Regulation of Postsurgical Fibrosis by the Programmed Death-1 Inhibitory Pathway

Matthew A. Holsti, Tanuja Chitnis, Ronald J. Panzo, Roderick T. Bronson, Hideo Yagita, Mohamed H. Sayegh and Arthur O. Tzianabos

*J Immunol* 2004; 172:5774-5781; doi: 10.4049/jimmunol.172.9.5774
http://www.jimmunol.org/content/172/9/5774

**References**
This article cites 41 articles, 14 of which you can access for free at:
http://www.jimmunol.org/content/172/9/5774.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Regulation of Postsurgical Fibrosis by the Programmed Death-1 Inhibitory Pathway

Matthew A. Holsti, Tanuja Chitnis, Ronald J. Panzo, Roderick T. Bronson, Hideo Yagita, Mohamed H. Sayegh, and Arthur O. Tzianabos

Surgical adhesions are a common and often severe complication of abdominal or pelvic injury that cause pelvic pain, bowel obstruction, and female infertility. Numerous postsurgical treatments and devices have been tested for their ability to abrogate adhesions or reduce their severity, but to date have been of limited effectiveness and are not widely used. The formation of an adhesion following surgery has been described as a detri
tive outcome of peritoneal wound healing, a complex process initiated by coagulation and inflammation. Tissue repair and remodeling ensue, involving cell growth and differentiation, angiogenesis, and extracellular matrix deposition. Little is known in detail, however, about the cellular and subcellular mechanisms underlying adhesion formation, although it has been hypothesized that reduced fibrinolytic activity, which correlates with tissue trauma, plays a key role.

We have focused our studies on the inflammatory response to abdominal surgery, hypothesizing that prolonged inflammation in the peritoneal cavity leads to the persistence of adhesions. Of the inflammatory cell types involved, the role of neutrophils and macrophages has been investigated, but the contribution of T cells had not been considered despite the central role they play in autoimmune and other inflammatory fibrotic diseases. We recently demonstrated that Th1 CD4+ αβ T cells are required for the development of postsurgical and infectious adhesions. Activated T cells home to the peritoneal cavity within a few hours of cecal abrasion surgery and are present in this site throughout the period of adhesiogenesis. These cells produce cytokines such as IL-17, which stimulate chemokine production, homing of other inflammatory cells, and prolonged fibrosis.

Here, we have extended these studies by focusing on the role that T cell costimulatory molecules play in the development of surgical adhesions. It is well-established that CD28 engagement by B7-1 and B7-2 costimulates T cell activation in vivo and that engagement by CTLA-4 is inhibitory. But, it has recently become clear that other accessory pathways regulate T cell responses, such as that involving the programmed death-1 (PD-1) molecule. PD-1 is expressed by activated mouse CD4+ and CD8+ T cells, and has been shown to inhibit IL-2 production and proliferation in vitro. The demonstration that mice genetically deficient in PD-1 expression develop autoimmunity suggests that the PD-1 molecule plays an inhibitory role in T cell responses in vivo, such as in the maintenance of peripheral tolerance.

Two ligands for PD-1 have been identified, PD-L1 (B7-H1) and PD-L2 (B7-DC). Expression of both molecules has been detected in a variety of nonlymphoid tissues as well as on activated macrophages and dendritic cells. Both stimulatory and inhibitory effects on T cell activation have been reported for both molecules; thus, their precise mode of action awaits further characterization.

In this work, we show that the B7-CD28 costimulatory pathway initiates the inflammatory response that leads to the development of surgical adhesions. Strikingly, CTLA-4 does not play a significant role in down-regulating the inflammatory response to cecal abrasion surgery. Instead, the engagement of PD-1 controls the severity of adhesiogenesis. In addition, we demonstrate for the first time that CD4+ cells home to the site of surgical adhesions. Our results suggest that the B7-CD28 and PD-1 pathways may serve as

Abbreviations used in this paper: PD-1, programmed death-1; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; IP-10, IFN-γ-inducible protein-10; KC, cytokine-induced neutrophil chemoattractant; MCP, monocyte chemoattractant protein; MIP, macrophage-inflammatory protein.

Received for publication October 28, 2003. Accepted for publication February 23, 2004.

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.
novel targets for therapies aimed at reducing the incidence and severity of surgical adhesions.

