Acquired Omenn-Like Syndrome, a Novel Posttransplant Autoaggression Syndrome Reversed by Rapamycin

Donald C. Vinh, Khalid Bin Dhuban, Helen Mason, Duncan Lejtenyi, SungMi Jung, Donald C. Sheppard, Damien Faury, Nada Jabado, and Ciriaco A. Piccirillo

Division of Infectious Diseases, Division of Allergy and Clinical Immunology, Department of Medicine, and Department of Medical Microbiology, McGill University Health Centre, Montreal, QC, Canada; Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada; FOCIS Center of Excellence, Research Institute of the McGill University Health Centre, Montreal, QC, Canada; Division of Pediatric Allergy and Clinical Immunology, Department of Pediatrics, McGill University Health Centre, Montreal, QC, Canada; and Department of Pathology, McGill University Health Centre, Montreal, QC, Canada

Graft-versus-host disease is uncommon in autologous hematopoietic cell transplantation (HCT) and is typically brief and mild. We report unusual, protracted, and severe Omenn syndrome-like autoaggression following autologous HCT. We identified a profound FOXP3+ regulatory T cell defect that coincided with hyperinflammatory T cell responses which were reversible with rapamycin in vitro.

CASE REPORT

A 55-year-old woman with Hodgkin’s disease underwent BEAM (carmustine, etoposide, cytarabine, and melphalan) conditioning for autologous hematopoietic cell transplantation (auto-HCT) with full engraftment after 2 weeks. However, over the subsequent year, her post-HCT course was complicated by anemia, thrombocytopenia, enteropathy (affecting the small intestine and colon), diffuse erythroderma with intermittent desquamation (Fig. 1A), hypogammaglobulinemia with the absence of circulating B cells, and inflammatory cholestatic liver injury. She was hospitalized at 5 months post-transplant with generalized deterioration. She suffered repeated bacterial (pneumonia and bacteremia), viral (cytomegalovirus [CMV] viremia), and fungal (probable invasive aspergillosis) infections. She had 4 further distinct episodes of clinical deterioration without an infectious focus, characterized by worsening erythroderma, increased diarrhea, hemodynamic instability, and cholestatic hepatitis, associated with a significant increase in circulating CD8+ T cells (range, 1,164 to 4,491 cells/ml; reference range, 127 to 735 cells/ml). Repeated investigations of bone marrow and lymph nodes did not reveal hematological malignancy.

At 12 months post-HCT, the patient again developed CMV viremia; ganciclovir treatment was started. Two weeks later, she developed severe neutropenia, possibly related to myelosuppression from the antiviral treatment. Ganciclovir was changed to foscarnet. Granulocyte colony-stimulating factor suppression from the antiviral treatment. Ganciclovir was started without improvement. At 12 months post-HCT, the patient again developed CMV viremia; ganciclovir treatment was started. Two weeks later, she developed severe neutropenia, possibly related to myelosuppression from the antiviral treatment. Ganciclovir was changed to foscarnet. Granulocyte colony-stimulating factor suppression from the antiviral treatment. Ganciclovir was started without improvement.

Based on in vitro data (see below), rapamycin was started after 8.5 months of hospitalization. After 1 week, the patient had no further diarrhea, no abdominal symptoms, and significant biochemical improvement of her cholestatic liver injury. Her family also reported subjective improvement in the rash. The pancytopenia persisted but did not worsen during rapamycin treatment. Unfortunately, on day 9 of rapamycin therapy, she developed catheter-related viridans group streptococcal bacteremia that progressed to septic shock with multiorgan failure, and she passed away. No autopsy was performed.

