Original Article

Expression of TIGIT on CD4$^+$ T Cells in Patients with Atopic Dermatitis

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Abstract

The T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) is a coinhibitory receptor mainly expressed on T cells. Although TIGIT plays an important role in various autoimmune diseases, its role in atopic dermatitis (AD) remains unclear. In this study, we examined the expression levels of TIGIT and their association with clinical features in patients with AD. TIGIT expression on $CD4^+$ T cells, central memory T cells, effector memory T cells, and regulatory T cells was determined by flow cytometry. $CD4^+$ T cells exhibited enhanced TIGIT expression in patients with AD compared with healthy individuals. In particular, effector memory T cells and regulatory T cells, but not central memory T cells, exhibited higher TIGIT expression in patients with AD than in healthy individuals. The frequency of $TIGIT^+$ cells among $CD4^+$ T cells was significantly increased in patients with mild AD compared with healthy individuals, while decreased in patients with severe AD. Consistently, the frequency of $TIGIT^+$ cells among $CD4^+$ T cells was negatively correlated with both serum thymus and activation-regulated chemokine levels and IgE levels in patients with AD. Furthermore, TIGIT expression on $CD4^+$ T cells inhibited cell proliferation in patients with AD. These results suggest that TIGIT expression on $CD4^+$ T cells in patients with AD may be increased to suppress chronic cutaneous inflammation. Moreover, TIGIT expression may be impaired in a subset of patients with AD, leading to a deterioration of skin inflammation. Our study may provide new insight into a TIGIT pathway-based therapeutic approach for AD.

Introduction

Atopic dermatitis (AD) is a pruritic inflammatory skin disease characterised by relapsing eczematous skin lesions which affects approximately 15-30% of children and 2-10% of adults.[1] Histologically, AD is characterised by acanthosis and spongiosis of the epidermis with marked infiltration of both $CD4^+$ and $CD8^+$ T cells, but with variable numbers of eosinophils and mast cells within the dermis.[2, 3] A growing body of evidence has demonstrated that AD is a T cell-driven disease.[4] Furthermore, interleukin (IL)-4, IL-5, and IL-13, which are hallmark cytokines of T helper (Th) 2 cells, predominate in early lesions of AD.[5, 6] Th2 cytokines not only promote B cell IgE production, but also decrease the expression of filaggrin, a key structural protein that contributes to the skin barrier and is found in keratinocytes, leading to skin barrier dysfunction.[7] In addition to Th2 cytokines, it has also been indicated that Th17 cytokines, namely, IL-17 and IL-22, are involved in the pathogenesis of AD.[6, 8] Thus, AD is regarded as a Th2/Th17-polarised inflammatory disease.

The T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT), a newly identified coinhibitory receptor, forms a pathway together with CD226 that delivers a positive costimulatory signal.[9, 10] TIGIT and CD226 share ligands, namely, CD155 and CD112, and compete with each other. TIGIT engagement of CD155 and CD112 augments T cell activation, whereas CD226 engagement of CD155 and CD112 suppresses T cell responses. Recent studies have revealed that the TIGIT pathway plays a crucial role in the regulation of autoimmune and inflammatory diseases. Deficiency or blockade of TIGIT augments disease severity in murine models of multiple sclerosis, rheumatoid arthritis, and graft-versus-host disease,[11, 12] while overexpression of TIGIT ameliorates the severity of rheumatoid arthritis in mice.[13] Therefore, we hypothesised that the TIGIT pathway may also play a role in the pathogenesis of AD. In this study, we examined the expression of TIGIT on T cell subsets from patients with AD.

Materials and Methods

Patients and clinical assessment

This study involved 17 patients with AD and 14 healthy individuals. All patients had been diagnosed with AD based on clinical features and had not received any immunosuppressant in the previous 6 months. The Severity Scoring of AD (SCORAD) scale was used to assess disease severity. All participants provided written informed consent before study participation. The study was approved by the Ethics Committee of The Jikei University in accordance with the Declaration of Helsinki.

