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# Th1 and Th2 $CD4^+$ T cells and Tc1 and Tc2 $CD8^+$ T cells of patients with Wegener's granulomatosis

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**Abstract**

A Th1/Th2 cytokine imbalance with a predominance of Th1 cytokines has been suggested to be of pathogenic importance in Wegener's granulomatosis. To evaluate the role of Th1/Th2 cytokines in Wegener's granulomatosis, the subsets of Th1, Th2, Tc1 and Tc2 cells from patients with active Wegener's granulomatosis were examined by intracellular cytokine flow cytometry. The population of Tc1 cells (72.0 ± 14.4 per cent) in Wegener's granulomatosis was significantly increased compared with Tc1 cells (37.3 ± 14.6 per cent) in control ($p<0.05$). Th1, Th2 and Tc2 cells in Wegener's granulomatosis were not significantly increased compared with the control cells. These results indicate that the predominance of Tc1 cells might contribute to the mechanism of the pathogenesis of Wegener's granulomatosis.

**Key words: Cytokines; Wegener's Granulomatosis; Flow Cytometry**

## Introduction

The importance of distinct subsets of $CD4^+$ T cells in the aetiology of a variety of immune-mediated diseases has become clear in the last 10 years. Recent studies have subdivided T helper (Th) cells into mutually exclusive Th1 (producing especially 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), both in mice and humans.[1–3] Th1 cells are primarily involved in cell-mediated immune responses, whereas Th2 cells fulfill an important role in humoral and allergic immune responses.[3] 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).[4,5]

Although a quantitative and functional disturbance of Th1 or Th2 cells is probably important for the pathogenesis of Wegener's granulomatosis, there have been few reports on the subject. The aim of this study was to examine the subsets of Th1, Th2, Tc1 and Tc2 cells from patients with active Wegener's granulomatosis.

## Material and methods

### *Patients*

Seven consecutive patients with Wegener's granulomatosis attending our department were studied. The median age of these patients was 49.5 (range 24–68) years with a median disease duration of 42 (range 14–128) months. At the time that blood was taken for analysis, patients were taking the following medication; prednisolone (n = 7) median dose (range) 15 (10–25) mg/day, oral cyclophosphamide (n = 5) median dose (range) 75 (50–100) mg/day. Clinical features of the patients are shown in Table I. Blood from 10 healthy controls matched for age and sex were used as a control.

### *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).

### *Single colour flow cytometry*

Peripheral blood monoclonal cells (PBMC) were isolated from the citrated plasma of the subjects by Ficoll-Hypaque, and incubated with indicated monoclonal antibody for 60 minutes 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 minutes and PI-positive cells were excluded from analysis. A minimum of 10 000 gated cells were analysed using a

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TABLE I
CLINICAL FEATURES OF THE WEGENER'S GRANULOMATOSIS PATIENTS

| | Sex | Age | Diagnosis | Clinical features | ANCA(IIF) |
|---|---|---|---|---|---|
| 1 | M | 25 | Gen | N, Ey, L | 8 |
| 2 | M | 46 | Lim | N | 16 |
| 3 | F | 31 | Lim | N, L | 0 |
| 4 | F | 63 | Lim | N | 32 |
| 5 | F | 65 | Lim | N, Ey | 64 |
| 6 | F | 52 | Gen | N, L, K | 16 |
| 7 | M | 62 | Gen | N, L, K | 8 |

Gen = general form; Lim = limited form = N = ear/nose/throat; L = lung; Ey = eye

fluorescence-activated flow cytometer (FACScan; BDIS).

*Intracellular cytokine analysis*

Peripheral mononuclear cells were isolated from heparinized blood, and suspended in RPMI-1640 medium supplemented with 10 per cent FBS. The cells were stimulated with PMA (10 ng/ml) plus ionomycin (1 μM) in the presence of brefeldin-A and incubated for four hours at 37°C in seven per cent $CO_2$ in air atmosphere. After stimulation, the cells were washed three times with PBS and incubated with PerCP-conjugated anti-CD4 or anti-CD8 monoclonal antibodies for 15 minutes 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) per instructions. Briefly, the washed cells were successively treated with FACS lysing solution (BDIS) for five minutes and FACS permeabilizing solution (BDIS) for 10 minutes, then stained with FITC-conjugated anti-INF-γ monoclonal antibody and PE-conjugated anti-IL-4 monoclonal antibody for 30 minutes at room temperature. The intensity of the fluorescence was measured using a FACScan flow cytometer and analysis was performed using Cell Quest Software (BDIS).

*Statistical analysis* Values are expressed as mean ± SD. Statistical analysis was performed using the Wilcoxon rank sum test and Mann-Whitney U test to determine correlations between study parameters. *P* values less than 0.05 were considered significant.