Autoaggression syndrome (or auto-graft-versus-host disease [auto-GVHD]) spontaneously occurs in ~10% of auto-HCT patients (5, 8, 9), is typically limited to the skin, and resolves within 1 to 3 weeks either spontaneously or after a short course of steroids (2). While this variant of autoaggression syndrome arises spontaneously, intentional induction of autoaggression syndrome with cyclosporine (CsA) has been attempted to promote antitumor activity. Clinical evidence of involvement of the gastrointestinal tract or liver is uncommon, and progression to a chronic phase is very rare (2). The features in our patient extended beyond involvement of organs typically involved in GVHD (i.e., the skin, gastrointestinal tract, and liver), producing hematopoietic impairment (anemia and thrombocytopenia in the absence of bone marrow failure or thrombotic microangiopathy) and immune defects (recurrent infections and abnormal lymphocyte phenotyping). An autoaggression syndrome manifesting similarly with these features of autoimmunity and immunodeficiency (Table 1) is Omenn syndrome (OS), a genetic severe combined immunodeficiency which can be caused by hypomorphic mutations in RAG genes. Cassani et al. have suggested that loss of tolerance in OS, associated with an impaired function in regulatory T (TReg) cells (3), may contribute to its immunopathology. Additionally, CsA-induced autoaggression syndrome is thought to occur because of preferential activation of autoreactive T cells in conjunction with impaired immunoregulatory systems (6, 7). Due to the glaring similarities between our patient’s condition and these syndromes, we postulated that at least some of her manifestations were consequential upon a TReg cell defect or dysfunction acquired following auto-HCT.

We examined the phenotype and function of circulating T...
cells by multiparametric flow cytometry. Analysis of T cell receptor (TCR) Vβ expression showed oligoclonal expansion of the T cell repertoire, with no evidence of monoclonality (Fig. 1C). Moreover, ex vivo analysis of patient-derived peripheral blood mononuclear cells (PBMCs) showed high frequencies of proliferating (Ki-67+) CD4+ T cells (19.2% versus 4.5%) and CD8+ T cells (43.7% versus 5.3%) compared to the healthy control (Fig. 2A). This hyperproliferation coincided with a significant increase in the production of the proinflammatory cytokines tumor necrosis factor alpha (TNF-α; with 89.9% versus 35.6% TNF-α+ cells) and gamma interferon (IFN-γ; with 28.0% versus 7.9% IFN-γ+ cells) (Fig. 2B). Moreover, patient-derived T cells were predominantly of the memory phenotype as indicated by the low frequency of CD45RA-expressing naïve cells (CD4+, 3.79% versus 34.9%; CD8+, 30.1% versus 54.2%) (Fig. 2C). Thus, the autoimmune features in this patient were associated with restricted T cell subsets demonstrating excessive activation and a hyperinflammatory state.

T<sub>Reg</sub> cells coexpressing the interleukin-2Rα (IL-2Rα) chain (i.e., CD25) and the transcription factor FOXP3+ are critical for controlling peripheral immune tolerance (14). Therefore, we asked whether a defect in the frequency of circulating T<sub>Reg</sub> cells underlay the excessive inflammation in the patient. Flow cytometric analysis of PBMCs revealed a severe deficiency in circulating CD25+ FOXP3+ T<sub>Reg</sub> cells in the patient compared to the healthy control (0.67% versus 5.64%) and an overall decrease in the frequency of FOXP3-expressing CD4+ T cells (1.12% versus 7.89%) (Fig. 2D). Thus, a profound FOXP3+ T<sub>Reg</sub> cell deficiency was coupled to the observed dysregulated hyperinflammatory T cell responses (Fig. 2A and B).

Our patient showed no improvement with steroids, the recommended treatment for auto-GVHD (2). Rapamycin (sirolimus) has been shown previously to promote the expansion of T<sub>Reg</sub> cells in vitro and in vivo (1, 12). We asked whether rapamycin treatment in vitro could modulate the hyperinflammatory responses observed in our patient by potentiating the development of T<sub>Reg</sub> cells. Upon in vitro rapamycin treatment, we detected a profound (2.43-fold) increase in the frequency of FOXP3-expressing CD4+ T cells.

