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Original Article

Peritoneal adhesions induce T_h17/Treg imbalance in mice

Linlin Dong[1], Xiaoyu Zheng[2], Guonian Wang[2,3]

[1]Department of Anesthesiology, Qi Lu Hospital of Shan Dong University, Jinan, Shandong, China; [2]Department of Anesthesiology, Harbin Medical University Cancer Hospital, Heilongjiang, China; [3]Department of Pain Research Institute of Heilongjiang Academy of Medical Sciences, Heilongjiang, China

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Abstract: Naïve $CD4^+$ T cells differentiate to a distinct subset to mount specific inflammatory responses while minimizing self-reactivity. Recent work has identified that an imbalance between T helper (T_h) 17 cell and regulatory T (Treg) cells is involved in the pathophysiology of tumor immune responses. The factors that modulate the development of T_h17 and Treg cells are variable but still unclear. Peritoneal adhesion is a common complication of surgery and peritonitis, which can lead to abdominal pain, intestinal obstruction, and infertility. In this study, we examined the role of peritoneal adhesions in development of T_h17 and Treg cells and discovered that adhesions reduced proliferation of T_h17 cells and promoted the Tregs. in particular we found that adhesion modulated the activity of signal transducers and activators of transcription (STAT) 5 which was critical for the development of T_h17 and Treg cells.

Keywords: T helper cell, regulatory T cell, peritoneal adhesion, signal transducers, activators of transcription (stat)

Introduction

To help initiate specific responses to pathogens while minimizing self-reactivity, naïve $CD4^+$ T cells differentiate into distinct subtypes of effector T helper (T_h) cells depending on the activation of specific TCRs and the context of cytokines and signals presented by antigen presenting cells (APCs) [1]. Among the $CD4^+$ T helper cell subtypes, T helper 17 (T_h17) cells represent a distinct subtype of interleukin (IL)-17A-producing $CD4^+$ T cells. T_h17 cells play an important role in the adaptive immune response against certain microbes, particularly extracellular bacteria and fungi [2, 3]. However, excessive T_h17-type responses are harmful to the host, leading to autoimmune diseases, colitis and allergies [4-6]. To orchestrate excessive T helper cell responses, regulatory T (Treg) cells, another subset of $CD4^+$ T cells, control a large part of the host's immune activation and are essential for immune tolerance by secretion of cytokines such as tumor growth factor (TGF)-β [7]. Treg cells have been considered to play a critical role in the prevention of tissue-specific and nonspecific inflammation, autoimmunity, transplant tolerance, and intestinal mucosal immunity [8, 9]. Under pathologic conditions, an enhanced Treg response is also detrimental. In cancer patients, increases in the number of Treg cells are destructive to the tumor microenvironment and are associated with tumor progression, clinical stage, and patient survival [10, 11]. Therefore, the balance of T_h17 and Treg cells is critically important for immune homeostasis and is also essential for orchestrating specific inflammatory responses to pathogens and minimizing self-reactivity [12]. However, this balance is disrupted in many immune diseases including tumors, surgical trauma, and autoimmune diseases, and the mechanisms that lead to T_h17/Treg cell imbalance are variable and unclear.

The peritoneum not only is a mechanical covering of digestive organs but is also a multilayered and complex immune system that defends against foreign materials. Peritoneal adhesions are a common complication after surgery, especially following laparotomy, and cause abdominal pain, intestinal obstruction, and infertility [13-15]. Duringsubsequent surgery, more than 95% of patients who have undergone a prior laparotomy were found to have adhesions [14]. Peritoneal adhesions are fibrous bands that attach and join together previously separated

organs [16], and destroy the defense system of the peritoneal cavity.

In addition to numerous mesothelial cells, the peritoneum consists of many immunobiologically associated cells, including peritoneal macrophages, neutrophils, mast cells, dendritic cells, NK cells, and T memory cells, which have been demonstrated to play important roles in anti-viral and bacterial infections, as well as tumor rejection [17, 18]. Although it is known that the peritoneum plays an important role in the immune response, few studies have explored the modulatory role of the peritoneum on host immune function, especially in terms of the development of $CD4^+$ T cell subtypes.

To evaluate the unappreciated immunomodulatory potential of adhesion formation involved in the generation of functionally opposing $CD4^+$ T-cell subtypes, we chose two distinct mouse models of adhesion and detected the development of T_h17 and Treg cells in splenocytes at two time points. As we hypothesized, there was a direct interaction between the adhesion and differentiation of Treg and T_h17 cells. Serious adhesions weaken T_h17 cell differentiation and enhance the development of Treg cells. The present study elucidates a new role for the peritoneal immune response in the imbalance of T_h17/Treg cells and will likely help identify new and potent therapies for alleviating T_h17/Treg mediated pathologies.