Cell isolation and flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinised whole blood using Lymphoprep (Axis-Shield PoC As, Oslo, Norway). The isolated PBMCs were resuspended (2×10^6 cells/ml) in complete medium (RPMI 1640; Sigma-Aldrich, St. Louis, MO) containing 10% foetal calf serum (Sigma-Aldrich), 110 U/ml penicillin (Gibco, Auckland, New Zealand), 110 μg/ml streptomycin (Gibco), and 0.3212 mg/ml L-glutamine (Gibco). T cells were phenotyped by flow cytometry (MACSQuant; Miltenyi Biotec, Auburn, CA) using fluorescein isothiocyanate-conjugated CD4 (BioLegend, San Diego, CA), allophycocyanin (APC)/Cy7-conjugated CD8a (BioLegend), APC-conjugated CD45RA (BioLegend), APC/Cy7-conjugated CD45RO (BioLegend), APC-conjugated CD197 (CCR7) (BioLegend), PE/Cy7-conjugated CD25 (BioLegend), PE-conjugated TIGIT (eBioscience, San Diego, CA) and PE-conjugated CD226 (BioLegend). All of these cell suspensions were incubated for 30 min on ice.

For intracellular staining, the cells were fixed and permeabilised with the FoxP3 Staining Buffer Set (Miltenyi Biotec, Auburn, CA) and stained with anti-human APC-conjugated Foxp3 (eBioscience) for 30 min on ice. All analyses were performed using fresh blood samples.

Proliferation assay

CD4 monoclonal antibody-coated microbeads (Miltenyi Biotec) were used to purify $CD4^+$ T cells by positive selection according to the manufacturer's instructions. When necessary, the cells were enriched a second time using a fresh magnetic-activated cell-sorting column to obtain > 95% $CD4^+$ cell purity. Purified $CD4^+$ T cells were labelled with 1.5 M carboxyfluorescein diacetate succinimidyl ester (CFSE) (eBioscience) for 10 min at room temperature in the dark. The labelled cells were resuspended in culture medium and co-cultured with human anti-CD3 and anti-CD28 antibodies in 96-well flat-bottom plates. After 4 days culture, the cells were harvested and stained using anti-human PE-vio770-conjugated CD4 and anti-human APC-conjugated TIGIT, and analysed for CFSE intensities.

Statistical analysis

Statistical analysis was performed using the Mann–Whitney *U* test for the comparison of two groups, the Kruskal–Wallis test and Bonferroni test for multiple comparisons, and the Wilcoxon matched-pairs signed-rank test for paired comparisons. All values are expressed as the mean ± standard error of the mean (SEM). A P value of < 0.05 was considered significant.

Results

TIGIT expression on $CD4^+$ T cells in patients with AD

The characteristics of patients with AD and healthy individuals enrolled in this study are shown in Table 1. We examined the expression of TIGIT on T cells in patients with AD and healthy individuals by using flow cytometry. Both $TIGIT^+$ $CD4^+$ T cell frequency and the mean TIGIT expression levels on $CD4^+$ T cells in patients with AD were significantly elevated compared with those in healthy individuals (37% increase; $P < 0.05$; Figure 1a). The frequency of $TIGIT^+$ $CD8^+$ T cells and mean TIGIT expression levels of $CD8^+$ T cells were not significantly different between patients with AD and healthy individuals (data not shown).

To further determine whether the augmented TIGIT expression on $CD4^+$ T cells differed among $CD4^+$ T cell subsets, we next examined TIGIT expression on $CD4^+CD45RO^+CCR7^-$ effector memory T cells (T_{EM}), $CD4^+CD45RO^+CCR7^+$ central memory T cells (T_{CM}), and $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs). Both the frequencies and mean TIGIT expression on T_{EM} and Tregs were significantly elevated in patients with AD compared with healthy individuals (57% and 42% increase, respectively; $P < 0.01$ for both; Figure 1b, d). By contrast, there were no significant differences in the frequency of $TIGIT^+$ cells and mean TIGIT expression levels of T_{CM} between patients with AD and healthy individuals (Figure 1c). Thus, T_{EM} and Tregs, but not T_{CM}, exhibited augmented TIGIT expression in patients with AD.

