

Profiles of Foxp3+ regulatory T cells in eczematous dermatitis, psoriasis vulgaris and mycosis fungoides

T. Fujimura, R. Okuyama, Y. Ito and S. Aiba

Department of Dermatology, Tohoku University Graduate School of Medicine, Seiryō-machi 1-1, Aoba-ku, Sendai 980-8574, Japan

Summary

Correspondence

Setsuya Aiba.

E-mail: aiba@mail.tains.tohoku.ac.jp

Accepted for publication

18 December 2007

Key words

dermatitis, Foxp3, mycosis fungoides, psoriasis, regulatory T cell

Conflicts of interest

None declared.

Background It is well known that regulatory T cells (Tregs), identified by their expression of CD4, CD25 and Foxp3, play a crucial role in maintaining peripheral tolerance. Recently, it has been demonstrated that a Treg population resides in normal human skin. However, only a few studies have demonstrated the presence of Foxp3+ Tregs in inflammatory skin disorders.

Objectives In this study, we immunohistologically examined the presence of CD4+ CD25+ Foxp3+ Tregs in the lesional skin of psoriasis vulgaris, mycosis fungoides and eczematous dermatitis.

Methods We used immunohistochemistry to examine the presence of Foxp3+ Tregs in fixed sections of the lesional skin from 16 patients with psoriasis vulgaris, 17 patients with mycosis fungoides and 18 patients with eczematous dermatitis in addition to 10 normal skin samples.

Results In normal skin, epidermal and dermal Foxp3+ cells were rare. The psoriasis vulgaris, mycosis fungoides and eczematous dermatitis samples contained substantial numbers of epidermal and dermal CD3+, CD4+ and CD25+ Foxp3+ Tregs. The epidermis contained a higher percentage of CD3+, CD4+ and CD25+ Foxp3+ cells than the dermis. The percentage of Foxp3+ cells among CD3+ or CD4+ cells was significantly lower in eczematous dermatitis than in psoriasis vulgaris or mycosis fungoides, and that of dermal Foxp3+ cells was significantly lower in psoriasis vulgaris than in eczematous dermatitis or mycosis fungoides.

Conclusions The lower percentage of epidermal or dermal Foxp3+ cells in eczematous dermatitis or psoriasis vulgaris, respectively, might contribute to their pathogenesis.

Immunological tolerance to self-antigen is a tightly regulated process. A primary mechanism for self-tolerance is deletion of self-reactive cells in the thymus. However, this mechanism is not perfect, and autoreactive clones do escape into the periphery. Tolerance is maintained in the periphery through a variety of mechanisms, including a population of regulatory T cells (Tregs) that actively suppress the function of autoreactive T cells. These Tregs are identified by their expression of CD4, the interleukin (IL)-2 receptor α chain (CD25), and the forkhead family transcription factor Foxp3. They can inhibit the development of autoimmunity when transferred into an appropriate host. Recent work has shown that Foxp3 is expressed predominantly in Tregs and is both necessary and sufficient for their development and function (reviewed by Sakaguchi¹).

The potential role of Tregs in human diseases is currently the focus of intensive research efforts.² Treg defects due to mutations in Foxp3 cause IPEX (immunodysregulation, polyendocrinopathy, enteropathy and X-linked inheritance

syndrome), a severe inflammatory syndrome in children.³ Furthermore, Treg defects have recently been reported in the peripheral blood of patients with multiple sclerosis,⁴ autoimmune polyglandular syndrome type II,⁵ type I autoimmune diabetes⁶ and psoriasis vulgaris.⁷ Moreover, functional defects of CD4+ CD25^{high} Tregs were observed directly in the thymus of patients with myasthenia gravis.⁸ It is also reported that Tregs are much more prevalent in the synovial fluid than in the peripheral blood in adult patients with rheumatoid arthritis.⁹ In juvenile idiopathic arthritis, patients with persistent oligoarticular juvenile idiopathic arthritis, which has a relatively benign, self-remitting course, display a significantly higher frequency of CD4+ CD25^{bright} T cells concomitantly with higher levels of mRNA Foxp3 in the peripheral blood than patients with extended oligoarticular juvenile idiopathic arthritis, which has a much less favourable prognosis.¹⁰

Recently, Clark and Kupper¹¹ succeeded in isolating and expanding the Treg population resident in normal human skin. These cells express high levels of CD25, L-selectin,

glucocorticoid-induced tumour necrosis factor receptor, Foxp3, intracellular cytotoxic T-lymphocyte-associated antigen-4, low levels of CD69, and high levels of the skin-homing addressins, cutaneous lymphocyte-associated antigen, CCR4 and CCR6. Moreover, RANKL expression by keratinocytes alters the function of epidermal Langerhans cells, resulting in systemic increases of Tregs,¹² which strongly suggests that Tregs have a crucial function in various inflammatory skin disorders or skin tumours. Despite these suggestions that Tregs are important in skin disorders, very few immunohistological studies have demonstrated that Tregs are present in the skin. Miyara *et al.*¹³ demonstrated that Foxp3+ CD3+ T cells accumulate in the sarcoid granuloma, while Bovenschen *et al.*¹⁴ found Foxp3+ Tregs in psoriatic lesional skin. In contrast, Verhagen *et al.*¹⁵ reported that Foxp3+ Tregs are not present in atopic dermatitis or psoriatic skin.

In this study, we used immunohistochemistry to examine whether CD4+ CD25+ Foxp3+ Tregs are present in the lesional skin of patients with eczematous dermatitis histologically showing spongiosis, psoriasis vulgaris or mycosis fungoides. We confirmed the specificity of an antibody (Ab) to Foxp3 by using it to label TL-Om1 cells that had been transfected with a human full-length Foxp3 cDNA. TL-Om1 is a leukaemic cell line isolated from a patient with adult T-cell leukaemia (ATL).¹⁶ Using this cell line, we also corroborated that the Ab to Foxp3 would still bind after formalin fixation. We then used the Ab to determine the presence of Tregs that were Foxp3+ and CD4+, CD3+ or CD25+ in tissue samples and found that the frequency of Tregs in the dermis and epidermis differed significantly among eczematous dermatitis, psoriasis vulgaris and mycosis fungoides.

