Effector Regulatory T Cells Reflect the Equilibrium between Antitumor Immunity and Autoimmunity in Adult T-cell Leukemia


Abstract

The regulatory T cells (Treg) with the most potent immunosuppressive activity are the effector Tregs (eTreg) with a CD45RA<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>CCR4<sup>+</sup> 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 case reports for two patients with ATL who responded to mogamulizumab but developed a severe skin rash and autoimmune brainstem encephalitis. Deep sequencing of the T-cell receptor 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. Cancer Immunol Res; 4(8); 644–9. ©2016 AACR.

Introduction

Human regulatory T cells (Treg) 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 (eTreg), which express a CD45RA<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>CCR4<sup>+</sup> 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<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> (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 (3). However, it sometimes causes adverse events, including a skin rash or Stevens–Johnson syndrome (14, 15), which may be attributed to Treg depletion. However, the association between therapeutic effects, adverse events, and Treg depletion remains unclear.

Here, we report on two mogamulizumab-treated ATL patients who developed severe skin rash and brainstem encephalitis. Deep sequence analysis of the T-cell receptor (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 eTreg recovered. Despite the fact that mogamulizumab suppresses ATL, it can induce serious...
immunopathology by depleting eTregs and it might suppress ATL until eTreg recovery.

Materials and Methods

Peripheral blood mononuclear cells (PBMC) were collected from the patients and sorted into specific cell fractions using a FACSARia cell sorter (Becton Dickinson). Sequencing of the TCRβ was performed at Repertoire Genesis Incorporation using the unbiased gene amplification method with Adaptor-Ligation PCR (Supplementary Table S1). About 10⁶ to 10⁷ valid reads were generated (Supplementary Table S2). Bioinformatics analysis was then performed using the repertoire analysis software, Repertoire Genesis (RG), provided by Repertoire Genesis Incorporation. 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 man 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. Histopathologic examination of the skin blood, he developed severe skin eruptions that required long-term corticosteroid therapy. Histopathologic examination of the skin revealed a marked dermal cellular infiltrate comprising mainly CD8+ T-cell–dominant lymphocytes and some eosinophils, without ATL cells (Supplementary Fig. S1A–S1K). A small percentage of CD3dimCD4+CDAD1+CD7− cells (P3 cells) still remained in the peripheral blood on day 234 after the patient started mogamulizumab (Fig. 1A). TCRβ repertoire analyses of the sorted cells revealed that these cells were exclusively clonal ATL cells: clone 1, TRAV9-2/J31, with CDR3 sequence CAPKNARLMF; and clone 2, TRAV13-1/J4, with CDR3 sequence CAAPGWYNNKLIF (Fig. 1A). While the skin eruption was active, CD3+CD4+CD45RA+CADM1+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 235 through day 438, which might reflect transient anti-ATL immunity. However, the patient's 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, with the former gradually becoming predominant during relapse (Fig. 1D). TCRβ 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). Although 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 man 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. S2A–S2F). 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). The patient also developed neurologic symptoms, including ataxia, diplopia, and hearing loss. 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 neurologic symptoms; therefore, the patient was diagnosed with autoimmune brainstem encephalitis. After steroid pulse and high-dose immunoglobulin therapy followed by prednisolone administration, the neurologic symptoms completely resolved and the high-intensity area on brain MRI disappeared (Fig. 2B: day 219). Two months later, the patient 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–S2L) with monoclonal integration of HTLV-1; therefore, a diagnosis of ATL with central nervous system 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. S2M). 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 between them. Given that ATL cells share the clonal TCR rearrangements, deep sequencing analysis of TCRβ 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 V_α and J_α chain combinations (TRAV9-2/J22; Fig. 1E), but not in terms of the β chain (Fig. 1F); the meaning of this observation remains unclear.

Figure 1.
Clinical course of case 1. A, TCR_α V and J assignment and sequencing of the CDR3 region from whole PBMCs on day 234 after the start of mogamulizumab therapy (top row) and sorted cells based on high-resolution flow cytometry (bottom row). CADM1^-CD7^+ cells (P1: 64.9%), CADM1^dim-CD7^+ cells (P2: 6.0%), CADM1^+CD7^- cells (P3 3.9%), and CADM1^dim-CD7^- cells (P4 6.2%) were sorted from the total CD4^+ T-cell population before analysis. Regarding TCR repertoire analysis, the upper 3D graphs show TRAV/J clones according to their percentage, and the lower tables show the ten major clones in each cell population. The two columns and bars highlighted in yellow (TRAV9-2/J31) and brown (TRAV13-1/J4) indicate ATL clones. B, high-resolution flow cytometric detection of eTregs (top row: CD45RA^-CD25^-Foxp3^++) and ATL cells (middle and bottom rows: CD3^dim-CD7^-) among the CD4^+ T-cell population in peripheral blood. Numbers represent the percentage of eTreg cells (top row), CD4^+ T cells (middle row), and CADM1^-CD7^- cells (bottom 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, TCR_α/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. A, 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 MRI of the brain. Diffusion-weighted images (DWI) are shown in the top row, and FLAIR images in the bottom 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 top row, the numbers indicate the percentage of CD45RA⁻Foxp3⁺⁺ cells (eTregs). In the bottom row, CD7⁻CADM1⁺ cells represent ATL cells. Days are counted from start of mogamulizumab treatment.
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 autoimmune disease. 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) reappeared 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 eTreg 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-Treg 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.

Disclosure of Potential Conflicts of Interest

S. Kimura reports receiving honoraria for service on the speakers bureau for Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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References


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