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Increase of Th2 and Tc1 cells in patients with Kimura's disease

Nobuo Ohta [a,*], Shigeru Fukase [a], Yusuke Suzuki [a], Tukasa Ito [a], Hiroshi Yoshitake [b], Masaru Aoyagi [a]

[a] *Department of Otolaryngology, Yamagata University School of Medicine, 2-2-2, Iida-nishi, Yamagata 990-9585, Japan*
[b] *Institute for Environmental and Gender Specific Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Tomioka, Urayasu City, Chiba, Japan*

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Abstract

Objective: A Th1/Th2 cytokine imbalance with a predominance of Th2 cytokines has been suggested to be of pathogenic importance in Kimura's disease.
Methods: To evaluate the role of Th1/Th2 cytokines in Kimura's disease, the subsets of Th1, Th2, Tc1 and Tc2 cells from patients with Kimura's disease were examined by intracellular cytokine flow cytometry. The expressions of IL-5, eotaxin and RANTES in the lesions were investigated by RT-PCR.
Results: The population of Th2 and Tc1 cells in Kimura's disease was significantly increased compared with these cells in control ($p < 0.05$). Th1 and Tc2 cells in Kimura's disease were not significantly increased compared with control subjects. The titers of IgE and the number of Th2 cells were correlated. The expression of IL-5 and RANES was observed in the lesions of patients with Kimura's disease.
Conclusion: These results indicate that the predominance of Th2 and Tc1 cells might contribute to the mechanism in pathogenesis of Kimura's disease.

Keywords: Th1; Th2; Tc1; Tc2; Kimura's disease

1. Introduction

Kimura's disease is an allergic, inflammatory disorder of unknown cause. The typical presentation is in a young Oriental male with nontender subcutaneous swellings in the head and neck region, lymphadenopathy, peripheral eosinophilia, and elevated serum IgE [1–6]. Recent studies reported that T helper (Th) cells subdivided into mutually exclusive Th1 (producing especially interleukin 2 (IL-2) and interferon gamma (IFN-γ)) and Th2 (producing especially IL-4, IL-5, IL-9, IL-10 and IL-13) subsets, together with cells that exhibit an unrestricted cytokine profile (namely Th0) [7–9]. Th1 cells are primarily involved in cell-mediated immune responses, whereas Th2 cells fulfill an important role in humoral and allergic immune responses [10,11]. Similarly, $CD8^+$ T cells have recently been subdivided into $CD8^+$ T cells secreting a Th1-like cytokine pattern, which are defined as Tc1 (T cytotoxic type 1) cells, versus $CD8^+$ T cells secreting a Th2-like pattern (Tc2 cells) [7–11].

A quantitative and functional disturbance of Th1 or Th2 cells is probably important for the pathogenesis of Kimura's disease, however, the works about this point have been rare. The aim of this study was to examine the subsets of Th1, Th2, Tc1 and Tc2 cells from patients with Kimura's disease.

2. Materials and methods

2.1. Patients

Seven consecutive patients with Kimura's disease attending our department were studied. The median age of these patients was 43 (range 21–72) years with median disease duration of 87.4 (range 12–240) months. Clinical

* Corresponding author. Tel.: +81 23 628 5380; fax: +81 23 628 5382.
E-mail address: noohta@med.id.yamagata-u.ac.jp (N. Ohta).

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Table 1
Clinical features of patients with Kimura's disease.

	Sex	Age	Site	Size (mm)	Eosinophils	IgE	Duration	Tx (mg)
1	M	72	Lt parotid	36 × 32	1830 (30%)	2000	10 years	None
2	F	31	Rt parotid	70 × 40	1650 (19%)	1300	5 years	None
3	M	62	Rt parotid	40 × 50	3660 (30%)	4350	20 years	None
4	M	21	Bil. neck	40 × 50	830 (10%)	2129	5 years	None
5	M	58	Rt parotid	65 × 50	522 (8%)	600	5 years	PSL (5)
6	M	29	Lt parotid	30 × 40	1840 (23%)	547	5 years	PSL (15)
7	M	28	Rt forearm	25 × 30	1512 (27%)	2100	1 year	PSL (5)

LT: left, Rt: right, Bil: bilateral, parotid: parotid gland, Tx: treatment, PSL: predonisolone, Tx: previous treatment.

features of the patients are shown in Table 1. The main findings were irregular subcutaneous nodules (25 mm × 70 mm in diameter) that were itchy, sometimes fluctuated in size, and were tender. There was some discoloration of the overlying skin. Blood from 10 healthy controls matched for age and sex were used as a control. The control group for RT-PCR study consisted of one male with tongue cancer, without metastastic lymph nodes.

