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Detection of IL-17A-producing peripheral blood monocytes in Langerhans cell histiocytosis patients

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Abstract Langerhans cell histiocytosis (LCH) is a rare disease of unknown cause with manifestations ranging from isolated granulomatous lesions to life-threatening multi-system organ involvement. This disorder is further characterized by infiltration of immune cells in affected tissues and an association with interleukin (IL)-17A has been reported. Here, we

Abbreviations: LCH, Langerhans cell histiocytosis; PBMCs, peripheral blood mononuclear cells; TNFα, tumor necrosis factor α; IL, interleukin; IFNγ, interferon γ; MMPs, matrix metalloproteinases; DCs, dendritic cells; moDCs, monocyte-derived DCs; IL-17RA, IL-17A receptor A; ROR, retinoic acid orphan receptor; PD, periodontitis; PMA, phorbol myristate acetate; CT, threshold cycle.

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investigated the presence of IL-17A-producing cells among peripheral blood mononuclear cells isolated from LCH patients and observed a high percentage of IL-17A$^+$ monocytes in peripheral blood of LCH patients compared to controls. The IL-17A$^+$ monocytes were also positive for the transcription factor retinoic acid orphan receptor (ROR) γt and showed increased mRNA levels for both IL-17A and RORγt. Notably, IL-17A was produced by all monocyte subsets and the expression level was positively associated with LCH disease activity. These data support a role for monocytes in the pathogenesis of LCH. Future therapeutic approaches may consider identification of patients who may benefit from IL-17A-targeted interventions.

1. Introduction

Langerhans cell histiocytosis (LCH) is a rare disease of unknown origin, but associated with inflammatory and tissue-remodeling responses in affected organs. Clinical manifestations range from asymptomatic, spontaneously regressing single lesion to a disseminated, life-threatening multi-system disorder [1]. Although it is not yet proven whether LCH is a neoplastic disease or a chronic inflammatory disease defined by a reactive process, a somatic *B-RAF* (*BRAF*) V600E mutation that encodes a known oncogenic form of the protein has been detected in 38–57% of the LCH granulomas studied [2–4]. This points towards a neoplastic component, whereas the formation of granulomas, which sometimes spontaneously regress, is indicative of a reactive inflammatory process. Only one out of the 56 peripheral blood mononuclear cell (PBMC) samples studied had a *BRAF* (germline) mutation [3]. Moreover, no somatic mutations could be detected in cells in the bone marrow or blood of patients whose lesions were positive for the mutation [3,4]. Still, constitutive activation of *BRAF* has been reported not only in patients with cancer, but also in patients with rheumatoid arthritis [5]. Thus, the contribution of *BRAF* mutations in LCH development needs further investigation and the cause of LCH remains unknown.

In LCH, upregulation of inflammatory cytokines, including tumor necrosis factor α (TNFα), various interleukins (IL), interferon γ (IFNγ), matrix metalloproteinases (MMPs) and granulocyte macrophage-colony stimulating factor, which are detected in lesions and blood [6–12], could explain the inflammation and other symptoms of LCH, such as osteolysis. Furthermore, some features of LCH, such as granuloma formation [13] and bone resorption [14,15], are shared with other IL-17A-associated diseases. IL-17A has been detected by immunofluorescence in LCH granuloma-associated dendritic cells (DCs) [9] and furthermore, higher levels of the IL-17A receptor A (IL-17RA) have been detected on DCs in lesions from patients with multisystem LCH as compared to those with single-system disease [16]. Although some of these observations point towards the IL-17A/IL-17RA axis as one component being involved in the pathogenesis of LCH, the role of IL-17A in LCH is debated [17]. In other inflammatory diseases however, subsets of human lymphocytes, lymphoid tissue inducer cells, as well as alveolar macrophages, neutrophils and eosinophils have been shown to produce IL-17A and confirmed to be associated with the pathogenesis of the disease [18]. In these cells, induction of transcription of the gene that encodes IL-17A is mediated by retinoic acid orphan receptor (ROR) γt, a transcription factor encoded by the gene *RORC* [18].

IL-17A plays a critical role in host defense against pathogens: it synergizes with other cytokines to amplify tissue inflammation via induced release of pro-inflammatory cytokines and chemokines and up-regulates growth factors, antimicrobial peptides and MMPs [19]. Furthermore, IL-17A promotes long-term survival and chemoresistance of in vitro generated monocyte-derived DCs (moDCs) [20]. Notably, endogenous production of IL-17A by moDCs was found to promote moDCs fusion and formation of multinucleated giant cells that expressed tissue destructive enzymes [9,21]. In plasma from LCH patients with active disease, IL-17A was detected by ELISA at relatively high [9,16] or low [22] levels, but no blood leukocytes stained positive for intracellular IL-17A analyzing 6 out of 13 patients [9]. Whether blood monocytes, similar to LCH moDCs, also have the capacity to produce IL-17A and express the IL-17RA in chronic inflammatory diseases has thus far not been reported.

In this study, we analyzed 22 LCH patients with different disease activity to explore whether IL-17A producing blood leukocytes could be detected in freshly isolated PBMCs. In this setting we show that both CD14^{high} and CD14^{low} IL-17A-producing monocytes were present in the blood of LCH patients with active disease. Furthermore, monocytes were the predominant population of IL-17A-producing cells in patients with detectable levels of IL-17A$^+$ leukocytes. Both IL-17A protein and mRNA were detected in monocytes and the proportion of IL-17A producing monocytes was related to the disease activity. To the best of our knowledge, this is the first report showing that monocytes produce IL-17A in a human disease, and that this is positively correlated with increased RORγt production by the monocytes. Future diagnostic and therapeutic approaches of LCH may therefore consider quantifying and targeting circulating monocytes in order to monitor and manage the over-production of IL-17A.

2. Material and methods

2.1. Blood samples

Altogether 22 patients (13 males and 9 females; Table 1) with a confirmed LCH diagnosis were enrolled in this study after providing informed consent. Patients were classified by disease activity, ranging from zero to three (0, Resolution; 1, Mild; 2, Moderate; 3, Marked disease), as previously reported [9]. Freshly collected peripheral blood samples from healthy adult volunteers (n = 22) were received from the Blood Transfusion Clinic at the Karolinska University