Materials and methods

Reagents

We used the following Abs for immunohistochemical staining: mouse monoclonal Abs for human CD3 and human CD4 (Nichirei Co., Tokyo, Japan), human CD8 (Dako A/S Inc., Glostrup, Denmark), human CD25 (Vector Inc., Burlingame, CA, U.S.A.), and rabbit antihuman Foxp3 Ab and immunoglobulin from a nonimmunized rabbit (BioLegend Inc., San Diego, CA, U.S.A.). Mouse IgG2a, IgG2b and IgG1 isotype controls were obtained from R&D Systems (Minneapolis, MN, U.S.A.).

Cell lines

The TL-Om1 cell line was derived from leukaemia cells isolated from a patient with ATL. TL-Om1 cells transfected with a full-length cDNA of human Foxp3, designated TL-Om1/Foxp3,¹⁶ were a kind gift from Prof. K. Sugamura and Dr N. Ishii, Department of Microbiology and Immunology, Tohoku University Graduate School of Medicine. These cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum.

Tissue samples

We collected archival formalin-fixed paraffin-embedded specimens of uninvolved lymph nodes from five patients with malignant melanoma or squamous cell carcinoma, and lesional skin specimens from 18 patients with eczematous dermatitis histologically showing spongiosis, 16 with psoriasis vulgaris, 17 with the patch or plaque stage of mycosis fungoides, and 10 normal skin specimens from the Department of Dermatology, Tohoku University Graduate School of Medicine.

Immunohistochemical staining for cytopsin preparation

Immunohistochemical analysis of Foxp3 expression was performed on cytopsin slides of TL-Om1 and TL-Om1/Foxp3 cells. The slides were first fixed with formalin for 30 min, then blocked with goat serum. The slides were incubated with rabbit antihuman Foxp3 Ab or immunoglobulin from a non-immunized rabbit. The staining for anti-Foxp3 Ab was developed using the biotin-alkaline phosphatase method for rabbit Abs (Histofine SAB-AP(R) kit; Nichirei Co.) and new fuchsin (Nichirei Co.) as the substrate. The slides were mounted in PermaFluor (Lipshaw Immunon, Pittsburgh, PA, U.S.A.).

Antigen retrieval treatment for immunohistochemistry for Foxp3

Formalin-fixed paraffin-embedded tissue samples were sectioned at 4 µm and deparaffinized. Deparaffinized sections were treated with one of the following three procedures: (i) incubation in 0.25% trypsin in 0.1 mol L⁻¹ phosphate-buffered saline containing 0.02% calcium chloride at pH 7.2 for 30 min; (ii) microwave heating for two cycles of 5 min each after preheating up to 95 °C for 10 min at 500 W; or (iii) autoclave heating at 121 °C for 5 min. After the pretreatment, the sections were cooled to room temperature prior to processing for immunohistochemistry.

Foxp3 single staining for lymph nodes

After the antigen retrieval treatment, the sections of lymph nodes were first blocked with goat serum for 10 min and then exposed to anti-Foxp3 Ab or immunoglobulin from a nonimmunized rabbit at 4 °C overnight. The staining for anti-Foxp3 Ab was developed using the biotin-alkaline phosphatase method for rabbit Abs (Histofine SAB-AP(R) kit; Nichirei Co.) and new fuchsin as the substrate. The slides were mounted in PermaFluor.

Immunohistochemistry for Foxp3 and CD3, CD4, CD8 or CD25

Sixteen psoriasis vulgaris samples, 17 mycosis fungoides samples and 18 eczematous dermatitis samples were processed for double staining of Foxp3 and CD3, CD4 or CD25. After the antigen retrieval treatment, the sections were rehydrated,

blocked with goat serum for 10 min, and then exposed to primary Abs at 4 °C overnight. Ab binding was demonstrated via peroxidase-conjugated antimouse immunoglobulins (Histofine SAB-PO(M) kits; Nichirei Co.) for the anti-CD3, anti-CD4, anti-CD8 or anti-CD25 Abs or their isotype controls and via alkaline phosphatase-conjugated antirabbit immunoglobulins (Histofine SAB-AP(R) kit) for anti-Foxp3 Ab or immunoglobulin from a nonimmunized rabbit. The mouse Abs were visualized with 3,3'-diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries, Ltd, Osaka, Japan), and the anti-Foxp3 Ab was developed with new fuchsin.

Assessment of immunohistochemical staining

In each section, three representative fields were selected from the epidermis and three that were associated with a dense dermal lymphoid infiltrate were selected from the dermis. The number of immunoreactive cells was counted using an ocular grid of 1 cm² at a magnification of $\times 400$. The Treg fractions of CD3, CD4, CD8 and CD25 were defined as the ratio (%) of CD3+ Foxp3+, CD4+ Foxp3+, CD8+ Foxp3+ and CD25+ Foxp3+ cells to the total number of CD3+, CD4+, CD8+ and CD25+ cells in each field, respectively. The data were expressed as the mean $\pm$ SD of the Treg fraction in each skin disorder.

Statistical analysis

To analyse the differences in the percentages of epidermal or dermal Foxp3+ cells in CD3+, CD4+ or CD25+ cells among the three skin disorders, we first examined the effects of the skin disorders on the difference in the percentages of Foxp3+ cells with two-way analysis of variance (ANOVA). When the analysis showed a significant difference, we identified the groups whose differences accounted for the significant P-value ($P < 0.05$) by two-way ANOVA.

Results

Anti-Foxp3 antibody stains adult T-cell leukaemia cell lines transfected with the Foxp3 gene

We first confirmed the specificity and the reactivity of anti-Foxp3 Ab with formalin-fixed TL-Om1 and TL-Om1/Foxp3 cells. The anti-Foxp3 Ab reacted with the nuclei of TL-Om1/Foxp3 cells in the cytospin preparation after a 30-min formalin fixation (Fig. 1a). No positive staining was obtained with control immunoglobulin from a nonimmunized rabbit (Fig. 1b) or in the cytospin preparation of untransfected TL-Om1 cells stained with the anti-Foxp3 Ab (Fig. 1c).