Materials and methods

Animals

Male BALB/c mice (six to eight weeks old) were purchased and maintained at the Experimental Animal Center of Second Hospital Afflicted to Harbin Medical University. All mice were bred under a 12 h:12 h light: dark cycle in a pathogen-free environment. Animals were randomly allocated to four groups: the Control group, Sham group, Adhesion group, and foreign materials group. This study was performed in accordance with the Animal Research Protocol guidelines and with the approval of the local ethics committee at the Harbin Medical University.

Procedure for surgical adhesion formation

The surgical adhesion formation model was created as previously described [19]. All mice were anesthetized with isoflurane and fixed in a supine position on a warm pad. In the adhesion group, the abdominal wall was disinfected with 75% alcohol and laparotomy was performed through a 2-cm midline incision at the lower part of the abdomen. The cecum was isolated and abraded with sterile dry surgical gauze until it was visibly damaged and bleeding. Then, the incision was closed in two layers with 4-0 silk sutures. Mice in the sham group received a 2-cm laparotomy, and the cecum was isolated without injury. Mice in the control group did not receive an incision. All animals were killed on Days 3 and 7 after the operation, and the adhesion scores were evaluated in a blinded fashion based on a widely used standard scoring system used in this area [20, 21]. The scoring system was as follows: score 0, no adhesion; score 1, one thin filmy adhesion; score 2, more than one thin adhesion; score 3, thick adhesion with a focal point; score 4, thick adhesion with plantar attachment or more than one thick adhesion with a focal point; and score 5, very thick vascularized adhesion or more than one plantar adhesion. The adhesion scores were evaluated by a single person blinded to the treatment of each group. The peritoneum with the adhesion tissue was excised and used for morphological examination. Hemorrhage is a factor that induces T_h17/Treg cell imbalance. To further elucidate the influence of adhesion on $CD4^+$ T cell subtypes, a new animal model of adhesion induction was used in this study. Foreign material is one of the main causes of postoperative adhesion [22]; therefore, a 1 × 1 cm piece of sterile surgical gauze was implanted into the abdominal cavity of mice in the foreign materials group. On Days 3 and 7 after surgery, adhesion formation was observed, and the adhesion scores were recorded.

Adhesion severity score and histological analysis

The mice were killed by cervical dislocation on Days 3 and 7 following the operation. The adhesion severity was scored using the scoring system mentioned above. For histopathologic studies, the peritoneum and adhesion tissues were dissected on Days 3 and 7 after surgery, and they were immersed in 4% buffered formalin for 24 h and then embedded in a paraffin

block. The histology slides were stained with hematoxylin and eosin to assess histological changes in the peritoneum and adhesions.

Splenocyte preparation and isolation of $CD4^+$ T cells

The spleens were removed and placed in 4°C PBS on ice to prepare a single-cell suspension. In brief, we cleaned the spleen from the connective tissue and disaggregated the spleen by applying an appropriate amount of pressure in a circular motion. We then filtered the cell suspension through a 70-μm cell strainer into a 2-ml tube and centrifuged it at 400 × g for 10 min at 4°C. The erythrocytes were hypotonically lysed with lysis buffer, and the remaining cells were washed with 1 × PBS buffer. Splenocytes were used for flow cytometric analysis, and the remaining cell pellets,s were divided into several parts and frozen at -80°C for further analysis. $CD4^+$ T cells were purified from the splenocytes using a mouse $CD4^+$ T Cell Isolation Kit according to the manufacturer's protocol (Miltenyi Biotec, Germany). Sorted $CD4^+$ T cells were frozen at -80°C until further analysis.

Flow cytometric analysis (FCS)

To measure the percentage of Treg cells, splenocytes were resuspended in FACS buffer at a density of 1×10^6 cells/100-μl and stained with FITC anti-mouse CD4, APC-Cy7 anti-mouse CD25. Then cells were incubated with PE labelled anti-Foxp3 antibody in permeabilization buffer for 20 mins at room temperature (BD Bioscience, USA). To detect the percentage of T_h17 cells, splenocytes were stimulated with a cell stimulation kit for four hours in the presence of 10-μg/ml brefeldin A (eBioscience, USA) and then stained with FITC anti-mouse CD4 in FACS buffer. To stain the IL-17, cells were fixed with 2% buffered formalin for 30 mins and then incubated with PE labelled anti-IL-17 antibody in permeabilization buffer for 20 mins at room temperature. The isotype-related antibodies were used as controls. A total of 10,000 events were analyzed with FACSCanto II (BD Bioscience, USA) and Flow Jo software.

