Cytokine 78 (2016) 22–26

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Short communication

Therapeutic potential of low-dose IL-2 in a chronic GVHD patient by *in vivo* expansion of regulatory T cells

Nayoun Kim [a,b], Young-Woo Jeon [a,b,c], Young-Sun Nam [a,b], Jung-Yeon Lim [a,b], Keon-Il Im [a,b], Eun-Sol Lee [a,b], Seok-Goo Cho [a,b,c,*]

[a] The Institute for Translational Research and Molecular Imaging, The Catholic University of Korea College of Medicine, Seoul, Republic of Korea
[b] Laboratory of Immune Regulation, Convergent Research Consortium for Immunologic Disease, Seoul, Republic of Korea
[c] The Catholic Blood and Marrow Transplantation Center, Seoul St. Mary's Hospital, The Catholic University of Korea College of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 22 July 2015
Received in revised form 17 October 2015
Accepted 18 November 2015
Available online 28 November 2015

Keywords:
Chronic graft-versus-host disease
Interleukin-2
Regulatory T cell
Immunotherapy
Allogeneic hematopoietic stem cell transplantation

ABSTRACT

Chronic graft-versus-host disease (cGVHD) is a common complication following allogeneic hematopoietic stem cell transplantation (HSCT), which is characterized by autoimmune like inflammatory responses and reduced levels of regulatory T cells (Tregs). Recently, the use of low-dose IL-2 has been reported to selectively increase Tregs and therefore facilitate immune regulation and promote clinical improvements in cGVHD patients. In this report, we describe the case of a cGVHD patient who was treated with daily low-dose IL-2 therapy. Our observations demonstrate that low-dose IL-2 could induce significant expansion of Tregs *in vivo* leading to improved Treg/Th17 ratios. The patient showed moderate clinical benefits suggesting that multiple factors may be involved in the immunological responses. Therefore, while the therapeutic potential of low-dose IL-2 is promising, strategic approaches may be needed to induce a clinically significant and sustained Treg effect.

1. Introduction

Chronic graft-versus-host disease (cGVHD) is a critical complication in long-term survivors of allogeneic hematopoietic stem cell transplantation (HSCT). Due to the widespread immunologic dysfunction, the treatment of cGVHD still remains one of the most important challenges in allogeneic HSCT [1]. Reduced frequency and impaired function of regulatory T cells (Tregs) following allogeneic HSCT are often associated with the pathogenesis of cGVHD [2,3] and thus, restoring the Treg balance may be crucial for effective treatment.

Recently, with the understanding that IL-2 signaling is essential for the development, function and homeostasis of regulatory T cells (Tregs), it has been reported that the use of low-dose IL-2 therapy preferentially induces Tregs *in vivo* [4,5]. Because Tregs express high levels of high-affinity IL-2 receptors, low doses of IL-2 can selectively expand Tregs while they are insufficient to activate alloreactive effector T cell populations *in vivo*. Previously, substantial increase in Treg frequency was observed in cGVHD patients following low-dose IL-2 therapy [4]. In this study, half of the patients demonstrated a partial response whereas others showed stable disease suggesting that multiple factors contribute to the therapeutic outcome of low-dose IL-2 therapy. Here, we describe the case of a 45-year-old man with severe cGVHD following allogeneic HSCT who was treated with daily low-dose IL-2 therapy. Through our longitudinal analysis of immunological responses following low-dose IL-2 therapy, we show the therapeutic potential of low-dose IL-2 therapy for severe cGVHD by *in vivo* expansion of Tregs and suggest the possibility of adjunctive approaches to enhance low-dose IL-2 therapy in future studies.

2. Materials and methods

2.1. Patient

In 2007, the patient was diagnosed with refractory anemia with excess blasts-2 and received HSCT from an unrelated female donor after conditioning with fludarabine (Fludara, 30 mg/m^2 for 5 days),

Abbreviations: cGVHD, chronic graft-versus-host disease; hematopoietic stem cell transplantation, HSCT; IL-2, interleukin-2; Treg, regulatory T cell.