Materials and Methods

Animals

C57BL/6 mice were purchased from Charles River Breeding Laboratories (Wilmington, MA). CD28−/− mice (B6.129S2-Cd28tm1Mak) and control C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were provided with food and water ad libitum and housed under specific pathogen-free conditions. The mice 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

Mice were anesthetized with a single injection i.p. of 0.2 ml of pentobarbital sodium (50 mg/ml; Abbott Laboratories, North Chicago, IL) diluted 1:5 v/v in PBS (10 mg/ml). Abdominal adhesions were induced by abrasion of the cecum and abdominal wall, as previously described (12). In some experiments, the degree of abrasion was moderated to induce adhesions of lesser severity. Animals were killed and examined for adhesion formation 6 days later by one of the authors (E. R. H.). The severity of adhesions in each animal was evaluated according to the following scoring system widely used in this field (23–25): 0, no adhesion; 1, one thin filmy adhesion; 2, more than one thin adhesion; 3, thick adhesion with focal point; 4, thick adhesion with planar attachment, or more than one thick adhesion with focal point; and 5, very thick vascularized adhesion or more than one planar adhesion. The median adhesion scores for the various experimental groups were compared using the Mann-Whitney U test. Differences between groups were considered significant at p < 0.05.

Antibodies

Mouse CTLA-4 Ig was obtained from Bristol-Myers Squibb (Princeton, NJ). Mice received 50 μg via the i.p. route immediately following surgery, and additional injections of 50 μg days 1 and 2 following surgery (26). The anti-mouse PD-1 mAb J43 (hamster IgG) has been described (15). The transcribed from a multiprobe template set and labeled with [32P]UTP 105/CCL2 RNAs using the BD RiboQuant MultiProbe RNAase Protection Assay System (BD Biosciences, San Diego, CA). Probes were transcribed from a multiprobe template set and labeled with [35S]UTP (3000 Ci/mm mol; Amersham Pharmacia Biotech, Piscataway, NJ). Protected probes were resolved on a denaturing 6.0% polyacrylamide gel (Invitrogen, Carlsbad, CA). Autoradiography was performed with Fuji Super RX medical X-ray film (Fuji Photo Film, Tokyo, Japan) using a Kodak BioMax cassette with intensifying screen (Eastman Kodak, Rochester, NY). Images of each autorad were generated using a Hewlett-Packard Scanjet (Palo Alto, CA), and the intensity of the bands was quantified with ImageJ software (http://rsb.info.nih.gov/ij; National Institutes of Health).

Histology

Samples of the abdominal wall comprising the muscle mass, peritoneum, and any tissue associated by adhesions were harvested, fixed in Bouin’s Fixative Solution, and mounted in parafilin. Sections (5–6 μm thick) were cut and stained with H&E. For immunohistochemistry, instead of fixation the samples were flash frozen in Tissue-Tek OCT compound (Sakura Finetek, Tokyo, Japan). Sections (5–6 μm thick) were cut and fixed with acetone. They were then stained using the biotin-avidin technique (Vector Laboratories, Burlingame, CA) and counterstained with hematoxylin. Primary Abs used were anti-CD4 (clone H129.19; BD Biosciences), anti-F4/80 (clone 33.2.15; eBioscience). Sections incubated with the biotinylated secondary Ab alone served as negative controls.

Results

Surgical adhesion formation requires engagement of the T cell costimulatory molecule CD28

Recent work in our laboratory demonstrated that Th1 CD4+ αβ T cells are necessary for the development of postsurgical and postinfectious adhesion formation (12). To determine whether the CD28 costimulatory pathway is essential for surgical adhesion formation, C57BL/6 mice were subjected to cecal abrasion surgery and treated either with saline or with monomeric CTLA-4 Ig, which binds to B7-1 and B7-2 molecules, disrupting their interaction with CD28. As shown in Fig. 1A, mice treated with saline developed cumulative adhesion scores ranging from moderate to high severity (median score 3). In marked contrast, all mice treated with

![FIGURE 1](http://www.jimmunol.org/) Effect of B7 and CTLA-4 blockade on adhesion formation in mice subjected to cecal abrasion surgery. Each point represents the adhesion score of an individual mouse. A line indicates the median score for each group. A, C57BL/6 mice subjected to cecal abrasion surgery were treated either with saline or with CTLA-4 Ig, α, p < 0.0025 compared with saline control (Mann-Whitney U test). B, C57BL/6 mice subjected to cecal abrasion surgery were treated with a control Ig or with anti-CTLA-4. In this experiment only mild abrasion was performed to induce adhesions of moderate severity in control animals, and thus to reveal any exacerbated response in the anti-CTLA-4-treated group. α, p = 0.8785 vs group treated with control Ig.
CTLA-4Ig failed to develop adhesions ($p < 0.0025$ vs control group treated with saline). These results strongly suggest that the physiologic engagement of CD28 by B7-1 and/or B7-2 is essential for adhesion formation, although it is possible that CTLA-4Ig treatment exerts its effects in part indirectly, via metabolic effects on B7-expressing APC (28).

