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Immunohistochemical Evidence of a Cytokine and Chemokine Network in Three Patients With Erdheim-Chester Disease

Implications for Pathogenesis

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Objective. **Erdheim-Chester disease (ECD) is a rare form of non–Langerhans' cell histiocytosis (LCH) of unknown etiology, characterized by diffuse histiocyte infiltration of bones and soft tissue. The purpose of this study was to assess cell proliferation and expression of cytokines, chemokines, and chemokine receptors that may potentially be important in histiocyte accumulation in ECD lesions.**

Methods. **Biopsies were performed on 3 patients with ECD. The diagnosis of the disease was based on clinical signs including typical radiologic osteosclerosis, and on the detection of foamy CD68+,CD1a− non–Langerhans' cell histiocytes on histologic examination. The expression of the proliferation marker Ki-67 as well as of selected chemokine/chemokine receptor pairs and cytokines was analyzed by immunohistochemistry.**

Results. **In all samples, Ki-67 was undetectable in CD68+ histiocytes. Conversely, these cells expressed the chemokines CCL2 (monocyte chemotactic protein 1), CCL4/macrophage inflammatory protein 1β (MIP-1β), CCL5/RANTES, CCL20/MIP-3α, and CCL19/MIP-3β, and their counter-receptors CCR1, CCR2, CCR3, CCR5, CCR6, and CCR7. Moreover, ECD histiocytes expressed interferon-γ–inducible 10-kd protein (CXCL10), which is specifically induced by interferon-γ, and interleukin-6 and RANKL, which are both implicated in bone remodeling. Finally, all cases showed a Th1-type lymphocyte infiltrate.**

Conclusion. **Our data indicate that, similar to LCH, ECD lesions are characterized by a complex cytokine and chemokine network, which may orchestrate histiocyte activation and accumulation through an autocrine loop and contribute to the pathogenesis of the disease.**

Erdheim-Chester disease (ECD) is a rare form of systemic non–Langerhans' cell histiocytosis (LCH), with ~250 cases described so far. The disease represents a distinct pathologic entity characterized by nearly pathognomonic osteosclerosis, especially of the long bones, and frequently by extraskeletal involvement. Histologically, the lesions consist of diffuse infiltration of bones and soft tissue (kidney, retroperitoneal space, skin, brain, and lung) by foamy CD68+,CD1a− non–Langerhans' cell histiocytes (1–3).

The etiopathogenesis of the disease is unknown; in particular, at variance with LCH (4), attempts to demonstrate the clonality of histiocytes in ECD have shown inconclusive results (5). Moreover, the mechanisms leading to histiocyte accumulation in ECD lesions have not been elucidated. In this regard, it is well known that leukocyte migration to tissues may be driven by chemokines via interaction with their specific receptors

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Table 1. Expression of chemokines and chemokine receptors by foamy macrophages in lesions from patients with Erdheim-Chester disease*

Patient	CD68	CCR1	CCL4	CCR2	CCL2	CCR3	CCL5	CCR7	CCL19	CXCR3	IP-10
1	72	83	69	>90	>90	>90	>90	66	21	>90	15
2	65	>90	>90	>90	>90	>90	>90	79	32	ND	22
3	45	>90	>90	>90	>90	>90	>90	15	12	>90	41

* Biopsy samples of the optic nerve sheath from patient 1, a bone lesion from patient 2, and a pulmonary lesion from patient 3 were evaluated by immunohistochemistry. Values for CD68 are the percentage of CD68+ macrophages in the inflammatory infiltrate (interstitial macrophages in the lung). All other values are the percentage of positive macrophages. The number of positive cells in 10 high-power fields (400×) was determined with the use of a 1-mm^2 optical microscope grid and is reported as the percentage of CD68+ cells, as evaluated in a comparable serial section. IP-10 = inducible protein 10; ND = not done.

(6–8). Indeed, recent evidence shows the expression of distinct patterns of chemokine/chemokine receptor pairs in the lesions of LCH, which may be responsible for the recruitment and retention of the pathologic Langerhans' cells (4,9). No similar information is as yet available for ECD histiocytes.

Here we report the results of the immunohistochemical analysis of expression of proliferation markers, cytokines, and chemokine/chemokine receptors in 3 patients with ECD.

PATIENTS AND METHODS

Patient 1 was a 45-year-old man with a 5-year history of diabetes insipidus, pulmonary fibrosis, and visual field loss. Cerebral magnetic resonance imaging (MRI) showed inflammatory infiltration surrounding the optic nerve bilaterally, skeletal radiographs showed symmetric osteosclerosis of the long bones of the lower limbs, and MRI of the abdomen showed retroperitoneal fibrosis. The diagnosis of ECD was confirmed by optic nerve sheath and transbronchial biopsies, and the patient was treated with steroids and alendronate.