* Corresponding author at: Department of Hematology, Catholic Blood and Marrow Transplantation Center, Laboratory of Immune Regulation, Convergent Research Consortium for Immunologic Disease (CRCID), Institute for Translational Research and Molecular Imaging, Seoul St. Mary's Hospital, The Catholic University of Korea, College of Medicine, #505, Banpo-Dong, Seocho-Ku, Seoul 137-040, Republic of Korea.
E-mail address: chosg@catholic.ac.kr (S.-G. Cho).

http://dx.doi.org/10.1016/j.cyto.2015.11.020
1043-4666/

busulfan (Busulfex, 3.2 mg/kg for 2 days) and total body irradiation (4 Gy). On day 63 after transplantation, the patient developed clinical manifestations of oral GVHD despite treatment with tacrolimus (TacroBell), mycophenolate mofetil (MMF; CellCept), and prednisolone (Solondo). The patient suffered from severe cGVHD for 7 years with serious manifestations involving lesions in the oral mucosa, skin sclerosis and cellulitis, cataract, and musculoskeletal impairments, which later required additional therapies such as dermatological ultraviolet ray therapy and cataract surgery.

2.2. Treatment

At 7 years post transplantation, daily treatment with subcutaneous IL-2 (Proleukin) was initiated at a dose of 1×10^6 IU per square meter for 8 weeks, discontinued for 18 weeks and was re-initiated at the same dose for another 8 weeks. In addition to IL-2, the patient received MMF (750–1000 mg/day during the first cycle and 250–500 mg/day during the second cycle) and prednisolone (7.5 mg/day) during both cycles. Tacrolimus (1 mg/ day) was only included during the second cycle. The patient signed an informed consent form approved by the Institutional Review Board of the Seoul St. Mary's Hospital, the Catholic University of Korea and followed the Declaration of Helsinki protocols.

2.3. Flow cytometry

Peripheral blood mononuclear cells were immunostained with various combinations of the fluorescence-conjugated antibodies: anti-CD3 Pacific Blue (clone UCHT1, eBioscience, San Diego, CA, USA), anti-CD4 APC-Cy7 (clone OKT4, eBioscience), anti-CD8 PerCP-Cy5.5 (clone SK1, eBioscience), anti-CD25 APC (clone BC96, eBioscience), anti-CD127 PerCP-Cy5.5 (clone eBioRDR5, eBioscience) for T-cell subsets; anti-CD3 Pacific Blue and anti-CD56 FITC (clone TULY56, eBioscience) or natural killer cells and natural killer T cells; and anti-CD19 APC (clone HIB19, eBioscience) for B cells. For intracellular cytokine and Foxp3 staining, surface-stained cells were processed with fixation and permeabilization buffer (eBioscience) according to the manufacturer's protocol and were incubated with anti-interferon gamma APC (clone 4S.B3, eBioscience), anti-IL-17 (clone eBio64D317, eBioscience), and anti-Foxp3 PE (clone PCH101, eBioscience). Prior to intracellular cytokine staining, cells were stimulated in culture medium containing phorbol myristate acetate (25 ng/mL; Sigma–Aldrich), ionomycin (250 ng/mL; Sigma–Aldrich), and monensin (GolgiStop, 1 μL/mL; BD PharMingen) in an incubator with 5% CO_2 at 37 °C for 6 h. Flow cytometry was performed on a fluorescence-activated cell sorting (FACS) Calibur cytometer (BD PharMingen) using FlowJo software (TreeStar, Ashland, OR, USA).

2.4. Enzyme-linked immunosorbent assay

Serum cytokine levels of IL-6, IL-10, IL-17, TNF-α, IL-2, and IFN-γ were determined by ELISA with Duoset cytokine assay reagents (Biolegend, San Diego, CA, USA) per manufacturer's protocol.

