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Defective Regulatory T Cells In Patients with Severe Drug Eruptions: Timing of the Dysfunction Is Associated with the Pathological Phenotype and Outcome

Ryo Takahashi,1,2* Yoko Kano,† Yoshimi Yamazaki,† Momoko Kimishima,† Yoshiko Mizukawa,† and Tetsuo Shiohara*†

Toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS) represent two ends of a spectrum of severe drug eruptions: DIHS is unique in that severe epidermal damage seen in TEN is absent, sequential reactivations of herpesviruses occur, and autoimmunity often ensues. To investigate whether changes in regulatory T (Treg) cell function would contribute to variability in the clinical manifestations, we examined the frequency, phenotype, and function of Treg cells both during the acute stage and again long after clinical resolution of both diseases. Dramatic expansions of functional Treg cells were found in the acute stage of DIHS. In contrast, Treg function was profoundly impaired in TEN, although present in normal frequency. Skin homing addresses were more preferentially expressed on Treg cells in DIHS than in TEN. Indeed, Treg cells were more abundantly present in the skin lesions of DIHS. Surprisingly, Treg cells contracted upon resolution of DIHS became functionally deficient, whereas their functional defects in TEN were restored upon recovery. These findings indicate that a transitory impairment in their function during the acute stage of TEN may be related to severe epidermal damage, while a gradual loss of their function after resolution of DIHS may increase the risk of subsequently developing autoimmune disease. The Journal of Immunology, 2009, 182: 8071–8079.

Drugs often induce various cutaneous adverse reactions of different severity, ranging from simple, uncomplicated papulomacular to potentially fatal, severe eruptions, such as Stevens-Johnson syndrome (SJS)3 and toxic epidermal necrolysis (TEN), in susceptible patients. Although many factors that cause variability in the clinical course have been suggested, it remains unknown which factors are predominantly involved in the process. Because the most prevalent severe drug eruptions are thought to be mediated by drug-reactive T cells (1–4), the phenotype and functions of these effector T cells are likely to determine the clinical picture of the disease. However, investigators need to be made aware of the alternative view that severe drug eruptions are due to a dysbalance of the immune system caused by excessive activation of effector T cells and an inadequately low function or number of T cells that can limit immunopathology. In this regard, regulatory T (Treg) cells are most likely candidates to search for inadequate down-regulation in severe drug eruptions. Indeed, Azukizawa et al. (5) reported that in an animal model of TEN Treg cells can prevent experimentally induced epidermal injury mimicking TEN (6), although the therapeutic effect cannot be observed. Thus, drug-induced immunopathology is likely to be subject to control by Treg cells. To date, however, data are not available on the presence and function of Treg cells in TEN: the degree to which Treg cells truly played a protective role during the actual disease process leading to severe immunopathology is largely unknown.

Much less attention has been focused on the role of Treg cells in the pathogenesis of drug-induced hypersensitivity syndrome (DIHS) or drug rash with eosinophilia and systemic symptoms representing another end of a spectrum of severe drug eruptions: this syndrome is characterized by delayed onset, multigain involvement, sequential reactivations of various latent herpesviruses (7–9), and hypogammaglobulinemia (10) during the active phase and autoimmune manifestations occurring as short-term or long-term sequelae of the disease, such as type 1 diabetes mellitus (11), autoimmune thyroiditis and systemic sclerosis-like manifestations (12), findings never reported in TEN. In view of a large body of evidence indicating the roles of viruses (13) and Treg cells (14) in several autoimmune diseases, this syndrome offers a unique opportunity to encompass viral infections, drug eruptions, and the subsequent development of autoimmune diseases. Given the capacity of Treg cells to prevent organ-specific tissue injury (15) and autoimmune diseases (14), we can hypothesize that Treg cells play a critical role in the disease process of the acute stage of both diseases.