**Blockade of PD-1, but not CTLA-4, exacerbates adhesions in wild-type and CD28-deficient mice**

The data demonstrating the requirement for CD28 engagement in adhesiogenesis led to the prediction that the inhibitory counterreceptor CTLA-4, which also binds B7-1 and B7-2, plays a role in resolving this inflammatory response. Surprisingly, C57BL/6 mice subjected to cecal abrasion surgery and treated with a blocking Ab to CTLA-4 developed adhesions that were similar in severity to those of mice treated with an irrelevant Ab ($p = 0.8785$; Fig. 1B).

We next assessed the effect of PD-1 blockade on adhesion formation. Treatment of mice subjected to cecal abrasion surgery with a blocking Ab to PD-1 (10) significantly exacerbated the severity of surgical adhesions ($p = 0.0047$ vs control IgG; Fig. 2A), indicating that the interaction of PD-1 with its ligands plays a role in limiting the inflammatory response associated with adhesion formation.

To determine whether the effect of PD-1 blockade depends upon the presence of a functional CD28 costimulatory pathway, we subjected mice genetically deficient in the CD28 molecule to cecal abrasion surgery and treated them with either anti-CTLA-4, anti-PD-1, or a control Ab. As shown in Fig. 2B, CD28-deficient mice had a median adhesion score of 0, whereas wild-type littermate control mice had severe adhesions (median score = 5). Treatment of CD28-deficient mice subjected to cecal abrasion surgery with a blocking anti-CTLA-4 Ab did not significantly increase adhesion scores ($p = 0.79$ vs CD28$^{-/-}$ mice treated with control Ig). These results strongly suggest that CTLA-4 has little, if any, role in the resolution of this inflammatory response. In contrast, treatment with the anti-PD-1 Ab significantly increased the severity of adhesions in CD28$^{-/-}$ mice compared with the group treated with control Ab ($p = 0.0087$). These results demonstrate that the PD-1 molecule exerts its inhibitory effects by a pathway largely independent from CD28- and CTLA-4-mediated signaling.

**Treatment with CTLA-4Ig or anti-PD-1 alters the expression of chemokine RNAs in the peritoneal cavity following cecal abrasion surgery**

Chemokines play a central role in orchestrating the cellular trafficking that leads to peritonitis (1, 29). We previously demonstrated that the chemokines MIP-2/CXCL8 and cytokine-induced neutrophil chemoattractant (KC)/CXCL1 are expressed in the peritoneal cavity shortly after cecal abrasion surgery, and that treatment with a blocking Ab specific to CXCR2, the receptor for MIP-2 and KC, significantly reduces adhesion formation (12). We hypothesized that blockade of T cell costimulatory molecules affects adhesion formation in part by altering the expression of chemokines by cells entering the peritoneal cavity. Accordingly, cells were isolated from mice by peritoneal lavage 5 days following cecal abrasion surgery. RNA was purified and tested for the presence of a panel of chemokine RNAs by RT-PCR analysis. As shown in Fig. 3A, peritoneal cells from mice subjected to cecal abrasion and receiving injections of saline expressed high levels of RNA for RANTES/CCL5, MIP-1α/CCL3, and MIP-2/CXCL8, and lower, but detectable levels of RNA for MIP-1β/CCL4, IP-10/CXCL10, and MCP-1/CCL2. RNA for lymphotxin and eotaxin/CCL11 was not detected (see Discussion). This pattern was indistinguishable from expression levels of mice receiving an irrelevant control Ab, demonstrating that the binding of Ab to Fc receptors of the cells does not itself affect chemokine RNA expression in this system (data not shown). In marked contrast, in mice treated with CTLA-4Ig, only low levels of RANTES and MIP-2 RNA were present; no RNA for MIP-1α, MIP-1β, IP-10, or MCP-1 was detected. This pattern was essentially the same as that for naive mice. Densitometric analysis showed that in mice treated with CTLA-4Ig there was a $\sim$2-fold reduction in RANTES RNA and a 6-fold reduction in MIP-2 RNA compared with the levels observed in saline-treated mice, and an absolute reduction of MIP-1β, MIP-1α, IP-10, and MCP-1 expression (Fig. 3B). These data correlate with the low adhesion scores observed in CTLA-4Ig-treated mice (see Fig. 1A), and support the hypothesis that blocking T cell activation with CTLA-4Ig inhibits the sustained expression of chemokines by cells entering the peritoneal cavity that is necessary for adhesion formation.