3. Results

Overall, IL-2 was well-tolerated without any infusion-related toxicities or adverse events. The patient showed partial response following IL-2 treatment with softening of the skin lesions and decreased erythema in the abdomen and groin (Fig. 1A). However, minimal change was noted in the in the cellulitis of the feet (Fig. 1B). Furthermore, the patient continuously presented complaints of severe pain associated with systemic GVHD symptoms. Following IL-2 treatment, we show that the percentage of Tregs, defined by $CD4^+Foxp3^{high}CD127^{low}$ cells, expanded up to 37.3% after one week of treatment (Fig. 1C). However, reduction in Tregs was noted during treatment despite continued administration of IL-2 (Fig. 1D).

In addition to the expansion of Tregs, we evaluated the ratios of Tregs to Th1 and Th17 cells involved in the immune responses following IL-2 treatment. The Treg/Th17 ratio (Fig. 2A and C) showed a similar pattern to the expansion of Tregs (Fig. 1D) suggesting that the control of Th17 may be strongly correlated with the presence of Tregs. In contrast, the Treg/Th1 ratio was low despite the presence of Tregs (Fig. 2B and D) suggesting a weak correlation between the balance of Treg and Th1 cells. Furthermore, immunological responses of IL-2 were analyzed through the measurement of inflammatory cytokine levels in the plasma (Fig. 2E). Overall, the different presentation of the cytokine levels between the first and second treatment cycle may be attributed to the addition of tacrolimus during the second cycle. Cytokines including IL-6, IL-10, IL-17, TNF-α, and IFN-γ were significantly suppressed during the second cycle; however, the overall reduction of inflammatory cytokines did not result in a dramatic clinical improvement of the patient. Finally, we confirmed that low-dose IL-2 preferentially induced Tregs without affecting other lymphocyte subsets (Fig. 2F).

4. Discussion

Koreth et al. had previously reported the significant expansion and maintenance of Tregs during IL-2 treatment in cGVHD [4]. Similarly, we show that the percentage of Tregs expanded significantly after one week of treatment. In contrast to the previous study in which the number of Tregs began to decline only after discontinuation of IL-2, reduction in Tregs despite continued administration of IL-2. While various factors, including the extent and severity of disease, may have affected this decline. Particularly, low-dose IL-2 therapy was initiated 7 years after onset of disease, which may have weakened the overall therapeutic effects. Recently, low-dose IL-2 therapy has been evaluated in the prevention of GVHD in HSCT patients and it is suggested that low-dose IL-2 in the form of GVHD prophylaxis may be more effective [6].

Furthermore, we evaluated whether the addition of calcineurin inhibitor had an additive effect during the second cycle. Calcineurin inhibition potently suppresses both IL-2 dependent Tcon and Treg proliferation; however, exogenous IL-2 can preferentially restore Tregs through high-affinity IL-2 binding [7,8]. Thus we initially hypothesized that the overall inhibition of T cells through calcineurin inhibition followed by subsequent low-dose IL-2 administration could preferentially rescue and expand Tregs [9]. However, in our patient, the addition of tacrolimus did not significantly change the frequency or durability of expanded Tregs. Correspondingly, it has also been demonstrated in pre-clinical models that mTOR inhibitors, in replacement of calcineurin inhibitor, may be a better choice for use in combination with IL-2 to allow expansion of Tregs [9]. Interestingly, sirolimus (rapamycin) was used in combination with low-dose IL-2 in half of the patients in the previous trial that had reported partial clinical response in a substantial proportion of patients [4]. However, the direct correlation between the use of sirolimus compared to tacrolimus, Treg expansion, and ultimately, clinical improvement remains to be elucidated, as it has yet to be thoroughly investigated in clinical studies.

In addition to the role of Tregs, recent data in humans have suggested that immune responses in cGVHD involve upregulated Th1 and Th17 levels caused by activated allogeneic T cells [2]. In the GVHD setting, activated Tcons also express high levels of CD25. Perol et al. demonstrated in a pre-clinical acute GVHD model that IL-2 treatment in mice induced sustained CD25 expression on allogeneic Tcons and therefore and the selectivity of IL-2 treatment on