PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 April 21; 12(15):2455-2458
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Increase of $CD4^+CD25^+$ T cells in $Smad3^{-/-}$ mice

Zi-Bing Wang, Yu-Fang Cui, Yu-Qing Liu, Wei Jin, Han Xu, Zhu-Jun Jiang, Ya-Xin Lu, Ying Zhang, Xiao-Lan Liu, Bo Dong

Zi-Bing Wang, Yu-Fang Cui, Wei Jin, Han Xu, Zhu-Jun Jiang, Ying Zhang, Xiao-Lan Liu, Bo Dong, Department of Immunology, Beijing Institute of Radiation Medicine, 27 Taiping Road, Beijing 100850, China
Yu-Qing Liu, Third Affiliated Hospital of Xinxiang Medical College, Xinxiang 453003, Henan Province, China
Ya-Xin Lu, Institute of Biotechnology, Beijing 100071, China
Correspondence to: Professor Yu-Fang Cui, Department of Immunology, Beijing Institute of Radiation Medicine, 27 Taiping Road, Beijing 100850, China. yufangc@vip.sina.com
Telephone: +86-10-66931353
Received: 2005-10-29 Accepted: 2005-11-18

Abstract

AIM: To investigate the changes of lymphocyte subpopulations, especially $CD4^+CD25^+$ T regulatory cells in $Smad3^{-/-}$ mice.

METHODS: Hematological changes and changes of lymphocyte subpopulations were detected in $Smad3^{-/-}$ mice using cell counter and flow cytometry, respectively, and compared to their littermate controls.

RESULTS: The numbers of neutrophils and lymphocytes in peripheral blood were significantly increased in $Smad3^{-/-}$ mice compared to littermate controls. $CD19^+$ expressing cells in blood and spleen, and $CD8^+$ T cells in thymus were all markedly decreased in $Smad3^{-/-}$ mice. More important, $Smad3^{-/-}$ mice had an increased population of $CD4^+CD25^+$ T cells in peripheral lymphoid tissues, including thymus, spleen, and lymph nodes.

CONCLUSION: These observations suggest that the changes of lymphocyte subpopulations might play a role in susceptibility to inflammation of $Smad3^{-/-}$ mice.

Key words: $CD4^+CD25^+$ T cells; Lymphocyte subpopulation; SMAD3; TGF-β signaling

Wang ZB, Cui YF, Liu YQ, Jin W, Xu H, Jiang ZJ, Lu YX, Zhang Y, Liu XL, Dong B. Increase of $CD4^+CD25^+$ T cells in $Smad3^{-/-}$ Mice. *World J Gastroenterol* 2006; 12(15):2455-2458

http://www.wjgnet.com/1007-9327/12/2455.asp

INTRODUCTION

TGF-β plays an important role in maintaining immune homeostasis. It signals through a set of transmembrane receptor serine/threonine kinases unique to the large superfamily of TGF-β related proteins [1]. As a downstream cytoplasm signaling element of TGF-β receptors, Smad3 mediates a positive signal pathway from the receptor serine/threonine kinases to the nuclei [2]. Previous reports revealed Smad3 plays an important role in mediating TGF-β signal in T lymphocytes and neutrophils, and demonstrated that Smad3 deficiency results in immune dysregulation and susceptibility to opportunistic infection [3].

The immune system discriminates between self and non-self, establishing and maintaining unresponsiveness to self. There is clear evidence that clonal deletion of self-reactive T and B cells is a major mechanism of self-tolerance [4]. However, the fact that potentially hazardous self-reactive lymphocytes are present in the periphery of normal adult individuals [5] reveals that the mechanisms that can prevent pathological autoimmunity exist. In recent years, a burst of papers are focused on a population of $CD4^+$ T cells that constitutively express the IL-2Rα (CD25) T cells and reveal them as key "actors" to self-tolerance[6,7]. A direct experiment to assess the regulatory role of $CD4^+CD25^+$ T cells in self-tolerance reported that the adoptive transfer of $CD4^+CD25^+$-depleted T cells could induce several organ-specific autoimmune diseases in immunodeficient animals [8]. $CD4^+CD25^+$ T cells also regulate antibody responses against self- and non-self-antigens by direct inhibitory effects on B cells or via inhibition of Th cell differentiation [9, 10]. In addition to self-tolerance and autoimmunity, there is evidence that $CD4^+CD25^+$ T cells are actively engaged in negative control of a broad spectrum of immune responses induced by microbial infection [11-13]. They can also mediate transplantation tolerance [14] and maternal tolerance to the foetus [15].