RNA isolation and RT-PCR

Total RNA was isolated from splenocytes with the Total RNA Isolation System (Sigma, USA) according to the manufacturer's guidelines. Normalization of the target gene mRNA expression was performed with β-actin. The primers were designed and synthesized by Invitrogen™. The primer sequences were as follows: retinoic acid-related orphan receptor (ROR)γt: sense: 5'-CCGCTGAGAGGGCTTCAC-3' and antisense 5'-TGCAG-GAGTAGGCCACATTACA-3'; fork-head/winged helix transcription factor (Foxp3): sense: 5'-CCCAGGAAAGACAG CAACCTT-3' and antisense 5'-TTCTCACAACCAGGCCACTTG-3'; and β-actin:sense:5'-AGAGGGAAATCGTGCGTGAC-3' and anti-sense: 5'-CAATAGTGATGACCTGGCCGT-3'. The RT-PCR was performed in an ABI PRISM 7500 fast system (Roche Life Science, USA). The cycling conditions were 50°C for 20 seconds, 95°C for 10 minutes, and 60°C for 1 minute, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The $2^{-\Delta\Delta Ct}$ method was used to quantitatively analyze the mRNA expression levels.

Enzyme linked immunosorbent assay (ELISA)

Blood (1 ml) was collected into a heparin-coated tube by cardiac puncture and centrifuged at 4000 g and 4°C for 15 min. Plasma was isolated and the plasma level of IL-17 and TGF-β was assessed by ELISA kits (ABclonal, China) according to the manufacturer's instructions. The standard curve was generated using standard protein provided by the manufacturer.

Western blot analysis

$CD4^+$ T cells were homogenized in cold RIPA lysis buffer containing protease inhibitors and then centrifuged at 14,000 g and 4°C for 10 min. Normalized proteins were separated by 10% SDS-PAGE and electrotransferred to a polyvinylidene difluoride (PVDF) membrane. The proteins were subsequently blocked with a 2% BSA solution. Primary antibodies against phospho-signal transducers and activators of transcription (pSTAT) 3 (1:1000), pSTAT5 (1:1000) and GAPDH (1:1000) were incubated with the membrane overnight at 4°C. All antibodies were purchased from ABclonal Biotechnology Corporation. The next day, the membranes were incubated with HRP-labeled secondary antibodies and visualized on an ABI Imaging System (LI-COR Bio-sciences, Bad Homburg, Germany).

