Cytokine 70 (2014) 194–197

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Short Communication

High serum osteopontin levels in pediatric patients with high risk Langerhans cell histiocytosis

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ARTICLE INFO

Article history:
Received 18 January 2014
Received in revised form 18 June 2014
Accepted 7 July 2014
Available online 30 July 2014

Keywords:
Langerhans cell histiocytosis
Osteopontin
Serum
Infant
Multisystem disease

ABSTRACT

Osteopontin (OPN) acts as an osteoclast activator, a proinflammatory cytokine, and a chemokine attracting histiocytes/monocytes and is abundantly expressed in Langerhans cell histiocytosis (LCH). We investigated whether serum OPN levels are related to disease types in LCH. Fifty-eight newly diagnosed LCH patients were studied; eight with risk organ (liver, spleen and/or hematopoietic) involvements positive multisystem (MS+) disease, 27 with risk organ involvement negative multisystem (MS−) disease and 23 with single system (SS) disease. Pediatric patients with non-inflammatory disease ($n = 27$) were used as controls. All of patients with MS+ disease were younger than 3 years. Serum OPN levels and 44 kinds of humoral factors were measured by ELISA and Bio-Plex suspension array system, respectively. In the patients younger than 3 years, the median serum OPN level (interquartile range) was 240.3 ng/ml (137.6–456.0) in MS+ ($n = 8$); 92.7 ng/ml (62.0–213.8) in MS− ($n = 14$) and 72.5 ng/ml (55.6–94.0) in SS ($n = 9$) and 74.4 ng/ml (42.2–100.0) in control ($n = 12$). The OPN values were significantly higher in the MS+ group than the MS−, SS and control groups ($p = 0.044$, $p = 0.001$ and $p = 0.002$, respectively), but not different between the MS−, SS and control groups. In the patients older than 3 years, the median level of serum OPN (IQR) was 56.2 ng/ml (22.9–77.5) in MS− ($n = 13$), 58.9 ng/ml (31.0–78.7) in SS ($n = 14$) and 41.9 (28.9–54.1) in control ($n = 15$). These values did not differ significantly between each group. The serum OPN levels were positively correlated with the serum IL-6, CCL2, IL-18, IL-8 and IL-2 receptor concentration. OPN may be involved in risk organ dissemination and poor prognosis of LCH through the function as inflammatory cytokine/chemokine.

1. Introduction

Langerhans cell histiocytosis (LCH) is a proliferative disease of immature dendritic cell (iDC), and has characteristics of a clonal and an inflammatory disease. Two disease types of LCH are known; multisystem disease (MS) and single system disease (SS). Infants with risk organ (liver, spleen and/or hematopoietic system) involvement positive-MS disease have a dismal prognosis, while children with SS disease have an excellent prognosis [1]. Many inflammatory cytokines and chemokines are involved in the pathogenesis of LCH [2] and recently Allen et al. reported that the pleiotropic cytokine osteopontin (OPN) is abundantly expressed by the abnormal LCH cells [3]. Furthermore, Prasse et al. found that OPN expression is upregulated in bronchoalveolar lavage cells in patients with pulmonary LCH and that overexpression of OPN in rat lungs induces lesions similar to pulmonary LCH [4]. OPN is expressed in various human cells and has a variety of functions that include promoting the generation of T helper (Th)1 and Th17 cells [5], recruiting histiocytes/monocytes [6,7] and activating osteoclasts (OCs) [8].

Abbreviations: OPN, osteopontin; LCH, Langerhans cell histiocytosis; MS, multisystem; SS, single system; iDC, immature dendritic cell; Th, T helper; OCs, osteoclasts; JLSG, the Japan LCH Study Group; MS+, multisystem disease with liver, spleen and/or hematopoietic system involvements; MS−, multisystem disease without liver, spleen or hematopoietic system involvements; IL, interleukin; IL-2R, IL-2 receptor α; IFN, interferon; TNF, tumor necrosis factor; CCL, CC chemokine ligand; CXCL, CXC chemokine ligand; IQR, interquartile range; MGCs, multinucleated giant cells.

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http://dx.doi.org/10.1016/j.cyto.2014.07.002
1043-4666/

Taken together, these findings imply that OPN must have a role in the pathogenesis as well as disease progression of LCH. In spite of data on OPN in *in vitro* system of LCH studies, clinical OPN data are still limited in patients with LCH [3]. In this study, we therefore investigated whether serum OPN levels are related to disease types, i.e. MS vs SS in patients with LCH.

2. Material and methods

2.1. Patients and sample collection

Blood samples and clinical information were obtained with informed consent from 58 newly diagnosed pediatric LCH patients through the Japan LCH Study Group (JLSG) registry, and from 27 pediatric patients with non-inflammatory diseases in stable state including inherited coagulopathy, thrombophilia and anemia, and resected benign tumor, as controls. The median age of LCH patients were 2.8 years (range: 0.4–12.0). A diagnosis of LCH was confirmed by immunohistochemical staining for CD1a antigen in biopsies of the affected organs. The patients consisted of two groups as follows: those with MS disease and those with SS disease. We defined MS as lesions in several organs, SS as lesions in only one organ. Of the 58 LCH patients, 35 were assigned to the MS group, 23 to the SS group. We divided MS patients into patients with liver, spleen and/or hematopoietic involvements (MS+) and patients without these lesions (MS−). Of the 35 MS patients, 8 were assigned to MS+ group, and all of these patients were younger than 3 years. All but three patients with SS disease had bone lesion(s). This study was approved by the ethics committee of the Jichi Medical University School of Medicine.

