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ORIGINAL ARTICLE

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Ornithine decarboxylase gene expression in Castleman's disease

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Abstract Castleman's disease (CD) is a rare atypical lymphoproliferative disorder that is clinically and histologically heterogeneous and is associated with the risk of developing malignant lymphoma. Based on pathological findings CD is divided into two types: a localized form and a multicentric form. The clinical course differs in these two forms. We examined the molecular mechanisms that lie between benign and malignant disease, evaluating a possible implication of oncogenes in the pathogenesis. Since deregulated expression of the gene for ornithine decarboxylase (ODC) has been observed in a variety of human malignancies, we compared *ODC* expression between the localized and multicentric forms. Using northern blot analysis we found that *ODC* gene expression clearly differs between the localized and multicentric forms. The findings in this report indicate that the variable pattern of *ODC* gene expression in the different types of CD could be useful for examining the evolution of this disease.

Key words Ornithine Decarboxylase · Castleman's disease · Multicentric form · Localized form

Abbreviations *CD:* Castleman's disease · *IL:* Interleukin · *ODC:* Ornithine decarboxylase · *PBS:* Phosphate-buffered saline

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Introduction

Castleman's disease (CD), also referred to as angiofollicular lymph node hyperplasia, is a rare lymphoproliferative disorder. The histology is characterized by the presence of lymphoid follicles, hyperplasia, and capillary proliferation with endothelial hyperplasia [1]. In about one-half of cases the prominent feature is the infiltration of plasma cells between the follicles. Based on either histological or clinical criteria CD is a heterogeneous syndrome. CD was initially divided into two histopathological types: the hyaline-vascular type, showing small hyaline follicles penetrated by numerous vessels, and the plasma cell type, characterized by florid hyperplastic follicles infiltrated by numerous plasma cells in interfollicular areas [2].

Clinically CD is divided into two forms: the localized form that can involve a single lymph node and the multi-

centric form that can involve several lymph nodes [3, 4]. The multicentric form usually develops in patients over 50 years old and systemic manifestations may include polyneuropathy, organomegaly, endocrinopathy, monoclonal gammapathy, skin abnormalities, autoimmune or hypochromic microcytic anemia, and hyper-γ-globulinemia [4]. A large number of the systemic manifestations can be associated with elevated interleukin-6 (IL-6) gene expression [5, 6]. The prognosis is important due to the frequent development of severe infection, Kaposi's sarcoma [7], B lymphoma [8], or myeloma [9]. The localized form is observed mainly in young patients. In this form systemic manifestations are inconsistent and, when present, patients recover after surgical resection of the affected lymph nodes. The favorable prognosis of this form of CD is correlated with the absence of associated malignancies. Thus the disease may constitute a spectrum of benign to malignant diseases. Although recent investigations have shed more light on the etiopathogenetic aspects involved in the various manifestations of CD, including increased IL-6 [5, 6] and IL-1 [10] production in the multicentric form, sometimes associated with decreased IL-2 production, decreased T cell colony formation, decreased natural killer cell activity and number, increased soluble IL-2 receptor, and decreased CD4/CD8 ratio [6]. Nevertheless the involvement of genes in the pathogenesis of the disease is unknown.

Emphasis has recently been placed particularly on the role of ornithine decarboxylase (ODC) activity during cell transformation [11]. This enzyme is the key regulator of the synthesis of polyamines; putrescine, spermidine, and spermine are normal constituents of prokariotyc and eucariotyc cells [12]. The functions of polyamines include stimulation of DNA, RNA and protein synthesis, and stabilization of membrane and cytoskeletal structures [13]. Expression of the *ODC* gene is sustained in rapidly proliferating cells, is transiently increased upon stimulation by growth factors [14], but becomes constitutively activated during cell transformation induced by carcinogens [15] or oncogenes [16]. Since cell transformation is associated usually with a large increase in ODC, we hypothesized that this enzyme is critical for malignant transformation. The aim of this study was to compare the pattern of *ODC* gene expression between the various forms of CD.

Patients and methods

Patients

Two patients suffering from CD were included in this study. Their main clinical and biological characteristics are summarized in Table 1. Patient 1, diagnosed with multicentric form of CD, was a 36-year-old man with a history of several months of asthenia, malaise, fever, dysphagia, dyspnea, edema, generalized peripheral lymphadenopathy, and abdominal dropsy. Patient 2, affected by a localized form of CD in the mammary region, was a 41-year-old woman, with a history of general fatigue and arthralgia. After lymph node resection, clinical and biological symptoms of patient 2 disappeared within several months. Both patients were seronegative for human immunodeficiency virus 1.

