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SHORT COMMUNICATION

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T-helper (Th)1 / Th2 imbalance in patients with previously untreated B-cell diffuse large cell lymphoma

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Abstract T-helper (Th)1/Th2 imbalance has been observed in a variety of pathological conditions, including malignant diseases. We evaluated the Th1/Th2 balance in peripheral blood Th cells by means of intracellular cytokine analysis in 19 patients with previously untreated B-cell diffuse large cell lymphoma (DLCL) and in 18 patients with B-cell DLCL who had achieved complete remission (CR) after chemotherapy. The mean percentage of Th2 in $CD4^+$ cells in patients with DLCL (5.00 ± 2.20) and that of Th1 in $CD4^+$ cells in patients in CR (32.42 ± 11.30) were significantly increased in comparison with those in healthy volunteers, respectively (Th1; 23.02 ± 9.45, Th2; 3.25 ± 0.90; $P < 0.01$). The mean ratio of Th1/Th2 was significantly lower in patients with DLCL (4.74 ± 0.52) than in patients in CR (9.31 ± 1.06; $P < 0.01$) and in healthy volunteers (7.25 ± 0.65; $P < 0.01$). We conclude that the Th1/Th2 balance was polarized to Th2 in untreated DLCL patients and to Th1 in patients in CR, which suggests that a Th1/Th2 imbalance could play a role in lymphomagenesis and durable remission.

Keywords Th1/Th2 balance · Diffuse large cell lymphoma · Non-Hodgkin's lymphoma

Introduction

An imbalance of T-helper (Th)1 and Th2 has recently been reported to be responsible for various pathological conditions [1]. In regard to malignant diseases, decreased Th1 responses and/or increased Th2 responses have been associated with various types of cancer [4, 8, 12]. However, the Th1/Th2 balance has not been fully investigated in patients with lymphoid malignancies, except in those with cutaneous T-cell lymphoma and Hodgkin's disease [2, 3, 6]. B-cell non-Hodgkin's lymphoma (NHL), particularly intermediate to high-grade NHL, frequently develops in immunocompromised hosts, including patients with acquired immunodeficient syndrome (AIDS), which suggests that disregulation of the endogenous immune system could play an important role in lymphomagenesis [9]. In this study, we evaluated the Th1/Th2 balance in patients with previously untreated B-cell diffuse large cell lymphoma (DLCL) by means of direct analysis of intracellular cytokines in peripheral blood Th cells.

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Materials and methods

Patients

Nineteen patients diagnosed with B-cell DLCL and 18 patients with B-cell DLCL who had achieved complete remission (CR) after chemotherapy at Keio University Hospital were included in the study. The patients in CR had been treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone)-based regimens. Patient characteristics are shown in Table 1. No significant characteristic differences were found between the two groups. Clinical stages were determined according to the Ann Arbor classification. All patients were negative for anti-human immunodeficiency virus (HIV) and anti-human T-cell leukemia virus (HTLV)-I antibodies.

Intracellular cytokine analysis

Intracellular cytokine analysis was performed as others and we have previously described [7,10]. In brief, heparinized whole blood was incubated with phorbol 12-myristate 13-acetate (25 ng/ml), ionomysin (1 μg/ml), and brefeldin-A (10 μg/ml) at 37 °C in a humidified 7% CO_2 atmosphere for 4 h. After being stained with peridinin chlorophyll protein conjugated anti-CD4 monoclonal antibody, cells were then permeabilized and incubated with fluorescein isothiocyanate-conjugated anti-interferon (IFN)-γ monoclonal antibody and phycoerythrin (PE)-conjugated anti-interleukin (IL)-4 monoclonal antibody. The antibodies used in this study

Table 1 Patent characteristics (*DLCL* diffuse large cell lymphoma, *CR* complete remission)

	DLCL ($n=19$)	DLCL in CR ($n=18$)
Sex (M/F)	10/9	12/6
Median age in years(range)	60.8 (30–83)	65.3 (37–77)
Clinical stage		
I	0	3
II	8	7
III	2	2
IV	9	6

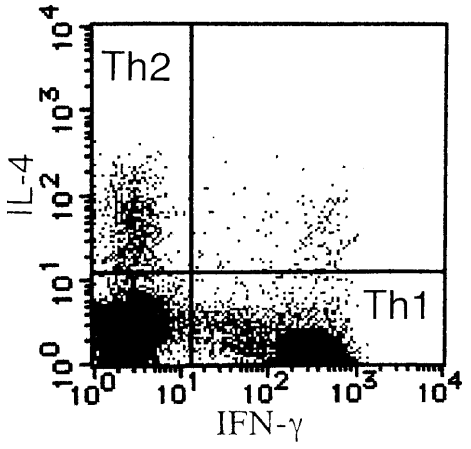

Fig. 1 Analysis of Th1/Th2 balance using intracellular cytokine analysis by flowcytometry. The results for a patient with diffuse large cell lymphoma are shown. After incubation with phorbol 12-myristate 13-acetate, ionomysin, and brefeldin-A, peripheral blood cells were stained with anti-CD4 antibody. Cells were then permeabilized and stained with IFN-γ and anti-IL-4 antibody. After $CD4^+$ cells were gated, the percentages of IFN-γ^+ IL-4^- cells (*Th1*) and IFN-γ^- IL-4^+ cells (*Th2*) in $CD4^+$ cells were determined

