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Thrombopoietin: a potential T-helper lymphocyte stimulator. Change in T-lymphocyte composition and blood cytokine levels in thrombopoietin cDNA transferred mice

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The aim of this study was to evaluate the effect of thrombopoietin (TPO) on T lymphocyte in Balb/c mice delivered hTPO cDNA with plasmid vector. Both mature and immature T lymphocytes in central organs increased, but only the CD4⁺ subset was preferably proliferated in circulation. High serum IFN-γ was coinciding with the declination of platelet counts, but TNF-α was positively associated with the platelet count, while high IL-2 level was similar to the course of TPO expression. Our data suggested that TPO is a stimulator for T lymphocytes, especially the CD4⁺ subset.

Accumulating materials have enlightened the participation of thrombopoietin (TPO) in immunological processes. TPO stimulates the proliferation of endothelial cells and enhanced their expression of cell adhesion molecules.[1] It also indirectly induces the production of interferon-α *in vitro*.[2] We previously observed macrophage proliferation and endothelial cell activation in the spleens of TPO gene therapy mice. Here we have investigated the T lymphocyte composition and cytokine production of mice with TPO cDNA delivery.

Female Balb/c mice were delivered with the pcDNA3/hTPO plasmid as previously described.[4] The dose was 60 μg plasmid DNA for each mouse. Null-treated mice as control. Mononuclear cells isolated from thymocytes, plenocytes, fumer marrow cells and blood cells were directly stained with FITC conjugated-anti-mouse CD4 (L3T4) and PE conjugated-anti-mouse CD8a (Ly-2)(Pharmingen). Samples were analyzed using a FacStar flow cytometer (Becton Dickinson). For each sample, 5,000 events were acquired. Sera collected from 5 mice at set times were analyzed in duplicate with IFN-γ, IL-2 ELISA kits (Genzyme) and TNF-α kits (Biotinge, China).

Both mature T lymphocytes ($CD4^+$ or $CD8^+$) and immature ones ($CD4^+CD8^+$) in central and peripheral immune tissues were affected. They assumed different characters in the 2nd week. As Figure 1 shows, in marrow and spleen, all the three T lymphocyte subsets increased, while only the immature cells increased in the thymus. However, $CD4^+$ subset predominantly and selectively increased in blood.

Serum IFN-γ, TNF-α and IL-2 concentrations began to change early within 24 hr. of gene delivery (Figure 2). IFN-γ peak was 9-12 folds that of the controls and maintained from the 2nd week. Striking peak TNF-α level was about 120 folds of the controls but only occurred in the first week. Meanwhile, IL-2 was high at the most times.

Our data indicated the stimulatory effect of TPO on T cell production. In this process, T helper subset was subject to be selectively enhanced, as implied by the increase in blood $CD4^+$ cells. Elevated immature but low mature T ratio in the thymus might suggest a speed-up development. Furthermore, high IFN-γ and IL-2 production implied activation of Th1 and possibly NK cells in the time.

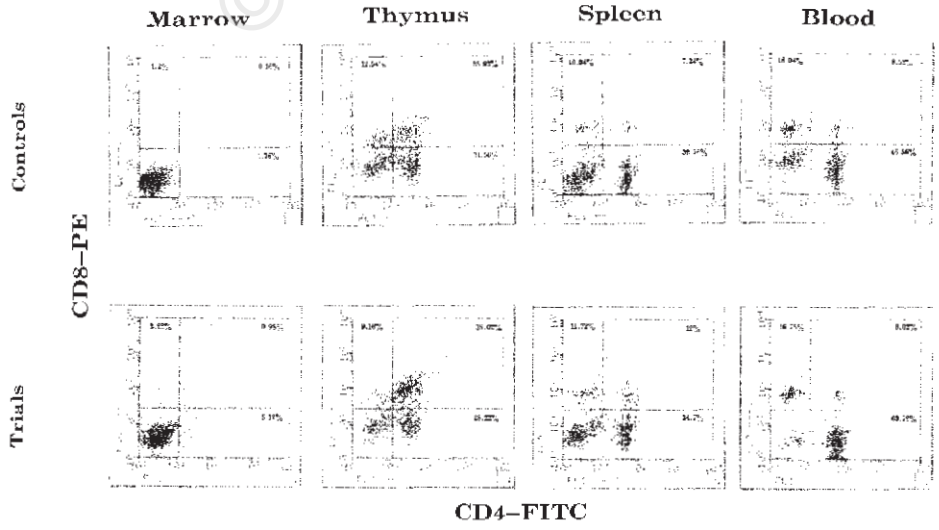

Figure 1. T-lymphocyte frequency alterations.

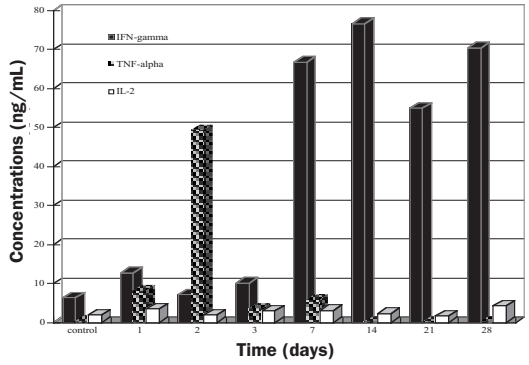

Figure 2. Serum cytokine kinetics of TPO gene delivery mice.

IFN-γ is inhibitory to CFU-Mk.[3] Although a contrary conclusion was recently gained,[4] its striking increases that coincide with the declination in platelet count and accompanying the fluctuation of platelet counts[5] inferred its down-regulatory role in thrombopoiesis. TNF-α can stimulate the proliferation of a human megakaryocytic cell line.[6] However, it showed little correspondence with platelet count in the later stages. The change of IL-2 was associated to TPO expression[7] that might support a direct stimulatory role of TPO on T lymphocytes. Although TPO indirectly induced IFN production *in vitro*[2] here, the proliferation of T lymphocytes might give a better explanation for the cytokine overproduction.

Several groups effectively promoted mice platelet production by TPO over-expression.[5,8,9] Recently, it was noticed that following platelet peak, the adenovector-mediated hTPO delivery had induced autoantibodies against TPO in Balb/c mice and resulted in pathological changes.[10] Was the activation of T lymphocyte part of such reactions? With the plasmid vector, first, we kept TPO expression for much longer than the platelet peak[7] without its being neutralized by the possible autoantibodies; second, our primary analysis of marrow megakaryocyte did not observed its reduction during this process. Furthermore, we recently observed that hTPO cDNA delivery thoroughly induced the turnover of tumor infiltration T lymphocyte phenotypes from $CD8^+$ to $CD4^+$ that accompanied significant retardation of the implanted tumor (unpublished data). The immunological responses did not seem auto-reactive.

Key words

Thrombopoietin, T lymphocyte, IFN-α, TNF-γ, IL-2

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Phenotypic changes in neutrophil granulocytes after G-CSF administration in patients with acute lymphoblastic leukemia under chemotherapy

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Phenotypic changes in neutrophil granulocytes (NG) after G-CSF have been scarcely studied. Using flow cytometry, we analyzed the changes of CD11b, CD14, CD33, CD71, HLA-DR, CD10, CD16 and CD15 on NG after G-CSF treatment in 6 patients with ALL receiving