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TARC and IL-5 expression correlates with tissue eosinophilia in peripheral T-cell lymphomas

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Abstract

The current study attempts to characterize the eosinophilia associated with T-cell lymphomas and to investigate its possible relationship with the secretion of eosinophil-stimulating factors by lymphoma cells and/or intra-tumoral surrounding cells.

Paraffin-embedded specimens from 50 patients diagnosed with peripheral T-cell lymphomas, either unspecified (PTCL-U, $n=30$) or angioimmunoblastic (AITL, $n=20$) were morphologically assessed for intra-tumoral eosinophilia and analyzed by immunohistochemistry using specific antibodies directed against TARC, IL-5, RANTES, and eotaxin.

The AITL and PTCL-U cases contained a mean of 147 ± 41 and 102 ± 37 eosinophils per 10 high power fields, respectively. Thirty-two of 47 cases (68%) showed IL-5-positive lymphoma cells while 15/50 (30%) tumors showed variable staining for TARC in scattered non-lymphoid cells with dendritic morphology. TARC and IL-5-positive cases possessed significantly more eosinophils.

Our data indicate that IL-5 and TARC expression highly correlate with eosinophilia in T-cell lymphomas, suggesting that these chemokines are involved in the recruitment of eosinophils into the tumors.

Keywords: T-cell lymphoma; Eosinophilia; Chemokine; Immunohistochemistry; TARC; IL-5; RANTES; Eotaxin

1. Introduction

Eosinophils (Eo) are non-dividing fully differentiated myeloid cells that participate in the innate immune response, and are normally not numerically prominent. Eosinophilia, defined as an abnormal accumulation of eosinophils in blood or tissues, is most commonly related to allergic processes and parasitic infections. Upon activation, eosinophils produce and secrete highly cationic proteins which display cytotoxic activity, pro-inflammatory cytokines, reactive oxygen species, and transforming growth factor (TGF-β) which all contribute to tissue damage and remodelling. They also produce arachidonic acid derivatives (prostaglandins and leukotrienes) which modulate smooth muscle tone in vessel and bronchial walls. The spectrum of mediators produced by eosinophils is very wide as are their potential functions in immune responses, all of which have recently been reviewed in [1].

The idiopathic hypereosinophilic syndrome (HES) is a rare condition characterized by persistent eosinophilia in blood, bone marrow, and multiple organs, which is diagnosed after exclusion of the usual illnesses associated with eosinophilia. Recent evidence has led to the description of two distinct underlying hematological disorders involving myeloid or lymphoid cells, and designated as the "myeloproliferative" (or more appropriately as chronic eosinophilic

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leukemia when clonal abnormalities are detected) or "lymphocytic" variants, respectively [2]. Most patients with the "lymphocytic" variant present a clonal expansion of CD3–CD4+ T-cells displaying a T-helper 2 (Th2) phenotype causally linked to the hypereosinophilia through the secretion of high levels of interleukin-5 (IL-5), and there is some evidence that they are an increased risk of developing peripheral T-cell lymphoma [3–5]. Interestingly, very high serum levels of thymus and activation-regulated chemokine (TARC/CCL17) are detected in these patients [6]. Antigen-presenting cells, mainly dendritic cells, are known to produce TARC and may be involved in the recruitment and activation of effector Th2 cells expressing CCR4 [7].

Tissue and/or blood eosinophilia is known to occur in association with various lymphoid malignancies: classical Hodgkin's lymphoma (HL) being the typical example, and divergent results have been reported regarding the correlation between eosinophilia and clinical outcome [8–10]. Eosinophilia is also commonly found in T-cell-derived neoplasms including adult T-cell leukemia/lymphoma, cutaneous T-cell lymphomas (CTCL) and nodal peripheral T-cell lymphomas [11–14].