In this regard, 26 years ago, Dosch et al. (16) provided interesting insight into the workings of hypogammaglobulinemia observed in patients with DIHS. They described an increased frequency of circulating suppressor T cells capable of inhibiting Ig production in these patients with transient Ab deficiency. Based on
this finding and our recent observation that in vitro activation of patients’ lymphocytes with the relevant drug resulted in profoundly decreased responsiveness in the acute stage but not in the resolution stage (17), we sought to compare the frequency and function of Treg cells in the peripheral blood at different time points after onset between patients with DIHS and those with TEN induced by the same drugs. Our study demonstrates that Treg cells with suppressive function were expanded at the acute stage of DIHS and that contraction of the Treg cell subset occurred coincident with resolution of the disease. In contrast, onset of TEN was associated with a functional defect of Treg cells, but this defect was eventually restored after clinical resolution. However, in patients with DIHS, a functional defect in the Treg cell population became evident upon clinical resolution despite their normal frequencies before the development of autoimmunity.

Materials and Methods

Subjects

Three groups of patients were included in this study: patients with DIHS (n = 12), patients with TEN (n = 11), patients with maculopapular drug eruption (n = 5), and healthy controls (n = 10). DIHS is characterized by high fever, a widespread erythematous eruption, lymphadenopathy, leukocytosis with atypical lymphocytosis and/or eosinophilia, and liver dysfunction (18); the diagnosis of DIHS was made based on the criteria established by a Japanese consensus group. In all patients with DIHS, HHV-6 reactivation as evidenced by the rise in serum HHV-6 IgG titers and HHV-6 DNA levels in the blood occurred commonly 2–3 wk after the onset of the illness. Criteria for the diagnosis of TEN were high fever, severe mucocutaneous lesions, and detachment of epidermal sheets above 30% of the body surface area and patients with areas of epidermal detachment between 10 and 30% were initially defined as SJS/TEN overlap syndrome (19). In this study, however, patients with overlap SJS/TEN were included into a “TEN” category, because there were no significant differences between the two in the frequency and function of Treg cells. The ages of patients ranged from 14 to 74 years (mean age, 46.8 ± 4.7 years in DIHS; 49.1 ± 5.0 years in TEN; and 45.2 ± 9.7 years in maculopapular drug eruption). Informed consent was obtained from each patient. These studies were approved by the review board at Kyorin University and followed the guidelines for the ethical conduct of human research. The causative drugs, most of which were either carbamazepine (CBZ) or phenobarbital (PB), were withdrawn when the diagnosis of DIHS, TEN, or maculopapular drug eruption was made.

Blood and serum samples were obtained from these patients on or near the day of the initial presentation before starting treatment, and additional samples were subsequently obtained from these patients on a monthly basis. A late follow-up sample (>6 mo after onset) was also collected in patients with DIHS and those with TEN after withdrawal of immunosuppressive agents for the treatment. For these studies, healthy control subjects, matched for sex and age, were selected for comparisons of frequencies, phenotype, and functions of Treg cells (n = 10; mean age, 40.6 ± 5.2). Biopsy specimens were obtained from skin lesions in each patient near the day of the initial presentation before starting treatment.

Abs and reagents

mAbs to human CD4 (SK3), CD8 (Leu-2a), CD25 (2A3), CCR4 (1G1), cutaneous lymphocyte-associated Ag (CLA; HECA-452), the isotype controls to these Abs, and 7-amino-actinomycin D were purchased from BD Biosciences. Goat F(ab’)_2 anti-human IgG Fc was purchased from...
FIGURE 2. Expression of skin-homing addressins on Treg cells expanded at the acute stage of DIHS. A, Representative flow cytometry dot plots showing the expression of Foxp3, CCR4, and ESL in CD4⁺ T cells in DIHS, TEN, and healthy controls. B, The mean frequency of Foxp3⁺ CD4⁺ Treg cells coexpressing CCR4 or ESL and ESL⁺ CCR4⁺ or ESL⁺ CLA⁺ CD4⁺ T cells in the acute and resolution stages of DIHS, TEN, and healthy controls. Results are expressed as the mean ± SEM. DIHS (acute stage, n = 8; resolution stage, n = 6), TEN (acute stage, n = 4; resolution stage, n = 5), and healthy controls (n = 10) (Student’s t test). C, Preferential expression of Foxp3 in the CCR4⁺ ESL⁺ fraction obtained at the acute stage of DIHS. Purified CD4⁺ T cells were stained by CCR4, ESL, and Foxp3 and then analyzed by FACS. Nine gates were set based on the intensity of ESL and CCR4 expression. The percentage of Foxp3⁺ cells differentially expressing ESL and CCR4 is shown in the gated subsets indicated. Results of one representative experiment from three independent experiments are shown.
from the addition of CD4\(^+\) T cells to the culture.