Table 1 Clinical characteristics of CD patients

	Patient 1	Patient 2
Age (years), sex	36, male	41, female
Affected lymph nodes	Multiple	Solitary
Hemoglobin (g/dl)	9.3	11.6
Erythrocyte sedimentation rate (mm/h)	122	53
Total protein (g/dl)	9.4	7.4
γ-Globulins (%)	44.0	38.6
IgG (mg/dl)	4830	4160
IgA (mg/dl)	987	322
IgM (mg/dl)	191	215
IgE (μg/ml)	16.2	11
C reactive protein (mg/dl)	3.4	0.6
Fibrinogen (mg/dl)	532	483

Cells

After informed consent, cells were obtained from peripheral blood, abdominal dropsy, and lymph nodes of patient 1 and from the peripheral blood and lymph nodes of patient 2. The cells from lymph nodes were obtained by cellular disintegration using Slocum-Pavelic's mechanical and enzymatic method. Cellular suspensions were diluted 1:3 with Hank's balanced salt solution, layered on Ficoll/Histopaque-1077 (Sigma) density gradient, and centrifuged for 20 min at 1200 rpm. The cells were washed three times with Hank's balanced salt solution and processed for RNA extraction.

Abdominal dropsy was withdrawn in a suitable heparinized tube; cellular suspensions were diluted 1:1 with phosphate-buffered saline (PBS) and centrifuged for 10 min at 1200 rpm. The cells were washed twice with PBS and processed for RNA extraction. Peripheral blood was diluted twofold with Ca^{2+}-, Mg^{2+}-free PBS and layered on Histopaque-1077 density gradient. After 30 min centrifugation at 1500 rpm the interface of peripheral blood mononuclear cells was collected, washed twice with PBS, and processed for RNA extraction. As a positive control for the expression of *ODC*, RNA from adherent monocytes obtained from healthy volunteers fresh buffy coats treated for 2 h with 50 U/ml human recombinant interferon-γ was used [17].

Histological findings

Lymph nodes were obtained by surgical biopsy. Tissue samples were fixed in neutral formalin and processed for histology. The resected lymph nodes from patient 1 displayed many lymph follicles with angiofollicular hyperplasia. Germinal centers were occasionally epithelioid in appearance, surrounded by small lymphocytes in an "onion skin pattern." Proliferation of small vessels was prominent in the interfollicular spaces that radially penetrated into the secondary follicles, giving a hyaline appearance. Interfollicular regions showed plasma cells and small lymphocytes.

The main histological characteristics of the lymph node from patient 2 were hyperplasia and interfollicular lymphoid cells containing numerous plasma cells and a few immunoblasts.

Immunohistochemical analysis

Immunohistochemical studies were performed on frozen material using an indirect immunoalkaline assay with antibodies against B-cell antigens CD19, CD20, CD22, against T-cell antigens CD3, CD4 and CD8, CD45RO, CDw75, against dendritic reticulum cell, CD35, and against macrophage antigen CD68.

RNA isolation and northern Blot analysis

The cells were washed twice with PBS and lysed by using guanidium isothiocyanate. RNA was purified according to the proce-

dure described elsewhere [18]. Of the total RNA 15 µg was denatured in the presence of 0.5 µg ethidium bromide and electrophoresed in 1.2% agarose, and 2.2 M formaldehyde. The gels were then capillary blotted onto nitrocellulose, prehybridized, and sequentially hybridized as previously described [17] with ^{32}P. The cDNA probes: human ODC [19] and glyceraldehyde-3-phosphate dehydrogenese [20] were labeled by the random primer method to a mean specific activity of $0.5–1.3\times10^9$ cpm μg^{-1} ml^{-1} DNA. Membranes were hybridized for 18–24 h and then washed twice for 5 min at room temperature with a mixture of 0.1× standard saline citrate and 0.1% sodium dodecyl sulfate. Membranes were exposed for 24–72 h to Kodak X-Omat X-ray films at –70°C with intensifying screens.

Results

Immunohistochemical analysis

Immunohistochemical analysis of patient 1 showed the presence of CD20 and CDw75 in the follicular centers, and the presence of CD3 in the parafollicular zones. The interfollicular areas revealed numerous B-cell follicles with a dendritic reticulum cell pattern (dendritic reticulum cells, CD35) and T cells CD45RO that predominated in the interfollicular areas. Immunohistochemical analysis of peritoneal effusion demonstrated the presence of CD35 and CD68. Immunohistochemical analysis in patient 2 presented follicular dendritic cells (labeled with an anti-dendritic reticulum cell monoclonal antibody) and B lymphocytes (labeled with anti-CD22 monoclonal antibody) in the mantle zone.

