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Modifications of lymphocyte subsets in autoimmune thrombocytopenic purpura patients submitted to splenectomy

We studied the behaviour of blood subset lymphocytes in 25 adult patients with autoimmune thrombocytopenic purpura (ATP) submitted to splenectomy. An increase of absolute concentrations of T-subset lymphocytes was observed in the different groups of splenectomized patients; in no responding/relapsing subjects an activation of T lymphocytes was demonstrated.

Sir,

Several studies have noted modifications in the cellular immunity in ATP patients.[1-7] We studied 25 patients (18 females, 7 males) with chronic ATP (thrombocytopenia lasting for more than 6 months), who had undergone splenectomy due to their unresponsiveness to corticosteroid therapy. These patients did belong to a more wide group of 94 splenectomized ATP subjects, recently evaluated as long-term follow-up.[8] Lymphocyte subset analysis was performed at a median time from the splenectomy of 10^8 months (12-252 months). By employing a Cytoron Absolute flow cytometer (Ortho Italia SpA, Milan, Italy) and Ortho monoclonal antibodies, the following parameters were evaluated: white blood cell count; lymphocyte and platelet count; absolute blood concentrations of T (CD3-positive), B (CD19-positive), helper-inducer (CD3/CD4-positive), suppressor-cytotoxic (CD3/CD 8-positive), activated T lymphocytes (CD3/HLA-DR-positive), T lymphocytes which express the receptor for interleukin-2 (IL-2) (CD3/CD25-positive) and natural killer (NK) cells (CD16-positive).

The data obtained were analyzed by comparing the groups of responding and non responding/relapsing patients to normal subjects (Table 1). The group of responding patients showed significant increases in the absolute count of lymphocytes, in the total number of T lymphocytes and in the main subsets of CD3-lymphocytes (CD3/CD4, CD3/CD8, CD3/HLA-DR-positive lymphocytes), compared to normal subjects. Similar results were obtained in the group of non responding/relapsing patients. A significant increase in the CD3/CD25-positive lymphocytes was also noted in no responding/relapsing patients as compared to the normal subjects. A more significant increase in the absolute values of

Table 1. Lymphocyte subsets in ATP patients.

	Normal subjects (n = 25)	Splenectomized responding pts. (n = 14)	ATP pts. non responding and relapsing (n = 11)	Non splenect. pts. (n = 15)
Lymph. total	1771±535.8	3,030.9±1,100.9 §#	3,100±674.7 *	1,700±544.4 °
CD3+	1364±422.4	2,347±784.4 §#	2,325±334.4 *	1,240±425.6 °
CD3/CD4+	798±262.9	1,301±412.8 §#	1,458±593.1 *	750±256.6 °
CD3/CD8+	544±190.4	855±364.1 §#	893±322.7 *	435±191.3 °
CD3/HLA-DR+	153± 81.4	253±215.9 §^	451±256.5 *	184±90.5 °#
CD3/CD25+	61±61.5	54±72.1 ^	147±164 *	60±80.8 °
CD16+	176±83.7	244±330.4	257±185.2	189±145
CD19+	231±106.6	191± 342.7	248±195.9	168±74.5
CD4/CD8	1.5±0.4	1.4±0.4	1.5±0.9	1.6±0.5

Blood lymphocyte subset concentrations are expressed as cells/mL. Statistically significant differences: *normal subjects vs no responder patients; §normal subjects vs responder patients; ^responder vs no responder patients; °no splenectomized patientsvs no responderpatients or †vs. normalsubjects or #vs.responder patients.

CD3/CD25-positive and CD3/CDHLA-DR-positive lymphocytes was noted in no responding/relapsing patients in relation to responding subjects. The results obtained in the splenectomized patients (responding and non responding/relapsing) were then compared with the data obtained in 15 non splenectomized ATP patients. Statistical analysis showed that there are no significant differences between lymphocyte subset concentrations of normal subjects and of non splenectomized ATP patients; a significant increase of T-lymphocytes and the relative subpopulations in the responding and no responding patients was observed compared to the no splenectomized patients, while the CD3/CD25-positive lymphocytes were significantly higher only in the non responding/relapsing group, as compared to no splenectomized patients.

In our patients an increase of absolute concentrations of T-subpopulation lymphocytes and an activation of T-lymphocyte system were observed in the different groups of splenectomized ATP patients, irrespective of the clinical results of the surgical operation. A similar result was obtained comparing the various groups of splenectomized patients to no splenctomized, no treated subjects affected by ATP, with platelet levels of $> 50x10^9$/L and $< 150x10^9$/L.

In conclusion, two possible modifications of the lymphocyte system might occur in ATP splenectomized patients: an increase of the T-lymphocyte subpopulations, a result more evident in no responding or relapsing subjects, and / or an alteration in the NK cell activity in spite of a normal their concentration.

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Key words

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Abnormal bleeding in a patient with chronic lymphocytic leukemia and acute hepatitis due to a circulating heparin-like anticoagulant

We report a case of a CLL patient with a history of abnormal bleeding. Laboratory tests were compatible with acute hepatitis. Coagulation assays were normal except a prolonged thrombin time (TT). The study of prolonged TT suggested a heparin-like anticoagulant activity as the cause. The TT was reduced progressively as hepatic enzymes returned to a normal range.

Sir,

A 56-year-old man with a refractory CLL was admitted to the hospital because of fever secondary to pnemococcal sepsis. This was resolved after treatment with cephotaxime. On the fifth hospital day the patient became icteric and laboratory tests showed altered liver function compatible with acute hepatitis. The HBsAg, anti-HBs and anti-HBc were positives. The patient was discharged home and fifteen days later he returns to the hospital because epistaxis and a large left costal tumor and needed rehospitalization for epistaxis. The patient had not past history of a bleeding disorder despite maintained low platelet count. On physical examination, petechiae were present on the trunk and extremities, left costal tumoration compatible with hematoma, generalised lymphadenopathy and hepatosplenomegaly. Laboratory studies showed a haemoglobin level of 5.9 g/dL; leukocyte count 191×10^9/L, of which 75% were lymphocytes; platelet count 24×10^9/L; AST 7400 U/L; ALT 4500 U/L; total bilirubine, 3 mg/L. There was no monoclonal protein on serum immunoelectrophoresis. A thoracic ultrasonograhy showed an image compatible with costal hematoma. The patient was treated with platelet transfusion, ε-aminoproic, steroids and vitamin K with control of bleeding syndrome. No heparin had been administered at any time.

Laboratory coagulation tests are summarised in Table 1. All coagulation assays were done in duplicate. Despite his mucocutaneous bleeding history, only the thrombin time (TT) was abnormal (with exception of first study, previous to vitamin K administration). The prolonged TT was partially corrected in the mixing study but totally corrected with toluidine blue and a heparinase I (Hepzyme™Dade®). A protamine titration suggested the presence of a heparin-like molecule. The in vitro addition of 50 µg/mL of protamine sulfate corrected the prolonged TT. Results of coagulation studies repeated a week later remained unchanged. The TT was