TIGIT expression on $CD4^+$ T cells is negatively correlated with disease severity

To assess whether TIGIT expression on $CD4^+$ T cells was associated with disease severity, we next compared TIGIT expression on $CD4^+$ T cells between the patients with severe AD (SCORAD index ≥ 50) and mild AD (SCORAD index < 50). The frequency of $TIGIT^+$ cells was significantly increased in patients with mild AD compared with healthy controls (60% increase; $P < 0.01$; Figure 2). Conversely, the frequency of $TIGIT^+$ cells was significantly decreased in patients with severe AD compared with healthy controls (32% increase; $P < 0.05$). Furthermore, the frequency

of $TIGIT^+$ cells was significantly decreased in patients with severe AD compared with mild AD (58% decrease; $P < 0.01$).

To further evaluate disease severity, we used serum levels of IgE and thymus and activation-regulated chemokine (TARC), a biological marker that reflects AD disease severity. The frequency of $TIGIT^+$ cells on $CD4^+$ T cells was negatively correlated with both serum TARC levels ($P < 0.05$, r = -0.54) and IgE levels ($P < 0.05$, r = -0.28) in patients with AD (Figure 3). These results suggest that the $TIGIT^+$ cell frequency was negatively correlated with disease severity in patients with AD.

TIGIT negatively regulate $CD4^+$ T cell proliferation in patients with AD

Cell proliferation analysis was performed in order to evaluate the relationship between TIGIT expression and the proliferation of $CD4^+$ T cells. After 4 days of stimulation, the proliferation of $CD4^+TIGIT^+$ T cells was significantly decreased in patients with AD compared with healthy individuals (23% decrease; $P < 0.05$; Figure 4). The proliferation of $CD4^+$ and $CD4^+TIGIT^-$ T cells also exhibited decreased proliferation in patients with AD compared with healthy individuals (20% decrease, $P = 0.06$; and 19% decrease, $P = 0.09$), although the difference was not statistically significant. Thus, TIGIT expression on $CD4^+$ T cells might play an inhibitory role in patients with AD.

Discussion

To our knowledge, this is the first study to evaluate the abnormalities of TIGIT expression on $CD4^+$ T cells in patients with AD. We demonstrated here that TIGIT expression on $CD4^+$ T cells was enhanced in patients with AD compared with healthy individuals. In particular, T_{EM} and Tregs, but not T_{CM}, exhibited higher TIGIT expression in patients with AD compared with healthy individuals. The frequency of $TIGIT^+$ cells was significantly increased in patients with mild AD compared with healthy individuals, while decreased in patients with severe AD. The frequency of $TIGIT^+$ cells among $CD4^+$ T cells was consistently negatively correlated with both serum TARC levels and IgE levels in patients with AD. Furthermore, TIGIT expression on $CD4^+$ T cells inhibited cell proliferation in patients with AD. These results suggest that TIGIT expression may be reduced in a subset of patients with AD, which leads to the deterioration of skin inflammation.

TIGIT engagement through CD155 not only inhibits cell proliferation but also the expression of transcription factors T-bet, GATA3, and RORc, which are specific for regulating Th1, Th2, and Th17, respectively.[14, 15] Furthermore, $TIGIT^+FoxP3^+$ Tregs exhibit a higher expression of coinhibitory molecules, such as cytotoxic T lymphocyte-associated antigen 4, programmed death-1, lymphocyte activation gene 3, and T-cell immunoglobulin and mucin domain 3, than do $TIGIT^-FoxP3^+$ Tregs.[16] Therefore, TIGIT expression on $CD4^+$ T cells in patients with AD may be increased to suppress chronic cutaneous inflammation. Consistent with this, TIGIT expression on $CD4^+$ T cells is elevated in patients with rheumatoid arthritis and systemic lupus erythematosus.[17-19] However, TIGIT expression on $CD4^+$ T cells was augmented in patients with mild AD but reduced in those with severe AD, suggesting that TIGIT expression may be impaired in a subset of patients with AD, leading to the deterioration of skin inflammation.