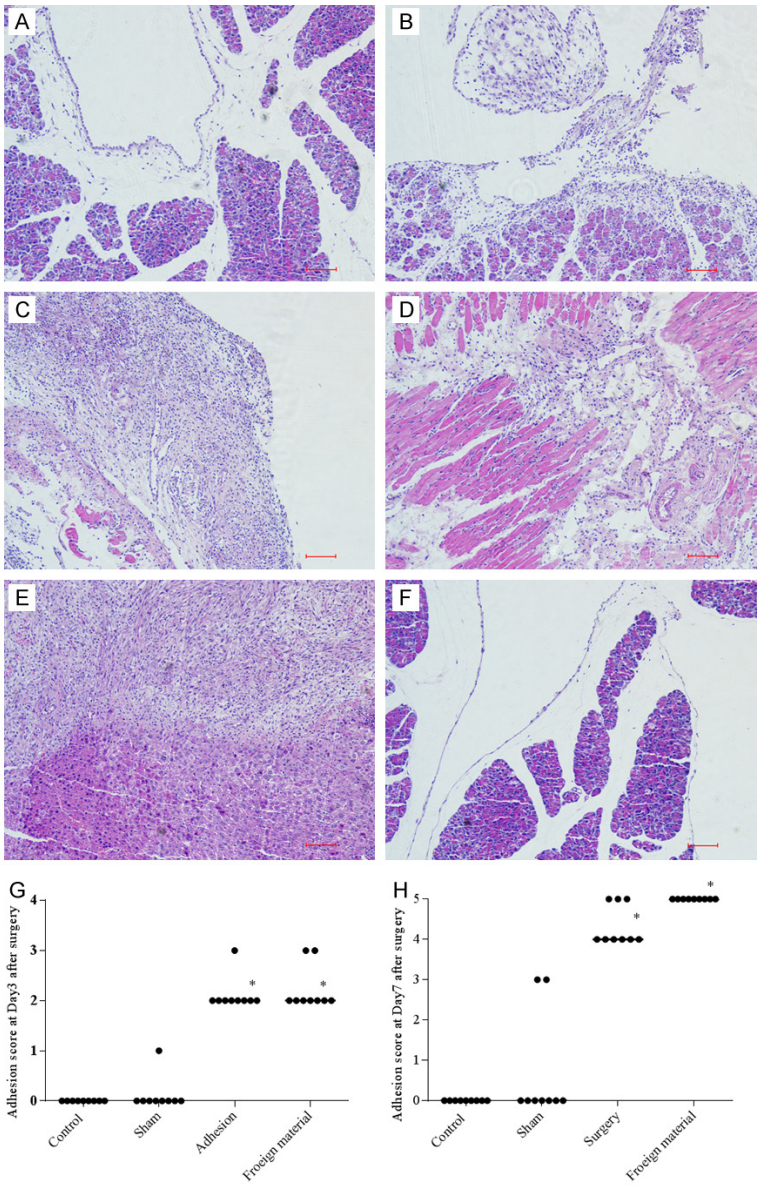

Figure 1. Peritoneal adhesion formation in the adhesion cecal abrasion and foreign material implantation mouse models. Adhesion scores for the control group, sham group, adhesion group and foreign material group on Days 3 and 7 after surgery. Each point represents an individual adhesion score for each mouse. A. Peritoneal histology in the cecal abrasion mouse model on Day 3. B. Peritoneumin the foreign material implantation mouse model on Day 3. C. Peritoneum in the cecal abrasion mouse model on Day 7. D. Peritoneum in the foreign material implantation mouse model on Day 7. E. The fibrotic peritoneum attached to the liver on Day 7 after surgery. F. The normal histology of peritoneum (original magnification 100 ×). G. The adhesion scores in the control group, adhesion group and foreign material group on Day 3 after surgery. H. The adhesion scores in the control group, adhesion group and foreign material group on Day 7 after surgery. The horizontal line represents the median values of each group. *$P < 0.001$ versus the control group (n = 9 in each group).

Statistical analysis

Data are expressed as the mean ± SD. Statistical significance was analyzed by one-way analysis of variance (ANOVA) with SPSS 19.0 software (SPSS, Chicago, USA). The Student t test was used to compare the differences between two groups. Adhesion scores were expressed as the median and compared using the Kruskal-Wallis test followed by the Mann-Whitney U test to compare differences between groups. A $p < 0.05$ was considered significant.

Results

Postsurgical adhesion formation in two distinct models

Three days following the operation, every mouse in the cecal abrasion group developed adhesions. The adhesions attached to various internal organs in the peritoneal cavity, including the abdominal wall, small intestine, colon, cecum, liver and spleen. Adhesions were membranous and easy to dissect from the organs on Day 3. On Day 7 after surgery, the fibrotic tissues were thick and difficult to remove from the involved organs, such as the liver. Histologic examination revealed early stage inflammatory cell infiltration (**Figure 1A**), which was followed by fibrosis (**Figure 1C**). In contrast, animals that underwent laparotomy without cecal manipulation developed only a few thick adhesions with focal points within the abdominal wall or lacked adhesions. There were no adhesions in the animals that did not undergo laparotomy.

In the foreign material-induced model of adhesion, gauze was attached to the abdominal wall and intestines, and we observed a larger amount of inflammatory cells infiltrate on Day 3 after surgery (**Figure 1B**). On Day 7, the adhesions were dense and highly fibrotic