ODC gene expression in the multicentric form of CD

ODC gene expression was analyzed by northern blot analysis in the peripheral blood and lymph nodes cells from the patient presenting a multicentric form of CD. Since patient 1 had abdominal dropsy at the same time, we also examined the *ODC* gene expression in these cells. *ODC* gene expression was detected in peripheral blood cells (Fig. 1A, lane 2), and in lymph node (Fig. 2A, lane 2); in parallel, *ODC* gene expression was pronounced in cells obtained from abdominal dropsy (Fig. 3A, line 2). As a positive control, adherent monocytes obtained from healthy volunteers fresh buffy coats treated for 2 h with 50 U/ml interferon-γ (Fig. 1A, 2A, 3A, lane 1) were used.

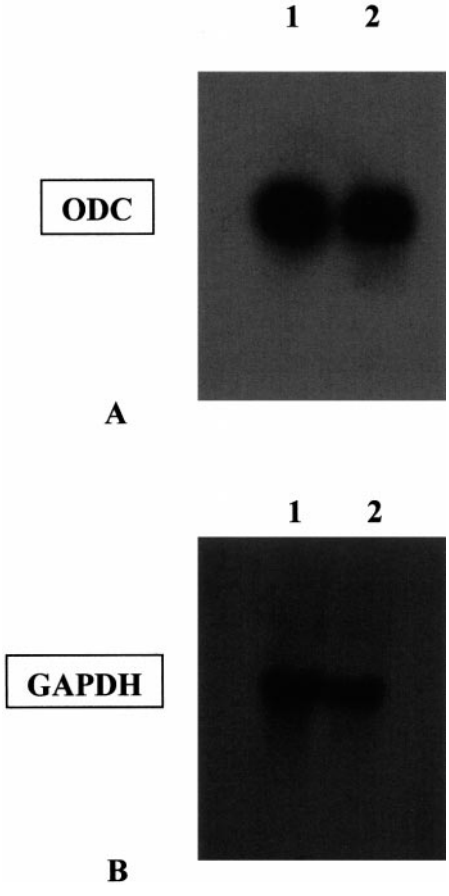

Fig. 1 **A** *ODC* gene expression analyzed by northern blot analysis in patient 1. *Lane 1* Monocytes treated with 50 U/ml interferonγ; *lane 2* cells from peripheral blood. **B** The same blot was reprobed with glyceraldehyde-3-phosphate dehydrogenese (*GAPDH*) to ensure that equal amounts of RNA were loaded into each lane

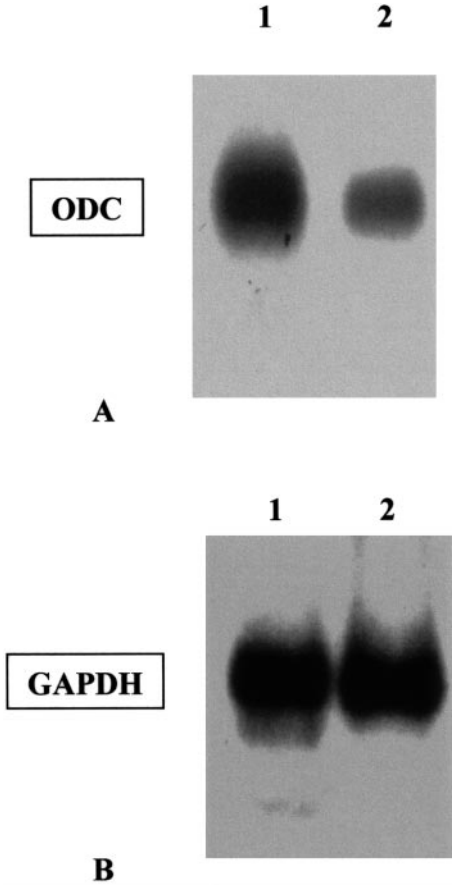

Fig. 2 **A** *ODC* gene expression analyzed by northern blot analysis in patient 1. *Lane 1* Monocytes treated with 50 U/ml interferon-γ; *lane 2* cells from lymph node. **B** The same blot was reprobed with glyceraldehyde-3-phosphate dehydrogenese (*GAPDH*) to ensure that equal amounts of RNA were loaded into each lane