**Cell isolation from peripheral blood**

PBMCs were prepared by density gradient separation (Lymphoprep; Axis-Shield) of peripheral blood of healthy donors, DIHS patients, and TEN patients. CD4\(^+\) T cells were isolated using magnetic beads (CD4 T cell isolation kit II; Miltenyi Biotec). CD4\(^+\) T cells thus isolated were incubated with anti-CD4-allophycocyanin and anti-CD25-FITC Abs, and subpopulations CD4\(^+\)CD25\(^+\) and CD4\(^+\)CD25\(^-\) of CD4 T cells were isolated by using FACSsaria (BD Biosciences).

To prepare T cell-depleted APCs, CD3\(^+\) T cells were depleted from PBMCs using CD3 magnetic beads (Miltenyi Biotec) according to the manufacturer’s recommendations, and then the T cell-depleted APCs were incubated with mitomycin C (Kyowa Hakko Kogyo) at 37°C for 45 min. To prepare T cell-depleted PBMCs for cytokine assay, CD25\(^+\) T cells were depleted from PBMCs using CD25 magnetic beads (Miltenyi Biotec) according to the manufacturer’s recommendations.

**T cell proliferation assay**

FACS-sorted CD4\(^+\)CD25\(^+\) effector T cells (3,000 cells/well) were cocultured with FACS-sorted Treg (CD4\(^+\)CD25\(^-\)) cells (3,000 cells/well) in the presence of allogeneic APCs; 25,000 cells/well obtained from healthy adult volunteers in U-bottom 96-well plates (Corning) as described previously (20–22). Cells were also cocultured at different Treg:effector cell ratios of 0.25:1 and 0.5:1. The cells were stimulated with 0.1 μCi of \(^{3}H\)thymidine (GE Healthcare) was added to each well. The cells were incubated with anti-CD4-allophycocyanin and anti-CD25-FITC Abs, and subpopulations CD4\(^+\)CD25\(^+\) and CD4\(^+\)CD25\(^-\) of CD4 T cells were isolated by using FACsaria (BD Biosciences).

**Flow cytometric analysis**

To simultaneously detect surface chemokine receptors and intracellular Foxp3 expression, fluorescence-conjugated anti-CD4-FITC, anti-chemokine receptor Abs, and 7-aminoactinomycin D was added to PBMCs and incubated for 30 min at room temperature. To simultaneously detect surface E-selectin ligand (ESL) and intracellular Foxp3 expression, CD4\(^+\) T cells isolated from PBMCs using a CD4 isolation kit were stained with anti-CD25-PE and ESL detection protocol, as described below. To detect intracellular Foxp3 expression, an alloxypcycocyanin anti-human Foxp3 staining set (eBioscience) was used according to the manufacturer’s instruction. Cells were analyzed with a FACs Calibur (BD Bioscience).

**Detection of ESL**

In this study, the ESL epitope is defined as a site specifically bound to \(\alpha\)E-selectin-IgG Fc chimera. The detection of ESL was performed by FACS analysis as described previously (23).

**Measurements of cytokine levels in culture supernatants**

TNF-α and IFN-γ levels were measured using cytometric bead arrays (BD Biosciences) according to the manufacturer’s protocol.

**Detection of Foxp3\(^+\) T cells in skin lesions by immunofluorescence**

Immunofluorescent detection of Foxp3 expression was performed as follows. The biopsy specimens were frozen immediately in liquid nitrogen, embedded in Sakura Tissue-Tek OCT Compound 4583 (Sakura Finetek) and stored at −80°C until used. Frozen sections 5-μm thick were air dried, fixed in acetone at 4°C for 10 min, and then followed with 4% paraformaldehyde at 4°C for 15 min. After Ag retrieval and incubation in blocking solution, samples were stained with anti-Foxp3 mAb at 4°C overnight and then followed by anti-mouse IgG/Fc-Alexa Fluor 488 or anti-mouse IgG/Fc-Alexa Fluor 355 at 37°C for 30 min. Finally, samples were stained by anti-human CD8 mAb-PE or anti-human CD4 mAb-FITC and anti-human CD8 mAb-PE.

