Local Accumulation of FOXP3⁺ Regulatory T Cells: Evidence for an Immune Evasion Mechanism in Patients with Large Condylomata Acuminata

Yuchun Cao, Jie Zhao, Zhang Lei, Shiqian Shen, Cong Liu, Dong Li, Jihong Liu, Guan-Xin Shen, Gui-Mei Zhang, Zuo-Hua Feng and Bo Huang

J Immunol 2008; 180:7681-7686; doi: 10.4049/jimmunol.180.11.7681
http://www.jimmunol.org/content/180/11/7681

Why The JI?
• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Speedy Publication! 4 weeks from acceptance to publication
*average

References  This article cites 42 articles, 18 of which you can access for free at:
http://www.jimmunol.org/content/180/11/7681.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

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2008 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Local Accumulation of FOXP3+ Regulatory T Cells: Evidence for an Immune Evasion Mechanism in Patients with Large Condylomata Acuminata

Yuchun Cao, 2* Jie Zhao, 2* Zhang Lei, 1 Shiqian Shen, 1 Cong Liu, 4 Dong Li, 1 Jihong Liu, 8 Guan-Xin Shen, 1 Gui-Mei Zhang, 1 Zuo-Hua Feng, 1 and Bo Huang3

Condylomata acuminata derived from the infection of human papillomavirus is a common sexually transmitted disease. Although T cell-mediated cellular immunity is considered as the main arm against such infection, the regulation of T cell immune responses in genital condylomata is unclear to date. In this study, we analyzed FOXP3+ regulatory T cells in genital condylomata of patients. The results show that FOXP3+ regulatory T cells with suppressive function accumulated in large warts. Consistently, the immunosuppressive milieu in large warts was characterized by high expression of IL-10 and TGF-β1 and low expression of IL-2 and IFN-γ. The responsiveness of wart-infiltrating T cells both in vitro and in vivo can be increased by depleting FOXP3+ T cells. The accumulation of FOXP3+ regulatory T cells in large warts can be partly ascribed to the chemotaxis of CCL17 and CCL22, derived from Langerhans cells and macrophages in wart. Although such accumulation favors the local immunosuppression, it seems not to influence the systemic immunity. In conclusion, these findings demonstrate that FOXP3+ regulatory T cells play an important role in genital condylomata, which has multiple implications in the comprehensive treatment of condylomata acuminata. The Journal of Immunology, 2008, 180: 7681–7686.

Condylomata acuminata (CA) is a common sexually transmitted disease, which is derived from the infection of human papillomavirus (HPV), typically types 6 and 11 (1, 2). HPV infects primitive basal keratinocytes and causes genital warts with a highly variable latent period. During such period, the host’s immune responses may lead to the spontaneous regression of the lesion. However, in many cases, the lesion evades the host’s immune surveillance and consequently grows into a wart. Responses of the immune system to HPV infection are largely T cell mediated (3, 4). During the initial stage, Langerhans cells (LCs) uptake viral Ags and present Ags to T cells in the draining lymph node, resulting in T cell activation. Then, the activated T cells migrate to the infection site and lyse the infected keratinocytes. This process may be impaired in different ways. Firstly, keratinocytes are destined for death and desquamation, thus HPV infection does not typically result in their cytopathic death (5), and may not cause the inflammation to the extent usually seen in other infected tissues. Such hypoinflammation attenuates the LC-mediated Ag presentation. Secondly, down-regulation of type I IFN induced by HPV may also attenuate antivirus immune responses (6–9), because IFN-α and IFN-β may activate immature dendritic cells and act as a bridge between innate and adaptive immunity (10–12). Nevertheless, it is not clear whether there exist other mechanisms underlying the immune evasion of HPV infection.

FOXP3+ regulatory T (Treg) cells exert a strong suppressive activity on multiple components of the immune system and are crucially involved in controlling the magnitude and type of immune responses against self- and nonself Ags (13–15). Documents well demonstrate that Treg cells are abundant in tumors and play a significant role in the suppression of antitumor immunity (16–18). Moreover, Treg cells are also involved in the pathophysiological process of various virus infections (19–22). The wart is the disease of HPV infection and resembles some features of a tumor due to unlimited proliferation of infected basal keratinocytes. Thus, it is reasonable to postulate that Treg cells also play a role in the development of CA. To date, it is not clear whether there exist Treg cells in warts and what role they play, if they exist. Clarifying these is valuable for both the elucidation of the mechanisms underlying immune evasion of CA and the immune therapy of CA. In this study, we hypothesized that FOXP3+ Tregs play important immunosuppressive role in patients with CA.