## Results

The difference in T cell subsets between patients with Wegener's granulomatosis and control subjects was not significant (Table II). There was no difference in the percentage of IFN-γ and IL-4 expressing $CD4^+$ T cell subsets between the patients with Wegener's granulomatosis and control subjects (Figure 1). However, the percentage of IFN-γ expressing $CD8^+$ T cell subsets was significantly increased in the patients with Wegener's granulomatosis compared with healthy individuals ($p<0.05$) (Figures 2 and 3).

TABLE II
T CELL SUBSETS OF THE WEGENER'S GRANULOMATOSIS

| | Control (n = 12) | WG (n = 7) | Statistics |
|---|---|---|---|
| $CD^{3+}$ | 59.6 ± 16.2 | 57.9 ± 15.8 | $p>0.05$ |
| $CD4^+$ | 35.2 ± 11.7 | 25.9 ± 13.8 | $p>0.05$ |
| $CD8^+$ | 26.9 ± 10.8 | 34.9 ± 16.8 | $p>0.05$ |

## Discussion

The purpose of this study was a detailed analysis of cytokine production in T cell subsets of Wegener's granulomatosis patients in comparison to healthy controls. Wegener's granulomatosis patients, there was a strong predominance of Tc1 cells and no significant difference was noted in the percentage of Th1, Th2 and Tc2 cells, that suggests that the predominance of Tc1 cells might contribute to the mechanism in the pathogenesis of Wegener's granulomatosis.

The existence of distinct cytokine-producing subsets within the $CD8^+$ T cell population is becoming increasingly accepted. However, little is known about the immunobiology of these subsets. The percentage of different cytokines in the T cell microenvironment appears to be the major factor that determines the differentiation of precursor T cells. IL-12, transforming growth factor-β, and IFN-γ induce differentiation of naive $CD4^+$ cells into Th1 but not Th2 cells, whereas IL-4 is essential for the differentiation of naive $CD4^+$ cells into Th2 cells and inhibits the development of Th1 cells.[6–10] IL-6 also has been implicated in Th2 differentiation.[11] IFN-γ and IL-4 also induce the differentiation of naive

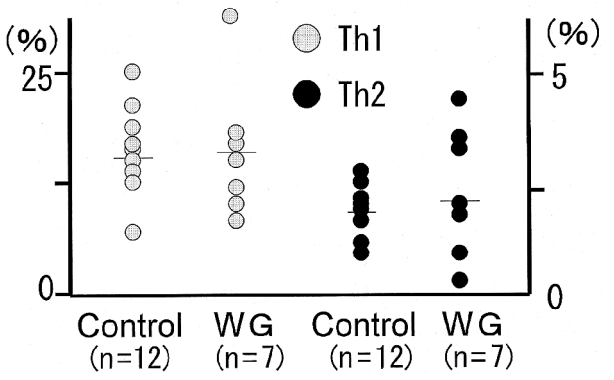


FIG. 1

Flow cytometric analysis of Th1 and Th2. No difference in the percentage of IFN-γ and IL-4 expressing $CD4^+$ T cell subsets was observed in Wegener's granulomatosis patients compared with control subjects.

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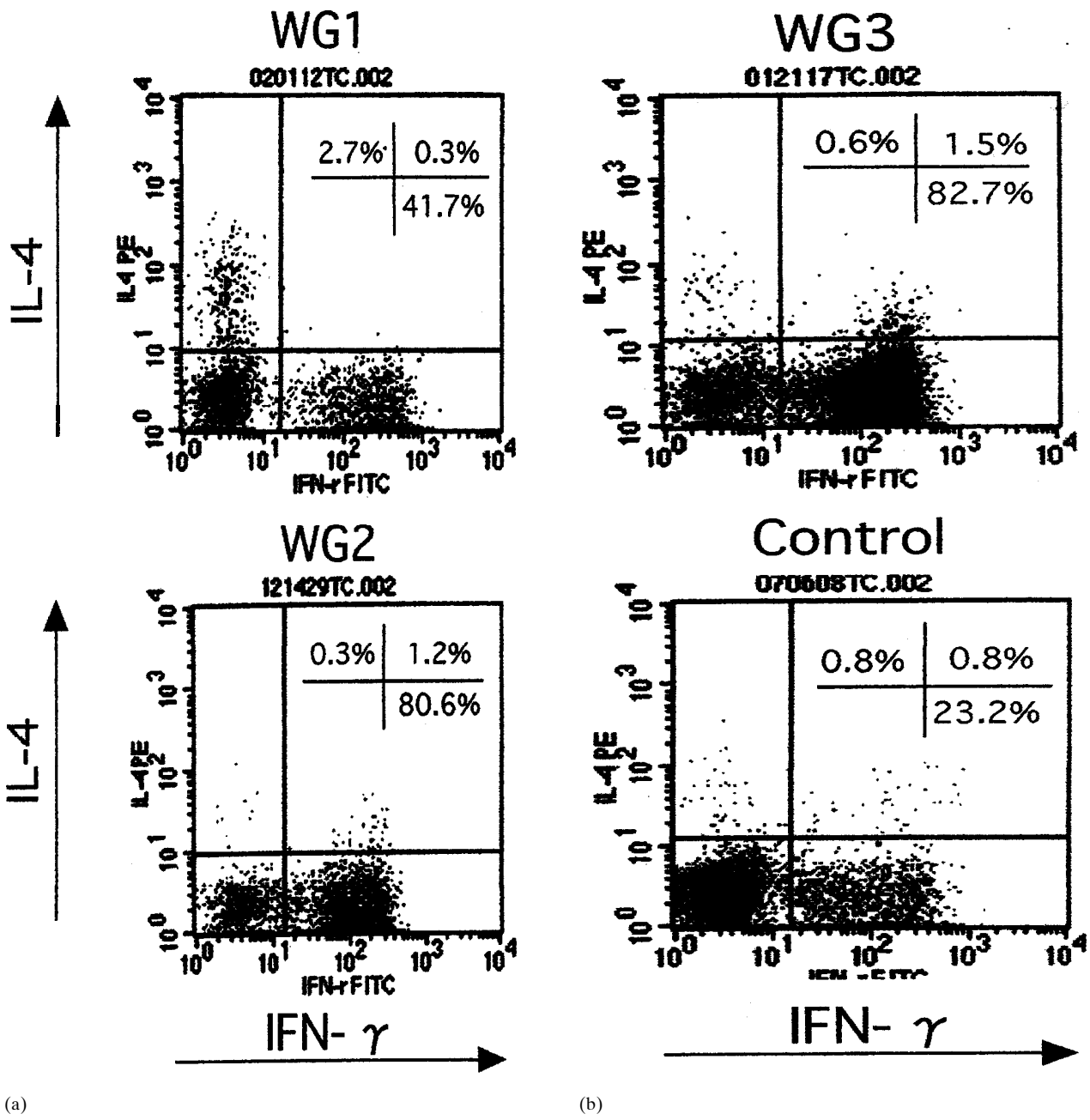