were purchased from Becton Dickinson (San Jose, Calif.). Samples were then analyzed on a FACScan (Becton Dickinson). After $CD4^+$ cells were gated, the percentages of IFN-γ^+ IL-4^- cells (Th1) and IFN-γ^- IL-4^+ cells (Th2) in $CD4^+$ cells were determined (Fig. 1).

Statistical analysis

The differences between the two groups were assessed by the Mann-Whitney test. *P* values less than 0.05 were accepted as statistically significant.

Results

The mean percentage of Th2 in $CD4^+$ cells in patients with DLCL was significantly increased (5.00 ± 2.20), compared with that in 20 healthy volunteers (3.25 ± 0.90; $P < 0.01$, Table 2). In contrast, the mean percentage of Th1 in $CD4^+$ cells in patients in CR was significantly increased (32.42 ± 11.30), compared with that in patients with DLCL (21.12 ± 8.97; $P < 0.01$) and that in healthy volunteers (23.02 ± 9.45; $P < 0.01$; Table 2). The mean ratio of Th1/Th2 [5, 11] was significantly lower in patients with DLCL (4.74 ± 0.52) than in patients in CR (9.31 ± 1.06; $P < 0.01$) and in healthy volunteers (7.25 ± 0.65; $P < 0.01$; Table 2). The difference in Th1/Th2 ratio between patients in CR and healthy volunteers was not significant ($P = 0.23$). No significant correlation between the Th1/Th2 balance and clinical stage was observed in patients with DLCL.

Discussion

B-cell NHL, particularly intermediate- to high-grade B-cell NHL, has been known to occur with increased frequency in a variety of immunodeficient conditions, including AIDS [9]. This strongly suggests that an impaired host immune system could contribute to lymphomagenesis. By means of intracellular cytokine analysis, we found that Th1/Th2 balances were significantly polarized to Th2 in patients with previously untreated B-cell NHL and to Th1 in patients who had achieved CR. Our observations were consistent with those of previous reports, which showed increased Th2 responses with or without suppressed Th1 responses in patients with cancer [4, 8, 12]. Regarding lymphoid malignancies, Th1/Th2 imbalances have previously been shown in patients with cutaneous T-cell lymphoma and Hodgkin's disease [2, 3, 6]. To the best of our knowledge, the present report is the first to clearly demonstrate Th1/Th2 imbalances in a series of patients with B-cell DLCL. The Th2 dominant states observed in patients with DLCL could be a cause of lymphomagenesis, but could also be the effects of the disease. In addition, we have shown the Th1 dominance in patients in CR. Th1 cytokines generate and activate cytotoxic T lymphocytes and natural killer cells, which play critical roles in anti-tumor immune responses [1]. Thus, it is conceivable that Th2 dominant states could contribute to lymphomagenesis and the progression of the disease

Table 2 Th1/Th2 balance in patients with DLCL and DLCL in CR. The percentages of Th1 and Th2 in $CD4^+$ cells were determined by intracellular cytokine analysis (*DLCL* diffuse large cell lymphoma, *CR* complete remission)

	DLCL ($n=19$)	DLCL in CR ($n=18$)	healthy volunteers ($n=20$)
Th1 (%)	21.12 ± 8.97	$32.42 \pm 11.30^*$	23.02 ± 9.45
Th2 (%)	$5.00 \pm 2.20^{**}$	3.92 ± 1.62	3.25 ± 0.90
Th1/Th2 ratio	$4.74 \pm 0.52^*$	9.31 ± 1.06***	7.25 ± 0.65

*Significant vs. other two groups respectively ($P < 0.01$)
**Significant vs. healthy volunteers ($P < 0.01$)
***Not significant vs. healthy volunteers ($P = 0.23$)

and that Th1 dominant states could contribute to durable remission. To further elucidate the role of Th1/Th2 balance in lymphomagenesis and response to chemotherapy, analyses of other Th1 and Th2 cytokines and of the Th1/Th2 balance in patients who fail to respond to chemotherapy are necessary.

Although we demonstrated only some aspects of impaired immune response in patients with DLCL, the results of this study may provide new insights into the pathogenesis of NHL. A better understanding of the pathogenetic role of Th1/Th2 imbalance in patients with NHL could contribute to the development of novel therapeutic strategies using Th1 cytokines.

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