**Results**

**Preferential expansion of Treg cells in PBMCs from patients with DIHS**

We initially assessed the frequencies of CD4\(^+\)CD25\(^+\)Foxp3\(^+\) T cells in total PBMCs of patients with DIHS and those with TEN at their acute and resolution stages, respectively. The frequency of Treg cells in total CD4\(^+\) T cells from patients with TEN at the acute stage was not significantly different from that in those from healthy controls (Fig. 1). In contrast, patients with DIHS at the acute stage showed significantly increased frequencies of Treg cells compared with healthy controls (Fig. 1). Patients with TEN at the acute stage was not significantly different from that in those from healthy controls (Fig. 1).

**Statistics**

Data are expressed as mean ± SEM and were determined using Student’s \(t\) test or Dunnett’s test.

**DYSFUNCTION OF Treg CELLS IN SEVERE DRUG ERUPTIONS**

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**FIGURE 3.** Longitudinal analysis of Treg cell levels at the acute and resolution stages of DIHS and TEN. Longitudinal monitoring of indicated subsets was performed in the same patients (DIHS, \(n = 6\); TEN, \(n = 4\); control, \(n = 10\); Student’s \(t\) test). Shown is the frequency of indicated subsets in CD4\(^+\) T cells.

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Beckman Coulter. mAbs to human CD3 (HIT3a), forhead box P3 (Foxp3; PCH101), and the isotype controls to Foxp3 were purchased from eBioscience. Human IgG Fc was purchased from Valeant Pharmaceuticals. Recombinant E-selectin-IgG Fc chimera and mAb to human CD28 (37407) were purchased from R&D Systems. mAbs to mouse IgG Fc (Alexa Fluor 488 and Alexa Fluor 355 labeled) was purchased from Invitrogen.

Flow cytometric analysis as described previously (23).}

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possibility is unlikely, however, because the total frequencies of CD25^{+} T cells were only increased in patients with maculopapular drug eruption but not in those with DIHS and TEN, compared with healthy controls. Thus, selective expansions of Treg cells in peripheral blood are likely a common feature in patients with DIHS at the acute stage.

**Trafficking receptors expressed on the expanded Treg cells**

Previous studies demonstrated that Treg cells preferentially express chemokine receptors and adhesion molecules required for skin homing, such as CLA and CCR4 (26, 27). We therefore investigated the expression profile of previously described chemokine receptors and adhesion molecules associated with Treg cells. In addition, we assessed whether the Treg cells could be able to bind rE-selectin-IgG chimera, as described in our previous studies on skin-homing T cells (23). Most of Treg cells expanded at the acute stage of DIHS expressed CCR4 (73.3 ± 5.4%, n = 10) (Fig. 2A, Foxp3 vs CCR4; B, Foxp3^{+}CCR4^{+} in CD4^{+} T cells), indicating a skin-homing phenotype. Nevertheless this skin-homing phenotype was also preferentially found in Treg cells in patients with TEN and healthy controls (Fig. 2B, Foxp3^{+}CCR4^{+} in CD4^{+} T cells). A substantial proportion of Treg cells expanded at the acute stage of DIHS coexpressed CCR4 and ESL, as assessed by the ability to bind rE-selectin-IgG chimera. The frequency of ESL^{+} cells in Treg cells was significantly higher in patients with DIHS at the acute stage than in those with TEN and healthy controls (Fig. 2B, Foxp3^{+}ESL^{+} in CD4^{+}Foxp3^{+}). Most of the Treg cells expanded at the acute stage of DIHS can be characterized by increased expression of ESL and
Cytotoxic T cells to the control of drug-induced immunopathology in DIHS (7, 18, 28). To directly assess the relative contribution of Treg cells in skin lesions of DIHS and TEN is not detected in the majority of skin lesions of TEN is not detected in the majority of skin lesions of DIHS and TEN. The most obvious difference in the skin lesions found between the two forms of drug reactions is the presence of a massive destruction of the epidermis: full-thickness epidermal necrosis typically observed in patients with DIHS at either the acute or resolution stage, or healthy controls were cocultured at different ratios with highly purified CD4+ CD25+ effector T cell populations from the same patients at the acute stage. Mean (n = 3–7) and SEM are shown. One-way ANOVA followed by Dunnett test was used to determine significant differences between patients and controls. In six patients with DIHS and four patients with TEN, frequencies of Treg cells and their CCR4 and ESL expression were reexamined >6 mo after the first sampling. As shown in Fig. 3, when compared with the frequency of Treg cells from the same patients at the resolution stage, three patients with DIHS at the acute stage showed a >3-fold expansion of Treg cells in peripheral blood CD4+ T cells. In contrast, four patients with TEN sampled twice on both occasions 6 mo apart showed identical values for both samples. Thus, the expanded Treg cells were contracted upon resolution in patients with DIHS and their preferential expression of ESL and CCR4 returned to normal.

