Available online at www.sciencedirect.com
ScienceDirect
Cellular Immunology 247 (2007) 1–11
Cellular Immunology
www.elsevier.com/locate/ycimm

Modulation of CLA, IL-12R, CD40L, and IL-2Rα expression and inhibition of IL-12- and IL-23-induced cytokine secretion by CNTO 1275

Manjula Reddy [a,*], Cuc Davis [a], Jackson Wong [a], Paul Marsters [b], Charles Pendley [a], Uma Prabhakar [a]

[a] *Department of Clinical Pharmacology and Experimental Medicine, Centocor, Inc., Malvern, PA, USA*
[b] *Department of Non-clinical Statistics, Centocor, Inc., Malvern, PA, USA*

Received 13 April 2007; accepted 25 June 2007
Available online 29 August 2007

Abstract

Cytokines interleukin (IL)-12 and IL-23 are implicated in the pathogenesis of psoriasis. IL-12 causes differentiation of CD4+ T cells to interferon-γ (IFN-γ)-producing T helper 1 (Th1) cells, while IL-23 induces differentiation to IL-17-producing pathogenic Th17 cells. The effects of the monoclonal antibody to IL-12/23 p40 subunit (CNTO 1275) on IL-12 receptor (IL-12R) expression, markers associated with skin homing, activation, and cytokine secretion were investigated *in vitro* using human peripheral blood mononuclear cells (PBMCs) from healthy donors. PBMCs were activated in the presence or absence of recombinant human (rh) IL-12 or rhIL-23, with or without CNTO 1275. CNTO 1275 inhibited upregulation of CLA, IL-12R, IL-2Rα and CD40L expression and also inhibited IL-12- and IL-23-induced IFN-γ, IL-17A, tumor necrosis factor (TNF)-α, IL-2, and IL-10 secretion. Thus, the therapeutic effect of CNTO 1275 may be attributed to the IL-12/23 neutralization, resulting in decreased expression of skin homing and activation markers, and IL-12- and IL-23-induced cytokine secretion.

Keywords: Cytokines; IL-12; IL-23; CNTO 1275; Psoriasis; IL-17

1. Introduction

Psoriasis is a chronic, T cell-mediated, inflammatory skin disease affecting approximately 1–3% of the world's population [1,2]. Both interleukin (IL)-12[1] and IL-23 are known to be involved in the pathogenesis of psoriasis [2–5], as well as other autoimmune diseases [6–8]. IL-12 is composed of the subunits p35/p40, while IL-23 is composed of the subunits p19/p40. Both cytokines bind via the p40 subunit to the IL-12 receptor β1 (IL-12Rβ1) located on the surface of T and natural killer (NK) cells [6]. The unique subunits of IL-12 (p35) and IL-23 (p19) bind to distinct receptors, IL-12Rβ2 and IL-23R, respectively [9]. While IL-12Rβ1 subunit is the ligand binding subunit, IL-12Rβ2 is the intra-cellular signaling component of the IL-12R complex, but both subunits are necessary for high affinity binding to IL-12 [10]. Similar to IL-12Rβ2, IL-23R is not detected on resting naïve T cells, but is induced rapidly upon activation [11]. IL-12 and IL-23 receptor binding initiates the respective cytokine-signaling cascades of IL-12 and IL-23.

The role of IL-12 in psoriasis including T cell differentiation towards T_H1 cytokine producing cells and facilitating T cell homing to the skin via induction of cutaneous lymphocyte antigen (CLA) has been well documented [3,12,13]. Less than 20% of peripheral blood T cells express

* Corresponding author. Address: Centocor Research and Development, Inc., 145 King of Prussia Road, Rm. 2512, Radnor, PA 19087-4517, USA. Fax: +1 610 651 6262.
E-mail address: MReddy@CNTUS.JNJ.COM (M. Reddy).

[1] *Abbreviations:* CLA, cutaneous lymphocyte antigen; IL-interleukin; IL-12R, interleukin-12 receptor; IFN-γ, interferon gamma; PBMCs, peripheral blood mononuclear cells; TNF-α, tumor necrosis factor-alpha.

0008-8749/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.cellimm.2007.06.006

CLA, while the majority of effector memory T cells in normal skin (under resting conditions) are CLA positive [14,15]. About 85% of T cells infiltrating psoriatic lesions and <5% of T cells in extracutaneous sites express CLA [14,16]. Evolving psoriatic lesions are reported to be enriched with CLA+ T cells preceding epidermal hyperproliferation. Also, a positive correlation was shown between disease severity and frequency of CLA expressing T cells in peripheral blood and lesions in psoriatic patients [13]. Several other activation (CD25) [17], co-stimulatory (CD40L) [18], and chemokine receptors (CXCR3) [19,20] have also been implicated in the pathogenesis of psoriasis.