Anti-Foxp3 Ab stains Foxp3-positive cells in paraffin-embedded sections of formalin-fixed lymph nodes

Next, we determined the best antigen retrieval method for obtaining specific signals for Foxp3 in formalin-fixed,

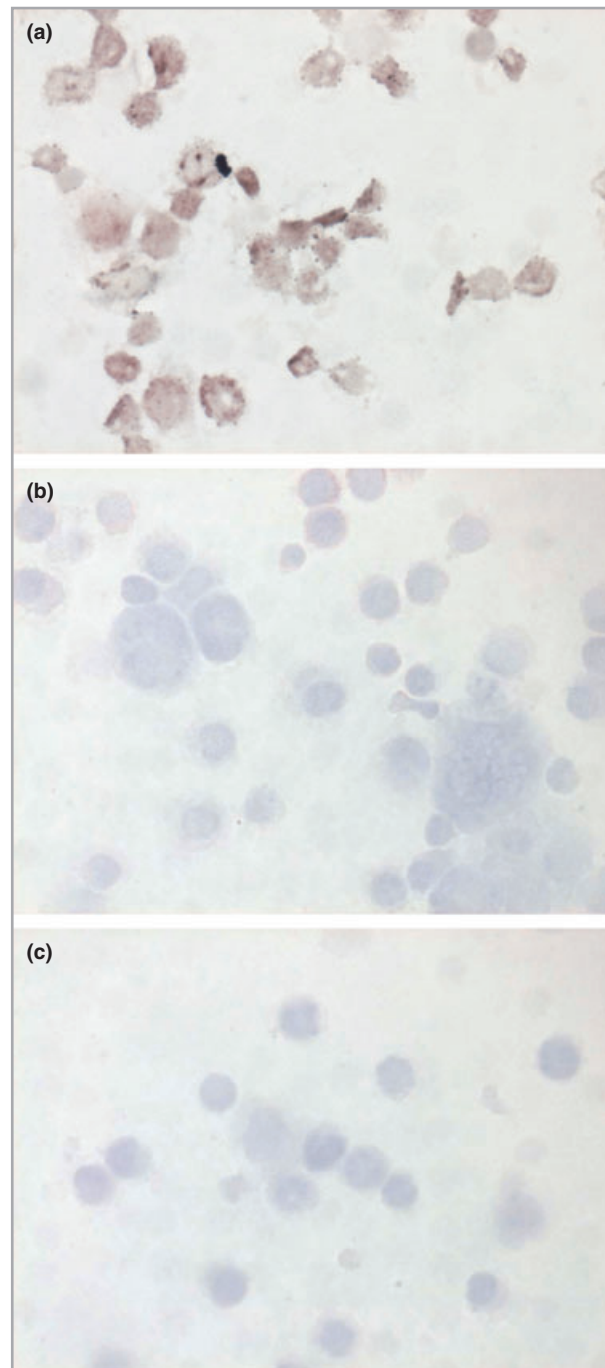


Fig 1. Anti-Foxp3 antibody (Ab) staining of adult T-cell leukaemia cell lines transfected with the Foxp3 gene. Immunohistochemical analysis of Foxp3 expression was performed on cytospin slides of TL-Om1 and TL-Om1/Foxp3 cells. The slides were fixed with formalin for 30 min, blocked with goat serum, and incubated with a rabbit antihuman Foxp3 Ab or immunoglobulin from a nonimmunized rabbit. The stain was developed with a biotin-alkaline phosphatase method with new fuchsin as the substrate. Original magnification $\times 400$. (a) TL-Om1/Foxp3 cells stained with an anti-Foxp3 Ab. (b) TL-Om1/Foxp3 cells stained with immunoglobulin from a nonimmunized rabbit. (c) TL-Om1 cells stained with anti-Foxp3 Ab.

paraffin-embedded tissue sections of lymph nodes with the anti-Foxp3 Ab. We compared three antigen retrieval methods: trypsin digestion, microwaving and autoclave heating. We found that the best results were obtained with autoclave heating, followed by microwave heating and then by trypsin digestion (data not shown).

Epidermal Foxp3⁺ T cells in eczematous dermatitis, psoriasis vulgaris and mycosis fungoides

Next, we evaluated the percentage of epidermal Foxp3⁺ cells among CD3⁺, CD4⁺, CD8⁺ and CD25⁺ cell populations in 10 samples of normal skin, 16 samples from patients with psoriasis vulgaris, 17 from patients with mycosis fungoides and 18 from patients with eczematous dermatitis. For each sample, we performed single staining for Foxp3, and double staining for CD3 and Foxp3, CD4 and Foxp3, CD8 and Foxp3, and CD25 and Foxp3. In normal skin, we found no Foxp3⁺ epidermal cells except in one sample (Fig. 2a,b). In contrast, we found substantial numbers of Foxp3⁺ cells in the epidermis of psoriasis vulgaris (Fig. 3a), mycosis fungoides (Fig. 4a) and eczematous dermatitis (Fig. 5a). Many cells in the psoriasis vulgaris samples, the mycosis fungoides samples and the eczematous dermatitis samples expressed CD3, CD4 and CD25 with Foxp3 (Figs 3b, 4b, 5b). The mean $\pm$ SD percentages of Foxp3⁺ cells in psoriasis vulgaris, mycosis fungoides and eczematous dermatitis were $45.9 \pm 3.7\%$, $44.6 \pm 3.7\%$ and $26.8 \pm 3.0\%$ among CD3⁺ cells; $50.0 \pm 4.7\%$, $47.6 \pm 5.4\%$ and $29.6 \pm 3.3\%$ among CD4⁺ cells; and $45.7 \pm 3.8\%$, $50.4 \pm 4.8\%$ and $40.7 \pm 3.1\%$ among CD25⁺ cells, respectively (Fig. 6a). As expected, there were no Foxp3⁺ cells among CD8⁺ cells in samples from any of the skin diseases we examined (Figs 4c, 5c). The percentage of Foxp3⁺ cells among CD3⁺ cells or CD4⁺ cells was signifi-

