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⁺ H11001 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 degrada-
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. αβ 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.

**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 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.

**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\(_4\)Cl. 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.edu; Scripps Research Institute). The absolute number of peritoneal cells collected was determined by trypan blue staining and a hemacytometer. The
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 Abraision.** CS7BL/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 CS7BL/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 CD3ε conjugated to cyochrome (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 CD3ε+ T cells that produce IL-17 compared with the proportion of non-CD3ε+4+ T cells that produce this cytokine.

**Generation of Marine 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**

**Posturgical 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 αβ 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).

**Cecal abrasion**  
**Control**

**Figure 1.** Posturgical 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.
Role of T Cells in Adhesions Induced by Intrabdominal 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 αβ TCR–/– mice. Wild-type and αβ 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 αβ TCR–/– mice did not (median score = 0; 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+ αβ T cell is the main 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 αβ 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 αβ TCR–/– mice to develop adhesions in a manner comparable with wild-type mice (P = 0.012 and 0.013, respectively, compared with αβ 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 αβ 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
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 without 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).
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 αβTCR<sup>−/−</sup> mice and littermate wild-type animals that underwent cecal abrasion surgery were compared. 6 h after surgery, αβTCR<sup>−/−</sup> 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<sup>+</sup> 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).

Discussion

These results demonstrate that CD4<sup>+</sup> αβ 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). Intrabdominal abscess formation is another example of a T cell–mediated host defense mechanism. CD4<sup>+</sup> T cell activation by the capsular polysaccharides of *Bacteroides fragilis* and *Staphylococcus aureus* mediates intrabdominal 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 αβTCR<sup>−/−</sup> 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-
et 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 αβTCR−/− 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 αβTCR−/− 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 αβ 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+ αβ 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.

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References
1. Weibel, M.A., and G. Majno. 1973. Peritoneal adhesions and their relation to abdominal surgery. A postmortem study. Ann. J. Surg. 126:345–353.
2. Menzies, D., and H. Ellis. 1990. Intestinal obstruction from adhesions—how big is the problem? Ann. R. Coll. Surg. Engl. 72:60–63.
3. Ellis, H., B.J. Moran, J.N. Thompson, M.C. Parker, M.S. Wilson, D. Menzies, A. McGuire, A.M. Lower, R.J. Hawthorn, F. O’Brien, et al. 1999. Adhesion-related hospital re-admissions after abdominal and pelvic surgery: a retrospective cohort study. Lancet. 353:1476–1480.
4. Ellis, H. 1997. The clinical significance of adhesions: focus on intestinal obstruction. Eur. J. Surg. Suppl. 163:5–9.
5. Trimbos-Kemper, T.C., J.B. Trimbos, and E.V. van Hall. 1985. Adhesion formation after tubal surgery: results of the eighth-day laparoscopy in 188 patients. Fertil. Steril. 43:395–400.
6. Ghellai, A.M., A.F. Stucchi, N. Chegini, C. Ma, C.D. Andry, J.M. Kasetta, J.W. Burns, K.C. Skinner, and J.M. Becker. 2000. Role of transforming growth factor beta-1 in peritonitis-induced adhesions. J. Gastrointest. Surg. 4:316–323.
7. Menzies, D. 1993. Postoperative adhesions: their treatment and relevance in clinical practice. Ann. R. Coll. Surg. Engl. 75:147–153.
8. Risberg, B. 1997. Adhesions: preventive strategies. Eur. J. Surg. Suppl. 163:32–39.
9. Holtz, G. 1984. Prevention and management of peritoneal adhesions. Fertil. Steril. 41:497–507.
10. Falk, K., P. Bjorquist, M. Stromqvist, and L. Holmdahl. 2001. Reduction of experimental adhesion formation by inhibition of plasminogen activator inhibitor type 1. Br. J. Surg. 88:286–289.
11. Holmdahl, L., B. Risberg, D.E. Beck, J.W. Burns, N. Chegini, G.S. diZerega, and H. Ellis. 1997. Adhesions: pathogenesis and prevention-panel discussion and summary. Eur. J. Surg. Suppl. 163:56–62.
12. Kuraoaka, S., J.D. Campeau, R.M. Nakamura, and G.S. diZerega. 1992. Modulation of postsurgical macrophage function by early postsurgical polymorphonuclear leukocytes. J. Surg. Res. 53:245–250.
13. Rodgers, K.E., and G.S. diZerega. 1993. Function of peritoneal...
exudate cells after abdominal surgery. *J. Invest. Surg.* 6:9–23.