In contrast to our current results, it has also been reported that TIGIT ligation on Tregs induces the expression of fibrinogen-like protein 2, which augments the suppression of Th1 and Th17 cell responses but not Th2 cell responses, leading to a

Th2-polarised condition.[16] Likewise, the blockade of TIGIT inhibits antigen-specific Th2 responses, while it does not affect Th1 and Th17 responses in a murine model of asthma,[20] indicating that TIGIT expression may enhance Th2 responses. Therefore, the role of TIGIT in Th2 responses may be different in each organ and disease. Further studies are required to clarify the role of TIGIT in various inflammatory and autoimmune diseases.

T_{CM} express CCR7, the lymph node-homing chemokine receptor, and migrate from blood to the lymph nodes.[21] By contrast, T_{EM} can migrate to peripheral tissue sites such as the skin, lung, and intestines.[22] In this study, we demonstrated that T_{EM}, but not T_{CM}, exhibited increased TIGIT expression in patients with AD compared with healthy individuals. Although the precise contributions of T_{EM} and T_{CM} to the pathogenesis of AD remain unclear, T_{EM} might be more pathogenic than T_{CM} in AD. Moreover, TIGIT expression could play a role, similar to a control valve, in suppressing excessive inflammation. However, we only examined TIGIT expression on circulating T cells, whereas most memory T cells reside in tissue sites including the skin.[21] The cellular infiltrate in the skin of patients with AD has been reported to consist mostly of memory $CD4^+$ T cells.[23] Additional studies will therefore be required to elucidate TIGIT expression on T cell subsets in the skin of patients with AD.

This study has several potential limitations that should be considered. First, the patient population was relatively small; a larger study is required in order to confirm our results. Second, the precise mechanism by which TIGIT expression is impaired in a subset of AD has not been elucidated. Third, it will be important to examine longitudinal changes of TIGIT expression on $CD4^+$ T cells in patients with AD and to assess their association with disease activity in future studies. Forth, we have not assessed the effects of TIGIT expression on cytokine production of $CD4^+$ T cells. Fifth, it will also be necessary to examine TIGIT expression on $CD4^+$ T cells in patients with other inflammatory skin diseases such as psoriasis and autoimmune bullous diseases. Nevertheless, our findings suggest that TIGIT expression may be

involved in the pathogenesis of AD. Our study may provide new insight into a TIGIT pathway-based therapeutic approach for AD.

Conflicts of interest

The authors have no conflicts of interest.

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None.

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Figure legends

Figure 1 TIGIT expression on $CD4^+$ T cell subsets in patients with AD. The frequency of $TIGIT^+$ cells and the mean TIGIT expression levels on (A) $CD4^+$ T cells, (B) $CD4^+CD45RO^+CCR7^-$ effector memory T cells (T_{EM}), (C) $CD4^+CD45RO^+CCR7^+$ central memory T cells (T_{CM}), and (D) $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs). The control group comprised healthy individuals. Samples from 17 patients with AD and 14 healthy individuals were analysed. Representative dot plots of TIGIT expressing cells on $CD4^+$ T cells, T_{EM}, T_{CM}, and Tregs are shown. The bars indicate the mean (± SEM) values in each group. Significant differences are noted between the sample means: $^{**}P < 0.01$.

Figure 2 The frequency of $TIGIT^+$ cells in $CD4^+$ T cells in patients with mild AD (SCORAD index < 50) and severe AD (SCORAD index ≥ 50). The control group comprised healthy individuals. Samples from 13 patients with mild AD, 4 patients with severe AD, and 14 healthy individuals were analysed. The bars indicate the mean (± SEM) values in each group. Significant differences are noted between the sample means: $^{*}P < 0.05$, $^{**}P < 0.01$.

Figure 3 Correlation of the frequencies of $TIGIT^+$ cells on $CD4^+$ T cells with serum TARC levels and IgE levels in patients with AD. Serum TARC and IgE levels were measured by using an enzyme-linked immunosorbent assay.

Figure 4 The effect of TIGIT expression on the proliferation of $CD4^+$ T cells in patients with AD. Purified $CD4^+$ T cells from healthy individuals and patients with AD were labelled with CFSE and stimulated with human anti-CD3 and anti-CD28 antibodies for 4 days. Samples from 8 patients with AD and 10 healthy individuals were analysed. The percentages of total $CD4^+$, $CD4^+TIGIT^+$, and $CD4^+TIGIT^-$ T cell proliferation are shown as the mean (± SEM). Significant differences between sample means are indicated: $^{*}P < 0.05$.