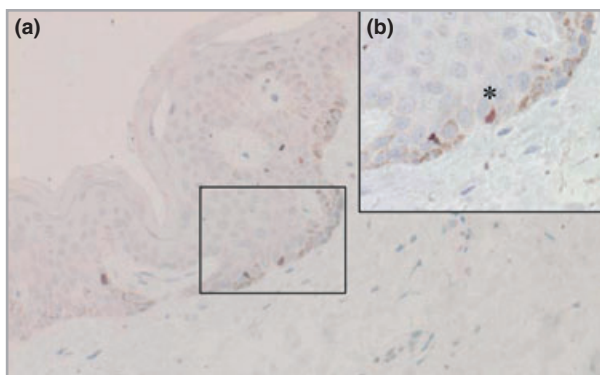


Fig 2. Anti-Foxp3 antibody (Ab) staining of normal skin. Immunohistochemical analysis of Foxp3 expression was performed on deparaffinized sections of normal skin. After autoclave heating at 121 °C for 5 min, the sections were reacted with anti-Foxp3 Ab and developed with new fuchsin by a biotin–alkaline phosphatase method. (a) Original magnification $\times 200$; (b) Higher magnification of the area marked in (a). Asterisk: Foxp3⁺ cell.

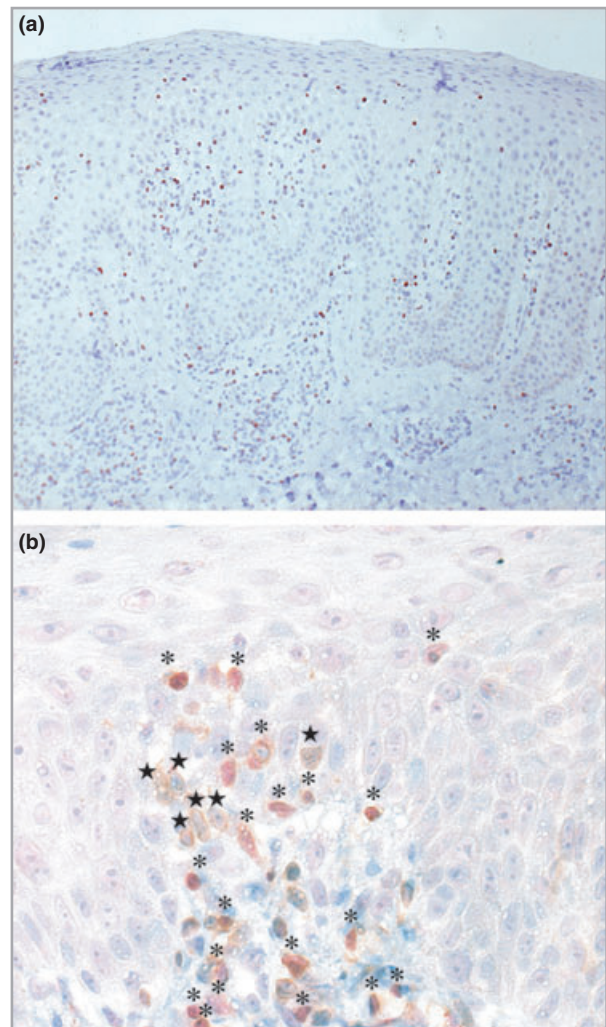


Fig 3. Anti-Foxp3 antibody (Ab) staining of psoriasis vulgaris. Formalin-fixed paraffin-embedded tissue samples from patients with psoriasis vulgaris were sectioned at 4 μ m and deparaffinized. Deparaffinized sections were treated with autoclave heating at 121 °C for 5 min. After pretreatment, the sections were stained with an anti-Foxp3 Ab (a), or with the combination of the anti-Foxp3 Ab and an anti-CD3 Ab (b). (a) The sections were developed with 3,3'-diaminobenzidine tetrahydrochloride. Original magnification $\times 200$. (b) The sections were developed with new fuchsin for Foxp3, and with 3,3'-diaminobenzidine tetrahydrochloride for CD3. Original magnification $\times 400$. Asterisks: CD3⁺ Foxp3⁺ cells. Stars: CD3⁺ Foxp3⁻ cells.

cantly lower ($P < 0.05$) in eczematous dermatitis than in psoriasis vulgaris or mycosis fungoides.

Dermal Foxp3⁺ T cells in eczematous dermatitis, psoriasis vulgaris and mycosis fungoides

We also examined the percentage of dermal Foxp3⁺ cells among perivascular CD3⁺, CD4⁺, CD8⁺ and CD25⁺ cells in the same set of samples using the same combinations of immunostaining. The mean $\pm$ SD percentages of Foxp3⁺ cells