Although great progress in $CD4^+CD25^+$ T cells study has been made in recent years, many issues remain to be solved. For example, the involvement of TGF-β in $CD4^+CD25^+$ T cell immunoregulatory function is still controversial [16-19]. In the present study, we examined the changes of lymphocyte subpopulations in peripheral lymphoid tissues of $Smad3^{-/-}$ mice as well as their controls. Our results showed that $Smad3^{-/-}$ mice were associated with an increased population of $CD4^+CD25^+$ T cells, suggesting that $CD4^+CD25^+$ T cells might play a role in susceptibility to inflammation of $Smad3^{-/-}$ mice.

MATERIALS AND METHODS

Mice

$Smad3^{-/-}$ mice were generated by targeted gene disruption

in murine embryonic stem cells by homologous recombination [3]. Both Smad3$^{-/-}$ mice and their littermate controls (wild-type, Smad3$^{+/+}$) were provided by Xiao Yang (Institute of Biotechnology, Beijing, China). The mice used in these experiments were 6-8 wk of age.

Antibodies and reagents

PE-anti-CD4, FITC-anti-CD8, FITC-anti-CD3, and PE-anti-CD19 were purchased from Southern Biotechnology Associates (Birmingham, USA). FITC-anti-CD25 was purchased from Biolegend (San Diego, CA).

Analysis of leukocytes in peripheral blood

Before mice were sacrificed, approximately 20 μL blood samples were collected through tail vein, diluted, and then analyzed on Sysmex F-820 semi-automatic analyzer (Japan).

Flow cytometry of lymphocytes

Peripheral blood, thymus, spleen and lymph nodes were harvested from mice. Single-cell suspensions were subjected to hypotonic lysis of red blood cells (Becton Dickinson), washed in phosphate-buffered saline, stained with fluorescein-conjugated antibodies according to standard protocols, and then analyzed on an FACScan (Beckman Dickinson). For isolation of peripheral blood mononuclear cells (PBMC), 2 mL of heparinised peripheral blood diluted 1:1 with PBS was layered onto an equal volume of Ficoll-Hypaque density gradient solution and centrifuged at 300 r/min at room temperature. The mononuclear cells were collected, washed twice with PBS.

Statistical analysis

Difference was defined as being statistically significant when P <0.05 was obtained using Student's t test.

RESULTS

Increased numbers of neutrophils and lymphocytes in Smad3$^{-/-}$ mice

We first compared the total numbers of white blood cells and differential distributions of leukocytes in peripheral blood samples from Smad3$^{-/-}$ mice and littermate control mice. A marked increase in absolute white blood cell counts was observed in Smad3$^{-/-}$ mice (P < 0.01). Accordingly, the numbers of neutrophils and lymphocytes were also elevated in Smad3$^{-/-}$ mice compared to their controls (Figure 1). These results are consistent with a previous report that Smad3$^{-/-}$ mice exhibited invasive mucosal infection involving multiple immune organs [3].

Changes of lymphocyte subpopulations

Susceptibility of Smad3$^{-/-}$ mice to infection and tissue inflammation [3] made us wonder whether quantitative changes of lymphocytes were present in these mice. The results showed that numbers of CD19^{+}-expressing cells (most B cells) in the peripheral blood and spleen were significanty decreased in Smad3$^{-/-}$ mice compared to their controls (Figures 2A and 2C). In addition, the number of CD8^{+} T cells was also reduced in thymus in Smad3$^{-/-}$ mice (Figure 2B). Analysis of lymph nodes did not reveal any significant difference between the mutant mice and littermate controls (Figure 2D).

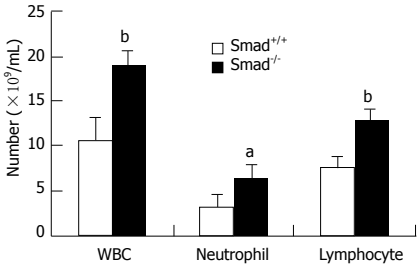

Figure 1 Total numbers and differential distributions of blood leukocytes in Smad3$^{+/+}$ and Smad3$^{-/-}$ mice. Peripheral blood samples were collected from tail veins of mice, and then analyzed on Sysmex F-820. Shown here are the means and standard deviations of total numbers and distributions of blood leukocytes from 4 wild-type and 4 mutant mice (^{a}P < 0.05, ^{b}P < 0.01).