Materials and Methods

Patient and normal donor samples

The diagnosis of condylomata acuminata was based on a clinically apparent wart of the external anogenital area and the positive detection by PCR of HPV type 6 DNA. The warts and peripheral blood were obtained from diagnosed patients after informed consent and with approval of the Clinical Research Ethics Board of the Tongji Medical College, including peripheral blood from normal donors. None of the patients had undergone any therapy for their warts at least 6 wk before sampling. PBMC from patients or normal donors were isolated by centrifugation over a Ficoll-Hypaque gradient. Genital warts were shaved off as close to

Copyright © 2008 by The American Association of Immunologists, Inc. 0022-1767/08/$2.00

www.jimmunol.org
the base as possible, using a sterile scalpel blade. The PBMCs and wart biopsies were either used directly or cryopreserved in liquid nitrogen.

**Immunochemistry**

The patient’s wart was surgically excised and embedded in optimum cutting temperature solution and cut into 4-μm sections. Sections were fixed by acetone. Before staining, the sections were incubated with 0.1% BSA to block the activity of endogenous peroxidase, then incubated overnight at 4°C with mouse anti-human CD3 or FOXP3 mAb diluted at 1/200. Biotinylated rat anti-mouse IgG was used as secondary Ab, followed by streptavidin-conjugated HRP at the third step. The Abs were purchased from Santa Cruz Biotecnology.

**Isolation of T cells, LCs, and macrophages from large wart**

To isolate immune cells, the large wart was digested by 500 μg/ml collagenase (Sigma-Aldrich), 500 μg/ml hyaluronidase (Sigma-Aldrich), and 5 U/ml DNase I at 37°C for 1 h. Cells were collected and filtered to remove undigested tissue. The dissociated cells were underlayered with 5 ml of lymphocyte separation solution (Mediatech) and centrifuged (2,200 rpm for 20 min). Wart-infiltrating mononuclear cells were harvested from the interface. T cells were isolated on a T cell enrichment column (R&D Systems); LCs were isolated from the left cells with human CD1a MicroBeads (Miltenyi Biotec); the final cells were used for the isolation of macrophages by human CD11b MicroBeads (Miltenyi Biotec).

In addition, normal PBMCs were used for the isolation of Treg cells with the Regulatory T Cell Isolation Kit (Miltenyi Biotec).

**Cyclophosphamide (CY) administration**

Patients with large CA (n = 10) were separated into two groups randomly. One group received CY at 50 mg orally, once a day for 1 wk. Another group received CY at 50 mg orally, once a day for 1 wk. The patient’s wart was surgically excised and embedded in optimum cutting temperature solution and cut into 4-mm sections. Sections were fixed by acetone. Before staining, the sections were incubated with 0.1% BSA to block the activity of endogenous peroxidase, then incubated overnight at 4°C with mouse anti-human CD3 or FOXP3 mAb diluted at 1/200. Biotinylated rat anti-mouse IgG was used as secondary Ab, followed by streptavidin-conjugated HRP at the third step. The Abs were purchased from Santa Cruz Biotecnology.

**Flow cytometry analysis and intracellular staining**

Flow cytometry was performed on a FACSCalibur (BD Biosciences) with CellQuest software using directly conjugated mAbs against the following markers: CD3-APC, CD4-FITC, and CD8-PE. CD19-PE-cy5, CD56-FITC with corresponding isotype-matched controls (eBioscience).

For intracellular staining, cells isolated from warts or peripheral blood were first dealt with surface staining, and then fixed and permeabilized with Fix/Perm solution (eBioscience). The cells were then resuspended in Perm Buffer and incubated with anti-human CCL1, CCL17, CCL22, and CCL19 neutralizing Abs (R&D Systems) for 30 min. After washing, the cells were used for flow cytometric analysis.