FIG 1  Autoimmune manifestations in acquired Omenn-like syndrome. (A) Diffuse erythroderma, appearing as an erythematous hue of the patient’s baseline pale brown complexion. (B) Immunohistochemistry analysis of a skin biopsy specimen demonstrating spongiform dermatitis with perivascular infiltration of lymphocytes, predominantly CD8+ T cells. (C) Clonogram of flow cytometric analysis of the TCR Vβ repertoire demonstrating abnormal, oligoclonal T cell expansion.
T cells (Fig. 2E), accompanied by substantial reductions in the frequencies of IFN-γ, TNF-α, and IL-17 CD4+ cells (1.5-, 1.6-, and 3-fold decreases, respectively) (Fig. 2E). Rapamycin not only reduced the proportions of proinflammatory cytokine-producing cells but also reduced the amounts of cytokine produced per cell, as indicated by the decrease in cytokine mean fluorescence intensity (Fig. 2F). Thus, rapamycin treatment in vitro ameliorates the T Reg cell deficiency by significantly augmenting the number of CD4+ FOXP3+ TReg cells and suppressing the associated T cell hyperinflammatory phenotype.

Our patient had most features of OS but did not demonstrate eosinophilia or elevated IgE. Further, clinical onset occurred only following auto-HCT, favoring acquired immunodeficiency. In support of this possibility, genomic sequencing of RAG-1 and RAG-2, the genes for which hypomorphic mutations are characteristically associated with OS (10), demonstrated no mutation. Hence, this appears to be the first report of an acquired OS-like state complicating auto-HCT. Definitive treatment of OS is allogeneic HCT. Unfortunately, the patient’s status precluded such aggressive therapy. Since immnosuppressants have been successfully used as a temporizing treatment in OS (11, 13), and given the demonstrated benefits of rapamycin for TReg cell development and function in vitro, we pursued a therapeutic trial of rapamycin in our patient. Clinically, our patient demonstrated noticeable, albeit moderate, improvement on rapamycin but succumbed to infection before we could determine further in vitro or in vivo evidence of amelioration. Whether TReg cell expansion from rapamycin paradoxically contributed to the fatal outcome is speculative. However, repeated bacteremias prior to initiation of rapamycin suggest an underlying infection diathesis unrelated to the drug. The severity of the streptococcal bacteremia was most likely related to the severe neutropenia that developed prior to rapamycin treatment.

In summary, we provide a seminal description of a progressive autoaggression syndrome acquired post-auto-HCT, mimicking the phenotype of OS and distinct from auto-GVHD by its chronicity and severity. We provide ex vivo evidence that the autoimmune features are related to excessive expansion, activation, and inflammatory function of oligoclonally restricted T cells in association with profound TReg cell deficiency. Moreover, we demonstrate that, at least in vitro, the TReg cell defect and accompanying hyperinflammatory state can be reversed with rapamycin. Despite its limitations, this primary report fundamentally urges the recognition and diagnosis of this potentially fatal immunodysregulatory complication. Increased awareness will undoubtedly permit its better understanding. Rapamycin may be a potential therapeutic agent for the treatment of this novel autoaggression syndrome.
FIG 2 Severe immune dysregulation coincides with profound T\(_{\text{reg}}\) cell deficiency reversible by rapamycin in vitro. The cellularity and phenotypes of CD4\(^{+}\) and CD8\(^{+}\) T cell subsets among PBMCs were assessed ex vivo (A to D), or the cells were activated for 7 days in the presence of soluble anti-CD3 (100 pg/ml), recombinant human IL-2 (100 U/ml), and the indicated doses of rapamycin added on days 0, 2, 4 and 6 of culture (E and F). (A) Percentages of proliferating (KI-67\(^{+}\)) cells among CD4\(^{+}\) and CD8\(^{+}\) T cells. (B) Inflammatory cytokine secretion by CD4\(^{+}\) T cells. (C) Percentages of naïve (CD45RA\(^{+}\)) CD4\(^{+}\) and CD8\(^{+}\) T cells. (D) Flow cytometric enumeration of T\(_{\text{reg}}\) cells expressing the CD25 and FOXP3 markers. (E) Effect of rapamycin on FOXP3 expression and inflammatory cytokine production on day 7 of culture. Upregulation of FOXP3 in response to rapamycin (right y axis) coincides with downregulation of the proportion of proinflammatory cytokine-producing T cells (left y axis), indicating recovery of T\(_{\text{reg}}\) cell function. The gray boxes indicate the change (n-fold) in the corresponding parameter at 100 nM rapamycin relative to untreated cells. (F) Rapamycin also reduces the amount of proinflammatory cytokines produced per cell, as indicated by the decline in mean fluorescence intensities (MFI) of individual cytokines after treatment (y axis). The decrease in MFI is quantified in the gray boxes.