2.2. Antibodies and reagents

FITC-conjugated monoclonal antibodies specific for human CD3, CD4, CD8, CD20, CD56 were purchased from Becton Dickinson Immunocytometry Systems (BDIS; San Jose, CA, USA). RPMI-1640, fetal bovine serum (FBS), and penicillin–streptomycin solution were obtained from Gibco BRL (Grand Island, NY, USA). Phorbol 12-myristate 13-acetate (PMA), ionomycin, and brefeldin-A were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phytohemagglutinin (PHA) and concanavalin A (Con-A) were obtained from Difco Laboratories (Detroit, MI, USA).

2.3. Single color flow cytometry

Peripheral blood monoclonal cells (PBMC) were isolated from the citrated plasma of the subjects by Ficoll-Hypaque, and incubated with the indicated monoclonal antibody for 60 min on ice and washed twice with phosphate buffered saline (PBS). To eliminate dying cells, the cells were incubated with propidium iodide (PI; 5 mg/ml) for 15 min and PI-positive cells were excluded from an analysis. At least 10,000 gated cells were analyzed using a fluorescence-activated flow cytometer (FACScan; BDIS).

2.4. Intracellular cytokine analysis

Peripheral mononuclear cells were isolated from heparinized blood, and suspended in RPMI-1640 medium supplemented with 10% FBS. The cells were stimulated with PMA (10 ng/ml) plus ionomycin (500 ng/ml) in the presence of brefeldin-A (10 μg/ml) and incubated for 4 h at 37 in 7% CO_2 in air atmosphere. After stimulation, the cells were washed three times with PBS and incubated with PerCP-conjugated anti-CD4 monoclonal antibody for 15 min at room temperature. They were again washed twice with PBS, and the staining of intracellular INF-γ and IL-4 was performed using Fastimmune IFN-γ FITC/IL-4 PE kit (BDIS) under the manufacture's recommendation. Briefly, the washed cells were successively treated with FACS Lysing Solution (BDIS) for 5 min and FACS permeabilizing solution (BDIS) for 10 min, then stained with FITC-conjugated anti-INF-γ monoclonal antibody and PE-conjugated anti-IL-4 monoclonal antibody for 30 min at room temperature. The intensity of the fluorescence was measured with the FACScan flow cytometer and analysis was performed using Cell Quest Software (BDIS).

2.5. Reverse-transcription-polymerase chain reaction (RT-PCR) for detection of cytokine mRNA

Total RNA was extracted from lymph node tissues using the RNeasy Mini Kit and QIAShredder (QIAGEN Inc., Chatsworth, CA) as outlined by the manufacturer. A first-strand complementary DNA (cDNA) was synthesized from 2 μg total RNA using a Omniscript RT^{TM} (QIAGEN Inc.). Polymerase chain reaction (PCR) was performed using 0.5 μl cDNA as template, 6.25 pM of each primers, 2.5 mM dNTPs, 0.125 U *Taq* DNA polymerase (Roche-Diagnostics Inc., Germany), 1× reaction buffer containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.01% gelatin in a 25 μl reaction volume. The amplification procedure, performed on a Gene Amp PCR System 9600-R (PerkinElmer Corp, Norwalk CT),was as follows: initial denaturation step at 94 °C for 5 min, denaturation step at 94 °C for 30 s, annealing step at 55 °C for 30 s, extention step at 72 °C for 30 s, respectively. Final extension was performed at 72 °C for 7 min. The reactions were subject to each cycles: β-actin; 22, IL-5, eotaxin and RANTES; 35. PCR products were visualized after electrophoresis on 3% agarose gels by staining with ethidium bromide.

Oligonucleotides sequences are as follows:

IL-5: forward primer 5′-GCTTCTGCATTTGAGTTTG-CTAGCT-3′
reverse primer 5′-TGGCCGTCAATGTATTTCTTTAT-TAAG-3′