**Results**

Results were expressed as mean value ± SD and interpreted by ANOVA test. Differences were considered to be statistically significant when p < 0.05.

**Results**

**FOXP3+ T cells accumulate in large CA**

We hypothesized that FOXP3+ Tregs may accumulate in genital warts. To test this, we first stained T cells in the tissues of genital warts from different patients. The results showed a number of CD3+ T cells infiltrating in the HPV-infected tissues (Fig. 1A). We also identified the Treg cells among them, we targeted FOXP3 protein, a specific marker for Treg cells, to stain the infected tissues. The brown FOXP3+ cells were observed in the infected epidermal site, and the number tended to increase in larger wart (Fig. 1A). We then further compared the expression level of FOXP3+ mRNA in genital warts of different sizes by real time RT-PCR. Consistently, the results showed very low or no expression of FOXP3 in small warts (size <5 mm), increased level of expression in middle warts (5 – 8 mm size), and high level of expression in large warts (size >8 mm), (Fig. 1B), suggesting the accumulation of FOXP3+ cells in large warts. We further confirmed this by the analysis of FOXP3+ CD3+ T cells by flow cytometry (Fig. 1C). We also found that most FOXP3+ CD3+ T cells (>94%) belonged to CD4+ T cells, and most of them coexpressed CD25 molecule (Fig. 1, D and E).

**T cells in large warts are hyporesponsive**

Because FOXP3+ CD3+ T cells accumulated in large warts, we wondered whether the responsiveness of the wart-infiltrated T cells was impaired. To address this, T cells were isolated from large warts and stimulated with anti-CD3 and anti-CD28 Abs. The result
showed a significantly decreased proliferation compared with autologous peripheral T cell control (Fig. 2A), indicating the hyporesponsiveness of T cells in large wart. We evaluated the status of the T cells in smaller warts by measuring both positive and negative immune cytokines, because the amount of T cells isolated from the smaller warts was not enough for the proliferation assay. We determined the expression of cytokines IFN-γ and IL-2 connected to the T cell immune responses in small warts by real time RT-PCR. The result showed much higher expression of IFN-γ and IL-2 in small warts, relative to that in large warts (Fig. 2B). IL-10 and TGF-β1 can be used to indirectly reflect the low responsiveness of T cells, and their lower expression suggests that T cells in small warts are more active. More convincingly, the flow cytometric analysis showed that there was much higher intracellular IFN-γ in T cells in small warts, compared with that in large warts (Fig. 2C).

FOX3+ T cells in wart are functional Tregs

The above data disclosed an inverse relationship between the number of FOX3+ T cells and T cell responsiveness, which caused us to speculate that FOX3+ T cells are Tregs and functionally suppress effector T cells in warts. To confirm this, we first isolated T cells from large warts, and depleted FOX3+ T cells by removing CD25+ T cells with human CD25 MicroBeads, because most of FOX3 Treg cells are CD25 positive cells. FOX3+ T cells were decreased by >10-fold after depletion, confirmed by flow cytometric analysis (Fig. 3A). The depletion of FOX3+ T cells not only significantly increased T cell responsiveness to the stimulation of anti-CD3 and CD28 Abs (Fig. 3B), but also enhanced the proliferation and effector function of HPV-specific T cells (Fig. 3C and D). Moreover, we analyzed two important inhibitory cytokines IL-10 and TGF-β1 before and after depletion. The RT-PCR results showed the decent expression of IL-10 and TGF-β1 in T cells before depletion, however, very weak expression after depletion (Fig. 3E), suggesting the expression of IL-10 and TGF-β1 by FOX3 Treg cells in large warts. Consistently, neutralization of IL-10 or TGF-β1 could augment the proliferation of T cells isolated from large wart (Fig. 3F).
To validate the in vitro data in vivo, we chose low dose CY to selectively deplete patients’ Treg cells (23–25). After 1-wk administration of metronomic CY, the expression of FOXP3 in the CY-treated subjects was strikingly down-regulated, compared with that in placebo subjects (Fig. 3). In line with the decreased FOXP3, the expression of IL-2 and IFN-γ was increased 3- and 2-fold, respectively, and the expressions of IL-10 and TGF-β were decreased by 2.6- and 1.5-fold, respectively (Fig. 3G). More importantly, the T cells had an enhanced proliferation in response to the stimulation of anti-CD3 and CD28 Abs. The suppressive effect of FOXP3 regulatory T cells in vivo. Patients with large warts were treated with cyclophosphamide (n = 5) or placebo (n = 5). After a 1-wk treatment, the wart was surgically excised. The expression of FOXP3, IL-2, IFN-γ, TGF-β, and IL-10 was analyzed with real time RT-PCR (G) or the T cells were isolated for proliferation in response to the stimulation of anti-CD3 and CD28 Abs. * p < 0.05, compared with placebo group (H).