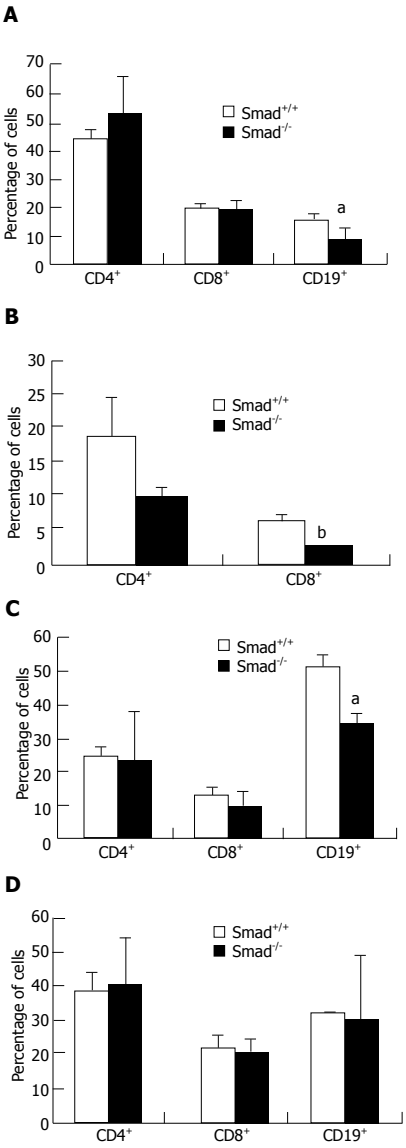

Figure 2 Percentage of lymphocyte subpopulations in peripheral blood (A), thymus (B), spleen (C) and lymph nodes (D) of wild-type and mutant mice. The cells were stained with PE-anti-CD4 and FITC-anti-CD8, or with FITC-anti-CD3 and PE-anti-CD19, and then subjected to cytometric analyses. Shown here are the means and standard deviations of percentage of lymphocyte subpopulations from 4 wild-type and 4 mutant mice (^{a}P < 0.05, ^{b}P < 0.01).

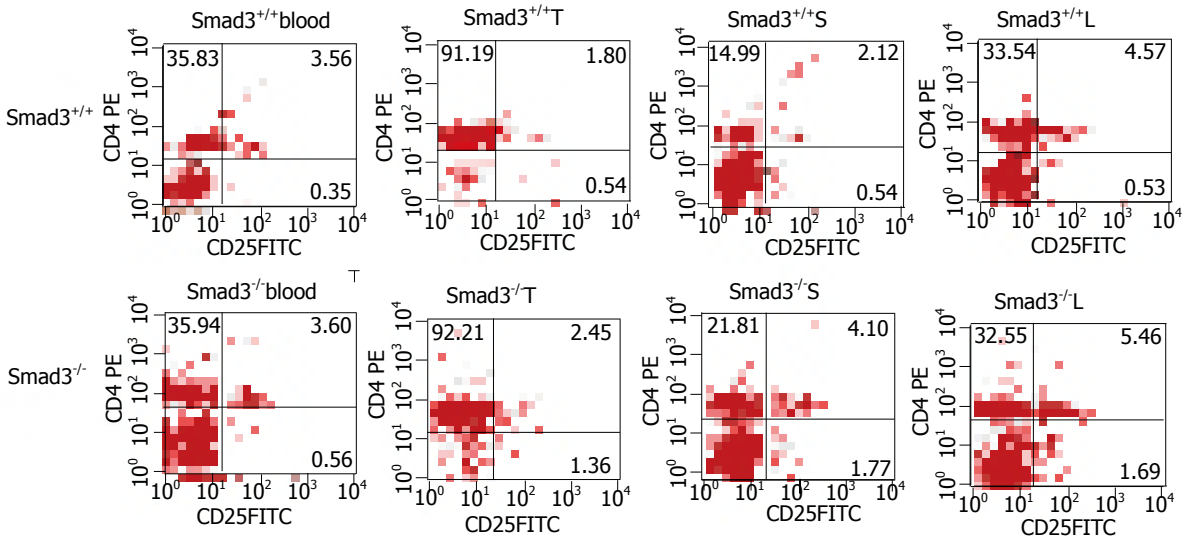

Figure 3 Percentage of CD4$^+$CD25$^+$ T cells in peripheral lymphoid tissues of Smad3$^{+/+}$ and Smad3$^{-/-}$ mice. PBMC (blood) or single-cell suspensions from thymus (T), spleen (S), and lymph nodes (L) were prepared according to "MATERIALS AND METHODS", co-labeled with PE-anti-CD4 and FITC-anti-CD25, and then analyzed by FACScan.

