonstrate for the first time that T cells play a role in this disease process and suggest a new strategy for preventing this type of postsurgical and postinfectious complication.

We thank Julie McCoy for editorial assistance.

This work was supported in part by National Institutes of Health/NIGMS grant 6M64805-01 to A.O. Tzianabos.

Submitted: 7 January 2002
Revised: 2 April 2002
Accepted: 29 April 2002

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