In a separate experiment, we tested whether treatment of mice subjected to cecal abrasion surgery and treated with the Ab to PD-1 would increase chemokine expression in cells entering the peritoneal cavity. As shown in Fig. 4A, peritoneal lavage cells of mice treated with anti-PD-1 expressed markedly increased levels of RNA for MIP-1β, MIP-1α, MIP-2, IP-10, and MCP-1 compared with mice treated with saline. Densitometric analysis showed that this increase ranged from greater than 2-fold for MIP-2, to 6-fold for MCP-1 (Fig. 4B). There was a more modest increase of RANTES RNA expression. The increased expression of chemokine RNA correlates with the higher adhesion scores of anti-PD-1-treated mice, and is consistent with the hypothesis that blocking the inhibitory action of PD-1 on T cells leads to increased...
chemokine expression by cells entering the peritoneal cavity after cecal abrasion surgery and a concurrent increase in the severity of adhesions.

To explore the mechanism by which the blockade of T cell costimulatory molecules affected chemokine RNA expression, cell counts were performed on peritoneal lavage samples from mice subjected to cecal abrasion surgery and treated with CTLA4-Ig, anti-PD-1, or an irrelevant control Ab. As shown in Fig. 5A, no significant difference in the average number of leukocytes was observed between the three groups. Differential cell counts from the same peritoneal lavage samples showed macrophages and lymphocytes in roughly equal proportion; few, if any, neutrophils were detected at this timepoint, consistent with our earlier observations (Fig. 5B) (12). There was no significant difference between the cellular composition of the three groups. These data indicate that the blockade of T cell costimulatory molecules does not affect the size or composition of the peritoneal infiltrate following cecal abrasion surgery and suggest that the activation state of the cells is...
affected, leading to reduced chemokine gene expression in CTLA-4g-treated mice and prolonged chemokine gene expression in anti-PD-1-treated mice.

**CD4+ T cells are present at the site of adhesions, colocalizing with F4/80+ macrophages and PD-L1 expression**

We next performed histological and immunocytochemical examination of the peritoneum of mice subjected to cecal abrasion surgery to compare normal wound healing with that which results in adhesion formation. Mice subjected to cecal abrasion surgery that had low adhesion scores showed marked peritonitis at the site in which abrasion of the peritoneum had taken place, with the formation of scar tissue as well as a marked inflammatory infiltrate (Fig. 6, B and C). Immunohistochemical analysis showed that among the inflammatory cells were significant numbers of CD4+ cells as well as many F4/80+ macrophages (Fig. 6, E and F). Many cells in the infiltrate expressed PD-L1; few, if any, expressed PD-L2 (Fig. 6, F and G). In contrast, mice subjected to cecal abrasion surgery and treated with the anti-PD-1 Ab consistently developed severe adhesions in which the peritoneum was replaced by the abdominal wall (Fig. 7A). Under higher magnification, extensive deposition of collagen was evident, as well as a marked inflammatory infiltrate (Fig. 7B). Many CD4+ cells were present within the adhesion (Fig. 7, D and E). In addition, large numbers of F4/80+ macrophages were also noted, which colocalized with extensive expression of PD-L1 (Fig. 7, F and G). Striated muscle fibers of the abdominal wall also reacted with the Ab to PD-L1; expression of PD-L1 has been detected in human skeletal muscle (21). Less intense staining of PD-L2 was observed on macrophages (Fig. 7H).

**Discussion**

Postoperative adhesion formation is a major complication of abdominal and gynecologic surgery. Severe surgical adhesions cause life-threatening bowel obstruction, organ failure, female infertility, and abdominal pain (1, 30). Recent studies have shown that a large majority of patients undergoing abdominal or pelvic surgery develop adhesions, and that greater than 30% are readmitted for disorders directly or possibly related to adhesions (30, 31). Efforts to prevent their formation have focused on the use of barrier devices or coating materials composed of bioreabsorbable films or gels, but these approaches have been of limited effectiveness and in some cases can promote infection (32).