Increased CD4$^+$CD25$^+$ T cells in Smad3$^{-/-}$ mice

CD4$^+$CD25$^+$ T cells play an important role in maintaining the equilibrium between immunity and tolerance[20,21]. Many papers have reported that this population of cells is able to suppress proliferation and effector function of CD4$^+$ and CD8$^+$ T cells [22-24]. To explore whether the decreased lymphocytes in Smad3$^{-/-}$ mice were related to the CD4$^+$CD25$^+$ T cells, we examined this population of cells in peripheral lymphoid tissues of Smad3$^{-/-}$ mice and littermate controls. Smad3$^{-/-}$ mice exhibited a greater percentage of CD4$^+$CD25$^+$ T cells in thymus, spleen and lymph nodes, compared to controls. In peripheral blood, however, no difference was observed between mutant mice and wild type in regarding to CD4$^+$CD25$^+$ T cell proportion (Figure 3).

DISCUSSION

TGF-β is an essential endogenous regulator of T-cell function [25]. It has been recently reported that TGF-β$^{-/-}$ mice have normal numbers of CD4$^+$CD25$^+$ T cells after birth, indicating that CD4$^+$CD25$^+$ T cells are able to develop in complete absence of endogenous TGF-β expression [16, 17]. This made us think whether quantitative or functional changes of CD4$^+$CD25$^+$ T cells occurred in Smad3$^{-/-}$ mice. Our main finding in this study is that Smad3$^{-/-}$ mice had increased CD4$^+$CD25$^+$ T cells compared to their littermate controls (Figure 3). Our results showed that neutrophil and lymphocyte numbers increased (Figure 1) and that lymphocyte subpopulation decreased in the peripheral lymphoid tissues of Smad3$^{-/-}$ mice (Figure 2), which are consistent with the previous reports [3,26].

During infection, the balance between self-reactive effector T cells and regulatory T cells could determine the time of onset, the intensity and duration of autoimmune response [27]. Recent studies have focused on a population of CD4$^+$ T cells that constitutively express CD25. CD4$^+$CD25$^+$ T cells comprise 5%-10% of the peripheral CD4$^+$ T cell pool of normal mice and humans and exhibit immunosuppressive abilities both *in vitro* and *in vivo* [28, 29]. Studies of human diseases indicate that the functional CD4$^+$CD25$^+$ T cells are enriched in inflamed joints of patients with rheumatoid arthritis [30] or with the juvenile idiopathic arthritis [31]. In our study, we showed that an increased population of CD4$^+$CD25$^+$ T cells was present in Smad3$^{-/-}$ mice, which could partially account for the susceptibility to inflammation of these mutant mice. However, our results and those of a previous study [3] did not reveal any significant difference of CD4$^+$ T cells of spleen and lymph nodes between the asymptomatic mice and littermate controls. A possible explanation for the increase of CD4$^+$CD25$^+$ T cells is that they are derived from the CD4$^+$CD25$^-$ cells under the condition of Smad3 gene mutation. It is well accepted that CD4$^+$CD25$^+$ T cells can be generated by the activation of mature, peripheral CD4$^+$CD25$^-$ T cells under different stimulatory conditions [28]. Further studies should concentrate on defining the functional characteristics of the CD4$^+$CD25$^+$ T cells in Smad3$^{-/-}$ mice to gain a better insight into the mechanisms of susceptibility to inflammation.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Xiao Yang for providing Smad-3 wild-type and mutant mice. In addition, the authors thank Drs. Qi-Hong Sun, Jian-Ping Mao and Jian-En Gao for their many helpful comments and suggestions on these experiments.

REFERENCES

1 **Massagué J**. TGF-beta signal transduction. *Annu Rev Biochem* 1998; **67**: 753-791
2 **Heldin CH**, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* 1997; **390**: 465-471
3 **Yang X**, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J* 1999; **18**: 1280-1291
4 **Kisielow P**, Blüthmann H, Staerz UD, Steinmetz M, von

Boehmer H. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4+8+ thymocytes. *Nature* 1988; **333**: 742-746

5 **Ota K**, Matsui M, Milford EL, Mackin GA, Weiner HL, Hafler DA. T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. *Nature* 1990; **346**: 183-187

6 **Sakaguchi S**, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; **155**: 1151-1164

7 **Shevach EM**. CD4+CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002; **2**: 389-400

8 **Asano M**, Toda M, Sakaguchi N, Sakaguchi S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med* 1996; **184**: 387-396

9 **Bystry RS**, Aluvihare V, Welch KA, Kallikourdis M, Betz AG. B cells and professional APCs recruit regulatory T cells via CCL4. *Nat Immunol* 2001; **2**: 1126-1132

10 **Curotto de Lafaille MA**, Muriglan S, Sunshine MJ, Lei Y, Kutchukhidze N, Furtado GC, Wensky AK, Olivares-Villagómez D, Lafaille JJ. Hyper immunoglobulin E response in mice with monoclonal populations of B and T lymphocytes. *J Exp Med* 2001; **194**: 1349-1359