Patient 2 was a 54-year-old man diagnosed as having diabetes insipidus at the age of 43, who then developed hypogonadotropic hypogonadism, periaortic fibrosis, and painful legs. Radiographs showed osteosclerosis of the long bones, and MRI showed a retroorbital mass surrounding the right optic nerve. A needle biopsy of the left femur yielded a diagnosis of ECD. The patient was then treated with steroids and bisphosphonates.

Patient 3 was a 52-year-old man with a 2-year history of panhypopituitarism, diabetes insipidus, pulmonary infiltration, and back pain. Cerebral MRI showed pituitary infiltration, but no bone lesion could be detected. A pulmonary biopsy showed histiocyte infiltrate. The patient was treated with steroids and bisphosphonates.

Histologic examination of biopsy samples of the optic nerve sheath from patient 1, a bone lesion from patient 2, and a pulmonary lesion from patient 3 revealed in all cases infiltration with CD1a−/S100−,CD68+ (Table 1) histiocytes with large foamy or eosinophilic cytoplasm, Touton giant cells, and loosely organized granulomas associated with lymphocytes and variable degrees of fibrosis. The patients were then diagnosed as having ECD.

Paraffin-embedded sections (5 μm) were dried, rehydrated, then subjected to heat-mediated antigen retrieval. The following antibodies were used: anti-CD68 (Dako, Carpinteria, CA), anti–interleukin-1 (IL-1), anti–tumor necrosis factor α (TNFα), anti–IL-10, anti–interferon-γ (IFNγ), anti-CCL2 (monocyte chemoattractant protein 1 [MCP-1]), anti–CCL4/macrophage inflammatory protein 1β (MIP-1β), anti-CCL5/RANTES, anti-CCL19 (Epstein-Barr virus–induced gene 1 ligand chemokine [ELC])/MIP-3β, CXCL10/IFN—inducible protein 10 (IP-10), anti-CCR1, anti-CCR2, anti-CCR3, anti-CCR5, anti-CXCR3 (all from R&D Systems, Minneapolis, MN), anti-CCR7 (eBioscience, San Diego, CA), and anti-RANKL (Santa Cruz Biotechnology, Santa Cruz, CA). After incubation with primary antibodies, a biotinylated secondary antibody was added, followed by streptavidin–horseradish peroxidase (Dako). Peroxidase activity was developed using H_2O_2 as a substrate and aminoethylcarbazole or diaminobenzidine (Dako) as chromogens.

The number of CD68+ cells and chemokine-positive and chemokine receptor–positive macrophages in 10 high-power fields (400×) that were recognized by morphology and CD68 expression on serial sections were counted by a pathologist (AS), using a 1-mm^2 optical microscope grid.

RESULTS

In order to investigate the mechanisms leading to histiocyte assembly in ECD lesions, biopsy samples from 3 patients (from the optic nerve sheath of patient 1, a bone lesion from patient 2, and a pulmonary lesion from patient 3) were analyzed morphologically for the presence of mitotic figures and by immunohistochemistry for the expression of proliferation markers and cytokines, chemokines, and chemokine receptors. The histiocytes were initially identified by CD68 expression. All macrophage types, epithelioid cells, tissue-infiltrating macrophages, Touton giant cells, and, particularly, foamy macrophages were stained by the antibody (results not shown). No mitotic figures were observed in different sections obtained from the 3 patients, and <1% of foamy macrophages stained with Ki-67 (results not shown), which is evidence against proliferation of precursors being a major determinant of histiocyte accumulation.

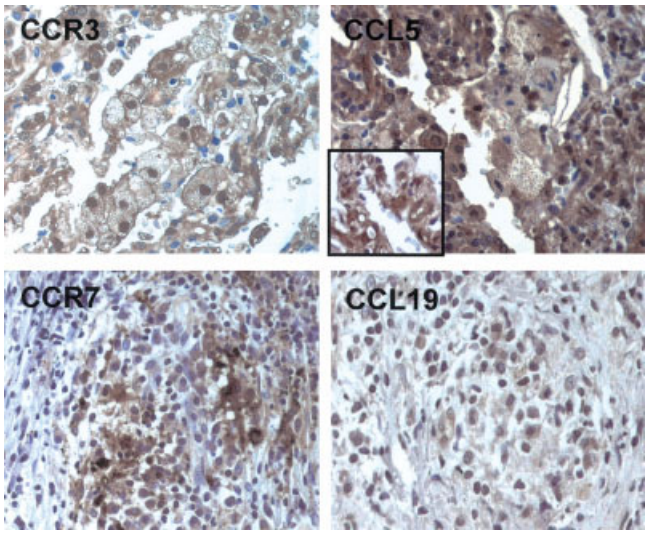

Figure 1. Chemokine and receptor expression in the Erdheim-Chester disease–affected lung of patient 3. The vast majority of the foamy macrophages, Touton giant cells, epithelioid macrophages of the granulomas, and, frequently, endothelial cells were strongly positive for inflammatory chemokines and their receptors. Reactivity of antibodies against the chemokine receptors CCR3 on the membranes of foamy and interstitial infiltrating macrophages and CCR7 on macrophages inside a granuloma, and against their respective ligands CCL5 and CCL19 in parallel tissue sections, is shown. CCL5 staining was also evident in the interstitial capillaries of a normal perilesional lung (boxed area). (Biotin/streptavidin–horseradish peroxidase–diaminobenzidine immunostained, hematoxylin counterstained; original magnification × 400.)

