Clinical Immunology (2013) 149, 111–118

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Psoriasis patients exhibit impairment of the high potency CCR5$^+$ T regulatory cell subset☆☆

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Received 19 March 2013; accepted with revision 25 June 2013
Available online 6 July 2013

KEYWORDS
Psoriasis;
Regulatory T cells;
Immunosuppression

Abstract CCR5 expression on CD4$^+$CD25^{high}Foxp3$^+$ regulatory T cells (Tregs) has been reported to be crucial for limiting Th1 inflammation associated with autoimmunity and bacterial infections. We inquired whether abnormalities in chemokine receptors expressed on Tregs might be involved in the psoriatic pathogenesis. Indeed, the proportion of CCR5$^+$ Treg was 58.8% in healthy individuals (n = 9), whereas only half as many CCR5$^+$ Treg cells were found in psoriatic individuals (29.1%, n = 8, $p < 0.01$). The flow-enriched control CCR5$^+$ Tregs consistently exceeded the suppressive capacity of unsorted Tregs in autologous MLR assays (n = 5, $p < 0.05$) showing that CCR5$^+$ Treg subset is a high potency regulatory T cell population. Interestingly, psoriatic CCR5$^+$ Treg cells exhibited significantly less migratory capacity toward CCR5 ligands MIP-1β and RANTES in vitro compared to CCR5$^+$ Treg controls (n = 3, $p < 0.05$). Our data demonstrate that psoriatic CCR5$^+$ Tregs cells are numerically-, functionally- and chemotactically-deficient compared to controls and may pose a triple impairment on the ability of psoriatic Tregs to restrain inflammation.

☆☆ Grant numbers and sources of support: National Institutes of Health (P30AR039750, P50AR055508; R01AR051498 KDC), Murdough Family Center for Psoriasis (HS, TSM, KDC).
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1521-6616
http://dx.doi.org/10.1016/j.clim.2013.06.007

1. Introduction

Human regulatory T cells (Tregs) are considered the immune system guardians against development of autoimmune disorders, being responsible for maintaining self-tolerance and playing an important role in limiting autoreactive and pathogenic cells. Tregs were initially characterized by expression of CD4 and high expression of the interleukin (IL)-2 receptor [alpha]-chain (CD25) [1]. Subsequent to this description, expression of the transcription factor Forkhead box P3 (Foxp3$^+$) was described to be the master transcriptional regulator of Tregs [2], with a concomitant lack of expression of the Interleukin-7 receptor subunit alpha (IL7R-α or CD127) [3]. Murine confirmation of the Treg master transcription factor Foxp3 was demonstrated when the mutation resembled human patients with immune dysregulation, polyendocrinopathy, enteropathy and X-linked syndrome (IPEX), diseases which carry a direct link to Foxp3 [4,5].

Recently, several studies have demonstrated that various chemokine receptors including CC chemokine receptor 5 (CCR5) are constitutively expressed on Tregs in humans and likely provide Tregs with a competitive advantage over naive T cells to migrate more efficiently to the periphery [6–9]. In addition to regulatory cell trafficking CCR5 also participates in control of effector functions of memory/effector T lymphocytes, macrophages, and immature dendritic cells (DCs). CCR5 is a G-Protein Coupled Receptor (GPCR) that binds the chemokines CCL5 (RANTES), CCL3 (MIP-1α) and CCL4 (MIP-1β) and is expressed on effector T cells that home toward peripheral sites of inflammation [10]. Deficiency in CCR5 leads to a reduced T cell infiltration in parasitic infections such as *Trypanosoma cruzi* [11,12] and *Toxoplasma gondii* [13], as well as viral infections [13].