Table 1. Clinical and laboratory findings in patients with AD compared with healthy individuals

	Patients with AD *n* = 17	Healthy individuals *n* = 14
Age at examination, years (mean ± SD)	38 ± 10	33 ± 6
Male : female	8 : 9	6 : 8
SCORAD, points (mean ± SD)	32.9 ± 18.4	–
Clinical features		
Asthma, no. (%)	4 (24)	–
Allergic rhinosinusitis, no. (%)	15 (88)	–
Laboratory findings		
Blood eosinophil, % (mean ± SD)	8.1 ± 5.0	–
Serum total IgE, IU/ml (mean ± SD)	6902 ± 109	–
Serum TARC levels, pg/ml (mean ± SD)	1510 ± 1620	–

Values were taken at the time of blood examination.

AD, atopic dermatitis; SCORAD, The Severity Scoring of AD; SD, standard deviation;

TARC, thymus and activation-regulated chemokine.

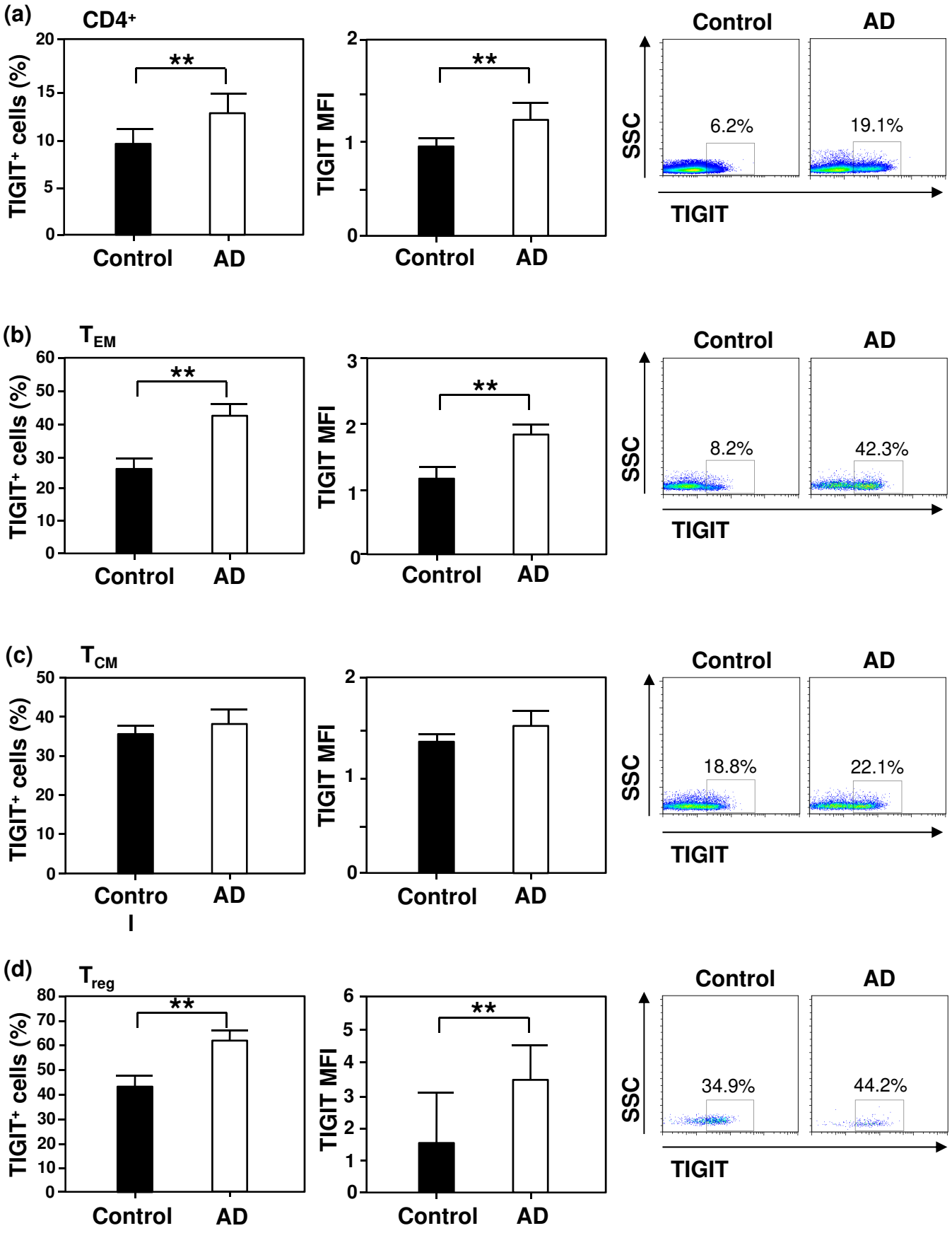

Figure 1
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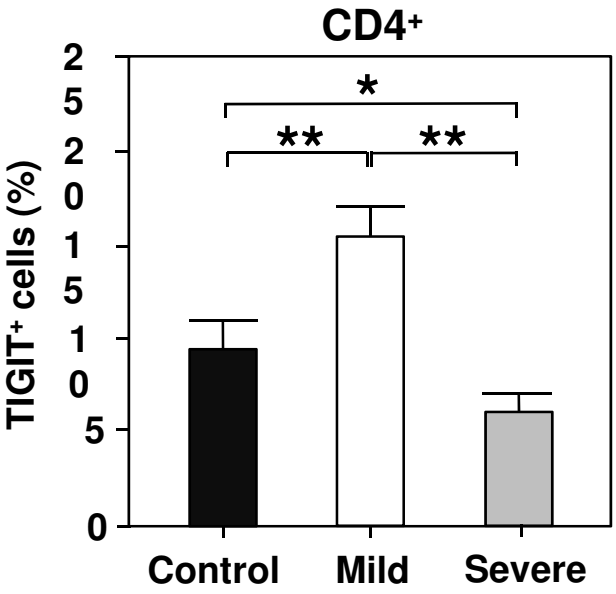

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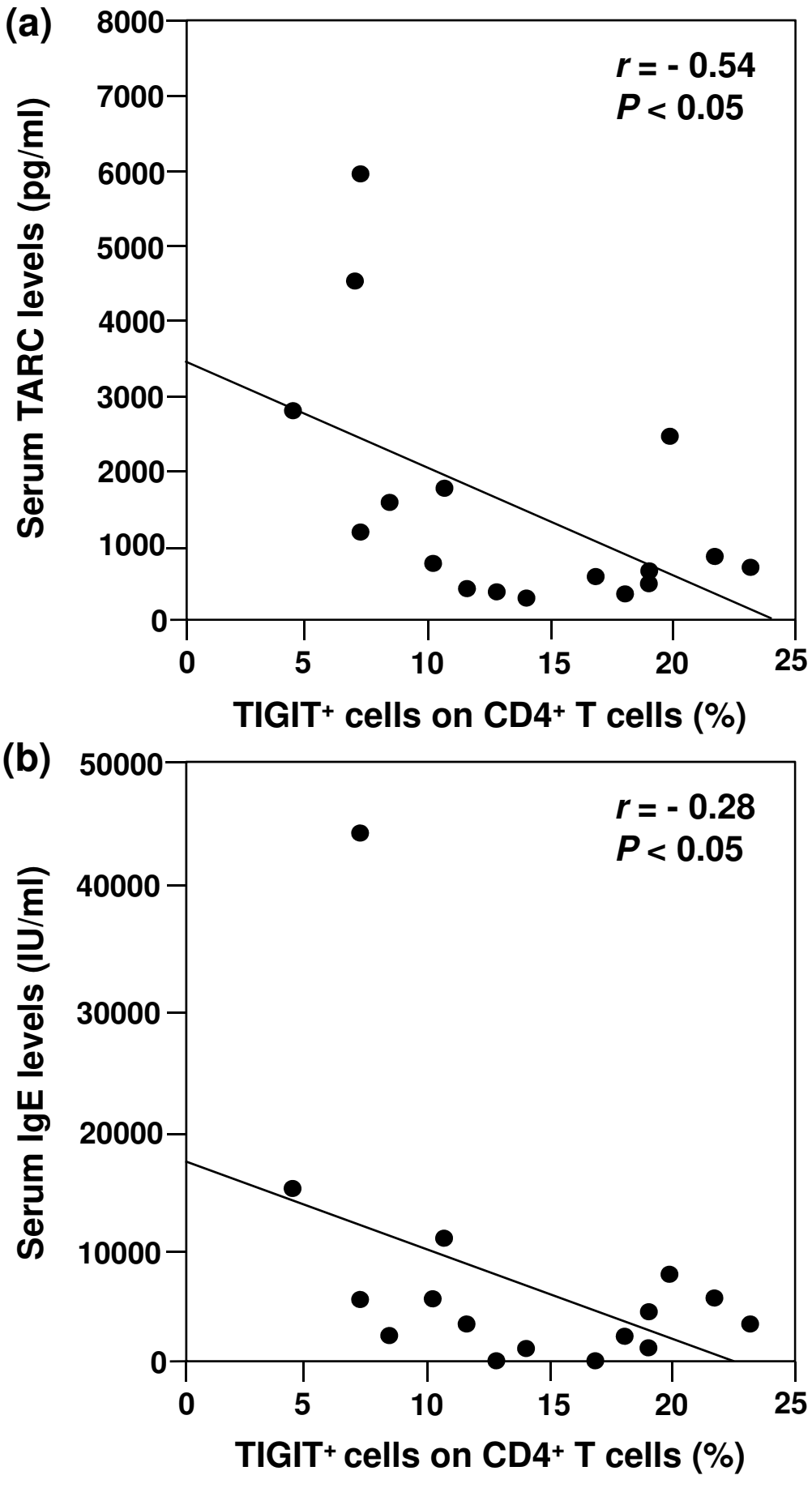