**Treg cells in skin lesions of DIHS and TEN**

The most obvious difference in the skin lesions found between the two forms of drug reactions is the presence of a massive destruction of the epidermis: full-thickness epidermal necrosis typically observed in TEN is not detected in the majority of skin lesions of DIHS (7, 18, 28). To directly assess the relative contribution of Treg cells to the control of drug-induced immunopathology in these skin lesions, we investigated whether the expanded Treg cells could migrate into the inflammatory sites to help limit epidermal damage caused by vigorous T cell activation. As shown in Fig. 4A, Foxp3+ T cells were frequently detected in the skin lesions of DIHS by immunofluorescence. Generally, Foxp3+ T cells were frequently detected close to where many CD8+ T cells were infiltrated (Fig. 4C). On the other hand, Foxp3+ T cells were much less frequently detected in skin lesions of TEN, where multiple blisters developed (Fig. 4B).

**Alterations in suppressive function of Treg cells depending on the stage in DIHS and TEN**

We next investigated whether the functional activity of Treg cells from patients with DIHS and TEN could be altered depending on the stage examined. Before determining the functionality of Treg cells, we examined whether effector T cells in PBMCs from these patients at the acute and resolution stages of DIHS could have the ability to make inflammatory cytokines in response to relevant Ag stimulation. As shown in Fig. 5A, effector T cells at either the acute or resolution stage produced detectable quantities of inflammatory cytokines only when stimulated with relevant drug Ag in vitro, although those at the resolution stage had a more potent ability. To test the suppressive function of these Treg cells, we initially analyzed the effects of depletion of Treg cells on cytokine production by drug Ag-specific effector T cells from these patients. In PBMCs...
obtained from patients with DIHS at the acute stage, Treg cell depletion induced a dramatic increase in IFN-γ and TNF-α production under either basal or Ag-stimulated conditions (Fig. 5B). In contrast, in those obtained from the same patients with DIHS at the resolution stage, Treg cell depletion did not induce a significant increase in the cytokine production under either basal or Ag-stimulated conditions. In the acute stage of TEN, Treg cell depletion from PBMCs did not result in a significant increase in cytokine production, as observed in the resolution stage of DIHS.

We next asked whether Treg cells obtained at the different stages could have the suppressive capacity to inhibit activation of effector T cells. To this end, we conducted a T cell proliferation assay using allogeneic APCs. As shown in Fig. 6A, on a cell-cell basis, Treg cells in patients with DIHS at the acute stage were found to retain the suppressive capacity, although less than that of healthy controls. However, surprisingly, their suppressive capacity became defective after clinical resolution of DIHS.

