Effector regulatory T cells reflect the equilibrium between antitumor immunity and autoimmunity in adult T cell leukemia

Hiroshi Ureshino1, Takero Shindo1, Hiroyoshi Nishikawa2*, Nobukazu Watanabe3, Eri Watanabe¹, Natsuko Satoh³, Kazutaka Kitaura4,5, Hiroaki Kitamura¹, Kazuko Doi6, Kotaro Nagase7, Hiromi Kimura7, Makoto Samukawa8, Susumu Kusunoki8, Masaharu Miyahara9, Tadasu Shin-I¹⁰, Ryuji Suzuki¹, Shimon Sakaguchi2, Shinya Kimura¹

¹Department of Hematology, Respiratory Medicine and Oncology, Saga University School of Medicine, Saga, Japan; ²Experimental Immunology, World Premier International Research Center, Immunology Frontier Research Center, Osaka University, Suita, Japan; ³Laboratory of Diagnostic Medicine, Division of Stem Cell Therapy, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; ⁴Department of Clinical Immunology, Clinical Research Center for Allergy and Rheumatology, Sagamihara National Hospital, Sagamihara, Japan; ⁵Repertoire Genesis, Inc., Ibaraki, Japan; ⁶Department of Dermatology, Karatsu Red Cross Hospital, Karatsu, Japan; ⁷Department of Dermatology, Saga University School of Medicine, Saga, Japan; ⁸Department of Neurology, Kinki University School of Medicine, Sayama, Japan; ⁹Department of Internal Medicine, Karatsu Red Cross Hospital, Karatsu, Japan; ¹⁰BITS Co. Ltd., Tokyo, Japan.

*Current Address:
Hiroyoshi Nishikawa
Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Tokyo, Japan

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Correspondence to:
Takero Shindo, M.D., Ph.D.
5-1-1 Nabeshima, Saga, 849-8501, Japan
Phone: +81-952-34-2366; Fax: +81-952-34-2017
E-mail: takeros@cc.saga-u.ac.jp

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Abstract

The regulatory T cells (Tregs) with the most potent immunosuppressive activity are the effector regulatory T cells (eTregs) with a CD45RA−Foxp3++CCR4+ phenotype. Adult T cell leukemia (ATL) cells often share the Treg phenotype and also express CCR4. Although mogamulizumab, a monoclonal antibody to CCR4, shows marked antitumor effects against ATL and peripheral T-cell lymphoma, concerns have been raised that it may induce severe autoimmune immunopathology by depleting eTregs. Here we present two ATL cases that responded to mogamulizumab, but developed a severe skin rash and autoimmune brainstem encephalitis. Deep sequencing of the TCR revealed that ATL cells and naturally occurring Tregs within the cell population with a Treg phenotype can be clearly distinguished according to CADM1 expression. The onset of skin rash and brainstem encephalitis was coincident with eTreg depletion from the peripheral blood, whereas ATL relapses were coincident with eTreg recovery. These results imply that eTreg numbers in the peripheral blood sensitively reflect the equilibrium between antitumor immunity and autoimmunity, and that mogamulizumab might suppress ATL until the eTreg population recovers. Close monitoring of eTreg numbers is crucial if we are to provide immunomodulatory treatments that target malignancy without severe adverse events.
Introduction

Human regulatory T cells (Tregs) suppress immune reactions, including autoimmunity and antitumor immunity; therefore, inhibiting Treg activity is becoming an important focus of cancer treatment (1). Tregs are classified into several subtypes, the most suppressive being effector Tregs (eTregs), which express a CD45RA Foxp3++ phenotype and the chemokine receptor CCR4 (2, 3). Adult T cell leukemia (ATL) is a therapy-resistant hematologic malignancy with a poor prognosis (4, 5). ATL occurs in about 5% of human T-lymphotropic virus type-1 (HTLV-1) carriers, and most ATL cells express CCR4 (6, 7). ATL cells often share the Treg phenotype, i.e., CD3+CD4+CD25+Foxp3+ (8, 9). Although ATL cells may be defined by their expression of CADM1 (10, 11), the overlap and differences between Tregs and ATL cells remain unclear.

Mogamulizumab is a defucosylated and humanized monoclonal antibody to CCR4 that shows marked effects against ATL (12) and peripheral T cell lymphoma (13). It works directly by depleting tumor cells and indirectly by increasing antitumor immunity through eTreg depletion via antibody-dependent cellular cytotoxicity (ADCC) (3). However, it sometimes causes adverse events, including a skin rash or Stevens-Johnson syndrome (14, 15), which may be attributed to Tregs depletion. However, the association between therapeutic effects, adverse events, and Tregs depletion remains unclear.