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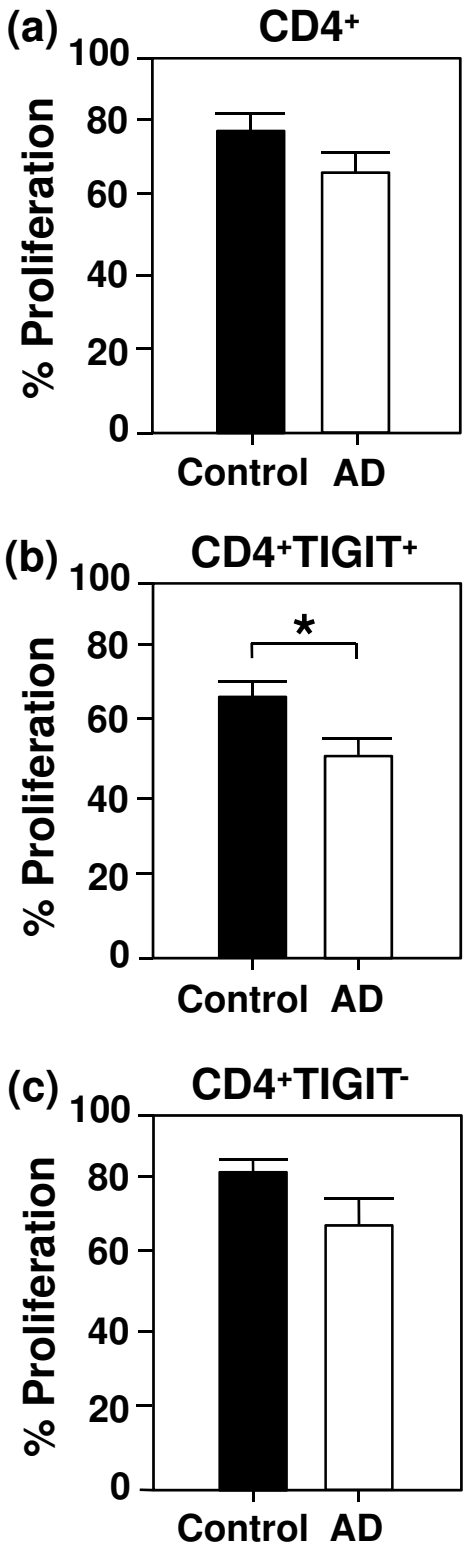

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