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ORIGINAL ARTICLE

Th1/Th2 balance: an important indicator of efficacy for intra-arterial chemotherapy

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Abstract
Purpose It has been reported that Th2 cytokines down-regulate antitumor immunity, while activation of type 1 T cells promotes antitumor immunity. However, the immunological features of liver cirrhosis (LC) patients with advanced hepatocellular carcinoma (aHCC) treated by intra-arterial chemotherapy are still unclear. The aim of this study was to assess the influence of intra-arterial combination chemotherapy on the Th1/Th2 balance in LC patients with aHCC.
Methods Twenty-one adult Japanese LC patients with aHCC were treated by intra-arterial combination chemotherapy. The control group was composed of 20 adult Japanese patients with chronic hepatitis C diagnosed from examination of liver biopsy specimens. All control patients were over 55 years old and were stage 1 according to the fibrosis score of Desment.
Results Thirteen of the 21 aHCC patients (group R) showed an objective response, but the other 8 patients (group N) showed no response. There were no significant differences of Th1 cells between group R and group N either before or after chemotherapy. Although there was no significant difference from group R, group N had a significantly higher percentage of Th2 cells than the control group both before and after chemotherapy ($p < 0.05$ by Tukey's test).
Conclusions These results indicate that the Th1/Th2 balance might be a useful indicator of the effect of intra-arterial combination chemotherapy in LC patients with aHCC. Inhibition of an increase of Th2 cells might be important for the efficacy of intra-arterial chemotherapy in such patients.

Keywords Th1/Th2 balance · Cytokine · Advanced HCC · Liver cirrhosis · Intra-arterial chemotherapy

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Introduction

Several therapeutic modalities, including surgery, PEI, TAE, microwave coagulation therapy, and radiofrequency ablation, are used to treat patients with small HCC. However, there are also a considerable number of patients with advanced HCC (aHCC), and intra-arterial chemotherapy is one of their few remaining options. The majority of patients with aHCC do not survive for longer than 6 months from the day of diagnosis [1], while other authors have found an average survival period of 4 months from the onset of symptoms and 2 months from the time of admission [2]. Improvement of implanted drug delivery systems has made it possible to administer repeated hepatic arterial infusion of anticancer agents to patients with aHCC, and such hepatic arterial infusion therapy has not only been found to improve survival but also QOL [3]. Continuous local intra-arterial infusion of 5-fluorouracil (5-FU) and cisplatin (CDDP) using an infuser pump and an implanted reservoir has been shown to prolong the survival of patients with aHCC [3–5]. We have also reported that the combination of intra-arterial low-dose 5-FU, CDDP, and leucovorin (LV) prolongs the survival of patients with aHCC [6], with continuous intra-arterial infusion for 24 h being more effective compared with infusion for 6 h in patients with aHCC and liver cirrhosis (LC) due to HCV infection, although 24-h infusion is associated with stronger hematologic toxicity [7].

The response of T cells to self and non-self antigens is controlled by a network of regulatory T (Treg) cells. $CD4^+$ cells that constitutively express CD25, the interleukin-2-receptor α-chain, are generally considered to be natural Treg cells, and account for 5–10% of peripheral $CD4^+$ T cells in healthy animals and humans [8–10]. Based on their cytokine production profiles, helper T cells can be divided into two distinct populations, which are type 1 helper T (Th1) cells and type 2 helper T (Th2) cells. Th1 cells produce interferon-γ (IFN-γ) and interleukin 2 (IL-2), and play a pivotal role in cell-mediated immunity, while Th2 cells produce interleukin 4 (IL-4), interleukin 10 (IL-10), and other cytokines that are essential for the regulation of humoral immunity [11, 12]. IFN-γ preferentially inhibits the proliferation of Th2 cells, while IL-4 and IL-10 secreted by Th2 cells down-regulate the secretion of IL-12, which is the critical cytokine for Th1 differentiation [13, 14]. Th1 and Th2 cross-regulate their own development. It has been Th2 type cytokines down-regulate antitumor immunity [15], while activation of Th1 responses promotes antitumor immunity [16–19]. However, the immunological background of LC patients with aHCC receiving intra-arterial chemotherapy is still unknown in detail. Accordingly, this study was performed to evaluate the effect of intra-arterial combination chemotherapy on the Th1/Th2 balance in LC patients with aHCC.