14. Ar’Rajab, A., W. Mileksi, J.T. Sentementes, P. Sikes, R.B. Harris, and I.J. Davidson. 1996. The role of neutrophils in peritoneal adhesion formation. *J. Surg. Res.* 61:143–146.

15. Ar’Rajab, A., I. Dawidson, J. Sentementes, P. Sikes, R. Harris, and W. Mileksi. 1995. Enhancement of peritoneal macrophages reduces postoperative peritoneal adhesion formation. *J. Surg. Res.* 58:307–312.

16. Baggiolini, M. 1998. Chemokines and leukocyte traffic. *Nature.* 392:563–568.

17. Kennedy, R., D.J. Costain, V.C. McAlister, and T.D. Lee. 2001. Type 1/type 2 cytokine paradigm and the role of neutrophils in intestinal inflammation: pathogenic and protective roles in a mouse colitis model. *Am. J. Physiol.* 276:G1317–G1321.

18. Witowski, J., K. Pawlaczyk, A. Breborowicz, A. Scheuren, M. Kuzlan-Pawlaczyk, J. Wisniewska, A. Polubinska, H. Friess, G.M. Gahl, U. Frei, et al. 2000. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO alpha chemokine from mesothelial cells. *J. Immunol.* 165:5814–5821.

19. De Winter, H., H. Cheroutre, and M. Kronenberg. 1999. Mucosal immunity and inflammation. II. The yin and yang of T cells in intestinal inflammation: pathogenic and protective roles in a mouse colitis model. *Am. J. Physiol.* 276:G1317–G1321.

20. Dixon, A.E., J.B. Mandac, D.K. Madtes, P.J. Martin, and J.G. Clark. 2000. Chemokine expression in Th1 cell-induced lung injury: prominence of IFN-γ-gamma-inducible chemokines. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 279:L592–L599.

21. Knott, P.G., P.R. Gater, P.J. Dunford, M.E. Fuentes, and C.P. Bertrand. 2001. Rapid up-regulation of CXC chemokines in the airways after Ag-specific CD4+ T cell activation. *J. Immunol.* 166:1233–1240.

22. 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 receptor 7 in the airways. *J. Immunol.* 162:2347–2352.

23. Witowski, J., K. Pawlaczyk, A. Breborowicz, A. Scheuren, M. Kuzlan-Pawlaczyk, J. Wisniewska, A. Polubinska, H. Friess, G.M. Gahl, U. Frei, et al. 2000. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO alpha chemokine from mesothelial cells. *J. Immunol.* 165:5814–5821.

24. 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.* 162:1246–1251.

25. Krause, T.J., N.K. Goldsmith, S. Ebner, G.A. Zazanis, and R.D. McKinnon. 1998. A peptide inhibitor of cell proliferation. *Immunity.* 4:313–319.

26. 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.* 165:5843–5848.

27. Kuzlan-Pawlaczyk, J. Wisniewska, A. Polubinska, H. Friess, G.M. Gahl, U. Frei, et al. 2000. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO alpha chemokine from mesothelial cells. *J. Immunol.* 165:5814–5821.

28. Kaplan, M.H., U. Schindler, S.T. Smiley, and M.J. Grusby. 2000. Type 1/2 cytokine paradigm and the role of neutrophils in intestinal inflammation: pathogenic and protective roles in a mouse colitis model. *Am. J. Physiol.* 276:G1317–G1321.

29. Moore, T.A., W.D. Widmann. 1998. Does lining polyethylene glycol 4000 and dextran 70 on adhesion formation in propylene with polyglactin mesh reduce intraperitoneal adhesion formation with cross-linked hyaluronic acid? *J. Surg. Res.* 84:45–50.

30. Moore, T.A., W.D. Widmann. 1998. Does lining polyethylene glycol 4000 and dextran 70 on adhesion formation in propylene with polyglactin mesh reduce intraperitoneal adhesion formation with cross-linked hyaluronic acid? *J. Surg. Res.* 84:45–50.

