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

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

Yuchun Cao, Z.C.; Zhang Lei, Shiqian Shen, Cong Liu, Dong Li, Jihong Liu, Guan-Xin Shen, Gui-Mei Zhang, Zuo-Hua Feng and Bo Huang

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 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.

Condylomata acuminata is a common sexually transmitted disease, which is derived from the infection of human papillomavirus (HPV). 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

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4 Abbreviations used in this paper: CA, condylomata acuminata; HPV, human papillomavirus; LC, Langerhans cell; Treg, regulatory T cell; CY, cyclophosphamide.
the base as possible, using a sterile scalpel blade. The PBMCs and wart biopsies were either used directly or cryopreserved in liquid nitrogen.

**Immunohistochemistry**

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. Bio- tinylated 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 underlaid 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 corresponding placebo. Informed consent was obtained according to the manufacturer’s instructions. For real time RT-PCR assays, the cDNA sequences of all detected genes were retrieved from National Center for Biotechnology Information database. The primers were designed with the Oligo Primer Analysis 4.0 software (National Biosciences) and the sequences were blasted (http://www.ncbi.nlm.nih.gov/BLAST/). The primer sequences were as follows: FOXP3, sense 5′-CAT GCCCTCCTCTCTCTCTG-3′, antisense 5′-GCTGTTGCGATAAATGTCG-3′; IL-2, sense 5′-CACAATGCTGACCTGTGTC-3′, antisense 5′-ATGCTCCAGTGTAAGCCTGTTG-3′; IFN-γ, sense 5′-CAGTGTATCTCGATTCTG-3′, antisense 5′-CCTTGGATGCTTCCACCTAC-3′; IL-10, sense 5′-TTGGCAACGCTCTGCTGAGATG-3′, antisense 5′-CACACGGGAAGAATTGATGC-3′; TGF-β1, sense 5′-CAAGAATCTGACATGTGAC-3′, antisense 5′-TTGACCTTCTCCTGGCAGTAC-3′; CCL1, sense 5′-GGAGACACTGCACTGATC-3′, antisense 5′-ATGAGAAAGTCTTGAGCG-3′; CCL17, sense 5′-CTTCTCTGCACGGCATACTC-3′, antisense 5′-GTTGACTGATGACATGT-3′; CCL22, sense 5′-CTCTAAAGCACTGAGGACAG-3′, antisense 5′-CAGTGAAGAATTGTTACACACG-3′, GAPDH, sense 5′-CCTCTTATGAGCTCCTACAT-3′, antisense 5′-GGTGTGGGTGATTCTTACGG-3′.

In brief, 100 ng of total RNA was used for reverse transcription using Superscript II RNase H reverse transcriptase (Invitrogen) in a volume of 25 μl. Then, 2 μl of cDNA was amplified with SYBR Green Universal PCR Mastermix (Bio-Rad) in duplicate. In addition, 1 and 10 ng of total RNA were used to calibrate each primer set. For sample analysis, the threshold was set based on the exponential phase of products, and CT value for samples was determined. The resulting data were analyzed with the comparative CT method for relative gene expression quantification against the housekeeping gene GAPDH.

**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 (BD Pharmingen) and incubated with anti-human FOXP3 or IFN-γ conjugated with FITC or PE, or isotype control (eBioscience) at room temperature in the dark for 20-min staining. After washing, the cells were used for flow cytometric analysis.

**Statistics**

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). To identify the Treg cells among them, we targeted FOXP3 protein, a specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues. The brown FOXP3-specific marker for Treg cells, to stain the infected tissues.

In vitro migration assay

Treg cells were isolated from patient peripheral blood by a Human Regulatory T Cell Isolation Kit (Miltenyi Biotec). The fresh wart tissue from patients were set in the lower chamber and 5 x 10⁶ Treg cells from the same patient were set in the upper chamber, in triplicate, of a 24-well transwell (BD Biosciences), and incubated at 37°C for 4 h. The Treg cells migrating into the lower chamber were enumerated under microscopy and the migrated cell percentage was calculated. Mouse-anti-human CCL1, CCL17, and CCL22 neutralizing Abs (R&D Systems) were used (10 μg/ml) in inhibition test, and corresponding IgG isotype Abs (R&D Systems) were used as control.

**Real-time RT-PCR**

Warts were homogenized in TRIzol reagent (Invitrogen) and total RNA was extracted according to the manufacturer’s instructions. For real time
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). In line with this, we also found lower expression of IL-10 and TGF-β1 in small 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 FOXP3+ T cells and T cell responsiveness, which caused us to speculate that FOXP3+ 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 FOXP3+ T cells by removing CD25+ T cells with human CD25 MicroBeads, because most of FOXP3+ Treg cells are CD25 positive cells. FOXP3+ T cells were decreased by >10-fold after depletion, confirmed by flow cytometric analysis (Fig. 3A). The depletion of FOXP3+ 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 FOXP3 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).