Several human autoimmune diseases exhibit a dysregulated T cell response in which the activity of pathogenic effector T cells (Teff) is inadequately controlled by naturally occurring CD4$^+$CD25^{high} Foxp3$^+$ CD127$^-$ Tregs [14,15]. In those cases, functional defects appear in Teff as well as Treg populations which lead to a loss of tolerance and subsequent activation and differentiation of pathogenic Th1 and Th17 cells [16,17]. Psoriatic Tregs are deficient in suppressing not only autoimmune Teffs but also normal (healthy) Teffs as shown in previous "criss-cross experiments" [18]. We also previously demonstrated that, as shown in the mouse [19] IL-6 could reverse the function of healthy human Tregs [20] Interleukin-6, which is highly elevated in psoriatic tissue, was demonstrated to induce Stat3 signaling prior to subsequent T cell activation, which results in the loss of functional Treg suppression [21]. However, the underlying mechanisms responsible for the suboptimal suppressive activity of psoriatic Treg remain incompletely defined. Recently CCR5 expression has been shown to be important for high potency Treg cells in a mouse model [22].

In the current manuscript, we show that the presence of the chemokine receptor CCR5 on the surface of regulatory T cells is associated with Tregs that exhibit enhancement of their suppressive potential in vitro. Indeed, Treg cells with higher CCR5 expression are superior suppressors of activated Teff cells and exhibit higher levels of the transcription factor Foxp3 as measured by flow cytometry and real-time PCR. Unfortunately for psoriasis patients CCR5 expression on Tregs is decreased compared to healthy controls, leading to a reduction in the CCR5$^+$Foxp3^{high} high potency Treg population. Chemotaxis assays confirmed that CCR5$^+$ Tregs isolated from healthy controls display superior migration toward the CCR5 ligands MIP1β and RANTES compared to CCR5$^+$ Tregs from psoriasis patients. These results suggest that impaired CCR5 likely contributes to the long-term failure of psoriatic Tregs to competently suppress the expanding effector cell response.

2. Materials and methods

2.1. Cell isolation and culture of human dermal cells and PBMCs

All studies involving human subjects were approved by the Institutional Review Boards of Case Western Reserve University and University Hospitals Case Medical Center. Punch biopsies and/or peripheral blood samples were obtained from healthy adult volunteers or patients with moderate plaque psoriasis following informed consent. Primary dermal cells were isolated as previously described [23] and cultured in complete medium (RPMI 1640 containing 10% FBS (Cambrex), L-glutamine, penicillin, streptomycin and 2 ME (Cellgro)). PBMCs were prepared from peripheral blood as previously described [18] and adhered to plastic for 1 h to enrich for the non-adherent, lymphocyte-containing fraction. Dermal cell suspensions were prepared from lesional skin biopsies as described previously, with minor modifications [23]. The DNCB exposure in psoriatic patients was a one-time pilot observational experiment. Viability was determined by trypan blue exclusion (70–90% range); CD4$^+$ cells were separated from PBMC's by negative selection on midiMACS columns (CD4$^+$ T cell isolation kit; Miltenyi Biotec) according to the manufacturer's instructions. After over-night incubation, CD4$^+$CD25^{high} (top 5%) CCR5$^+$ cells were sorted using a BD FACS Aria flow cytometer and FoxP3 was analyzed.

2.2. Proliferation assays

CD4$^+$CD25$^-$ Teff, 2 × 10^4 cells were cultured 6–7 days in round-bottom, 96-well plates (Costar) in the presence or absence of various dilutions of Treg cells. 1 × 10^5 plastic-adherent 3000-rad irradiated PBMCs were used as APCs per well. Cells were pulsed with 1 μCi/well [3H] thymidine for the last 16 h before harvesting on the day described.

2.3. Antibodies and flow cytometry

Surface markers on blood or skin cells were detected by incubation with CD4-APC and CD25-PE monoclonal antibodies (mAbs) (BD), followed by fixation in 4% paraformaldehyde. To detect intracellular antigens, cells were permeabilized using a perm/fix kit (BD Biosciences). Analytical samples were analyzed on a BD LSR II flow cytometer (BD Biosciences). The following chemokine receptor mAbs were used for analyzing sorted Tregs: anti-CD62L-PECy5, anti-CLA-FITC, anti-CCR4-Pecy7, anti-