We have focused our efforts on understanding the cellular inflammatory response in the peritoneal cavity that leads to adhesiogenesis. Some recent initial studies investigated the role of neutrophils and macrophages in adhesion formation (5, 6), but the involvement of T cells, which play a central role in the pathogenesis of numerous autoimmune and fibrotic tissue disorders, has until recently been uncharacterized. We previously showed that Th1 CD4+ T cells are required for the development of postsurgical and infectious adhesions, and that these T cells produce the proinflammatory cytokine IL-17, which stimulates the production of neutrophil-specific chemokines MIP-2 and KC. Neutralization of IL-17 or the receptor for these chemokines, CXCR2, markedly reduces the severity of adhesions, thus confirming the key role these immune modulators play in adhesiogenesis (12).

In this study, we have focused on the role of costimulatory pathways that control T cell activation in adhesiogenesis, demonstrating that blockade of the interaction of CD28 with its ligands B7-1 and B7-2 completely abrogates adhesion formation following cecal abrasion surgery. Mice treated with CTLA-4g developed no detectable adhesions; moreover, cells in the peritoneal cavity of these mice expressed only low to undetectable amounts of a panel of chemokine RNAs compared with the amounts detected in untreated mice. In addition, CD28-deficient mice developed few or no adhesions. These results indicate that engagement of the CD28 costimulatory pathway plays a central role in adhesiogenesis. Nevertheless, the fact that some CD28-deficient mice develop moderate adhesions suggests that other costimulatory molecules, whether cell surface proteins or cytokines, are involved as well, as has been reported for the induction of experimental autoimmune encephalomyelitis in mice (33).

Given the role of the CD28 costimulatory pathway in adhesiogenesis, it was somewhat surprising that treatment of mice, whether wild type or CD28 deficient, with an Ab to the inhibitory receptor CTLA-4 did not significantly exacerbate the development of surgical adhesions following cecal abrasion surgery. Evidence is accumulating, however, that pathways distinct from that induced by CTLA-4 engagement are involved in down-regulating inflammatory responses (10). These results led us to explore whether other inhibitory molecules could be involved in the resolution of the inflammatory response in the peritoneal cavity. Importantly, an Ab to the PD-1 molecule markedly increased the severity of adhesions in both wild-type and CD28-deficient mice. In addition, blockade of PD-1 in wild-type mice led to markedly increased levels of chemokine RNA in cells homing to the peritoneal cavity. Histological examination of adhesions from anti-PD-1-treated wild-type mice revealed the presence of infiltrating CD4+ T cells as well as F4/80+ macrophages at this site. These cells colocalized with extensive expression of one of the ligands of PD-1, PD-L1/B7-H1, as well as more modest expression of PD-L2/B7-DC. Unlike B7-1 and B7-2, which are expressed only by professional
APC, expression of PD-L1 and PD-L2 has been detected in parenchymal tissues as well as on activated mouse T cells, B cells, peritoneal macrophages, and dendritic cells (17, 21, 34). Thus, it will be of interest to determine which cells expressing PD-L1 serve to down-regulate the T cell response in the peritoneum through PD-1, to determine how inflammation leading to adhesion formation is resolved in the periphery. It should be noted that the recent characterization of the B and T lymphocyte attenuator, an inhibitory receptor structurally related to CTLA-4 and PD-1 (35), and its ligand of the B7 family, B7-H4/B7S1 (36, 37), indicates that other inhibitory receptors could well play a role in limiting adhesiogenesis.

Because we have shown that chemokines such as MIP-2 and KC play a critical role in the development of adhesions (12), it was important to evaluate how blockade of the CD28 and PD-1 pathways would affect chemokine gene expression during adhesiogenesis. As shown above, we observed that treatment of mice subjected to cecal abrasion surgery with CTLA-4Ig reduced the expression of a number of chemokine RNAs in peritoneal cells to levels observed in resident peritoneal cells of naive mice. In contrast, chemokine RNA levels were markedly increased in peritoneal cells from anti-PD-1-treated mice. The fact that treatment with CTLA-4Ig or anti-PD-1 did not significantly alter the size or composition of the cells in the peritoneal cavity (Fig. 5) suggests that the differences in RNA expression can be attributed to the activation state of the cells themselves, which could be expected to determine both the rate of transcriptional initiation as well as mRNA stability (38). Experiments are currently in progress to further address this question.