Here, we report two mogamulizumab-treated ATL patients who developed severe skin rash and brainstem encephalitis. Deep sequence analysis of the TCR
repertoire revealed that CADM1 expression specifies minimal residual ATL cells within the cell population with a Treg phenotype. While the immunopathology was closely associated with eTreg depletion, ATL relapsed as eTregs recovered. Despite the fact that mogamulizumab suppresses ATL, it should be noted that it can induce serious immunopathology by depleting eTregs, and that it might suppress ATL until eTreg recovery.
Materials and methods

Peripheral blood mononuclear cells (PBMCs) were collected from the patients and sorted into specific cell fractions using a FACSARia cell sorter (Becton Dickinson, Franklin Lakes, NJ). Sequencing of the TCRα/β was performed at Repertoire Genesis Incorporation (Osaka, Japan) using the unbiased gene amplification method with Adaptor-Ligation PCR (Supplementary Table 1). About $10^3$–$10^4$ valid reads were generated (Supplementary Table 2). Bioinformatics analysis was then performed using the repertoire analysis software, Repertoire Genesis (RG), provided by Repertoire Genesis Incorporation (Osaka, Japan). RG assigns TRV and TRJ alleles to queries and then generates CDR3 sequences, finally aggregating their combination patterns. Out-of-frame sequences were excluded from the analyses. The details of these analyses have been described previously (16). All the studies using patient samples were performed in accordance with the guidelines set out in the Declaration of Helsinki and were approved by the institutional review board.
Case presentations and results

Case 1: A 77-year-old male with acute ATL received mogamulizumab. Although ATL cells immediately disappeared from the peripheral blood, he developed severe skin eruptions that required long-term corticosteroid therapy. Histopathological examination of the skin lesions revealed a marked dermal cellular infiltrate comprising mainly CD8+ T cell–dominant lymphocytes and some eosinophils, without ATL cells (Supplementary Fig. S1A–K). A small percentage of CD3dimCD4+CADM1+CD7– cells (P3 cells) still remained in the peripheral blood on day 234 after starting mogamulizumab (Fig. 1A). TCRα repertoire analyses of the sorted cells revealed that these cells were exclusively clonal ATL cells: clone 1, TRAV9-2/J31, with CDR3 sequence CAPKNNARLMF and clone 2, TRAV13-1/J4, with CDR3 sequence CAAIPWGYNKLIF (Fig. 1A). While the skin eruption was active, CD3+CD4+CD45RA–Foxp3++ eTregs almost disappeared (Fig. 1B and C: day 213). When the eTregs recovered, the ATL cell population (CD4+CADM1+CD7– cells) gradually increased (Fig. 1B: days 282 and 325). The severity of the skin eruption was closely associated with the percentage of eTregs (Fig. 1C). The eruption was mild on day 192 (%eTreg 2.12%) but became severe on day 213 (%eTreg 0.55), before resolving on day 282 (%eTreg 5.93). Notably, the percentage of ATL cells spontaneously fluctuated from day 325 through day 438, which might reflect transient anti-ATL immunity. However, his ATL clinically relapsed as multiple mediastinal lymphadenopathy, which required further treatment on day 430. This time, mogamulizumab had little effect. Following several courses of cytotoxic chemotherapy, the patient died on day 644. Simultaneous staining for Foxp3 and CADM1 revealed that
the Foxp3⁺ cell population contained both CADM1-positive and -negative cells; the former gradually becoming predominant during relapse (Fig. 1D). TCRα/β and CDR3 sequencing of CADM1-positive and -negative CD3⁺CD4⁺CD25⁺⁺ cells collected on day 417 revealed that CADM1-positive cells exclusively contained one of the two ATL clones (Fig. 1A and E). Though CADM1-negative cells were predominantly TRAV9-22/J22 (Fig. 1E), they had diverse CDR3 sequences (Fig. 1E) and TRBV/J patterns (Fig. 1F), meaning that they were not clonal. These results show that, even among cells with the Treg phenotype, ATL and non-ATL cells can be distinguished according to CADM1 expression.