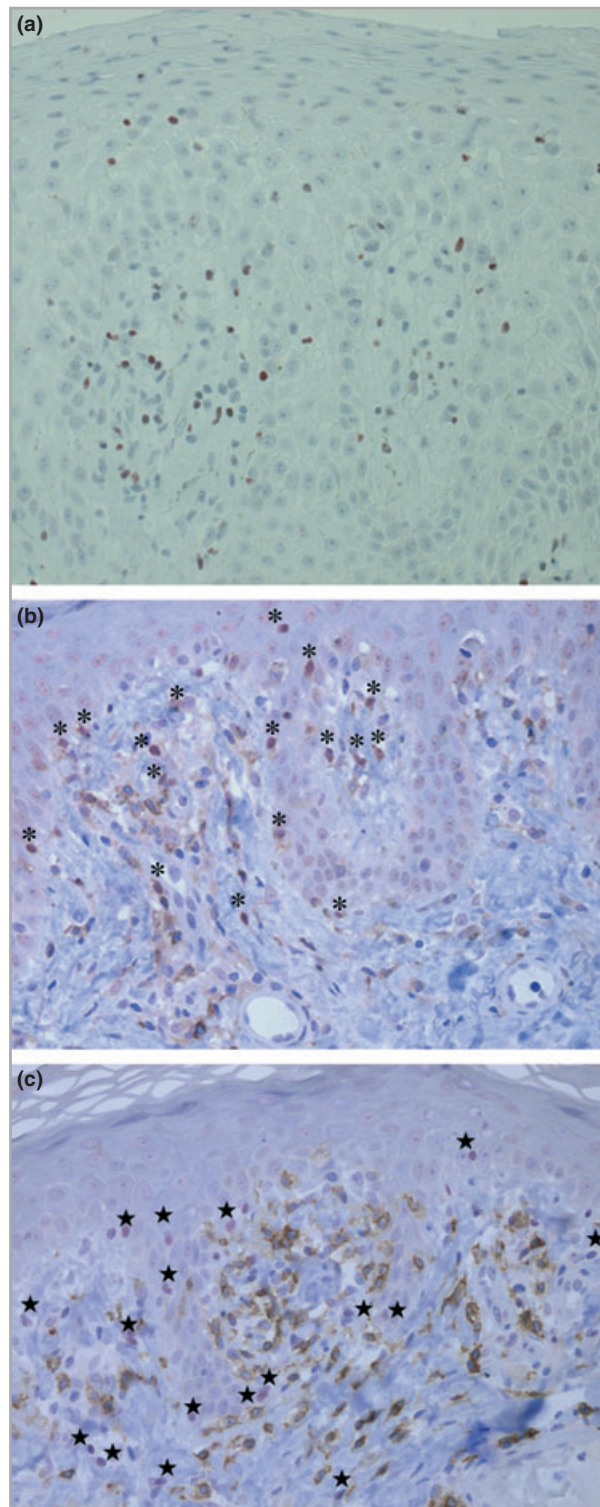


Fig 4. Anti-Foxp3 antibody (Ab) staining of mycosis fungoides. Formalin-fixed paraffin-embedded tissue samples from patients with mycosis fungoides were sectioned at 4 μ m and deparaffinized. Deparaffinized sections were treated with autoclave heating at 121 $^{\circ}$ C for 5 min. After pretreatment, the sections were stained with an anti-Foxp3 Ab (a), or with the combination of the anti-Foxp3 Ab and an anti-CD4 Ab (b) or with the combination of the anti-Foxp3 Ab and an anti-CD8 Ab (c). (a) The sections were developed with 3,3'-diaminobenzidine tetrahydrochloride. Original magnification $\times$ 200. (b) The sections were developed with new fuchsin for Foxp3 and with 3,3'-diaminobenzidine tetrahydrochloride for CD4. Original magnification $\times$ 200. (c) The sections were developed with new fuchsin for Foxp3 and with 3,3'-diaminobenzidine tetrahydrochloride for CD8. Original magnification $\times$ 200. Asterisks: CD4⁺ Foxp3⁺ cells. Stars: CD8⁺ Foxp3⁺ cells.

The percentage of Foxp3⁺ cells among CD3⁺, CD4⁺ or CD25⁺ cells was significantly lower ($P < 0.05$) in the psoriasis vulgaris samples than in those from eczematous dermatitis or mycosis fungoides. In addition, in these three skin diseases, the percentage of dermal Foxp3⁺ cells was always significantly smaller ($P < 0.05$) than that of epidermal Foxp3⁺ cells.

Discussion

In this study, we examined Treg compartmentalization in the skin of three representative skin disorders: eczematous dermatitis, psoriasis vulgaris and mycosis fungoides. We found significant numbers of epidermal as well as dermal CD3⁺, CD4⁺ and CD25⁺ cells that were Foxp3⁺. To our knowledge, this is the first quantitative analysis showing the percentages of Foxp3⁺ cells among infiltrating T cells in these disorders. In psoriatic skin, Sugiyama *et al.*⁷ have estimated the percentage of Tregs among epidermal and dermal CD4⁺ cells, although they used high CD25 expression as a marker for Tregs. Their data demonstrated that Tregs were approximately 35% and 14% of psoriatic epidermal and dermal CD4⁺ cells, respectively. Although their method of counting CD25^{high} cells using flow cytometry of epidermal or dermal cell suspensions completely differed from our method of immunohistological staining, their estimated percentages are similar to ours. Unfortunately, although Bovenschen *et al.*¹⁴ demonstrated Foxp3⁺ Tregs in psoriatic lesional skin, they did not count the percentage of Foxp3⁺ cells among various T-cell populations.

Several chemokine receptors, selectins and integrins on Tregs have been suggested to play a role in trafficking the Tregs to bone marrow, lymph nodes, tumours or inflammatory tissues. E- or P-selectin ligands¹⁷ and CD103¹⁸ are crucial for the migration of Tregs in the inflamed murine skin. These studies suggest that the substantial numbers of Tregs in the skin of patients with eczematous dermatitis and psoriasis vulgaris that we observed in this study may be the result of the activity of chemokines, selectin ligands or CD103, although further experiments will be necessary to identify the specific molecules involved. Interestingly, Clark and Kupper¹¹ reported

in the dermis of psoriasis vulgaris, mycosis fungoides and eczematous dermatitis were $5.2 \pm 2.4\%$, $19.1 \pm 5.1\%$ and $14.9 \pm 2.3\%$ among CD3⁺ cells; $2.8 \pm 2.0\%$, $19.5 \pm 5.1\%$ and $13.2 \pm 1.7\%$ among CD4⁺ cells; and $3.3 \pm 2.8\%$, $29.9 \pm 5.6\%$ and $14.3 \pm 2.7\%$ among CD25⁺ cells, respectively (Fig. 6b). Again, there were no Foxp3⁺ cells among CD8⁺ cells in the dermis of any skin diseases we examined.