It is notable that the levels of individual chemokine RNAs in the samples from saline-treated mice were different in the experiments shown in Figs. 3 and 4 (compare Fig. 3, lane 2, with Fig. 4, lane...
A possible explanation is that from experiment to experiment there is variability in the composition and/or the activation state of the different cell types making up the peritoneal population, due to variation in the kinetics of the overall inflammatory response. We previously showed (12) that the cellular composition of the peritoneal infiltrate changes markedly during the course of the inflammatory response to cecal abrasion surgery. In the experiment shown in Fig. 4, the mice were subjected to a relatively mild degree of abrasion to induce moderate adhesion formation; thus, the kinetics of this milder inflammatory response might differ from those in the experiment of Fig. 3, leading to differences in the expression of individual chemokines between the two experiments.

It will be of interest to further elucidate how the different cell types entering the peritoneal cavity, and their activation states, affect the overall pattern of chemokine expression during this complex inflammatory response.

Several recent studies of both human and mouse polarized Th1 and Th2 lymphocytes as well as human T cell clones have shown a preferential expression of particular chemokine receptors, and thus differential responsiveness to their cognate chemokines. In particular, Siveke and Hamann (39) demonstrated that mouse Th1-polarized T cells respond preferentially to MIP-1α, MIP-1β, and RANTES. In addition, Bonecchi et al. and Sallusto et al. (40, 41) demonstrated that Th1 cells respond preferentially to IP-10. Both groups also reported a preferential responsiveness of Th2 cells to eotaxin. Using Stat 4- and Stat-6-deficient mice, we previously...
demonstrated that adhesion formation is mediated by a Th1 re-
sponse (12). In the experiments shown in this work, we consis-
tently detected significant levels of MIP-1α, MIP-1β, RANTES, and
IP-10; no etoxin was detected (Fig. 3A). Moreover, by use of
confocal microscopy, our preliminary data indicate that the CD4+ 
T cells infiltrating adhesions express CCR5, a receptor for MIP-
1α, MIP-1β, and RANTES; and CXCR3, the receptor for IP-10.
These results are thus consistent with our earlier experiments, as
well as the other published reports mentioned above, that the in-
flammatory response leading to adhesions is a Th1-type response.

We have thus demonstrated for the first time that the regulation
of T cell function profoundly influences chemokine expression in
the peritoneal cavity as well as the severity and duration of surgical
adhesions in mice. CD4+ T cells home to the adhesion site and
appear to have local control of the inflammatory process. We have
demonstrated that the CD28 pathway plays a central role in the
initiation of adhesiogenesis. In addition, we have demonstrated
that the inhibitory receptor PD-1, rather than CTLA-4, plays a
central role in resolving the inflammatory response in the perito-
neal cavity following abdominal surgery and in limiting the sever-
ity of the adhesions that develop. Our results indicate that blocking
the activity of critical chemokines (12) or, as indicated in this
study, treatment with agents that block the CD28 pathway or stim-
ulate the PD-1 pathway, could significantly reduce the incidence
and severity of surgical adhesions.

Acknowledgments
We thank Matthew Adamowicz and Andrew Thompson for technical assistance.

References
1. Chegini, N. 2002. Peritoneal molecular environment, adhesion formation and
4. Hellebrekers, B. W. J., T. C. M. Trimbos-Kemper, J. B. M. Z. Trimbos,
¨
E. H. Sallusto, F. C. R. Mackay, and A. Lanzavecchia. 1998. Flexible pro-
duction of interleukin-12 by murine T cells and APC.
Sokmensier. 1999. The role of neutrophils in the formation of peritoneal adhe-
Evidence supporting a direct role for peritoneal macrophages in healing injured
adhesion-related hospital readmissions after abdominal and pelvic surgery: a retrospective
M. Crowe. 2000. The impact of adhesions on hospital readmissions over ten
century years after 8849 open gynecological operations: an assessment from the Surgical
9. Tsuzuki, T., N. Naja, K. Abdallah, V. Dong, H. Yagita, M. H. Sayegh, and
S. J. Khoury. 2001. CD28-independent induction of experimental autoimmune
family member that negatively regulates T cell activation.
the B7 family, negatively regulates T cell immunity. Immunity 18:849.
family member that negatively regulates T cell activation. Immunity 18:853.
2003. Regulation of chemokine mRNA stability by lipopolysaccharide and IL-10.
17. Benacerraf, R., G. Bianchi, P. P. Bordignon, D. A’Brosio, R. Lang, A. Barsotti,
Differential expression of chemokine receptors and chemotactic responsiveness of
type 1 T helper cells (Th1s) and Th2s. J. Exp. Med. 187:1279.
18. Pili, L., F. Di Lello, C. Pizzuto, and A. Lazzarini. 2003. Flexible pro-
duction of chemokine receptor expression on human polarized Th1 helper 1 and