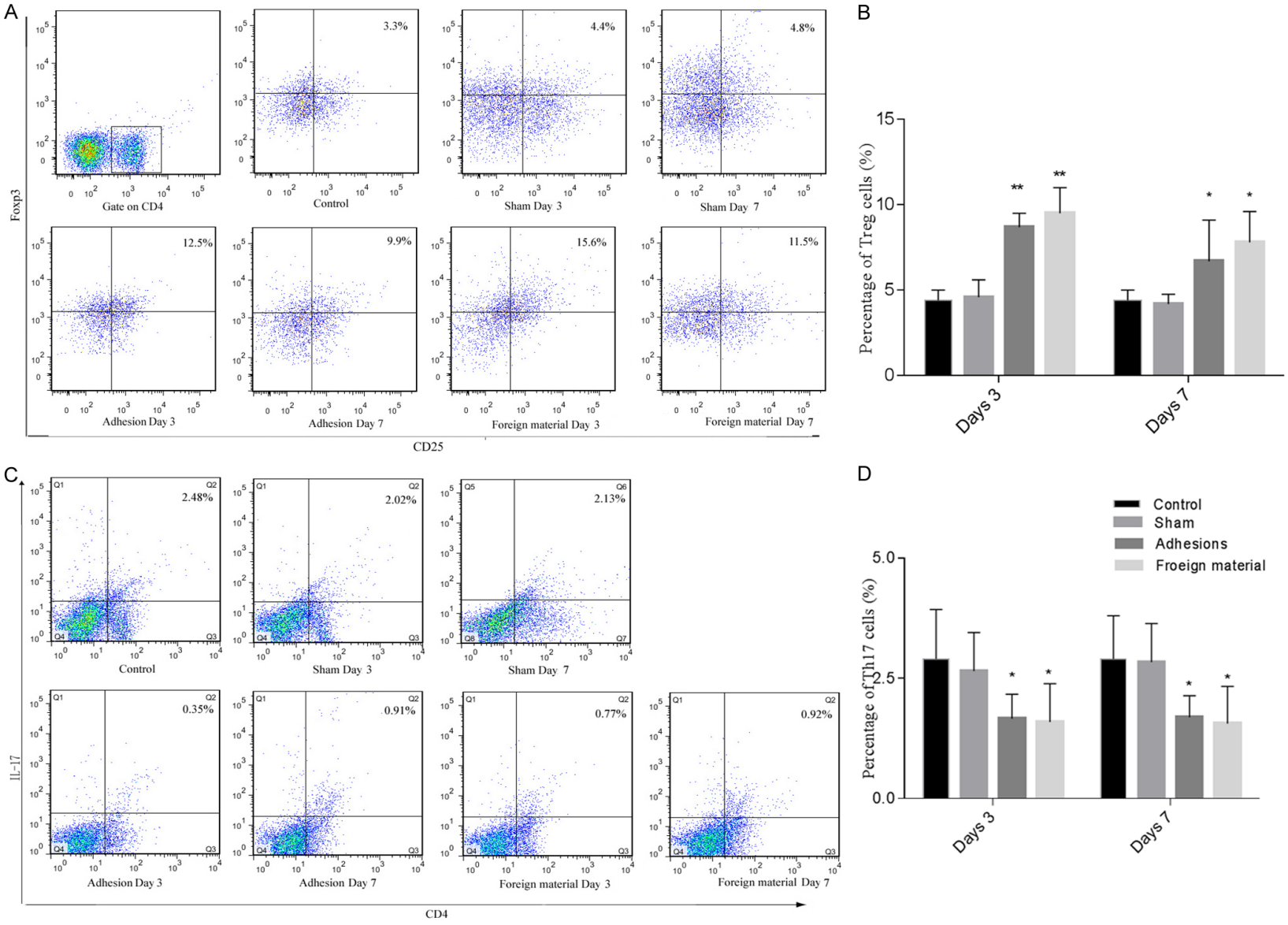

Figure 2. Adhesion formation played a critical role in the differentiation of T helper (T_h) 17 and regulatory T (Treg) cells. A. Flow cytometric analysis for Treg cells in control group, sham group, adhesion group and foreign material group on Days 3 and 7 after surgery. B. The percentage of Treg cells in control group, sham group, adhesion group and foreign material group on Days 3 and 7 after surgery. C. Flow cytometric analysis for T_h17 cells in control group, sham group, adhesion group and foreign material group on Days 3 and 7 after surgery. D. The percentage of T_h17 cells in control group, sham group, adhesion group and foreign materials group on Days 3 and 7 after surgery. Data are presented as the mean ± SD. *P < 0.05; **P < 0.01; compared with the control group (n = 9 in each group).

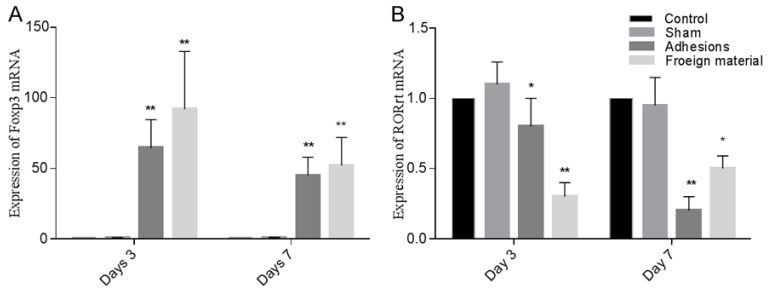

Figure 3. The effects of adhesion formation on the mRNA expression of Foxp3 and retinoic acid-related orphan receptor (ROR)γt in splenocytes. A. The mRNA expression of fork-head/winged helix transcription factor (Foxp3) in the four groups on Days 3 and 7; B. The mRNA expression of RORγt in the four groups on Days 3 and 7. Data from four different experiments are presented as the mean ± SD and are shown in the figure. **P* < 0.01 compared with the control group; ***P* < 0.001 compared with the control group (n = 9 in each group).