REFERENCES
1. Battaglia M, et al. 2006. Rapamycin promotes expansion of functional CD4\(^{+}\) CD25\(^{+}\) FOXP3\(^{+}\) regulatory T cells of both healthy subjects and type 1 diabetic patients. J. Immunol. 177:8338–8347.
2. Bolaños-Meade J, et al. 2007. Induction of autologous graft-versus-host disease: results of a randomized prospective clinical trial in patients with poor risk lymphoma. Biol. Blood Marrow Transplant. 13(10):1185–1191.
3. Cassani B, et al. 2010. Defect of regulatory T cells in patients with Omenn syndrome. J. Allergy Clin. Immunol. 125(1):209–216.
4. de Villartay J-P, Schwarz K, Villa A. 2006. V(D)J recombination defects, p 153–168. In Ochs HD, Smith CI, Puck JM (ed), Primary immunodeficiency diseases: a molecular and cellular approach. Oxford University Press, New York, NY.
5. Drobyski WR, Hari P, Keever-Taylor C, Komorowski R, Grossman W. 2009. Severe autologous GVHD after hematopoietic progenitor cell transplantation for multiple myeloma. Bone Marrow Transplant. 43(2):169–177.
6. Hess AD. 2010. Reconstitution of self-tolerance after hematopoietic stem cell transplantation. Immunol. Res. 47(1–3):143–152.
7. Hess AD, Thoburn CJ. 2006. Immune tolerance to self-major histocompatibility complex II antigens after bone marrow transplantation: role of regulatory T cells. Biol. Blood Marrow Transplant. 12(5):518–529.
8. Hood AF, Vogelsang GB, Black LP, Farmer ER, Santos GW. 1987. Acute graft-vs-host disease. Development following autologous and syngeneic bone marrow transplantation. Arch. Dermatol. 123(6):745–750.
9. Martin RW, III, Farmer ER, Altomonte VL, Vogelsang GB, Santos GW. 1995. Lichenoid graft-vs-host disease in an autologous bone marrow transplant recipient. Arch. Dermatol. 131(3):333–335.
10. Matangkasombut P, et al. 2008. Lack of iNKT cells in patients with combined immune deficiency due to hypomorphic RAG mutations. Blood 111(1):271–274.
11. Meyer-Bahlburg A, et al. 2002. Treatment with cyclosporin A in a patient with Omenn’s syndrome. Arch. Dis. Child. 87(3):231–233.
12. Monti P, et al. 2008. Rapamycin monotherapy in patients with type 1 diabetes modifies CD4\(^{+}\) CD25\(^{+}\) FOXP3\(^{+}\) regulatory T cells. Diabetes 57:2341–2347.
13. Rego S, Kemp A, Wong M, Knight P. 2006. Omenn syndrome: therapeutic effects of cyclosporine. J. Paediatr. Child Health 42(5):319–320.
14. Sakaguchi S, Miyaara M, Costantino CM, Hafier DA. 2010. FOXP3\(^{+}\) regulatory T cells in the human immune system. Nat. Rev. Immunol. 10:490–500.