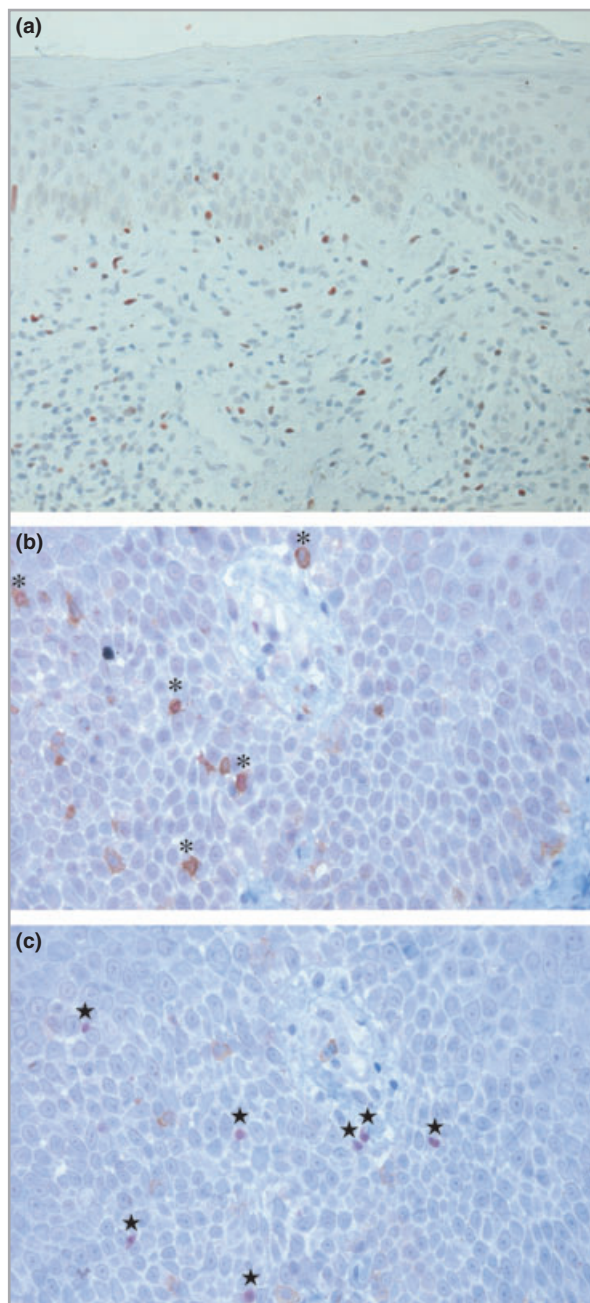


Fig 5. Anti-Foxp3 antibody (Ab) staining of eczematous dermatitis. Formalin-fixed paraffin-embedded tissue samples from patients with eczematous dermatitis were sectioned at 4 μ m and deparaffinized. Deparaffinized sections were treated with autoclave heating at 121 $^{\circ}$ C for 5 min. After pretreatment, the sections were stained with an anti-Foxp3 Ab (a), or with the combination of the anti-Foxp3 Ab and an anti-CD25 Ab (b) or with the combination of the anti-Foxp3 Ab and an anti-CD8 Ab (c). (a) The sections were developed with 3,3'-diaminobenzidine tetrahydrochloride. Original magnification $\times$ 200. (b) The sections were developed with new fuchsin for Foxp3 and with 3,3'-diaminobenzidine tetrahydrochloride for CD25. Original magnification $\times$ 200. (c) The sections were developed with new fuchsin for Foxp3 and with 3,3'-diaminobenzidine tetrahydrochloride for CD8. Original magnification $\times$ 200. Asterisks: CD25⁺ Foxp3⁺ cells. Stars: CD8⁺ Foxp3⁺ cells.

that there are natural Tregs or their precursors in human skin and that dermal fibroblasts in combination with IL-15, which is expressed by dermal fibroblasts stimulated with tumour necrosis factor- α ,¹⁹ can expand Tregs derived from the skin. They hypothesized that local proliferation of Tregs might occur within inflamed skin. Therefore, the mechanism for skin compartmentalization of Tregs in the skin disorders remains to be elucidated.

The percentage of dermal Foxp3⁺ cells was significantly smaller in psoriasis vulgaris than in eczematous dermatitis. Sugiyama *et al.*⁷ have reported that Tregs in peripheral blood of patients with psoriasis are deficient in their suppressor activity, although their percentage in the peripheral blood does not significantly differ from that of healthy controls. Although the pathogenesis of this disease is not well understood, various studies have suggested that intralesional activated T cells produce cytokines that trigger primed basal keratinocyte stem cells to proliferate and perpetuate the disease (reviewed by Nickoloff and Nestle²⁰ and by Krueger and Bowcock²¹). In addition, psoriasis is characterized by the inflammation sustained by the ongoing activation of pathogenic effector T cells. The lower frequency of dermal Foxp3⁺ cells in psoriatic skin and the functionally impaired Tregs in peripheral blood may therefore play an important role in the pathogenesis of psoriasis vulgaris.

Higher percentages of CD3⁺, CD4⁺ and CD25⁺ T cells expressed Foxp3 in the epidermis than in the dermis. Although it has been speculated that inflamed keratinocytes produce chemokines that attract Tregs, a recent observation by Loser *et al.*¹² suggested that RANKL expression on keratinocytes may stimulate Langerhans cells to induce Tregs. Interestingly, in our study, the percentages of epidermal CD3⁺ Foxp3⁺ and CD4⁺ Foxp3⁺ cells were significantly smaller in eczematous dermatitis than in psoriasis vulgaris, although the difference in CD25⁺ Foxp3⁺ cells did not differ significantly between these disorders. Animal models in allergic contact dermatitis elucidated the nature of the effector T-cell population in allergic contact dermatitis. Allergic contact dermatitis is primarily mediated by CD8⁺ T cells and is negatively regulated by CD4⁺ T cells.²² In addition, recent studies of nickel allergic contact dermatitis have confirmed that the pathophysiology of allergic contact dermatitis in humans is similar to that of allergic contact dermatitis in mice and involves CD8⁺ effector T cells and CD4⁺ Tregs.²³ In the context of this paradigm, our data are rather surprising; however, haptens stimulate Langerhans cells to induce several costimulatory molecules and pro-inflammatory cytokines.^{24,25} These activated Langerhans cells may suppress the trafficking of Tregs or the induction of Tregs in the epidermis.