CCR4 signal pathway mediates the migration of Treg cells to wart

Next, we wondered about the underlying mechanism of FOXP3 Treg accumulation in large warts. We hypothesized that the migration of Treg cells to wart tissue was mediated by chemokine/chemokine receptor pathway. We paid the attention on CCR4 and CCR8, the specific chemokine receptors for Treg cells (26, 27), and the ligands CCL17, CCL22, and CCL1 (28–30). Using a Transwell system, we found that Treg cells effectively migrated toward wart tissue-containing medium, and the migration was significantly inhibited by anti-human CCL22 or CCL17 (ligands for CCR4), but not CCL1 (ligand for CCR8), neutralizing Abs (p < 0.05, Fig. 4A), suggesting that the migration of Treg cells to wart is mediated by the CCL22 or CCL17/CCR4 signal pathway. Furthermore, we also analyzed the transcripts of CCL22, CCL17, and CCL1 in warts. Consistently, large warts but not small warts expressed a

To validate the in vitro data in vivo, we chose low dose CY to selectively deplete patients' Treg cells (23–25). After 1-wk administration of metronomic CY, the expression of FOXP3 in the CY-treated subjects was strikingly down-regulated, compared with that in placebo subjects (Fig. 3G). In line with the decreased FOXP3, the expression of IL-2 and IFN-γ was increased 3- and 2-fold, respectively, and the expressions of IL-10 and TGF-β were decreased by 2.6- and 1.5-fold, respectively (Fig. 3G). More importantly, the T cells had an enhanced proliferation in response to the stimulation of anti-CD3 and CD28 Abs. The suppressive effect of FOXP3 regulatory T cells in vivo. Patients with large warts were treated with cyclophosphamide (n = 5) or placebo (n = 5). After a 1-wk treatment, the wart was surgically excised. The expression of FOXP3, IL-2, IFN-γ, TGF-β, and IL-10 was analyzed with real time RT-PCR (G) or the T cells were isolated for proliferation in response to the stimulation of anti-CD3 and CD28 Abs. * p < 0.05, compared with placebo group (H).

CCR4 signal pathway mediates the migration of Treg cells to wart

Next, we wondered about the underlying mechanism of FOXP3 Treg accumulation in large warts. We hypothesized that the migration of Treg cells to wart tissue was mediated by chemokine/chemokine receptor pathway. We paid the attention on CCR4 and CCR8, the specific chemokine receptors for Treg cells (26, 27), and the ligands CCL17, CCL22, and CCL1 (28–30). Using a Transwell system, we found that Treg cells effectively migrated toward wart tissue-containing medium, and the migration was significantly inhibited by anti-human CCL22 or CCL17 (ligands for CCR4), but not CCL1 (ligand for CCR8), neutralizing Abs (p < 0.05, Fig. 4A), suggesting that the migration of Treg cells to wart is mediated by the CCL22 or CCL17/CCR4 signal pathway. Furthermore, we also analyzed the transcripts of CCL22, CCL17, and CCL1 in warts. Consistently, large warts but not small warts expressed a