Methods

Patients

Twenty-one adult Japanese patients who had aHCC and LC due to HBV, HCV, or non B-non C infection were treated with the intra-arterial combination of low-dose 5-FU, CDDP, and LV at our hospital between 2005 and 2006. All of the patients were more than 55 years old. Their tumors were inoperable on the basis of computed tomography findings. Blood samples were collected from the patients in the early morning both before and after chemotherapy. The control group was composed of 20 adult Japanese patients with chronic hepatitis C diagnosed from examination of liver biopsy specimens. All control patients were also older than 55 years and had stage 1 disease according to the fibrosis score of Desment.

Chemotherapy

All patients were treated with 24-h intra-arterial infusion chemotherapy (LV at 12 mg/h, CDDP at 10 mg/h, and 5-FU at 250 mg/m^2/22 h). Continuous infusion was performed via the proper hepatic artery every 5 days for 4 weeks using a catheter connected to a subcutaneously implanted drug delivery system [6, 7].

Infusion system

In all patients, an intra-arterial catheter was inserted via the femoral artery and was attached to a subcutaneous reservoir [20]. In principle, the gastroduodenal artery and right gastric artery were occluded with steel coils to prevent gastroduodenal injury by the anticancer agents. Written informed consent was obtained from all of the patients.

Evaluation of therapeutic efficacy

On CT scans obtained after 4 weeks of treatment, the size of the intra-hepatic tumors was measured as the product of the two longest perpendicular diameters of the largest tumor. A complete response (CR) was defined as disappearance of the tumor, while a partial response (PR) was defined as more than 50% reduction of the product of the two longest diameters. An increase of the product by more than 25% was defined as progressive disease (PD), and changes between PD and PR were defined as stable disease (SD).

Analysis of CD4-positive T cell subsets

CD4-positive T cell subsets in the peripheral blood were analyzed after non-specific stimulation with phorbol 12-myristate 13-acetate (PMA), ionomycin, or brefeldin A (Sigma Chemical Co., St Louis, MO, USA), according to the modified method of Jung et al. [21, 22].

Flow cytometry was used to detect IFN-γ and IL-4 in the cytoplasm of peripheral blood CD4-positive T cells after culture and staining, as reported previously [21]. Results were expressed as the percentage of cytokine-producing cells among the CD4-positive T cell population, such as IFN-γ-positive/IL-4-negative (Th1) cells or IFN-γ-negative/IL-4-positive (Th2) cells (Fig. 1).

Statistical analysis

Tukey's test was used to compare patient characteristics among the groups. Results are expressed as the mean ± SD. Probability values of less than 0.05 were considered to indicate statistical significance.

Results

The patients were divided into two groups. Thirteen of the 21 patients showed an objective response (group R: PR or SD), while 8 patients (group N) showed no response. The control group was composed of 12 men and 8 women aged

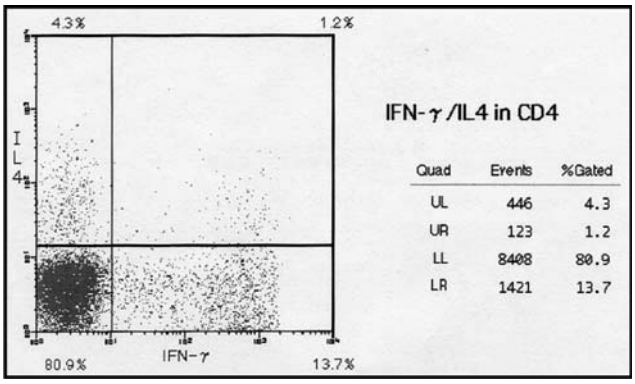

Fig. 1 Flow cytometric detection of interferon (IFN)- γ and interleukin (IL)-4 in CD4-positive T cells. *Upper left*: IFN-γ negative and IL-4 positive cells (Th2); *lower right*: IFN-γ positive and IL-4 negative cells (Th1); *upper right*: IFN-γ positive and IL-4 positive cells (Th0)

Table 1 Clinical characteristics of the 41 patients with liver cirrhosis

	Control	Group N	Group R
No. of patients	20	8	13
Mean age	61.6 ± 4	61.3 ± 5	65.6 ± 5
Gender (M/F)	12/8	7/1	12/1
Type of cirrhosis (HBV/HCV/alcohol)	0/20/0	3/5/0	0/10/3
Child-Pugh classification (A/B/C)		5/3/0	7/5/1
Stage (III/IVA/IVB)		2/4/2	4/5/4
		(vv2:1, vv3:1)	(vv2:1, vv3:1, vv4:2)
		(vp2:1, vp3:1)	(vp3:1, vp4:2)
JIS score (2/3/4/5)		1/3/4/0	3/5/4/1