A recent study using an *in vitro* photopheresis model suggested that tumour cells in cutaneous T-cell lymphoma can be induced to adopt a Treg phenotype and function.²⁶ Our data demonstrating that the dermal infiltrate of mycosis fungoides contains higher percentages of Tregs than that of psoriasis vulgaris or eczematous dermatitis is consistent with this report, although our study did not discriminate Foxp3⁺ tumour cells

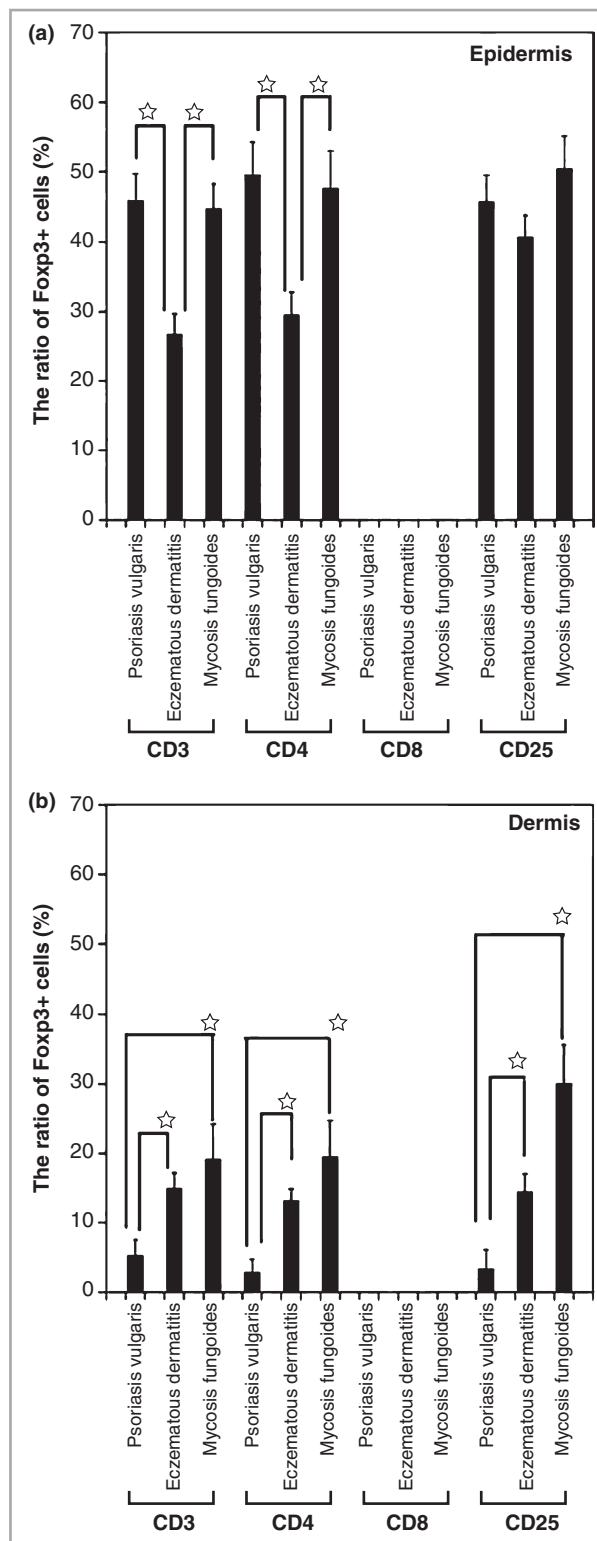


Fig 6. Summary of the percentage of Foxp3+ cells among epidermal or dermal CD3+, CD4+, CD8+ or CD25+ T cells in lesional skin from patients with psoriasis vulgaris, mycosis fungoides and eczematous dermatitis. Three representative fields of each section were selected from the epidermis and from the dermis associated with a dense dermal lymphoid infiltrate. The number of immunoreactive cells was counted using an ocular grid of 1 cm² at a magnification of $\times 400$. The regulatory T cell (Treg) fraction of CD3+, CD4+ or CD25+ cells was defined as the ratio (%) of CD3+ Foxp3+, CD4+ Foxp3+ or CD25+ Foxp3+ cells to the total number of CD3+, CD4+ or CD25+ cells in each field, respectively. The data are expressed as the mean $\pm$ SD of the Treg fractions in each skin disorder. (a) Epidermis; (b) dermis. Stars: $P < 0.05$.

receptor and anti-CD28 Abs in the presence of transforming growth factor- β .^{16,27,28} These so-called adaptive CD4+ CD25+ Tregs exhibit phenotypic and functional characteristics similar to those of natural CD4+ CD25+ Tregs, including the expression of Foxp3.²⁹ The relationship between natural and adaptive CD4+ CD25+ Treg populations is of significant interest. As our study did not examine the cytokines produced by Foxp3+ infiltrating cells, it is uncertain whether they are adaptive Tregs or natural Tregs. In addition, our studies did not examine the dynamics of Tregs in the progression of these three skin disorders, but such a study will be indispensable in the future to understand the role of Tregs in skin.

Acknowledgments

This study was supported in part by the 21st COE program of Tohoku University and by Japan Chemical Industry Association (a Long-range Research Initiative), and in part by a grant-in-aid for scientific research from the Japan Society for the Promotion of Science (17790749).