**FIGURE 1.** Accumulation of FOXP3+ Tregs in large warts of HPV-infected patients. A, Different size of warts were used for immunohistochemical analysis (200×). Among them, a–d were stained with anti-CD3 Ab, and e–h were stained with anti-FOXP3 Ab as described in Materials and Methods. The data were the representative from nine total patients (each size with three patients). B, FOXP3 transcripts in warts were analyzed with real time RT-PCR. C, Expression of FOXP3 in wart T cells. Single cells in warts were stained with anti-CD3 and FOXP3 Abs for flow cytometric analysis. The representative result was shown by analysis of gated CD3+ cells. D and E, Expression of CD4 and CD25 by FOXP3+ T cells. The representative flow cytometric result was shown by analysis of gated CD3+CD4+ cells.

**FIGURE 2.** T cells in large wart are hyporesponsive. A, T cells in large wart have the attenuated ability of proliferation. T cells were isolated from three patients’ large warts and autologous peripheral blood, respectively. One × 10^6 T cells were used for proliferation in response to the stimulation of anti-CD3 and CD28 Abs as described in Materials and Methods. T cell proliferation was determined by [3H]-thymidine incorporation assay. Stimulation index (SI) was calculated by dividing the proliferation count in the presence of peptide complexes or Abs by that in the absence of peptide complexes or Abs, *p < 0.05, compared with peripheral T cell group. B, Expression of IL-2, IFN-γ, TGF-β1, and IL-10 in warts was analyzed with real time RT-PCR. The normal skin tissue was used as control. C, Analysis of intracellular IFN-γ in T cells from wart by flow cytometry.
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 were 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 (Fig. 3H), suggesting 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>10:8</td>
<td>7:7</td>
</tr>
<tr>
<td>Age (y)</td>
<td>34 (19–60)</td>
<td>32 (22–54)</td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt;CD19&lt;sup&gt;+&lt;/sup&gt; T cell</td>
<td>68.4 ± 7.5%</td>
<td>62.3 ± 8.1%</td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt;CD19&lt;sup&gt;+&lt;/sup&gt; B cell</td>
<td>16.3 ± 2.6%</td>
<td>18.6 ± 3.4%</td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt;CD4&lt;sup&gt;+&lt;/sup&gt;CD8&lt;sup&gt;+&lt;/sup&gt; T cell</td>
<td>34.7 ± 4.3%</td>
<td>32.1 ± 5.2%</td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt;CD4&lt;sup&gt;+&lt;/sup&gt;CD8&lt;sup&gt;+&lt;/sup&gt; T cell</td>
<td>25.2 ± 3.1%</td>
<td>24.4 ± 3.7%</td>
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<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt;CD56&lt;sup&gt;+&lt;/sup&gt; NK cell</td>
<td>14.2 ± 4.2%</td>
<td>17.5 ± 3.8%</td>
</tr>
<tr>
<td>CD3&lt;sup&gt;+&lt;/sup&gt;FOXP3&lt;sup&gt;+&lt;/sup&gt; Treg cells</td>
<td>4.3 ± 1.1%</td>
<td>4.8 ± 1.6%</td>
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<sup>a</sup> 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<sup>+</sup>, and CD4<sup>+</sup>IL-10<sup>+</sup> Treg cells, may participate in the regulation of immune responses to viral infections. CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in the Treg cell family are likely the major player in CA, because depletion of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells can reverse the suppression of effector T cells in large warts. There are two subpopulations of CD4<sup>+</sup>CD25<sup>+</sup> 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<sup>+</sup>CD25<sup>+</sup> naive 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 T 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<sup>+</sup> 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<sup>+</sup> 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...
Therefore, direct application of agent to the wart surface to select CA patient is very useful for CA immunotherapy. Local anti-HPV and are a potent treatment target. Thus, Treg cells may act as an important obstacle in the suppression of anti-HPV immunity in genital warts. Therefore, administration of CY may possibly be a means of systemic immunotherapy. However, such strategy possesses the risk of 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 CCL2, TGF-β1, and so on. It has been well documented that anti-CD25 deletion Ab efficiently eradicates CD4+CD25+ Treg cells in mice (40–42). Because most CD25+ T cells in warts are FOXP3+ Tregs, using an anti-CD25 deletion Ab may eliminate Treg cells. Recent reports show that the low dose of CY selectively depletes human CD4+CD25+ 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.

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Disclosures
The authors have no financial conflict of interest.

References

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