Several factors promote eosinophil chemotaxis, including IL-5 (also known as eosinophil-differentiation factor), regulated on activation normal T cell expressed and secreted (RANTES/CCL5) and eotaxin (CCL11). Th2 cells are the predominant source of IL-5. This cytokine plays a crucial role on the differentiation of eosinophils in the marrow; it stimulates their release into the peripheral blood and participates in eosinophil recruitment to tissues in synergy with eotaxin. IL-5 also activates eosinophils and prolongs their life span through the prevention of apoptosis *in vitro* [15–17]. RANTES displays chemoattractant activity for eosinophils, T lymphocytes, monocytes, and basophils and is produced by a variety of cell types, including T cells, fibroblasts, and epithelial cells [18–20]. Eotaxin has received considerable attention in the setting of eosinophil-mediated disorders because, unlike other factors, it appears to signal through a single receptor, CCR3, which is expressed at high levels on eosinophils and Th2 cells, but not on neutrophils or monocytes [21,22]. Cellular sources of eotaxin include fibroblasts, endothelial cells, eosinophils, and lymphocytes. In HL, numerous cytokines and chemokines (including IL-5, RANTES, and eotaxin) have been detected at the transcriptional and/or protein level in either Reed–Sternberg cells or in non-neoplastic surrounding cells, contributing to pathological features namely tissue eosinophilia (reviewed in [23]).

The mechanisms of eosinophilia in T-cell lymphomas have not been largely assessed so far. Here, we examined the possible correlation between the level of eosinophilia in T-cell lymphomas and the production of factors acting on eosinophils by lymphoma cells or intra-tumoral surrounding cells. We selected for this study 50 cases of angioimmunoblastic and unspecified peripheral T-cell lymphomas, which are the two most common types of nodal T-cell lymphomas in western countries [24]. Eosinophilia was quantified and immunohistochemistry using specific antibodies directed against TARC, IL-5, RANTES, and eotaxin was performed.

2. Materials and methods

2.1. Patient characteristics and tumor samples

This study included 50 patients diagnosed with peripheral T-cell lymphoma (unspecified (PTCL-U), $n = 30$ or angioimmunoblastic (AITL), $n = 20$) between 1992 and 2006. The cases were retrieved from the files of the Departments of Pathology of the CHU Sart-Tilman, Liège, Belgium and of the CHU Farhat Hached, Sousse, Tunisia. Clinical data including age, sex, and disease stage were retrieved from the clinical charts at the time of diagnosis. The age of patients ranged from 14 to 84 years (mean: 55 years). The sex distribution was M/F = 31/19. All cases were reviewed by two pathologists (LdL and VR) and classified according to the WHO classification criteria [25] after precise immunohistochemical evaluation. The staining panel included at least CD20 and CD3, and for most AITL a FDC marker (CD21 and/or CD23 and/or CNA-42) and an EBV marker (LMP-1 (latent membrane protein-1) and/or EBERs (EBV-encoded small RNAs)). FDC expansion and EBV-infected cells were demonstrated in 10/19 and 14/20 AITL, respectively. A variable expression of CD10 and BCL6 in tumor cells was demonstrated in 4/5 and 10/15 AITL with interpretable staining.

This study was approved by the Ethical Commission of the Faculty of Medicine of the University of Liège.

2.2. Eosinophilia

The extent of eosinophilic infiltration was quantitatively evaluated on Hematoxylin–Eosin stained paraffin section. The absolute number of eosinophils was evaluated in 10 randomly selected high power fields (HPF 400×). The sum of 10 fields was calculated [8] and absolute counts/10 HPF were considered for statistical analysis.

2.3. TARC, IL-5, RANTES, and eotaxin immunohistochemistry

Cases were stained with specific antibodies against TARC, IL-5, RANTES, and eotaxin. Five-micrometer, formalin-fixed, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated in graded alcohols. After blocking of the endogenous peroxidase activity with 4.5% hydrogen peroxide in methanol for 5 min, the sections were subjected to heat-induced antigen retrieval and allowed to cool down before incubation with primary antibodies. Clone designations, sources, pretreatments, and working dilutions for the antibodies are summarized in Table 1. Revelation of IL-5 and eotaxin was performed using a standard three-step technique with LSAB-2 system (Dako, Heverlee, Belgium) and diaminobenzidine tetrahydrochloride (DAB) (Vel, Leuven, Belgium) as the chromogen in presence of hydrogen peroxide. For TARC and RANTES detection, after antigen retrieval, the sections were pre-incubated with PBS containing 1% BSA and 3% normal rabbit serum (X0902, Dako) prior to incubation with anti-RANTES or TARC antibodies. Visualization of the primary antibody was performed using HRP-coupled rabbit anti-goat Ig 1:500 (ref. P0160, Dako) and DAB. Negative controls were obtained by omitting the first antibody.