Differential expression of CCR6 by the lesional cells in pulmonary Langerhans cell histiocytosis

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Abstract

Solitary pulmonary Langerhans cell histiocytosis occurs predominantly in young adults, who are frequently heavy smokers. Besides the strong association to smoking, it differs from childhood Langerhans cell histiocytosis as well in that it is a polyclonal disorder, and the lesional Langerhans cells in this form of disease are reported to display mature markers. Thus, in this study we set out to analyse the chemokine receptor expression pattern of CCR6 and CCR7, chemokine receptors associated with immature or mature dendritic cells, respectively. This study is a follow up of a previous report that has shown that in childhood Langerhans cell histiocytosis CD1a LCH cells always express CCR6 and lack CCR7. The current study showed that there is a differential expression of CCR6 in pulmonary Langerhans cell histiocytosis lesions, ranging from lesions with all CD1a LCH cells expressing CCR6, to lesions where there is a partial population of CD1a LCH cells that express CCR6, to lesions where all CD1a LCH cells lack CCR6 expression. In addition, CCR7 was always absent on the CD1a cells even on those LCH cells that lacked CCR6. Thus, in contrast to childhood LCH lesions where LCH cells always express CCR6, pulmonary LCH lesions display a differential pattern of expression of chemokine receptor CCR6.

Introduction

Pulmonary involvement with Langerhans cell histiocytosis (LCH) can be observed in patients of any age. Multifocal and systemic forms of the disease are usually seen in infants and children, and pulmonary involvement is often not a prominent feature. In contrast, isolated pulmonary LCH (pLCH) occurs predominantly in young adults with a peak frequency between 20 and 40 years of age (1-3). The main epidemiological factor associated with pLCH is smoking: 90-100% of patients have been current smokers in almost all series and tend to be heavy smokers (4-6). Localized pLCH is actually the form most frequently encountered by specialists in pulmonary medicine. It has several unique clinical and epidemiological features that justify its classification as a distinct clinicopathological entity. The natural history of pLCH remains poorly defined and no treatment has been found to be efficacious. The characteristic lesion of pLCH is composed of activated Langerhans cells (LCs) organized into a loose granuloma and associated with lymphocytes and inflammatory cells, particularly eosinophils and macrophages (7-9). LCs in pLCH express a unique surface phenotype, which strongly suggests that these cells are activated. They express CD80, CD86 and CD40, which are not present on normal pulmonary LCs or other pulmonary lesions such as lung cancer, and are thus likely important in the pathogenesis of LCH (10). Under normal circumstances, upon exposure to antigens, LCs respond to
various stimuli, such as TNF-a, LPS, and migrate to the regional lymphoid organs, where they stimulate antigen-specific T cells (11, 12). This migration process is strongly influenced by the chemokine gradient between the afferent lymph ducts and the antigen introduction site. During migration, LCs lose their expression of Birbeck granules and switch their surface expression of the chemokine receptor CCR6, typically expressed by immature dendritic cells (DCs), to CCR7, typically expressed by mature DCs, and thus become responsive to the CCR7 ligands CCL19/MIP-3b and CCL21/6Ckine (13, 14). In a previous study it was shown that regardless of the tissue site of LCH lesions, CCR6 is expressed by CD1a+ LCs in bone, skin and lymph node LCH lesions (15). Due to the fact that pLCH is a different form of LCH as, among other factors, LCs here are described to be more mature, we set out to investigate the presence of CCR6 and CCR7 in pLCH lesions. For this purpose, we carried out a combined immunohistochemical analysis for CCR6 and CCR7 with the marker for LCH cells, CD1a, in several pLCH lesions. This will hopefully reveal any potential differences between this form of disease and childhood LCH, which may help corroborate the already described differences.

Materials and methods

Patients
Paraffin biopsies from eleven pulmonary LCH patients were used in this study. This tissue was obtained from the pneumologie service, Paris, France. In all cases the diagnosis was reviewed and confirmed by immunohistochemistry for S-100 and CD1a.

Immunohistochemistry analysis
Paraffin sections were cut at 4 μm and double and triple immunofluorescence stainings were carried out, according to Annels et al. (15). Mouse monoclonal antibodies to CD1a (O10) from Neomarkers, CCR6 (53103.111) from R&D Systems and CD68 (514H12) from Serotec, and rabbit polyclonal antibody to CCR7 (E271) from AbCam, were used in this study.

Results

Differential expression of CCR6 in pLCH
In order to analyse the expression of CCR6 in LCH lesions, triple immunofluorescence stainings were carried out on eleven pulmonary LCH cases. The CCR6 antibody was combined with CD1a, for detecting LCH cells, and CD68, for detecting macrophages. A differential expression of CCR6
by the CD1a LCH cells in the pLCH lesions was observed. In 4 out of 11 pLCH all CD1a LCH cells expressed CCR6 (Figure 1 A, Table 1), in 2 out of 11 cases we found that between 30 and 50% of the CD1a LCH cells expressed CCR6 and the remaining CD1a+ cells lacked the expression of this chemokine receptor (Figure 1 B), and finally in 5 out of 11 cases all CD1a LCH cells lacked the expression of CCR6 (Figure 1 C).

**Figure 1.** Differential expression of CCR6 in pulmonary Langerhans cell histiocytosis. Immunofluorescence staining of three representative lung LCH lesions using antibodies specific for CD1a (red), CCR6 (green) and CD68 (blue). Triple immunofluorescence staining shows that all the CD1a+ cells are positive for CCR6 which appear yellow in 4 out of 11 lesions analysed (A), between 30 and 50% of the CD1a+ cells are positive for CCR6 in 2 out of eleven cases analysed (B), and no CD1a+ cells are positive for CCR6 in 5 out of 11 cases analysed (C). Original magnification 400X.