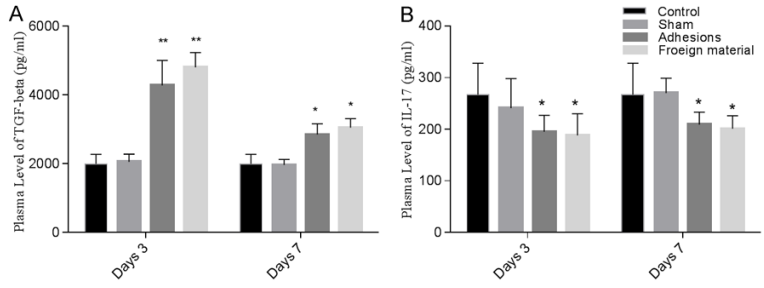

Figure 4. The effects of adhesion formation on the plasma levels of transforming growth factor (TGF)-β and interleukin (IL)-17. Blood samples were collected by cardiac puncture and centrifuged at 4000 g for 10 min. The levels of TGF-β and IL-17 in the plasma were analyzed with the enzyme linked immunosorbent assay (ELISA). A. Plasma TGF-β levels in the four groups on Days 3 and 7; B. Plasma IL-17 levels in the four groups on Days 3 and 7. Data from four different experiments are presented as the mean ± SD and are shown in the figure. **P* < 0.05 compared with the control group; ***P* < 0.001 compared with the control group (n = 9 in each group).

(**Figure 1D**), and could not be completely dissected from the abdominal wall and liver (**Figure 1D** and **1E**).

The adhesion scores of all mice are shown in **Figure 1G** and **1H**. On Day 3 after surgery, the mice in the adhesion group had much higher scores than the control and sham groups (**Figure 1G** and **1H**; *P* < 0.001), and the scores increased to 4 or 5 after 7 days. In the sham group, the scores of most mice were 0 on Days 3 and 7 following laparotomy.

In the adhesion model of foreign material implantation, the scores ranged from 3 to 4 at 3 days after surgery, and 7 days after surgery, the scores reached 5. These scores were much higher than in the control group (*P* < 0.001; **Figure 1G** and **1H**).

Peritoneal adhesion increased the percentage of Treg cells and decreased the percentage of T_h17 cells

To evaluate their effect in surgical adhesion formation, the percentages of T_h17 and Treg cells were analyzed by FCS. As shown in **Figure 2**, the percentage of T_h17 cells had significantly decreased, and the percentage of Treg cells increased on Days 3 and 7 after surgery in the adhesion and foreign material groups compared with the control group (*P* < 0.05, **Figure 2A** and **2B**). There were no significant differences in the percentages of T_h17 and Treg cells between the control and sham groups (*P* > 0.05).

Peritoneal adhesion promoted the expression of Foxp3 and decreased the expression of RORγt

Treg cells are characterized by the specific expression of the Foxp3 transcription factor, which is a key factor for differentiation and maintaining the function of Treg cells [23]. RORγt is the critical transcription factor required for T_h17 development and IL-17 production [24]. To further investigate the influence of peritoneal adhesion on the differentiation of T_h17/Treg cells, transcription factors Foxp3 and RORγt were detected by RT-PCR. Consistent with the effect of adhesion on the percentages of T_h17 and Treg cells, the expression of Foxp3 was much higher (**Figure 3A**, *P* < 0.001) and the expression of RORγt was lower in the adhesion and foreign material groups than in the control group on Days 3 and 7 (*P* < 0.01; **Figure 3B**). The expression levels of both Foxp3 and RORγt were not different between the control and sham groups (*P* > 0.05, **Figure 3**). These results

