spleens removed were classified into two groups according to their effect; namely low responders in which the platelet count did not change or changed by less than 100×10^9/L after a splenectomy, and high responders which showed an improvement in their platelet count of more than 100×10^9/L after a splenectomy. As shown in Fig. 4, the number of $CD4^+CD25^+$ cells was significantly higher in the high responders than that in the low responders ($P=0.0006$).

Relationship with the number of $CD4^+CD25^+$ cells and the anti-platelet antibodies

In many ITP patients various anti-platelet antibodies which recognize such platelet membrane glycoproteins as GpIIb-IIIa, GpIa-IIa, GpIb/IX, and so on, have been reported [16-18]. The most common target recognized by anti-platelet antibodies in ITP patients is GPIIb-IIIa. Antibodies against platelet membrane GpIIb-IIIa were detected in 6 of 33 patients (18.2%). Although no statistical significance was observed, the number of $CD4^+CD25^+$ cells tended to be low in the patients who were positive for antibody against GpIIb-IIIa ($P=0.078$) (Fig. 5).

Expression mRNA levels of Foxp3 in PBMC from patients with ITP

Since CD25 is also expressed on activated T cells. It is necessary to identify a unique cell marker which is expressed by regulatory T cells. Foxp3 is found in regulatory T cells and cells transfected with Foxp3 are known to have a regulatory function. Therefore, the expression of Foxp3 is considered as a specific marker for regulatory cells. Finally, the expression levels of Foxp3 in PBMC from ITP were investigated using real-time RT-PCR. As shown in Fig. 6, the expression mRNA level of Foxp3 in PBMC was significantly higher in the patients with more than 50×10^9/L of platelet count than in those with less than 50×10^9/L ($P=0.05$). All together, the expression levels of Foxp3 correlated well with the number of $CD4^+CD25^+$ cells.

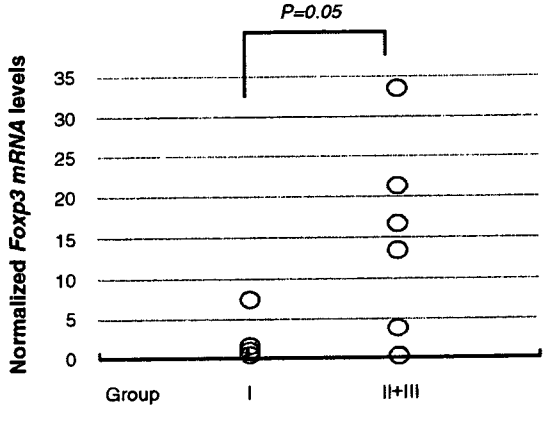

Figure 6 Quantification of relative Foxp3 mRNA levels in the patients with ITP. cDNA samples were subjected to real-time quantitative PCR analyses using primers and an internal fluorescent probe specific for Foxp3 or GAPDH. The relative quantity of Foxp3 in each sample was normalized to the relative quantity of GAPDH.

Discussion

The mechanisms to maintain immunological self-tolerance in the peripheral blood and thymus, as the clonal deletion of self reactive T cells in the thymus (negative selection) and anergy in peripheral blood (passive tolerance), are present. Nevertheless, potentially pathogenic autoreactive T cells which avoid these mechanisms are present in the peripheral blood of healthy individuals. The dominant mechanisms to regulate autoreactive T cells and prevent autoimmune disease exist. Recently, some investigators have described the role of $CD4^+CD25^+$ T cells in the regulation self-reactive T cells in the murine models.

Chronic immune thrombocytopenic purpura (ITP) is an autoimmune disease characterized by an increased platelet clearance caused by anti-platelet autoantibodies, which bind to circulating platelets, thus resulting in the destruction of platelet autoantibody complex by the reticuloendothelial system [19,20]. The major targets of anti-platelet antibodies have been reported to be platelet membrane GPs, including GPIIb-IIIa, GPIb-IX and GPIV [21], however, the mechanisms of onset and maintenance of the disease are still unclear. In 1998, Kuwana et al. reported that $CD4^+$ T cells to GPIIb-IIIa are involved in production of anti-platelet autoantibody in ITP patients and are related to the pathogenic process in chronic ITP [15]. From these findings, it is speculated that a disorder of the regulatory T cells which regulate autoreactive T cells thus exists regarding the onset and maintenance of disease in ITP as other autoimmune diseases.

In this study, the numbers of $CD4^+CD25^+$ in the patients with ITP showed a very wide distribution. However, no reduction in the number of $CD4^+CD25^+$ cells was found in the group with a low platelet count including the onset cases, thus suggesting that the number of $CD4^+CD25^+$ T cells are not related with the onset of ITP. However, the number of $CD4^+CD25^+$ cells was significantly high in the cases with a platelet count of more than 100×10^9/L. In all such cases, the recovery of the platelet count occurred after various types of medication, thus suggesting that $CD4^+CD25^+$

T cells may be related to the effect of these medications. However, the number of $CD4^+CD25^+$ T cells was not significantly high in the patients not demonstrating a recovery of the platelet count. These findings suggest that $CD4^+CD25^+$ T cells play an important role in the recovery of the platelet count.

It is interesting that the number of $CD4^+CD25^+$ cells was found to be significantly high in the patients treated with a splenectomy [22]. Although the detailed mechanisms for the increase of $CD4^+CD25^+$ cells after a splenectomy remain to be elucidated, our results suggest that an increased number of $CD4^+CD25^+$ T cells may possibly have an immunosupressive effect on ITP.

Foxp3, which encodes a forkhead/winged-helix transcription factor designated as Scurfin [23-26]. IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), which is characterized by multi-organ autoimmune diseases, allergy and IBD, and XLAAD (X-linked autoimmunity-allergic dysregulation syndrome) in human, or scurfy, a mouse mutant strain which succumbs to X-linked recessive autoimmune/inflammatory disease, are caused by a mutation of Foxp3 [27]. Recently, Foxp3 has been shown to be specifically expressed in $CD4^+CD25^+$ T cells in thymus and peripheral blood of normal mice, and it is a key regulatory gene for the development of regulatory T cells [28].

In this study, the expression levels of Foxp3 were significantly high in cases with improved platelet counts. In this investigation, $CD4^+CD25^+$ cells closely correlated with Foxp3. These results also suggested that regulatory T cells were thus related to the activity of ITP.

ITP is a very heterogenous disease, which has various clinical features and responses to different therapies. Therefore, further studies are needed to elucidate the role of regulatory T cells on ITP.

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