<table>
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<th>Patients</th>
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<th>Outcome</th>
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<th>CCR7 expression</th>
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-, lack of expression; +, expression; + and -, partial expression; N.A, data not available.

*All patients were heavy smokers.
Lack of CCR7 expression in pLCH lesions

As it has previously been reported that LCH cells in pLCH lesions have a more mature phenotype and in the current study we observed that in at least 7 out of 11 cases of pLCH all or part of the CD1a LCH cells were lacking CCR6, we were interested to look at whether these cells had up-regulated CCR7. Interestingly, we found that all CD1a LCH cells that were lacking CCR6 also lacked CCR7 expression (Figure 2).

Discussion

In childhood LCH lesions it has been shown that CD1a+ LCs always express CCR6, a chemokine receptor expressed by immature LCs (15). These findings contrast with the results from another study which showed that LCH cells co-express CCR6 and CCR7 (16). This difference may be due to different technical approaches. However, the results from the first study are in keeping with other reports that show additional evidence that these cells combine an early stage of activation with an immature phenotype. In contrast, in pulmonary LCH (pLCH), LCH cells were reported to express surface markers associated with activation, that are not present on normal pLCs and that are likely important in the pathogenesis of LCH (10). In light of the previous report by Annels et al. in which only CCR6 was observed by the pathologic LCH cells in childhood LCH and the report by Tazi et al. which demonstrated a more mature phenotype of the LCH cells in pLCH the current study set out to investigate the expression of CCR6 and CCR7 in pLCH. The results showed that in pLCH lesions there is a dif-

Figure 2. Lack of CCR7 expression in all pulmonary Langerhans cell histiocytosis lesions. Immunofluorescence staining on a pLCH lesion using antibodies specific for CD1a (green) and CCR7 (red). All CD1a+ cells in lung LCH lesions showed no expression of CCR7. It is possible to see that other cells in the lesion (arrow) are positive for CCR7. Original magnification 400X.
ferential expression of CCR6, ranging from the totality of CD1a LCH cells being CCR6 positive, to a mixture of CCR6 positive and CCR6 negative CD1a LCH cells, and finally to all of CD1a LCH cells being CCR6 negative.

These differences of expression of CCR6 by the CD1a+ LCH lesions may reflect the gradual stages of the disease, clinically observed by the destruction of the epithelium. In pLCH early lesions are responsible for eccentric infiltration of the walls of terminal and respiratory bronchioles, which undergo gradual destruction. LCs are abundant at this stage and form a compact central granuloma with a large number of lymphocytes located between the LCs and at the periphery of the lesion. Later in the process the LCs are less abundant and form clusters surrounded by lymphocytes and inflammatory cells. Finally, in advanced disease there is few or no LCs (17, 18).

LCH cells in pLCH lesions have been shown to display a more mature phenotype than LCH cells in childhood LCH, as they express B7-1 and B7-2 molecules (10). The microenvironment in which cells lie exerts a strong influence on dendritic cell function at all steps of the immune response and influences the elicitation of an efficient immune response or tolerance. In fact, the profile of cytokines expressed in pLCH lesions corresponds to one that has been shown by in vitro studies to induce the maturation of LCs into cells with strong lymphostimulatory activity (10, 19). Epithelial cells are able to produce a variety of cytokines including factors that influence the proliferation, survival and differentiation of LCs. In this regard, bronchiolar epithelial cells overlying early LCH granulomas produce greater amounts of GM-CSF than epithelial cells in adjacent uninvolved bronchioles (20-22). This supports the observation of greater amount of LCs observed in earlier LCH lesions in comparison to the lesser number of LCs observed in a later stage of pLCH (18). It may also explain the differential expression of CCR6 in the pLCH lesions.

Although LCs in pLCH lesions display a mature surface phenotype, no functional studies such as T cell stimulation have been performed to confirm the fact that these cells are fully mature cells. Interestingly, in all the lesions studied CD1a LCH cells always lacked CCR7 expression, even in the lesions where CCR6 was totally or partially absent. This is quite surprising as once dendritic cells down-regulate CCR6 they up-regulate CCR7. Thus, it appears that, like in childhood LCH, there is a blockade in the up-regulation of CCR7 in pLCH. In fact, these cells may be similar to semi-mature DCs described by Steinman et al., which are actually tolerogenic DCs (23).

The clusters of LCH cells typically observed in pLCH lesions appears to be due to accumulation instead of local proliferation. In fact, LCs in pLCH lesions have a rather low rate of proliferation, considerably less than that of carcinoma cells (24).

The absence of CCR6 expression in some LCH lesions may suggest that another recruitment pathway (than CCL20-CCR6) is involved in the accumulation of CD1a cells in these specimens. In fact, CCR2 was shown to directly control the accumulation of DCs into allergic lungs (25). Likewise, Chiu et
al. has shown that CCR2 knockout mice confers an intrinsic DC activation defect (26). Thus, further studies on chemokine ligand and receptor profiles in pLCH lesions will confirm whether other sets of chemokines have an important role in the recruitment of DCs into the lungs in LCH lesions.

There is still much speculation concerning initiating triggers in pLCH. Tobacco smoke in itself induces an increase in LC numbers in the airway epithelium. However, the transition to unbridled accumulation of LCs likely requires additional factors, which could be genetic predisposition, acquired mutations such as allelic loss at the level of tumor suppressor genes, or maybe another environmental trigger, such as viral infection. In this study we aimed at analysing the CCR6 and CCR7 expression in pLCH lesions in order to gain further understanding of the pathogenesis of LCH by comparing with the expression of these chemokine receptors previously reported in childhood LCH. However, more studies need to be carried out in order to complete this knowledge.

References