References

- 1 Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005; **6**:345–52.
- 2 Baecher-Allan C, Hafler DA. Human regulatory T cells and their role in autoimmune disease. *Immunol Rev* 2006; **212**:203–16.
- 3 Gambineri E, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr Opin Rheumatol* 2003; **15**:430–5.
- 4 Viglietta V, Baecher-Allan C, Weiner HL *et al.* Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J Exp Med* 2004; **199**:971–9.
- 5 Kriegel MA, Lohmann T, Gabler C *et al.* Defective suppressor function of human CD4+ CD25+ regulatory T cells in autoimmune polyglandular syndrome type II. *J Exp Med* 2004; **199**:1285–91.
- 6 Lindley S, Dayan CM, Bishop A *et al.* Defective suppressor function in CD4(+)CD25(+) T-cells from patients with type 1 diabetes. *Diabetes* 2005; **54**:92–9.
- 7 Sugiyama H, Gyulai R, Toichi E *et al.* Dysfunctional blood and target tissue CD4+CD25high regulatory T cells in psoriasis:

from Foxp3+ reactive cells. These data may suggest that skin supplies a favourable environment for maintaining Treg phenotypes of tumour as well as normal T cells.

Finally, in addition to natural CD4+ CD25+ Tregs, another species of CD4+ CD25+ Tregs can be derived *in vitro* through treatment of peripheral CD4+ CD25– T cells with anti-T-cell

- mechanism underlying unrestrained pathogenic effector T cell proliferation. *J Immunol* 2005; **174**:164–73.
- 8 Balandina A, Lécart S, Darteville P *et al.* Functional defect of regulatory CD4(+)CD25⁺ T cells in the thymus of patients with autoimmune myasthenia gravis. *Blood* 2005; **105**:735–41.
 - 9 Cao D, Borjesson O, Larsson P *et al.* FOXP3 identifies regulatory CD25bright CD4⁺ T cells in rheumatic joints. *Scand J Immunol* 2006; **63**:444–52.
 - 10 de Kleer IM, Wedderburn LR, Taams LS *et al.* CD4⁺CD25⁺ bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. *J Immunol* 2004; **172**:6435–43.
 - 11 Clark RA, Kupper TS. IL-15 and dermal fibroblasts induce proliferation of natural regulatory T cells isolated from human skin. *Blood* 2007; **109**:194–202.
 - 12 Loser K, Mehling A, Loeser S *et al.* Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. *Nat Med* 2006; **12**:1372–9.
 - 13 Miyara M, Amoura Z, Parizot C *et al.* The immune paradox of sarcoidosis and regulatory T cells. *J Exp Med* 2006; **203**:359–70.
 - 14 Bovenschen HJ, van Vlijmen-Willems IM, van de Kerkhof PC *et al.* Identification of lesional CD4⁺ CD25⁺ Foxp3⁺ regulatory T cells in psoriasis. *Dermatology* 2006; **213**:111–17.
 - 15 Verhagen J, Akdis M, Traidl-Hoffmann C *et al.* Absence of T-regulatory cell expression and function in atopic dermatitis skin. *J Allergy Clin Immunol* 2006; **117**:176–83.
 - 16 Chen S, Ishii N, Ine S *et al.* Regulatory T cell-like activity of Foxp3⁺ adult T cell leukemia cells. *Int Immunol* 2006; **18**:269–77.
 - 17 Siegmund K, Feuerer M, Siewert C *et al.* Migration matters: regulatory T-cell compartmentalization determines suppressive activity in vivo. *Blood* 2005; **106**:3097–104.
 - 18 Suffia I, Reckling SK, Salay G *et al.* A role for CD103 in the retention of CD4⁺CD25⁺ Treg and control of *Leishmania major* infection. *J Immunol* 2005; **174**:5444–55.
 - 19 Rappl G, Kapsokafalou A, Heuser C *et al.* Dermal fibroblasts sustain proliferation of activated T cells via membrane-bound interleukin-15 upon long-term stimulation with tumor necrosis factor- α . *J Invest Dermatol* 2001; **116**:102–9.
 - 20 Nickoloff BJ, Nestle FO. Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. *J Clin Invest* 2004; **113**:1664–75.
 - 21 Krueger JG, Bowcock A. Psoriasis pathophysiology: current concepts of pathogenesis. *Ann Rheum Dis* 2005; **64** (Suppl. 2):30–6.
 - 22 Bour H, Peyron E, Gaucherand M *et al.* Major histocompatibility complex class I-restricted CD8⁺ T cells and class II-restricted CD4⁺ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene. *Eur J Immunol* 1995; **25**:3006–10.
 - 23 Cavani A, Albanesi C, Traidl C *et al.* Effector and regulatory T cells in allergic contact dermatitis. *Trends Immunol* 2001; **22**:118–20.
 - 24 Aiba S, Terunuma A, Manome H *et al.* Dendritic cells differently respond to haptens and irritants by their production of cytokines and expression of co-stimulatory molecules. *Eur J Immunol* 1997; **27**:3031–8.
 - 25 Aiba S, Manome H, Nakagawa S *et al.* p38 mitogen-activated protein kinase and extracellular signal-regulated kinases play distinct roles in the activation of dendritic cells by two representative haptens, NiCl₂ and DNCB. *J Invest Dermatol* 2003; **120**:390–8.
 - 26 Berger CL, Tigelaar R, Cohen J *et al.* Cutaneous T-cell lymphoma: malignant proliferation of T-regulatory cells. *Blood* 2005; **105**:1640–7.
 - 27 Fantini MC, Becker C, Monteleone G *et al.* Cutting edge: TGF- β induces a regulatory phenotype in CD4⁺CD25[–] T cells through Foxp3 induction and down-regulation of Smad7. *J Immunol* 2004; **172**:5149–53.
 - 28 Zheng SG, Wang JH, Gray JD *et al.* Natural and induced CD4⁺CD25⁺ cells educate CD4⁺CD25[–] cells to develop suppressive activity: the role of IL-2, TGF- β , and IL-10. *J Immunol* 2004; **172**:5213–21.
 - 29 Chen W, Jin W, Hardegen N *et al.* Conversion of peripheral CD4⁺CD25[–] naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF- β induction of transcription factor Foxp3. *J Exp Med* 2003; **198**:1875–86.