56–68 years (mean ± SD, 61.6 ± 4 years). There were 7 men and 1 woman aged 58–70 years (mean ± SD, 61.3 ± 5 years) in group N, while 12 men and 1 woman aged 59–73 years (mean ± SD, 65.6 ± 5 years) formed group R. There were three patients with HBV cirrhosis and five patients with HCV cirrhosis in group N, while there were ten patients with HCV cirrhosis and three patients with non B-non C cirrhosis in group R. The Child-Pugh class was A for five patients in group N and seven patients in group R, while it was B for three and five patients, respectively, and was C for one patient in group R. There were two patients with stage III disease, four patients with stage IVA disease, and two patients with stage IVB disease in group N, while the respective numbers were 4, 5, and 4 in group R. One patients had a Japan Integrated Staging (JIS) score [23] of 2, three patients had a score of 3, four patients had a score of 4, and no patients had a score of 5 in group N, while the respective numbers were 3, 5, 4, and 1 in group R. In group N, one patient had tumor thrombi in major branches of the portal vein and one patient had tumor thrombi in the first branch, while there was one patient with tumor invasion into a branch of the right hepatic venin and one with invasion into the right hepatic vein itself. In group R, there was also one patient with tumor thrombi in major branches of the portal vein and two patients with thrombi in the portal trunk, while one patient had tumor invasion into the first branch of the right hepatic vein, one patient had invasion of the right hepatic vein itself, and two patients had invasion of the main hepatic trunk (Table 1).

Serum aminotransferases

Figure 1 summarizes the comparison of serum aminotransferases between each group. There were no significant differences among the three groups with respect to serum alanine aminotransferase (ALT) or serum aspartate aminotransferase (AST), and also there were no significant differences of serum aminotransferases between before and after chemotherapy in each group (Fig. 2).

Peripheral blood Th1 and Th2 cells

There were no significant differences of Th1 cells between group R and group N either before or after chemotherapy. There were also no significant differences of Th1 cells between the control group and group N either before or after chemotherapy. Furthermore, there were no significant differences of Th1 cells between the control group and group R either before or after chemotherapy (Fig. 3).

There were no significant differences of Th2 cells between group R and group N either before or after chemotherapy. However, the percentage of Th2 cells in group N were significantly higher than that in the control group both before and after chemotherapy ($p < 0.05$), although there were no significant differences of Th2 cells between group R and the control group either before or after chemotherapy (Fig. 4).

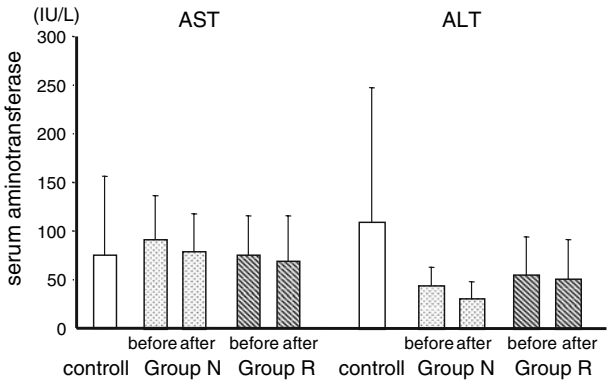

Fig. 2 Comparison of the serum aminotransferases before and after treatment in group N, group R, and the control group. There were no significant differences

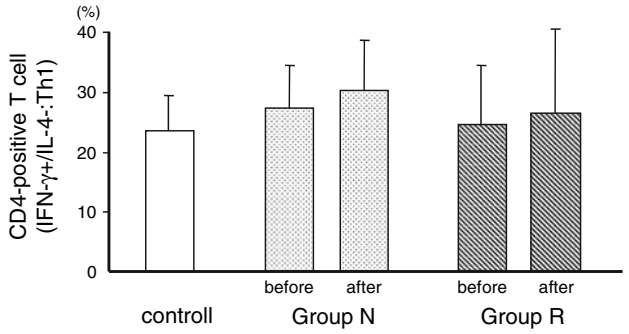

Fig. 3 Comparison of the relative prevalence of CD4-positive T cells (IFN-γ positive and IL-4 negative cells: Th1 cells before and after treatment in group N, group R, and the control group. There were no significant differences