In addition to cell surface expression markers, a complex network of stimulatory and inhibitory cytokines has been associated with psoriatic lesions with elevated levels of IL-12 (p40), IL-23, interferon-gamma (IFN-γ), IL-17A, tumor necrosis factor-alpha (TNF-α), IL-10, IL-5, IL-1, IL-2, IL-6, and IL-8 [3,13,21–24]. Recently, IL-23 has also been reported as a key cytokine in the chronic inflammation associated with psoriasis mediated by the IL-23-induced pro-inflammatory cytokine, IL-17 [4,25]. Also, Chan et al. showed that injection of IL-23 into the skin of mice promoted greater epidermal thickness than the injection of IL-12 [24]. Further, Zheng et al. indicated that IL-12 injections resulted in increased IFN-γ levels, whereas IL-23 increased the levels of IL-17A, IL-22, and other cytokines, but not IFN-γ [26]. Therefore, a therapy based on the neutralization of the common subunit of IL-12 and IL-23 (p40) should alter the downstream cytokine induction pathways leading to improved clinical efficacy in the treatment of psoriasis.

The first-in-human phase I [27] and phase II [28] clinical trials using the fully human IgG1 monoclonal antibody specific for the p40 subunit of IL-12 and IL-23, designated CNTO 1275 (Centocor, Inc., Malvern, PA), showed a significant improvement in psoriatic lesions in patients with moderate-to-severe psoriasis; CNTO 1275 binds to the IL-12/23 p40 subunit with high affinity, thereby blocking the IL-12Rβ1 receptor binding and preventing subsequent IL-12 and IL-23 receptor mediated signaling. The study presented here focused on the *in vitro* effects of CNTO 1275 on pro-inflammatory (IFN-γ, IL-17A, TNF-α) and anti-inflammatory (IL-10 and IL-5) cytokine secretion by polyclonal activators in the presence of exogenous IL-12 and IL-23. CNTO 1275 was shown to neutralize IL-12 and IL-23 in activated human peripheral blood mononuclear cells (PBMCs), which resulted in decreased expression of CLA, CD25, CD40L, and IL-12R, and inhibition of pro-inflammatory cytokine secretion. In the current study, we show that IL-12 and IL-23 exert differential cytokine secretion profiles and induction of T cell markers indicating that regardless of their individual contributions, targeting both cytokines may be a more effective therapeutic strategy. Notable clinical efficacy data from psoriasis phase I [27] and phase II [28] clinical studies using CNTO 1275 in psoriasis, supports this strategy.

2. Results

2.1. Recombinant human IL-12 (rhIL-12)-induced upregulation of CLA expression on activated T cells is inhibited by CNTO1275

To determine the effects of CNTO 1275 on rhIL-12-induced expression of CLA, PBMCs were isolated from healthy donors and stimulated *in vitro* with either the mitogen phytohemaglutinin (PHA), anti-CD3 + anti-CD28 (α-CD3 + α-CD28), or the superantigen Staphylococcus Enterotoxin B (SEB), in the presence or absence of exogenous rhIL-12 or rhIL-23, and with or without CNTO 1275. Expression of CLA was increased by activation and further increased by the addition of exogenous rhIL-12, and inhibited by CNTO 1275 (Fig. 1a). The induction of CLA expression was specific for IL-12 because IL-23 (Fig. 1b), IL-6, and TNF-α did not affect CLA expression (data not shown). Co-stimulation of PBMCs with PHA and rhIL-12 enhanced CLA expression by 30% compared with unstimulated (non-PHA activated or non-IL-12 activated) levels, as measured by percent positive cells (Fig. 2a) and antigen density (mean fluorescent intensity, MFI; Fig. 2b).

The addition of CNTO 1275 to the PBMC cultures inhibited the expression of CLA induced by PHA and rhIL-12 (Fig. 1a and Fig. 2a), and decreased its antigen density (MFI; $p = 0.056$; Fig. 2b). Pre-blocking of CNTO 1275 with a neutralizing anti-idiotypic antibody (NAb) (CNTO 1438, Centocor) specific to CNTO 1275 fully reversed the inhibitory effect of CNTO 1275 (MFI; $p = 0.034$; Fig. 2b), whereas a non-NAb (CNTO 8612, Centocor) did not alter the inhibitory effects of CNTO 1275 (Fig. 2a and b). When PBMCs were activated with α-CD3 + α-CD28 and rhIL-12, similar inhibitory effects of CNTO 1275 were observed ($p = 0.002$; data not shown). Additionally, SEB alone induced significant CLA expression that was inhibited by CNTO 1275, with or without exogenous rhIL-12 ($p = 0.048$ and 0.027, respectively), as superantigens themselves are potent inducers of IL-12 (data not shown).

2.2. Effects of CNTO 1275 on IL-12R expression

To determine the effect of CNTO 1275 on IL-12R expression, PBMCs were activated with PHA in the presence or absence of rhIL-12, with or without preincubation with CNTO 1275. While unstimulated T cells expressed moderate levels of IL-12Rβ1 and very low to undetectable levels of IL-12Rβ2, activation with PHA (and also α-CD3 + α-CD28 or SEB; data not shown) upregulated expression of both IL-12Rβ1 (Fig. 3a) and IL-12Rβ2 (Fig. 3b). As expected, PHA and exogenous rhIL-12 (Fig. 3c) had a negligible effect on IL-12Rβ1 expression ($p = 0.372$) and significantly increased IL-12Rβ2 receptor expression by >30% compared with unstimulated levels ($p = 0.006$). The addition of CNTO 1275 had a negligible effect on IL-12Rβ1 expression ($p = 0.690$), but significantly