**Case 2:** A 66-year-old male with acute ATL received mogamulizumab. Despite the rapid disappearance of the ATL cells from the peripheral blood, he developed a severe skin rash on the trunk (Fig. 2A). Skin biopsy and immunohistochemical staining revealed both CD4 and CD8 T cell infiltration of the dermal layer, but no ATL or Foxp3⁺ cells were detected (Supplementary Fig. 2A–F). At that time, Foxp3⁺ Tregs (especially CD3⁺CD4⁺CD45RA⁻Foxp3⁺⁺ eTregs) were depleted in the peripheral blood (Fig. 2C: days 121 and 149 after mogamulizumab administration).

He also developed neurological symptoms, including ptosis, diplopia, and hearing loss. Magnetic resonance imaging (MRI) of the brain on day 149 revealed a high-intensity area in the brainstem on diffusion-weighted images and fluid attenuated inversion recovery images (FLAIR) (Fig. 2B: day 149). Tests of the cerebrospinal fluid revealed a mild increase in protein levels (62 mg/dL), but an absence of ATL cells. Anti-ganglioside GM2 IgM was detected in the serum, and intrathecal administration of
methotrexate and cytarabine did not improve the neurological symptoms; therefore, he was diagnosed with autoimmune brainstem encephalitis. After steroid pulse and high-dose immunoglobulin therapy followed by prednisolone administration, the neurological symptoms completely resolved and the high-intensity area on brain MRI disappeared (Fig. 2B: day 219).

Two months later, he developed progressive drowsiness. Brain MRI revealed a broad high-intensity area in the right frontal and temporal lobes on FLAIR; no abnormal signals were detected in the brainstem (Fig. 2B: day 270). Steroid pulse and high-dose immunoglobulin therapy did not improve the symptoms, and biopsy of the frontal lobe revealed diffuse infiltration by atypical CD3+CD4+CD25+ lymphocytes (Supplementary Fig. S2G–L) with monoclonal integration of HTLV-1; therefore, a diagnosis of ATL with central nervous system (CNS) involvement was made. Notably, eTregs in the peripheral blood had recovered at that time (Fig. 2C: day 275) and Foxp3+ cells were focally detected in the brain (Supplementary Fig. 2M). The CD4+Foxp3+ T cells in peripheral blood were negative for CADM1 (Fig. 2C: day 275), indicating that they were not ATL cells, but naturally occurring Tregs. High-dose methotrexate/cytarabine and mogamulizumab ameliorated the symptoms, and both the abnormality detected on MRI and the CD4+CD45RA-Foxp3++ cells in the peripheral blood rapidly disappeared (Fig. 2C: day 310). At the time of the study, the patient showed no signs of ATL relapse.
Discussion

Based on our sequencing and phenotypic analyses of these two patients over time, we can come to some conclusions about the overlap and differences between ATL cells with the Treg phenotype and non-leukemia “true” eTregs. As these two populations share the same phenotype of CD3⁺CD4⁺CD25⁺Foxp3⁺, it is actually difficult to discriminate them. Given that ATL cells share the clonal TCR rearrangements, deep sequencing analysis of TCRα/β chains and CDR3 diversity confirmed that the CD3⁺CD4⁺CADM1⁺CD7⁻ cells were exclusively ATL cells. Among cells with the Treg phenotype only the CADM1⁺ cells shared the same clonality as ATL cells. The data suggest that ATL cells with the Treg phenotype have a different cellular origin from that of naturally occurring Tregs, and that transition between the two subsets is unlikely. CADM1-negative CD3⁺CD4⁺CD25⁺ cells appeared clonal in terms of Vα and Jα chain combinations (TRAV9-2/J22) (Fig. 1E), but not in terms of the β chain (Fig. 1F); the meaning of this remains unclear.

The frequency of eTregs was closely associated with adverse events and ATL relapses. Depletion of eTregs was coincident with severe skin rash and autoimmune brainstem encephalitis, suggesting that depletion of eTregs causes severe autoimmunity. On the other hand, eTregs recovery was coincident with ATL relapse. In addition, at the time case 1 relapsed, CADM1⁻Foxp3⁺ cells (naturally occurring Tregs) re-appeared first, followed by the re-appearance of CADM1⁺Foxp3⁺ cells (ATL cells), which then gradually became dominant (Fig. 1D). These observations raise a hypothesis that mogamulizumab suppresses ATL until eTregs recovery, which weakens anti-ATL
immunity and results in relapse of ATL.