We then evaluated the expression of chemokine/chemokine receptor pairs selected for monocyte migration activity, including the inflammatory chemokines CCL2 (MCP-1), CCL4/MIP-1β, CCL5/RANTES, and their receptors CCR1, CCR2, CCR3, and CCR5 (6–8). As shown in Table 1, expression of chemokines and receptors was comparable in the 3 samples and independent of the organ location of the lesion. The vast majority of macrophages (the foamy type, the Touton giant cells, and the epithelioid macrophages of the granulomas) expressed CCR1, CCR2, CCR3, and CCR5 (Table 1 and Figure 1) and were also strongly positive for their respective ligands CCL4, CCL2, and CCL5 (Table 1 and Figure 1). The same chemokines were also expressed by the endothelial cells of the lesion's vessels. Interestingly, in the lung biopsy, where the perilesional normal tissue was sufficient to be analyzed, the interstitial capillaries were also found to be positive for CCL5/RANTES (boxed area in Figure 1). Moreover, a sizable fraction of CD68+ histiocytes expressed CCR7 and its ligand, CCL19/MIP-3β (Table 1, and Figure 1, lower panels), a chemoattractant for B and T lymphocytes and dendritic cells, which displays the unique capability to attract macrophage progenitors among myeloid progenitor cells (10). Finally, CCR6 and its ligand, CCL20/MIP-3α, exhibited similar expression patterns (data not shown).

Chemokine production can be activated by inflammatory cytokines, and elevated serum levels of IL-6 have been reported in ECD (11). Sections of the 3 biopsies were therefore stained with antibodies against IL-6, IL-1α, TNFα, IL-10, and IFNγ. IL-1α, TNFα (results not shown), and IL-6 were indeed strongly expressed in the lesions; in particular, as shown in Figure 2, macrophages, hyperplastic pneumocytes, and endothelial cells of the lesion vessels in the lung lesion were stained for IL-6 and, as observed with CCL5/RANTES, the peripheral healthy lung vessels also showed IL-6 staining. Altogether, ECD histiocytes shared features with the so-called polarized M1 macrophages that represent effector cells integrated in Th1 immune responses (12). Accordingly, staining of lung sections with antibodies against IFNγ and IL-10, which are prototypical cytokines of either a Th1- or a Th2-polarized microen-

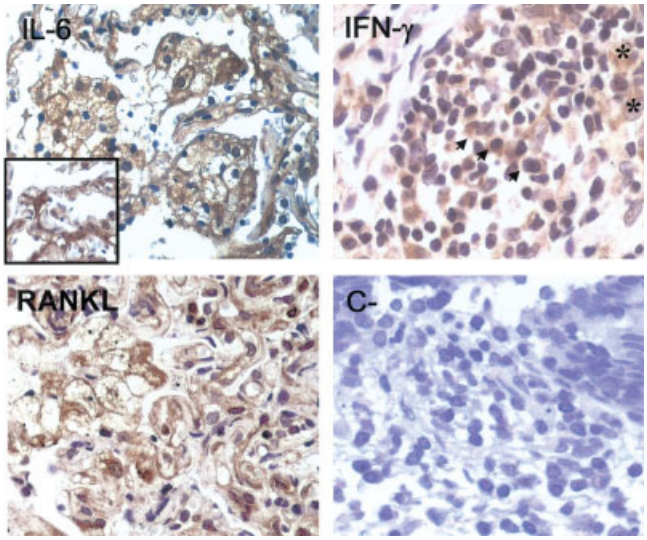

Figure 2. Cytokine expression in the Erdheim-Chester disease–affected lung of patient 3. Strong staining for interleukin-6 (IL-6) was observed in the foamy macrophages and the endothelial cells of normal lung vessels (boxed area). A large number of cells with lymphocyte morphology (**arrows**) were positive for interferon-γ (IFNγ); some macrophages (**asterisks**) were also stained. The foamy histiocytes were also stained with anti-RANKL antibody. C− = isotype-negative control. (Biotin/streptavidin–horseradish peroxidase–diaminobenzidine immunostained, hematoxylin counterstained; original magnification × 400.)

vironment (13), revealed only rare macrophages that express IL-10 (results not shown); conversely, a high number of cells, mostly with lymphocyte morphology, were IFNγ-positive (Figure 2). Moreover, a significant fraction of CD68+ cells (15% in patient 1, 22% in patient 2, and 41% in patient 3) (Table 1) expressed the chemokine CXCL10/IP-10 (Figure 2), which is specifically induced by INFγ (6–8), and its receptor CXCR3 (Table 1), further supporting the presence of a Th1-polarized microenvironment in ECD lesions.