31. Moore, T.A., M.W. Newstead, R.M. Strieter, B. Mehrad, B.L. Beam, and T.J. Standiford. 2000. Bacterial clearance and survival are dependent on CXCL chemokine receptor-2 ligands in a murine model of pulmonary Nocardia asteroides infection. *J. Immunol.* 164:908–915.

32. Mehrad, B., R.M. Strieter, T.A. Moore, W.C. Tsai, S.A. Lira, and T.J. Standiford. 1999. CXCL chemokine receptor-2 ligands are necessary components of neutrophil-mediated host defense in invasive pulmonary aspergillosis. *J. Immunol.* 163:6086–6094.

33. Moore, T.A., M.W. Newstead, R.M. Strieter, B. Mehrad, B.L. Beam, and T.J. Standiford. 2000. Bacterial clearance and survival are dependent on CXCL chemokine receptor-2 ligands in a murine model of pulmonary Nocardia asteroides infection. *J. Immunol.* 164:908–915.

34. Hebert, C.A., A. Chuntharapai, M. Smith, T. Colby, J. Kim, D.C. Gruenert, B.E. Skoogh, and A. Linden. 1999. Neutrophil recruitment by human IL-17 via C-X-C chemokine receptor 7 in the airways. *J. Immunol.* 162:2347–2352.

35. Kuzlan-Pawlaczyk, J. Wisniewska, A. Polubinska, H. Friess, G.M. Gahl, U. Frei, et al. 2000. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO alpha chemokine from mesothelial cells. *J. Immunol.* 165:5814–5821.

36. Kennedy, R., D.J. Costain, V.C. McAlister, and T.D. Lee. 1996. Prevention of experimental postoperative peritoneal adhesions by N,O-carboxymethyl chitosan. *Surgery.* 120:866–870.

37. Kocak, I., C. Ulu, Y. Akcan, and K. Yakin. 1999. Reduction of adhesion formation with cross-linked hyaluronic acid after peritoneal surgery in rats. *Fertil. Steril.* 72:873–878.

38. Nagelschmidt, M., and S. Saad. 1996. Influence of polyethylene glycol 4000 and dextran 70 on adhesion formation in rats. *J. Surg. Res.* 67:113–118.

39. Dasika, E.U., and W.D. Widmann. 1998. Does lining polypropylene mesh reduce intraperitoneal adhesions? *Am. Surg.* 64:817–819.

40. Onderdonk, A.B., R.L. Cisneros, R. Finberg, J.H. Crabb, and D.L. Kasper. 1990. Animal model system for studying virulence of and host response to *Bacteroides fragilis.* *Rev. Infect. Dis.* 12(Suppl):S169–S177.

41. Strieter, R.M., S.L. Kunkel, M.D. Burdick, P.M. Lincoln, and A. Walz. 1992. The detection of a novel neutrophil-activating peptide (ENA-78) using a sensitive ELISA. *Immunol. Invest.* 21:589–596.

42. Moore, T.A., W.D. Widmann. 1998. Does lining polyethylene glycol 4000 and dextran 70 on adhesion formation in propylene with polyglactin mesh reduce intraperitoneal adhesion formation with cross-linked hyaluronic acid? *J. Surg. Res.* 84:45–50.

43. Moore, T.A., W.D. Widmann. 1998. Does lining polyethylene glycol 4000 and dextran 70 on adhesion formation in propylene with polyglactin mesh reduce intraperitoneal adhesion formation with cross-linked hyaluronic acid? *J. Surg. Res.* 84:45–50.

44. Moore, T.A., W.D. Widmann. 1998. Does lining polyethylene glycol 4000 and dextran 70 on adhesion formation in propylene with polyglactin mesh reduce intraperitoneal adhesion formation with cross-linked hyaluronic acid? *J. Surg. Res.* 84:45–50.

45. Moore, T.A., W.D. Widmann. 1998. Does lining polyethylene glycol 4000 and dextran 70 on adhesion formation in propylene with polyglactin mesh reduce intraperitoneal adhesion formation with cross-linked hyaluronic acid? *J. Surg. Res.* 84:45–50.

46. Moore, T.A., W.D. Widmann. 1998. Does lining polyethylene glycol 4000 and dextran 70 on adhesion formation in propylene with polyglactin mesh reduce intraperitoneal adhesion formation with cross-linked hyaluronic acid? *J. Surg. Res.* 84:45–50.