Clinical Immunology (2010) **135**, 430–439

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Monocytes differentiated with IL-15 support Th17 and Th1 responses to wheat gliadin: Implications for celiac disease

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Received 3 November 2009; accepted with revision 12 January 2010
Available online 11 February 2010

KEYWORDS
IL-15;
IL-23;
Innate immunity;
Th17;
Th1;
Celiac Disease;
Autoimmunity

Abstract Interleukin (IL)-15 contributes to the immunopathogenesis of Celiac disease (CD). However, it is not clear how IL-15 affects APC that shape adaptive immune responses to the dietary antigen, gliadin. Using PBMC from healthy individuals, we show that monocytes differentiated with IL-15 (IL15-DC) produced IL-1β, IL-6, IL-15, IL-23, TNFα and CCL20 in response to pepsin-trypsin digested gliadin (PTG) and activated contact-dependent Th17 and Th1 responses from autologous $CD4^+$ T cells. Lower concentrations of IL-15 augmented IFNγ responses to PTG in PBMC from CD patients compared to controls. Thus, IL-15 supports Th17 and Th1 responses to a dietary antigen that is normally well-tolerated in healthy individuals by generating IL15-DC. These potentially pathogenic immune responses may result in CD patients and not healthy individuals as a consequence of IL-15 hypersensitivity. Therefore, genetic and/or environmental factors that control IL-15 expression and responsiveness in the intestine likely participate in the pathogenesis of CD.

Introduction

Th17 cells are memory $CD4^+$ T cells implicated in the pathogenesis of an increasing number of tissue-specific autoimmune diseases [1–6]. Circulating human Th17 cells are characterized by the expression of the surface markers CD45RO, CD161, IL-23R, CCR6 and CCR4 [7,8]. Th17 cells are thought to perpetuate inflammation by secreting the proinflammatory mediators IL-17, IL-21, IL-22 and IFNγ that act on immune and nonimmune cells within the local tissue [8]. Although it is not yet clear how human Th17 cells develop from naïve $CD4^+$ T cells *in vivo*, *in vitro* studies indicate that memory Th17 responses are induced by interactions with activated monocytes or their $CD14^+$ tissue progeny and the proinflammatory mediators IL-1β, IL-6, IL-15, IL-23, TNFα and CCL20 that are produced in response to danger signals [3,9–13]. Surprisingly, the relationship between Th17 cells and IL-15 was identified in rheumatoid arthritis (RA) before the discovery of IL-23 and the Th17 hypothesis, and yet the role of IL-15 in the generation, activation and/or expansion of this T helper subset remains to be determined [1,14].

IL-15 is a pleiotropic cytokine related to innate immunity, which has been associated with numerous inflammatory

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1521-6616/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2010.01.003

conditions, including celiac disease (CD), Crohn's disease, psoriasis, RA, type 1 diabetes and multiple sclerosis [3,15–19]. IL-15 provides activating and survival signals to CTL and is thought to indirectly modulate $CD4^+$ T cell responses by acting on APC [20,21]. During inflammation, monocytes infiltrate the affected tissue and differentiate into various types of inflammatory APC depending on the cytokine milieu. In the presence of IL-15, monocytes differentiate into potent APC that exhibit a unique phenotype, coexpressing surface markers thought to distinguish monocytes/macrophages (CD14) from myeloid (CD86, CD209) and plasmacytoid (BDCA2, CD123) DC [21,22]. Therefore, IL-15 in the inflamed tissues of individuals with autoimmune diseases may promote Th17 responses by generating this unique subset of $CD14^+$ APC [10,23,24].

Among the spectrum of autoimmune disorders associated with IL-15 and the Th17 axis, CD is the only one in which the major genetic (HLA-DQ2/8) and environmental factors (dietary glutens) are known [2,6,25]. CD is an enteropathy generally considered to be mediated by gliadin-specific IFNγ-producing $CD4^+$ T cells that are restricted by the disease associated alleles, HLA-DQ2 and HLA-DQ8 [26]. Still, the majority of HLA-$DQ2^+/8^+$ individuals ($\sim$35% of the general U.S. population) tolerate dietary glutens [27] and "humanized mice" engineered to express HLA-DQ2 did not develop the intestinal pathology that is seen in CD even though gliadin-specific $CD4^+$ T cell responses occurred in these animals [28]. Thus, factors in addition to $CD4^+$ T cells that recognize peptide restricted HLA alleles are likely required for CD. Accumulating evidence indicate that IL-15 is one of these factors.