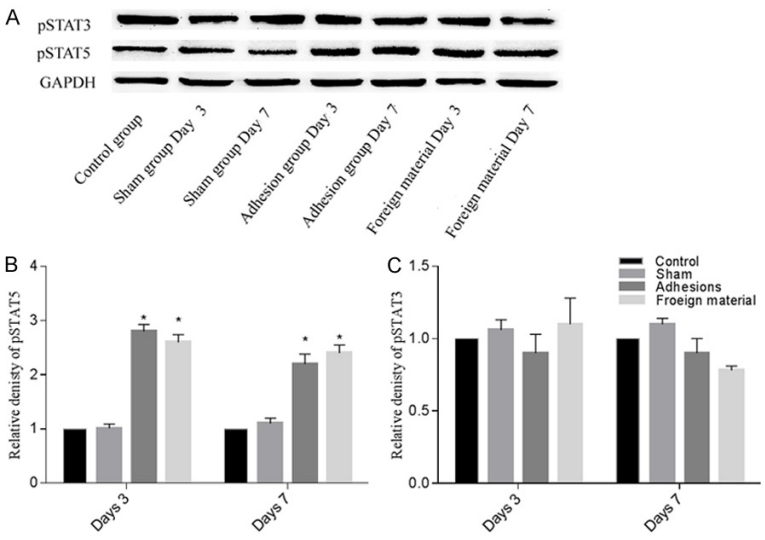

Figure 5. Expression of phospho-signal transducers and activators of transcription (pSTAT) 3 and pSTAT5 in the control group, sham group, adhesion group and foreign material group. Proteins from the $CD4^+$ T cells were separated by SDS-PAGE and electrotransferred to polyvinylidene difluoride (PVDF) membranes. The primary antibodies against pSTAT3 (1:1000), pSTAT5 (1:1000) and GAPDH (1:10,000) were incubated. HRP-labeled secondary antibodies were incubated the next day, followed by visualization on an ABI Imaging System. A. The protein levels of pSTAT5 and pSTAT3 in the four groups on Days 3 and 7. B. Relative density of pSTAT5 for the four groups on Days 3 and 7; C. Relative density of pSTAT3 in the four groups on Days 3 and 7. Data are shown as the mean ± SD. **P* < 0.01 compared with the control group (n = 9).

demonstrated that adhesions enhanced the development of Treg cells and repressed T_h17 cell development.

Peritoneal adhesion increased the plasma level of TGF-β and decreased the level of IL-17

We also detected the production of TGF-β and IL-17 in the plasma using ELISA. As shown in **Figure 4**, compared to the control and sham groups, the plasma level of TGF-β was significantly increased in the adhesion and foreign material groups on Days 3 and 7 (*P* < 0.001, **Figure 4A**). The level of IL-17 were lower on Days 3 and 7 in foreign material group and adhesion group than that in the control group (*P* < 0.05, **Figure 4B**). We did not find significant difference between the control and sham groups.

Peritoneal adhesion modulated the expression of pSTAT5

It is well known that STAT pathways are linked to the differentiation of T_h17 and Treg cells, especially STAT3 and STAT5 [25]. Therefore we detected the protein levels of pSTAT3 and pSTAT5 using western blot. As shown in **Figure 5**, the expression of pSTAT5 in $CD4^+$ T cells was higher in both the adhesion and foreign material groups than in the control group on Days 3 and 7 (*P* < 0.01, **Figure 5A** and **5B**). However, the expression of pSTAT3 in $CD4^+$ T cells was not significantly different among the four groups (*P* > 0.05, **Figure 5A** and **5C**).

Discussion

Adhesion is a common complication of surgery and indicates a poor prognosis. Here, we identified a new influence of adhesion on the development of T_h17 and Treg cells. In both cecal abrasion and foreign material implantation animals, the differentiation of Treg cells increased while T_h17 cell differentiation decreased. We first demonstrated that adhesion was an independent factor that promoted the development of Treg cells, while it constrained the development of T_h17 cells. Our data also showed higher activity of the STAT5 pathway which may play an important role in this process.

Postoperative adhesion formations are observed in more than 90% of patients recovering from abdominal surgery, and this remains a prevalent and costly problem [21, 26]. In cancer patients, the formation of adhesions after surgery accelerates peritoneal dissemination [27, 28]. Adhesions are the result of fibrin deposits attached to organs. In the present study, adhesions were observed in mice that underwent cecal abrasion. On Day 3 of cecal manipulation and foreign material implantation, adhesions were observed in all animals and the adhesion tissues attached to various internal organs in the peritoneal cavity, such as abdominal wall, bowel and liver. On Day 7, the fibrotic tissues were dense and difficult to remove from the organs. In animals implanted

with gauze, we observed many neoformative blood vessels. Meanwhile, histologic examination showed that the peritoneum was highly fibrotic in mice that underwent cecal abrasion. The normal structures of the peritoneum were replaced by fibrotic tissues. These histologic changes in the peritoneum are consistent with previously described adhesions in rodents [29].