Treg deficiency induces a severe autoimmune pathology called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, and X-linked) syndrome (17, 18), whereas abundant Tregs in tumor tissues are associated with a poor prognosis (19, 20); however, it is unclear whether the percentage of Tregs in the peripheral blood has an inverse impact on immunopathology (21-23). This may be because the Foxp3⁺ cell population contains some non-Tregs cells (CD45RA⁺Foxp3dim cells) that do not have suppressive activity (2). We found that the depletion and recovery of eTregs in the peripheral blood, but not that of the whole Treg population, were coincident with adverse events and ATL relapse, respectively.

In conclusion, the eTreg population reflects the equilibrium between antitumor immunity and autoimmunity. Currently, many monoclonal antibodies that increase antitumor immunity (including immune checkpoint blockers) are approved for clinical use (24). Although these reagents enable deep suppression of Tregs or enhancement of antitumor immunity, they may increase the risk of severe autoimmune disease. To optimize the effects (and avoid serious adverse events) of the currently available monoclonal antibodies that modulate antitumor immunity, close observation and analyses of the correlation between eTreg numbers, antitumor immunity, and autoimmunity are warranted.
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References


Abbreviations

eTreg, effector regulatory T cell
ATL, adult T cell leukemia
HTLV-1, human T-lymphotropic virus type-1
ADCC, antibody-dependent cellular cytotoxicity
FLAIR, fluid attenuated inversion recovery images
MRI, magnetic resonance imaging
IPEX; immune dysregulation, polyendocrinopathy, enteropathy, and X-linked
**Figure legends**

**Figure 1. Clinical course of Case 1.**

(A) TCRα V and J assignment and sequencing of the CDR3 region from whole PBMCs on day 234 after the start of mogamulizumab therapy (upper row) and sorted cells based on high-resolution flow cytometry (lower row). CADM1 CD7+ cells (P1: 64.9%), CADM1dimCD7+ cells (P2: 6.0%), CADM1+CD7- cells (P3 3.9%), and CADM1dimCD7- cells (P4 6.2%) were sorted from the total CD4+ T cell population before analysis. Regarding TCR repertoire analysis, the upper 3D graphs show TRA V/J clones according to their percentage, and the lower tables show the ten major clones in each cell populations. The two columns and bars highlighted in yellow (TRA V9-2/J31) and brown (TRA V13-1/J4) indicate ATL clones. (B) High-resolution flow cytometric detection of eTregs (upper row: CD45RA-Foxp3++) and ATL cells (middle and lower rows: CD3dimCADM1+CD7-) among the CD4+ T cell population in peripheral blood. Numbers represent the percentage of eTreg cells (upper row), CD4+ T cells (middle row), and CADM1+CD7- cells (lower row). (C) Time course of skin eruptions. (D) Flow cytometric analysis of CADM1-positive and -negative cells within the CD3+CD4+Foxp3+ cell population. (E and F) TCRα/β V and J assignment and sequencing of the CDR3 region in sorted CADM1-positive and -negative cells with the Treg phenotype (CD3+CD4+CD25+). Days are counted from start of mogamulizumab treatment.

**Figure 2. Clinical course of Case 2.**
Sequential emergence of symptoms followed by improvement: ptosis, diplopia, hearing loss, skin rash, and drowsiness. Administration of therapeutic reagents is also shown: mogamulizumab (Moga), prednisolone (PSL), methylprednisolone (mPSL), intravenous immunoglobulin (IVIG), and methotrexate/cytarabine (MTX/AraC). (B) Representative magnetic resonance images (MRI) of the brain. DW images are shown in the upper row and FLAIR images in the lower row. The high-intensity area in the right frontal lobe on the day 314 DW image indicates hematoma after the biopsy. (C) The expression of CD45RA, Foxp3, CADM1, and CD7 by peripheral blood CD3^+CD4^+ T cells was repeatedly analyzed by flow cytometry. In the upper row, the numbers indicate the percentage of CD45RA^+Foxp3^+++ cells (eTregs). In the lower row, CD7^+CADM1^+ cells represent ATL cells. Days are counted from start of mogamulizumab treatment.
Figure 2

A

ptosis/diplopia

hearing loss

skin rash

drowsiness

PSL

Days after Mogamulizumab

21 50 80 121 149 178 190 219 270 314 340

Moga

mPSL

IVIG

MTX/AraC

B

Day 149  Day 161  Day 178  Day 219  Day 270  Day 314  Day 344

DWI

FLAIR

C

Day 121  Day 149  Day 163  Day 191  Day 254  Day 275  Day 310

CD45RA

1.68%

1.08%

0.27%

0.39%

1.90%

16.0%

1.41%

Foxp3

CD7

99.0%

1.0%

CD7

98.9%

1.1%

CADM1
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