Increased levels of IL-15 have been detected in the intestinal epithelium and lamina propria of patients with untreated CD compared to patients in remission and healthy controls [29]. Furthermore, it has been shown that the p31-43 peptide in α-gliadin rapidly induced $CD68^+$ APC to produce IL-15 in intestinal explants from CD patients and not healthy controls. Importantly, this IL-15 response was found to modulate IEL-mediated epithelial destruction and Th1 activation *ex vivo* [25]. More recently, the pathologic features of CD were recapitulated in the proximal small intestine of mice engineered to over express IL-15 in enterocytes *in vivo* [30]. Together, these findings indicate that production of IL-15 in an unidentified subset of $CD68^+$ (monocyte-derived) APC in the mucosa is fundamental to the pathogenesis of CD.

Although augmented levels of IL-15, increased numbers of $CD14^+$ cells and higher expression of Th17 and Th1 promoting factors have been described in the intestine of untreated CD by independent reports, a direct relationship between these separate observations has not been examined [2,6,24,29,31].

We previously reported that a pepsin–trypsin digest of gliadin (PTG) induced PBMC from patients with CD as well as PBMC from healthy controls to secrete cytokines associated with the generation of Th17 cell responses [32]. The difference between PBMC from healthy individuals compared to PBMC from CD patients was significantly higher levels of proinflammatory cytokines produced by the latter. In addition, we determined that circulating monocytes ($CD14^+$) were the source of the observed cytokine profile induced by PTG. These observations together with the data cited above regarding IL15-DC and the key role of IL-15 in the pathogenesis of CD, prompted us to test the hypothesis that IL-15 promotes Th17 and Th1 responses by differentiating monocytes into IL15-DC ($CD14^+$) that produce activating signals upon encounter with PTG. For practical reasons, the majority of these studies were carried out using PBMC from healthy individuals since we found similar qualitative responses with these cells to PTG as that found in PBMC from CD patients. The rationale for using monocytes is justified by the accumulation of monocyte-derived cells that has been described in the inflamed intestine of CD patients on a gluten-containing diet [24,31].

Monocytes differentiated into $CD11c^+CD14^+CD209^+$HLA-DQ^+ cells in the presence of GM-CSF and IL-15 (IL15-DC) and produced IL-15, IL-23 and other memory Th17 and Th1 promoting cytokines in response to PTG. Conversely, monocytes that were differentiated into immature DC ($CD11c^+CD14^-CD209^+$) with GM-CSF and IL-4 (IL4-DC) did not. The IL15-DC loaded with PTG induced secretion of IL-17 and IFNγ in autologous $CD4^+$ T cells from healthy individuals, which normally tolerate this common dietary antigen. Secretion of IL-17 and IFNγ was cell-contact dependent and in addition, IFNγ production required surface expression of IL-15, similar to the mechanism reported in RA by Miranda-Carus et al. [19]. Notably, PBMC from CD patients had significantly higher IFNγ responses to PTG compared to PBMC from controls when exposed to 10-fold lower levels of IL-15. Together, these results indicate that IL-15 supports Th17 and Th1 responses by skewing monocytes into IL15-DC ($CD14^+$ APC) and in addition, offer insight as to how IL-15 expression and responsiveness may orchestrate the immune response to gliadin in individuals with and without CD.

Materials and methods

Cells

PBMC from CD patients and healthy donors were isolated by density gradient centrifugation in Lymphocyte Separation Medium (ICN Biomedicals Inc.) according to manufacturer's instructions. Purified lymphocytes and monocytes were obtained from healthy donors as above followed by counter-current centrifugal elutriation. All individuals gave informed consent. The study protocol was approved by the Institutional Review Board at the University of Maryland School of Medicine.

DNA extraction and HLA typing

DNA was extracted from PBMC using the QIAamp DNA Mini Kit (Qiagen). HLA alleles were determined by One Lambda Micro SSP™ ABDR Typing Kit (One Lambda) and DQA1 and DQB1 SSP UniTray® Kits (Dynal Biotech) per manufacturers' instructions.

Reagents

PTG was prepared by enzymatic digestion as follows. 50g of crude gliadin (Sigma) was dissolved in 500 ml of 0.2 N HCl for