11 **Kullberg MC**, Jankovic D, Gorelick PL, Caspar P, Letterio JJ, Cheever AW, Sher A. Bacteria-triggered CD4(+) T regulatory cells suppress Helicobacter hepaticus-induced colitis. *J Exp Med* 2002; **196**: 505-515

12 **Singh B**, Read S, Asseman C, Malmström V, Mottet C, Stephens LA, Stepankova R, Tlaskalova H, Powrie F. Control of intestinal inflammation by regulatory T cells. *Immunol Rev* 2001; **182**: 190-200

13 **Hesse M**, Piccirillo CA, Belkaid Y, Prufer J, Mentink-Kane M, Leusink M, Cheever AW, Shevach EM, Wynn TA. The pathogenesis of schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. *J Immunol* 2004; **172**: 3157-3166

14 **Wood KJ**, Sakaguchi S. Regulatory T cells in transplantation tolerance. *Nat Rev Immunol* 2003; **3**: 199-210

15 **Aluvihare VR**, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004; **5**: 266-271

16 **Piccirillo CA**, Letterio JJ, Thornton AM, McHugh RS, Mamura M, Mizuhara H, Shevach EM. CD4+CD25+ regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. *J Exp Med* 2002; **196**: 237-246

17 **Mamura M**, Lee W, Sullivan TJ, Felici A, Sowers AL, Allison JP, Letterio JJ. CD28 disruption exacerbates inflammation in Tgf-beta1-/- mice: in vivo suppression by CD4+CD25+ regulatory T cells independent of autocrine TGF-beta1. *Blood* 2004; **103**: 4594-4601

18 **Nakamura K**, Kitani A, Fuss I, Pedersen A, Harada N, Nawata H, Strober W. TGF-beta 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. *J Immunol* 2004; **172**: 834-842

19 **Huber S**, Schramm C, Lehr HA, Mann A, Schmitt S, Becker C, Protschka M, Galle PR, Neurath MF, Blessing M. Cutting edge: TGF-beta signaling is required for the in vivo expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. *J Immunol* 2004; **173**: 6526-6531

20 **Shevach EM**. Regulatory T cells in autoimmmunity*. *Annu Rev Immunol* 2000; **18**: 423-449

21 **Maloy KJ**, Salaun L, Cahill R, Dougan G, Saunders NJ, Powrie F. CD4+CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. *J Exp Med* 2003; **197**: 111-119

22 **Takahashi T**, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S. Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int Immunol* 1998; **10**: 1969-1980

23 **Thornton AM**, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* 1998; **188**: 287-296

24 **Wing K**, Ekmark A, Karlsson H, Rudin A, Suri-Payer E. Characterization of human CD25+ CD4+ T cells in thymus, cord and adult blood. *Immunology* 2002; **106**: 190-199

25 **Kulkarni AB**, Thyagarajan T, Letterio JJ. Function of cytokines within the TGF-beta superfamily as determined from transgenic and gene knockout studies in mice. *Curr Mol Med* 2002; **2**: 303-327

26 **Bommireddy R**, Engle SJ, Ormsby I, Boivin GP, Babcock GF, Doetschman T. Elimination of both CD4+ and CD8+ T cells but not B cells eliminates inflammation and prolongs the survival of TGFbeta1-deficient mice. *Cell Immunol* 2004; **232**: 96-104

27 **Schwartz M**, Kipnis J. Autoimmunity on alert: naturally occurring regulatory CD4+CD25+ T cells as part of the evolutionary compromise between a 'need' and a 'risk'. *Trends Immunol* 2002; **23**: 530-534

28 **Piccirillo CA**, Thornton AM. Cornerstone of peripheral tolerance: naturally occurring CD4+CD25+ regulatory T cells. *Trends Immunol* 2004; **25**: 374-380

29 **Fehérvari Z**, Sakaguchi S. CD4+ Tregs and immune control. *J Clin Invest* 2004; **114**: 1209-1217

30 **Sullivan KE**, McDonald-McGinn D, Zackai EH. CD4+ CD25+ T-cell production in healthy humans and in patients with thymic hypoplasia. *Clin Diagn Lab Immunol* 2002; **9**: 1129-1131

31 **Prakken BJ**, Samodal R, Le TD, Giannoni F, Yung GP, Scavulli J, Amox D, Roord S, de Kleer I, Bonnin D, Lanza P, Berry C, Massa M, Billetta R, Albani S. Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. *Proc Natl Acad Sci U S A* 2004; **101**: 4228-4233

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Zhang Y