2.2. Measurement of osteopontin and other humoral factors in serum

Serum samples were stored at −80 °C prior to assay and not subjected to freeze–thaw cycles. Serum OPN levels were measured using the human OPN Quantikine ELISA Kit (R&D Systems, Minneapolis, USA). Forty-four kinds of serum humoral factors, including interleukin (IL)-1β, IL-1 receptor antagonist, IL-2, IL-2 receptor α (IL-2R), IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, IL-18, interferon (IFN)-α2, IFN-γ, tumor necrosis factor (TNF)-α, TNF-β, macrophage inhibitory factor, granulocyte-colony stimulating factor, macrophage-colony stimulating factor, granulocyte–macrophage colony-stimulating factor, stem cell factor, leukemia inhibitory factor, fibroblast growth factor basic, hepatocyte growth factor, nerve growth factor-β, vascular endothelial cell growth factor, tumor necrosis factor-related apoptosis inducing ligand, CC chemokine ligand (CCL)2, CCL3, CCL4, CCL7, CCL11, CCL27, CXC chemokine ligand (CXCL) 1, CXCL9, CXCL10 and CXCL12, were measured using Bio-Plex suspension array system (BIO-RAD Laboratories, Hercules, USA).

2.3. Statistical analysis

A Student's *t*-test was used for analyzing normally distributed data (age), and the Mann–Whitney U test for non-normally distributed data (serum OPN level). $P < 0.05$ was regarded as significant. All the data were analyzed using Statcel3 software (OMS Publishing Inc., Saitama, Japan).

3. Results and discussion

The scattergram of serum OPN level and age of the MS, SS and control groups is shown in Fig. 1A. Patients with remarkably high levels of serum OPN were infants younger than 3-years-old with MS disease. Therefore, we analyzed serum OPN levels separately in the two groups: younger or older than 3 years. The younger group consisted of 8 MS+, 14 MS−, 9 SS and 12 controls, while the older group consisted of 13 MS−, 14 SS and 15 controls. In the younger group, the median level of serum OPN was 240.3 ng/ml (interquartile range (IQR), 137.6–456.0) in MS+, 92.7 ng/ml (IQR, 62.0–213.8) in MS−, 72.5 ng/ml (IQR, 55.6–94.0) in SS ($n = 9$) and 74.4 ng/ml (IQR, 42.2–100.0) in control ($n = 12$) groups, respectively. The serum OPN level in MS+ patients was significantly higher than that in MS−, SS and control ($p = 0.044$, $p = 0.001$ and $p = 0.002$, respectively) (Fig. 1B), but did not differ significantly between MS−, SS and control (MS− vs SS: $p = 0.284$, MS− vs control: $p = 0.181$ and SS vs control: $p = 0.239$). In the older group, the median level of serum OPN was 56.2 ng/ml (IQR, 22.9–77.5) in MS−, 58.9 ng/ml (IQR, 31.0–78.7) in SS and 41.9 ng/ml (IQR, 28.9–54.1) in control patents, respectively. The serum OPN level did not differ significantly between these patents (Fig. 1C).

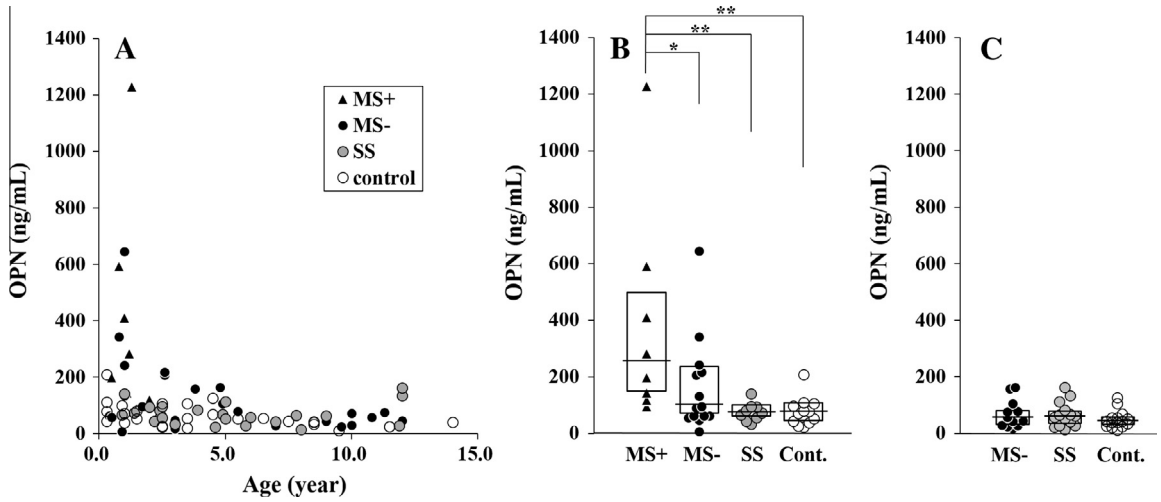

Fig. 1. Serum OPN levels are high in the MS+ patients. (A) Scattergram of serum OPN level and age in all patients. Black triangles, MS+ patients ($n = 8$); black circles, MS− patients ($n = 27$); gray circles, SS patients ($n = 23$); open circles, control ($n = 27$). (B) Comparison of serum OPN levels in patients younger than 3 years. MS + patients ($n = 8$), MS− patients ($n = 14$), SS patients ($n = 9$), control ($n = 12$). $^{*}p < 0.05$; $^{**}p < 0.001$. Bars in boxes indicate the median value, boxes indicate the interquartile range. (C) Comparison of serum OPN levels in patients older than 3 years. MS− patients ($n = 13$), SS patients ($n = 14$), control ($n = 15$). Bars in boxes indicate the median value, boxes indicate the interquartile range.