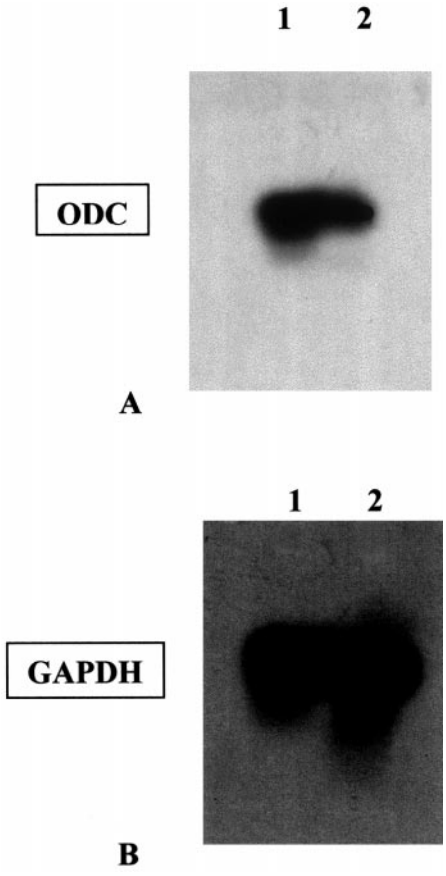

Fig. 3 **A** *ODC* gene expression analyzed by northern blot analysis in patient 1. *Lane 1* Monocytes treated with 50 U/ml interferonγ; *lane 2* cells from abdominal dropsy. **B** The same blot was reprobed with glyceraldehyde-3-phosphate dehydrogenese (*GAPDH*) to ensure that equal amounts of RNA were loaded into each lane

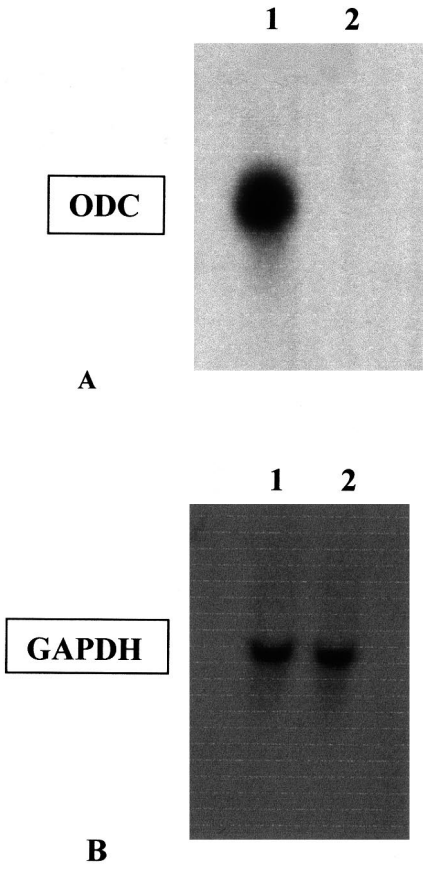

Fig. 4 **A** *ODC* gene expression analyzed by northern blot analysis in patient 2. *Lane 1* Monocytes treated with 50 U/ml interfereon-γ; *lane 2* cells from peripheral blood. **B** The same blot was reprobed with a with glyceraldehyde-3-phosphate dehydrogenese (*GAPDH*) to ensure that equal amounts of RNA were loaded into each lane

The same blot was reprobed with a glyceraldehyde-3-phosphate dehydrogenese probe to ensure that equal amounts of RNA were loaded into each lane (Fig. 1B, 2B, 3B)

ODC gene expression in the localized form of CD

RNA was extracted from the peripheral blood and from the lymph node of patient 2 to examine *ODC* gene expression in the localized form of CD. *ODC* expression was absent both in peripheral blood (Fig. 4A, lane 2) and in lymph node cells (Fig. 5A, lane 2). As a positive control, adherent monocytes obtained from healthy volunteers fresh buffy coats treated for 2 h with 50 U/ml interferon-γ (Fig. 4A, 5A, lane 1) were used. The same blot was reprobed as for the multicentric form (Fig. 4B, 5B)

Discussion

We examined *ODC* gene expression in two different forms of CD. An increased level of *ODC* gene expression was detected in the peripheral blood in the peritoneal effusion and in lymph nodes cells of the patient suffering from a multicentric form of CD. In contrast, *ODC* gene expression did not occur in the peripheral blood or the lymph node cells of the patient affected by the localized form of CD. The *ODC* gene plays an important role in cell growth and proliferation. Moreover, increased levels of ODC enzyme followed by increased levels of polyamine are involved in promotion [21]. The *ODC* gene has been recognized as a member of cellular proto-oncogenes [11]. In fact, deregulated expression of ODC and ODC polyamine metabolism has been observed in a variety of animal and human malignancies, such as colon carcinoma [22], gastrointestinal malignancy [23], breast cancer progression [24], human squamous cell carcinomas of the head and of the neck [25], Ehrlich ascitic carcinoma [26], and human gliomas [27].