In contrast, Treg cells obtained from patients with TEN at the acute stage were found to be profoundly defective in their capacity to suppress T cell proliferation (Fig. 6B); the degree of functional defect in TEN was directly related to the severity of epidermal damage. Nevertheless, their defective capacity at the acute stage was restored upon clinical resolution (Fig. 6B). To exclude the possibility that subtle changes in Treg cell function were exaggerated in our T cell proliferation assay because the CD25^− effector population was also potentially changing depending on the stage examined, add-back experiments using the CD4^+CD25^− effector cells from the constant stage and the CD25^+ Treg cells from the different stages were performed for some patients with DIHS: these populations at the different stages were obtained from the same patients. As shown in Figs. 6, C and D, the impairment in suppressive function of Treg cells was specifically observed at the resolution stage regardless of whether the effector population at either the acute (Fig. 6C) or resolution (Fig. 6D) stage was used.

**Discussion**

In this study, we for the first time demonstrated that CD4^−CD25^+ Foxp3^+ Treg cells are expanded at the acute stage of DIHS while such expansions are never observed in patients with other types of drug eruptions such as TEN and maculopapular drug eruption: in the acute stage of TEN, although Treg cells can be present in normal numbers in the blood, their capacity to migrate into the skin and to suppress the activation of effector T cells is profoundly impaired, thereby allowing drug-specific T cells to function in an uncontrolled fashion, as demonstrated in experimental animals (5, 6, 29). A marked increase in the frequency of Treg cells at the acute stage of DIHS raises the question of whether a simple accumulation of Treg cells in the peripheral blood has occurred as a consequence of their defective migration to the inflammatory skin sites. However, this possibility is unlikely, because this cell compartment expanded in the acute stage of DIHS is further characterized by an increase of Treg cells coexpressing ESL and CCR4, well-known markers associated with skin-homing T cells (23, 27, 30). Indeed, our immunohistochemical study showed that Foxp3^+ Treg cells migrated to the skin lesions of DIHS at much higher frequencies than those in the lesions of TEN. An influx of Treg cells with suppressive properties into the skin lesions of DIHS would serve to alleviate lesion severity. However, the “dark side” of reduced lesion severity could be reactivation of viruses persisting in a latent state, which is typically observed in patients with DIHS as well as those with graft-versus-host disease (7–9, 31).

Thus, this expanded Treg cell population might serve to prevent the activation and expansion of antiviral T cells, thereby reducing lesion severity and possibly allowing latent herpesviruses to reactivate in an uncontrolled fashion.

Our in vitro Treg cell depletion study and T cell proliferation study clearly demonstrated that the expanded Treg cells displayed a strong inhibitory function on cytokine production and proliferation of effector CD4^+CD25^+ T cells on a per-cell basis. These results indicate that the expanded CD4^+CD25^+Foxp3^+ T cells were fully functional Treg cells and not effector T cells. Although their suppressive function was not assessed using an Ag-driven T cell proliferation or cytokine production assay due to a limited number of Treg cells available, we reasoned that the T cell proliferation assay using allogeneic APCs is sufficient to assess the function of Treg cells to suppress Ag-driven T cell activation at a single-cell level. Given an increased frequency of these cells in peripheral blood, the overall inhibitory effect of the expanded Treg cells would be even more pronounced in vivo, indicating that these T cells would limit the severity of a T cell-mediated immunoinflammatory response to drug. These findings provide an explanation for why severe epidermal damage cannot be detected in the skin lesions of DIHS, unlike TEN lesions, why the onset of this disease is delayed in relation to the introduction of the causative drug, and why proliferation of drug-specific T cells can only be detected at the resolution stage of DIHS, but not at the acute stage, whereas this can be detected at the acute stage of other types of drug eruptions, such as TEN, as evidenced by the results of lymphocyte transformation tests (17, 31). Hypogammaglobulinemia and a profound decrease in B cell numbers specifically observed at the onset of DIHS (10) may be related to expansions of functional Treg cells, because Treg cells have been shown to have the ability to induce B cell death (32).

In contrast, Treg cells detected in the resolution stage of DIHS were found to be functionally impaired in their in vitro suppressive activity despite their normal frequencies, although all of the patients examined had not developed autoimmune diseases at the time of sampling. Consistent with this finding, some recent studies have demonstrated the existence of Treg cells with a reduced in vitro suppressive function in patients with type 1 diabetes and other autoimmune diseases (33–35); interestingly, some of which were reported to develop as long-term sequelae of DIHS (11, 12, 36, 37). An important question to be resolved is whether the expanded Treg cells in patients with DIHS could be driven eventually to exhaustion during the disease course or replaced by other subsets of Treg cells with lower functional activity.