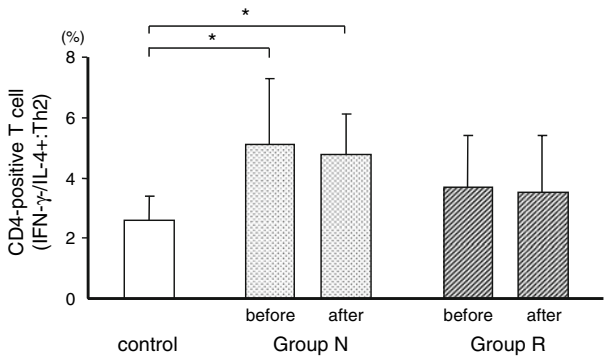

Fig. 4 Comparison of the relative prevalence of CD4-positive T cells (IFN-γ negative and IL-4 positive cells: Th2 cells before and after treatment in group N, group R, and the control group. The percentage of Th2 cells was significantly higher in group N than in the control group before or after treatment ($p < 0.05$, Tukey's test)

Discussion

When chemotherapy is given to LC patients with aHCC, we must consider both tumor factors and host immunity. In the present study, we examined the effect of intra-arterial combination chemotherapy on the Th1/Th2 balance in LC patients with aHCC. We previously reported that Th1 and Th2 cells in the peripheral blood show a significant increase with age in healthy volunteers [24], and also that the prevalence of peripheral blood CD4-positive cell subsets was not significantly different between the various histological grades or stages of chronic hepatitis C [25]. Therefore, we used patients aged more than 55 years as the control group, all of who had chronic hepatitis C diagnosed from examination of liver biopsy specimens. All control patients were in stage 1 according to the fibrosis score of Desment and their livers were nearly normal.

Before and after chemotherapy, the percentage of Th1 cells in each tumor group showed no significant different from the control group. However, the percentage of Th2 cells was significantly higher in group N before and after chemotherapy compared with the control group, although there were no significant differences of Th2 cells between group R and the control group either before or after chemotherapy. It has been reported that Th2 cytokines down-regulated antitumor immunity [15], and this report might support our findings. Because Th2 cells were significantly higher in group N than in the control group before and after chemotherapy, Th1 cells were not dominant before or after chemotherapy among CD4-positive T cells in these patients. Our results indicate that intra-arterial combination low-dose 5-FU, CDDP, and LV chemotherapy might not demonstrate an effect in patients with aHCC who have a high percentage of Th2 cells among CD4-positive T cells.

CD8+ tumor-infiltrating lymphocytes (TILs) play an important role in host defenses against tumor progression. Some studies have indicated that there is a positive correlation between an increase of CD8+ TILs and the occurrence of tumor cell apoptosis [26, 27]. Ikeguchi et al. reported the detection of significant infiltration of CD8+ T cells in the fibrous tissue and sinusoidal capillaries of the non-cancerous liver, as well as around the tumor, although the average number of CD8+ T cells within the tumor was significantly lower than that in the non-cancerous liver tissue and the extent of CD8+ T cell infiltration into non-cancerous hepatic lobules was not correlated with the severity of liver fibrosis [28]. CD4+CD25+ regulatory T cells (Treg) have an important role in maintaining self-tolerance and regulating the immune response under both physiological condition and in disease statuses [29]. It has been reported that Treg are increased in the peripheral blood and/or tumors of HCC patients and that this increase of Treg suppresses CD4+ helper T-cell responses and appears to promote the progression of HCC [30–32]. Accordingly, the percentage of Th2 cells may have been significantly higher in group N with loss of Th1 dominance due to a increase or malfunction of Treg. Moreover, these changes may have induced a decrease of CD8+ TILs and a decrease of infiltrating CD8+ T cells around the tumor in group N.

In present study, group N included 3 aHCC patients with HBV, although group R did not. We have previously shown that intra-arterial low-dose 5-FU, CDDP, and LV chemotherapy might be more useful for aHCC patients with HCV or non B-non C cirrhosis than for patients with HBV cirrhosis, although there were no significant differences of survival time between each subgroup of patients [6]. Lars et al. studied Treg cells in patients with HCC and controls, including healthy donors, patients with chronic hepatitis due to HBV and HCV, and patients with non-viral LC. They reported that patients with HCC have an increase of Treg in their peripheral blood compared with healthy control, although there were no significant differences compared with HCV or HBV patients [31]. However, it is not

known whether Th2 cells are higher in LC patients with HBV than in LC patients with HCV infection, and we also could not examine this in the present study.

In conclusion, these results suggest that the Th1/Th2 balance might be a useful indicator of the effect of intra-arterial combination chemotherapy in LC patients with aHCC. Inhibition of an increase of Th2 cells might be important for the activity of intra-arterial chemotherapy in these patients.

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