CD is a clinically and histologically heterogeneous syndrome associated with a risk of developing malignant lymphoma. Most localized lesions tend to be transformed into the multicentric form, associated with severe systemic manifestations and an inexorable clinical course. The association of multicentric CD and malignancies may depend on the involvement of *ODC* overexpression in addition to a cytokine overproduction [5, 6,

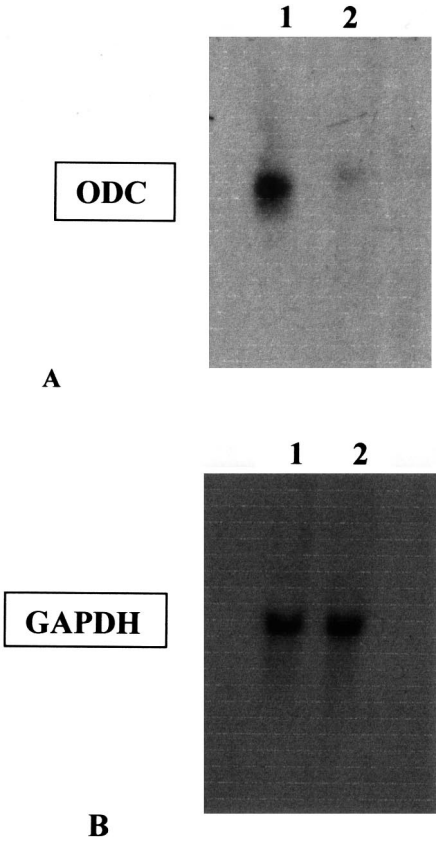

Fig. 5 **A** *ODC* gene expression analyzed by northern blot analysis in patient 2. *Lane 1* Monocytes treated with 50 U/ml interferon-γ; *lane 2* cells from lymph node. **B** The same blot was reprobed with a with glyceraldehyde-3-phosphate dehydrogenese (*GAPDH*) to ensure that equal amounts of RNA were loaded into each lane

10]. In fact, elevated serum levels of IL-6 have previously been reported in patients with multicentric CD, suggesting a role for IL-6 in the pathogenesis of the disease [5, 6, 10]. IL-6 behaves as an important cofactor in the expression of this disease [28]. Moreover, factors other than IL-6 have recently been considered necessary for the full expression of multicentric CD. Such factors include other cytokines such as IL-1 [10], which is an early mediator of inflammation produced by monocytes/macrophages, and tumor necrosis factor [29]. IL-1 and tumor-necrosis factor participate in the monokine network in which they stimulate IL-6 [30]. The abnormal expression of *ODC* genes could in part be related to the presence of inflammatory and immune stimuli associated with systemic manifestations. This is further supported by our previous findings that the *ODC* expression in human macrophages of healthy volunteers is induced in vitro following stimulation with factors mimicking inflammatory processes such as lipopolysaccharide, interferon-γ, and tumor-necrosis factor [17, 31]. Polyamines and cytokines with their inflammatory as well as their regulatory activities may play a role in the perpetuation and possibly the initiation of inflammation in this disease and its local and/or systemic complications. An overexpression of these factors could result in dramatic phenotypic changes, including rapid proliferation and malignant transformation since as they control cycle progression and tumorigenesis [11]. Multicentric CD is a late, well identified complication in patients with long-standing localized CD. We show here a high production of ODC for the first time in a patient with multicentric CD. Our results suggest that aberrant *ODC* expression is not merely a coincidence but may be a critical factor contributing to transformation of premalignant into a malignant lesion. Tumor progression may be accelerated by cytokine-induced inflammation. It is important to underline that the evaluation of *ODC* activity can be used as a prognostic factor for the evolution of this disease since many of the variants of CD begin as the localized form and then transform into the multicentric form, associated with multisystemic manifestations and neoplastic degeneration. The *ODC* gene should therefore be recognized as a member of the growing family of cellular proto-oncogenes. Positioning of *ODC* at the convergence point of the signaling pathway of many oncogenes suggests that *ODC* transduces their transforming activity, which would make it a possible target for novel therapeutic strategies against CD. Because of the great difficulties in finding a larger number of patients, additional studies are needed. The goal of this paper is to bring attention to this condition and to offer guidelines for analysis of a larger series so that a early evaluation of *ODC* gene expression can be used as marker of malignant development.

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