To validate the in vitro data in vivo, we chose low dose CY to selectively deplete patients’ Treg cells (23–25). After 1-wk administration of metronomic CY, the expression of FOXP3 in the CY-treated subjects was strikingly down-regulated, compared with that in placebo subjects (Fig. 3G). In line with the decreased FOXP3, the expression of IL-2 and IFN-γ was increased 3- and 2-fold, respectively, and the expressions of IL-10 and TGF-β were decreased by 2.6- and 1.5-fold, respectively (Fig. 3G). More importantly, the T cells had an enhanced proliferation in response to the stimulation of anti-CD3 and CD28 Abs. The suppressive effect of FOXP3 regulatory T cells in vivo. Patients with large warts were treated with cyclophosphamide (n = 5) or placebo (n = 5). After a 1-wk treatment, the wart was surgically excised. The expression of FOXP3, IL-2, IFN-γ, TGF-β, and IL-10 was analyzed with real time RT-PCR (G) or the T cells were isolated for proliferation in response to the stimulation of anti-CD3 and CD28 Abs. * p < 0.05, compared with placebo group (H).

CCR4 signal pathway mediates the migration of Treg cells to wart

Next, we wondered about the underlying mechanism of FOXP3 Treg accumulation in large warts. We hypothesized that the migration of Treg cells to wart tissue was mediated by chemokine/chemokine receptor pathway. We paid the attention on CCR4 and CCR8, the specific chemokine receptors for Treg cells (26, 27), and the ligands CCL17, CCL22, and CCL1 (28–30). Using a Transwell system, we found that Treg cells effectively migrated toward wart tissue-containing medium, and the migration was significantly inhibited by anti-human CCL22 or CCL17 (ligands for CCR4), but not CCL1 (ligand for CCR8), neutralizing Abs (p < 0.05, Fig. 4A), suggesting that the migration of Treg cells to wart is mediated by the CCL22 or CCL17/CCR4 signal pathway. Furthermore, we also analyzed the transcripts of CCL22, CCL17, and CCL1 in warts. Consistently, large warts but not small warts expressed a
Table I. Analysis of peripheral lymphocyte phenotype

<table>
<thead>
<tr>
<th>Groups</th>
<th>Patients n = 18</th>
<th>Normal donors n = 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (Male:Female)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>34 (19–60)</td>
<td>32 (22–54)</td>
</tr>
<tr>
<td>CD3+CD19+ T cell</td>
<td>68.4 ± 7.5%</td>
<td>62.3 ± 8.1%</td>
</tr>
<tr>
<td>CD3+CD19+ B cell</td>
<td>16.3 ± 2.6%</td>
<td>18.6 ± 3.4%</td>
</tr>
<tr>
<td>CD3+CD4+CD8+ T cell</td>
<td>34.7 ± 4.3%</td>
<td>32.1 ± 5.2%</td>
</tr>
<tr>
<td>CD3+CD4+CD8+ T cell</td>
<td>25.2 ± 3.1%</td>
<td>24.4 ± 3.7%</td>
</tr>
<tr>
<td>CD3+CD56+ NK cell</td>
<td>14.2 ± 4.2%</td>
<td>17.5 ± 3.8%</td>
</tr>
<tr>
<td>CD3+FOXP3+ Treg cells</td>
<td>4.3 ± 1.1%</td>
<td>4.8 ± 1.6%</td>
</tr>
</tbody>
</table>

*Age was shown as median (range). Other data were expressed as means ± SEM.

Discussion

Tregs play an important role in a large number of infections, including life-threatening diseases such as AIDS, hepatitis C virus infection, and malaria (31–33). In the present study, we additionally demonstrate that Treg cells are also involved in condylomata acuminata derived from HPV infection. In general, Treg cells migrate to the infected sites to exert their suppressive function by inhibiting both effector cells and APCs. However, the migration of Treg cells to warts may have more significant implications, due to that 1) the number of Treg cells is in parallel with the size of wart; 2) Treg cells suppress anti-HPV immune responses; and 3) the accumulation of Treg cells in warts does not influence systemic immunity. Therefore, blockade of Treg cell migration pathway may be a promising strategy to modulate the immune responses against CA.