Finally, to address the bone affinity of ECD macrophages, we assessed the expression of RANKL, which is involved in bone remodeling (14). Foamy and tissue macrophages, both in the bone lesion (58%) and surprisingly also in the lung (15%), were stained (Figure 2).

DISCUSSION

ECD is a rare form of non-LCH, characterized by the infiltration of bones and of different organs by foamy histiocytes (1–3). The diagnosis of the disease relies upon the typical radiologic osteosclerosis and histologic features; additionally, clinical signs such as exophthalmos and diabetes insipidus suggest a histiocytic disorder (1). We describe 3 patients with ECD. All 3 patients had diabetes insipidus; in the case of patients 1 and 2, the diagnosis of ECD was supported by the finding of symmetric long-bone osteosclerosis upon skeletal radiograph examination. Moreover, histologic examination of biopsies from all 3 patients showed infiltration by foamy histiocytes that expressed CD68 and lacked CD1a and Birbeck granules, pointing to a diagnosis of non-LCH (4).

The etiopathogenesis of ECD is unknown. At variance with nonpulmonary diffuse LCH (4), we could not detect expression of the proliferation marker Ki-67 by ECD histiocytes, both in lung and extrapulmonary lesions. The consensual absence of mitotic figures in the same cell types also argues for the limited pathogenetic relevance of proliferation. On the other hand, we provide evidence of an inflammatory milieu in ECD lesions, characterized by the expression of abundant and multiple cytokines and chemokines. Accordingly, an inflammatory phenotype has been reported in peripheral blood from 1 patient with ECD (15). Expression of distinct chemokine/chemokine receptor pairs has been described in LCH lesions (4,9), and has been proposed to function as an autocrine recruitment/retention factor for LCH cells. A similar circuit might be operating in ECD; indeed, a wide array of chemokines and receptors that may be responsible for macrophage migration/retention is expressed at the lesion site. Of interest is the concomitant expression of CCR6 and CCR7 by ECD histiocytes, which has been found in other forms of histiocytosis, including LCH, Rosai-Dorfman disease, and hemophagocytic syndrome, at variance with the mutually exclusive expression in normal tissue macrophages and normal LC (9).

In addition to contributing to an autocrine loop, chemokines expressed by ECD histiocytes, CCL5, IP-10, and, in particular, CCL19 and CCL2, may be responsible for the recruitment of other cellular components bearing the specific counterreceptors, especially lymphocytes (5–7). Consistent with these findings, a lymphocyte infiltrate was detectable in the 3 biopsy samples. T lymphocytes displayed a Th1 profile, as indicated by the prominent IFNγ staining. In turn, Th1 cells might drive activation and chemokine production by ECD histiocytes, as can be argued because of the up-regulation of the chemokine IP-10, which is specifically induced by IFNγ. CCL19/MIP-3β is also expressed by macrophages when activated, and can be induced by proinflammatory cytokines, including IFNγ (10).

Finally, we could detect IL-6 expression by ECD histiocytes. IL-6 is produced by macrophages and promotes activation and differentiation of T lymphocytes and macrophages. The cytokine is also involved in osteoclast differentiation and bone resorption (14), and elevated serum levels were reported in ECD, in association with biochemical markers of bone turnover (11). We show an increased production of IL-6 by ECD histiocytes, together with expression of RANKL, which is also responsible for osteoclast differentiation (14). The presence of a similar cytokine profile in bone lesions would suggest that the typical osteosclerosis observed in ECD is related to an imbalance between mechanisms leading to bone resorption versus bone formation. Further studies of the expression of IL-6, RANK/RANKL, and its decoy receptor osteoprotegerin (14) in ECD bone lesions will help to clarify this.

In conclusion, our data suggest that, similar to LCH (4,9), a cytokine and chemokine network exists in ECD lesions, that may contribute to the pathogenesis of the disease. Expression of distinct chemokine/chemokine receptor pairs may function as an autocrine recruitment/retention factor for ECD histiocytes. Moreover, chemokines expressed by ECD histiocytes may attract inflammatory cells and particularly Th1-type T lymphocytes that express the relevant chemokine receptors (6–8), which are able, in turn, to drive activation and chemokine production by histiocytes. A better under-

standing of such a network might provide insights into the development of targeted therapies.

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