Surgery remains the main therapy for cancer patients. Nevertheless, surgical trauma-induced postoperative immune suppression has been reported to accelerate tumor recurrence and metastasis, which are the primary causes of cancer-related death. T_h17/Treg cell imbalance is an important factor contributing to tumor metastasis after surgery [30]. Postoperative immune suppression and adhesion formation are two major clinical complications after the removal of malignant tumors and both jeopardize the beneficial effects of surgery. Despite many studies on this topic, little is known on the relationship between these two processes. Data from our previous study showed that an imbalance of T_h17 and Treg cells was more evident in mice with severe adhesion formation after gastric surgery. These findings led us to speculate that adhesion may be associated with the development of T_h17 and Treg cells. Consistent with our hypothesis, the proportion of Tregs increased, and the proportion of T_h17 decreased. Foxp3 and RORγt are specific transcription factors of Treg and T_h17 cells, respectively. Increased expression of Foxp3 and decreased expression of RORγt further supported our findings. Moreover, we also detectedthe plasma levels of TGF-β and IL-17 and found that there was a significantly increased level of TGF-β and decreased IL-17 in mice with adhesion formation, which suggested at least partly that Treg function was promoted and the T_h17 was inhibited.

A previous study has shown that hemorrhage affects the differentiation of both Treg and T_h17 cells [31]. In a cecal abrasion model, hemorrhage is inevitable. To minimize the influence of hemorrhage on the development of T_h17 and Treg cells, we used a new adhesion model. Foreign material is an important factor contributing to adhesion formation. Glove lubricants, gauze lint, anti-microbial agents, absorbable hemostatics, and necrotic tissue and blood, are thought to act as foreign materials in the peritoneum. All of these materials induce adhesion formation. Hence, we chose to implant sterile gauze into the peritoneal cavity to induce adhesion formation. We found that mice in the foreign material group had a higher adhesion score. The morphology of the adhesion tissues was consistent with the morphology observed in mice that underwent cecal abrasion. We also found increased Treg-type response with a decreased T_h17-type response. These results clearly indicated the importance of adhesions in the development of T_h17 and Treg cells in mice.

To further determine the mechanism of adhesion on the T_h17/Treg cell balance, the intracellular signals were also examined. STAT proteins are critical for determining the fate of T helper cells and master transcription factor expression [32]. STAT3 binds to the IL-17 promoter and promotes the induction of IL-17. Deletion of STAT3 in mice results in the loss of IL-17 production, while a constitutively active STAT3 increases IL-17 expression [33]. STAT5, another subset of the STAT family, promotes the expression of Foxp3 and is required for Treg development and its suppressive function [34]. STAT5 directly binds to the Foxp3 promoter and enhances the differentiation of Treg cells and its suppressive function [35, 36]. STAT5 is also important in limiting T_h17 differentiation. Arian et al [34] showed that STAT5 attenuates IL-17 production by directly binding to the IL17a promoter. Deletion of STAT5 via transgenic expression favored IL-17 production. Considering the critical role of cytokines in the STAT3 and STAT5-related induction of T_h17 and Treg cells, we measured the protein levels of pSTAT5 and pSTAT3 using western blot. Our experiments demonstrated that adhesion formation promoted the activity of pSTAT5 in $CD4^+$ T cells, but with no influences on the activity of pSTAT3. These data partially implied that the activity of STAT5 may be responsible for the imbalance of T_h17 and Treg cells induced by adhesion.

In this study, we mainly focused on how adhesions influence the development of T_h17 and Treg cells. The peritoneum is one of the most important immune tissues. It has a large surface are (1.7 m^2) and a large number of immune cells, especially innate immune cells, such as macrophages, dendritic cells and NK cells. These innate immune cells are important APCs

with multiple immune functions such as antigen recognition and presentation and cytokine secretion [37, 38]. However, few studies have evaluated the immunomodulatory function of the peritoneum in the host immune system. In pathologic conditions, such as adhesions and acute or chronic peritonitis, the structures of the peritoneum are destroyed, but the effect of this damage on immune function is not well understood and requires further investigation.

In conclusion, our research showed that adhesions disrupted the delicate balance between T_h17 cells and the Treg cell. This effect is at least partially due to the modulation of pSTAT5. These data demonstrated, for the first time, that adhesions play an important role in orchestrating the host immune response. This expands our knowledge of the peritoneal immune system and immune homeostasis.

Conclusion

Our research showed that adhesions disrupted the delicate balance between T_h17 cells and the Treg cell. This effect is at least partially due to the modulation of pSTAT5. These data demonstrated, for the first time, that adhesions play an important role in orchestrating the host immune response. This expands our knowledge of the peritoneal immune system and immune homeostasis.

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Disclosure of conflict of interest

None.

Address correspondence to: Guonian Wang, Department of Anesthesiology, Harbin Medical University Cancer Hospital, Heilongjiang, China; Department of Pain Research Institute of Heilongjiang Academy of Medical Sciences, 6 Baojian Road, Nangang District, Harbin 150081, Heilongjiang, China. Tel: 86451-86298882; Fax: 86-451-86298811; E-mail: wangguonian609cn@aliyun.com

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