Although other types of Tregs, such as Tr1, Th3, CD8+, and CD45RBlow Treg cells, may participate in the regulation of immune responses to viral infections. CD4+CD25+ Treg cells in the Treg cell family are likely the major player in CA, because depletion of CD4+CD25+ Treg cells can reverse the suppression of effector T cells in warts. There are two subpopulations of CD4+CD25+ Treg cells, the natural Treg cells that emerge from the thymus and the inducible Treg cells that are induced and converted in the periphery from CD4+CD25+ naïve T cells (14, 15). However, it was difficult to elucidate the origin and the specificity of wart-infiltrating Treg cells in this study, because polyclonal and HPV-specific Treg cells are possibly coexistent in the wart. Several mechanisms may be involved in the accumulation of Treg cells in the wart. The tissue damage by HPV infection results in the release of endogenous TLR ligands (such as HSPs, HMGB1), which trigger the signal for Treg cell induction; HPV Ags mimic self Ags and can engage with TCR to induce virus-specific Treg cells; virus-induced cytokines (such as IL-10, TGF-β) help Treg cell induction.

CCR4 signaling has been demonstrated to guide Treg cells to sites of Ag presentation in inflamed areas to attenuate T cell activation (24, 25). In this study, we also confirm the migration of Treg cells to HPV-infected epidermal tissue through the same pathway. The LCs and macrophages in lesions release the chemokines CCL17 and CCL22, the ligand for CCR4 (25, 27), to mediate the trafficking of Treg cells to the HPV-infected site. In our study, the effective trafficking only happened in large warts, but not small warts. This may be due to the CCL17 and CCL22 level. One possible reason is the low number of LCs and macrophages in small warts. Another possibility is the low expression of CCL17 and CCL22 in these cells.

The suppressive cytokines IL-10 and TGF-β have been implicated in a functional mechanism for FOXP3+ Treg cells (34–36). In this study, we found that Treg cells in warts also express IL-10 and TGF-β. We draw this conclusion from real time PCR results before and after Treg depletion (Fig. 3E). Differently, the FOXP3+ Treg cells in peripheral circulation express very weak IL-10 and TGF-β (our unpublished data). This is possibly attributed to the up-regulation of IL-10 and TGF-β expressions by Treg cells in the inflammatory microenvironment of warts. The HPV-associated inflammation is limited to the local site of infection, thus cannot influence the peripheral circulating Treg cells. Thus, the presence of IL-10 and TGF-β in large warts may be an important mechanism for Treg-mediated T cell suppression. They may also play a crucial role in prevention of LC maturation. Moreover, TGF-β, IL-10, and pathogenic Ags together create a microenvironment that may favor the survival of Treg cells in the site of infection (37). Therefore, blocking the function of IL-10 and TGF-β by...
local administration of neutralizing Ab might be beneficial to the immunotherapy of CA.

Multiple treatments have been applied against genital warts, including clyotherapy, surgical excision, electrocautery, and laser therapy (38). However, all of these tend to be associated with high recurrence rates (39), especially if latent HPV is present in the clinically normal epithelium outside the treatment area. Alternatively, immunotherapy is a promising treatment for HPV infection. The ideal immunotherapy comprises both increasing anti-HPV immunity and decreasing HPV infection-induced immunosuppression. The present study provides the first evidence that FOXP3+ Tregs play an important role in the suppression of anti-HPV immunity in genital warts. Thus, Treg cells may act as an important obstacle in the immunotherapy of CA and are a potent treatment target.

The goal of immunotherapy of CA is to create the most potent anti-HPV responses. The integrative systemic immunity status in the CA patient is very useful for CA immunotherapy. Local anti-HPV immunity can be augmented without necessarily enhancing the systemic immunity so as to avoid the induction of autoimmunity. Therefore, direct application of agent to the wart surface to selectively eradicate Treg cells may be an alternative to the aforementioned neutralization of local chemokines or inhibitory cytokines, such as CCL22, TGF-β1, and so on. It has been well documented that anti-CD25 depletion Ab efficiently eradicates CD4+ Treg cells in mice (40–42). Because most CD25+ T cells in warts are FOXP3+ Tregs, using an anti-CD25 depletion Ab may eliminate Treg cells. Recent reports show that the low dose of CY selectively depletes human CD4+ Treg cells (21–23). Therefore, administration of CY may possibly be a means of systemic immunotherapy. However, such strategy possesses the risk of induction of autoimmunity.

Acknowledgments

We thank the patients for cooperation and confidence.

Disclosures

The authors have no financial conflict of interest.

References