The studies on an animal model of TEN suggested the importance of Treg cells in protecting susceptible patients from the development of TEN (6), although their protective role has never been examined during the actual disease process. In this regard, our data show that the functionality of Treg cells at the acute stage of TEN was impaired, on a per-cell basis, in agreement with the present finding that severe epidermal damage can be seen in skin lesions of TEN patients, in whom circulating Treg cells are present in normal frequency. These data suggest that the major difference in Treg cells between patients with TEN and healthy controls is qualitative and not quantitative. It should be noted, however, that our data do not provide an answer to the important question of how Treg cells become functionally impaired at the onset of TEN. Given the ability of Treg cells to sense pathogens directly through TLR such as TLR2 and consequently modify their function (38), along with the fact that TLR2 is critical in the initiation of innate immune responses to herpesviruses and Mycoplasma (39, 40), it is reasonable to speculate that the suppressive function of Treg cells...
might be temporarily impaired during the acute phase of the infection. In support of this possibility, infections with these pathogens have been implicated in the pathogenesis of severe drug eruptions (41). Addressing this question will require longitudinal numerical/functional analyses of Treg cells at different stages of infection.

Similar expansion of Treg cells with the antiproliferative activity during the acute phase and their subsequent contraction upon resolution have been also reported to occur in patients with sarcoidosis (42). Interestingly, the clinical features of both DIHS and sarcoidosis fit within the spectrum of immune reconstitution syndrome (IRS), which is characterized by a paradoxical deterioration of clinical symptoms or laboratory parameters attributable to restoration of a valid immune response against previously unrecognized pathogens (31, 43, 44). Although the IRS was originally defined in HIV-infected patients, it was necessary to broaden the concept of IRS to include diverse infectious or autoimmune diseases such as herpes zoster and Graves’ disease that occur following the tapering or discontinuation of immunosuppressive drugs (31, 41, 42). Our kinetic analyses of circulating lymphocyte count in patients with DIHS showed that a lymphopenic state immediately preceded lymphocytosis, a finding typically seen at the onset of DIHS, and that Treg cells were expanded during the recovery from lymphopenia, coincident with the onset (data not shown). Indeed, reconstitution of T cell-deficient mice by an adoptive transfer of mature peripheral lymphocytes was accompanied by rapid expansions of Treg cells that facilitated CD4+ T cell clonal anergy induction probably to prevent the development of overt autoimmunity in hosts recovering from lymphopenia (45).

In summary, our findings suggest that the timing of a dysfunction of Treg cells in individuals with drug-specific T cells in their T cell repertoire is associated with the pathologic phenotype and outcome of T cell-mediated drug eruptions, although it remains unclear whether a dysfunction in Treg cells is causative in the induction of the disease. Expansions of Treg cells at the acute stage of DIHS may reflect an attempt to limit collateral tissue damage induced by activation and migration of effector T cells while allowing latent herpesviruses to reactivate in a sequential manner. However, a resultant loss of their suppressive function would increase the susceptibility of these patients to autoimmune diseases. In contrast, their functional defect at the acute stage of TEN would have detrimental effects on patients to autoimmune diseases. In contrast, their functional depressive function would increase the susceptibility of these patients to autoimmune diseases such as herpes zoster and Graves’ disease that occur following the tapering or discontinuation of immunosuppressive drugs (31, 41, 42). Our kinetic analyses of circulating lymphocyte count in patients with DIHS showed that a lymphopenic state immediately preceded lymphocytosis, a finding typically seen at the onset of DIHS, and that Treg cells were expanded during the recovery from lymphopenia, coincident with the onset (data not shown). Indeed, reconstitution of T cell-deficient mice by an adoptive transfer of mature peripheral lymphocytes was accompanied by rapid expansions of Treg cells that facilitated CD4+ T cell clonal anergy induction probably to prevent the development of overt autoimmunity in hosts recovering from lymphopenia (45).

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