FIG. 2

Flow cytometric analysis of Tc1 and Tc2. The percentage of IFN-γ expressing $CD8^+$ T cells subsets was increased in Wegener's granulomatosis patients compared with control subjects.

$CD8^+$ cells into type 1 and type 2, respectively, whereas IL-12 promotes the development of Tc1 cells.[12] After differentiation, effector T cells show a stable cytokine pattern and rarely, if ever, switch to the opposite phenotype or revert to their precursor state. However, some modifications have been described. Th0 cells can shift toward Th1 or Th2 in response to cytokines, and human Th2 clones can transiently express IFN-γ after IL-12 treatment.[13–16] Th1 cells can produce IL-4 when stimulated in the presence of IL-4.[15] IL-4 also inhibits the ability of differentiated Tc1 cells to synthesize IL-2 and down-regulates other functions, including cytokine synthesis, proliferation, and long-term cytotoxicity.[17,18] In addition to their classic role in the killing of infected cells, CD8 T cells play a role in the regulation of activation and differentiation of CD4 cells. This regulation could be mediated through secreted products (cytokines, chemokines) or by cell-cell interaction. CD8 T cells can alter the balance of Th1/Th2 responses *in vivo* by influencing the development of IL-4 or IFN-γ secreting CD4 cells.[19–22] In addition, CD8 T cells appear to play a role in the development of CD4 perforin-mediated cytotoxicity and also have been reported to suppress CD4 proliferative responses through the inhibition

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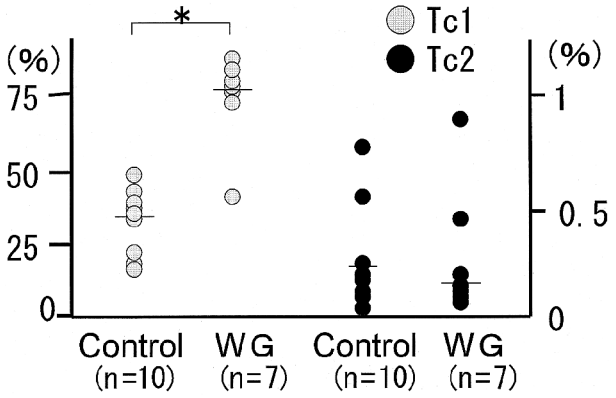


FIG. 3

Flow cytometric analysis of Tc1 and Tc2. The percentage of IFN-γ expressing $CD8^+$ T cells subsets was significantly increased in Wegener's granulomatosis patients compared with control subjects.

of co-stimulatory interactions.[21,23,24] CD8 cells are also capable of influencing other components of the immune responses, such as the recruitment of eosinophils into the lungs during respiratory syncytial virus infection or allergic asthma, activation of macrophages, and regulation of antibody production by B cells.[25–31]

Our findings showed Tc1 cell predominance in patients with Wegener's granulomatosis, which emphasizes the involvement of Tc1 cells in the pathogenesis of Wegener's granulomatosis. To identify the precise functional properties, which would greatly enhance our understanding of their pathogenetic role, detailed phenotypic and functional analysis of this cytotoxic Tc1 subset is warranted.

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