Case S1

An 8-month-old girl with a history of recurrent respiratory infections, including *Pneumocystis carinii* pneumona (PCP)[1] at the age of 6 months, was admitted to the hospital. Physical examination was remarkable for small stature, but was otherwise normal. Laboratory examination revealed a WBC of 10,600, which were 80% lymphocytes. An HIV test was negative. Flow cytometric analysis of her mononuclear cells is depicted in Fig. 1. *In vitro* lymphocyte proliferative responses were absent to phytohemagglutinin, pokeweed mitogen, and concanavalin A (lectins that induce lymphocyte proliferation), as well as anti-CD3 mAb. However, the patient's lymphocytes proliferated normally to phorbol myristate acetate (an activator of protein kinase C) plus ionomycin (a calcium ionophore that causes an influx of calcium). Lymphocytes were isolated from the patient's peripheral blood and lymph node and were examined for calcium fluxes in responsed to anti-CD3 mAbs (Fig. 2). From these data, it was determined that the patient was likely to have a mutation in the gene coding for ZAP-70; this was confirmed by DNA sequencing. An immunoblot of detergent extracts derived from the patient's peripheral blood lymphocytes demonstrated complete absence of ZAP-70. At one year of age, the patient received a T cell-depleted bone marrow transplant from her mother.

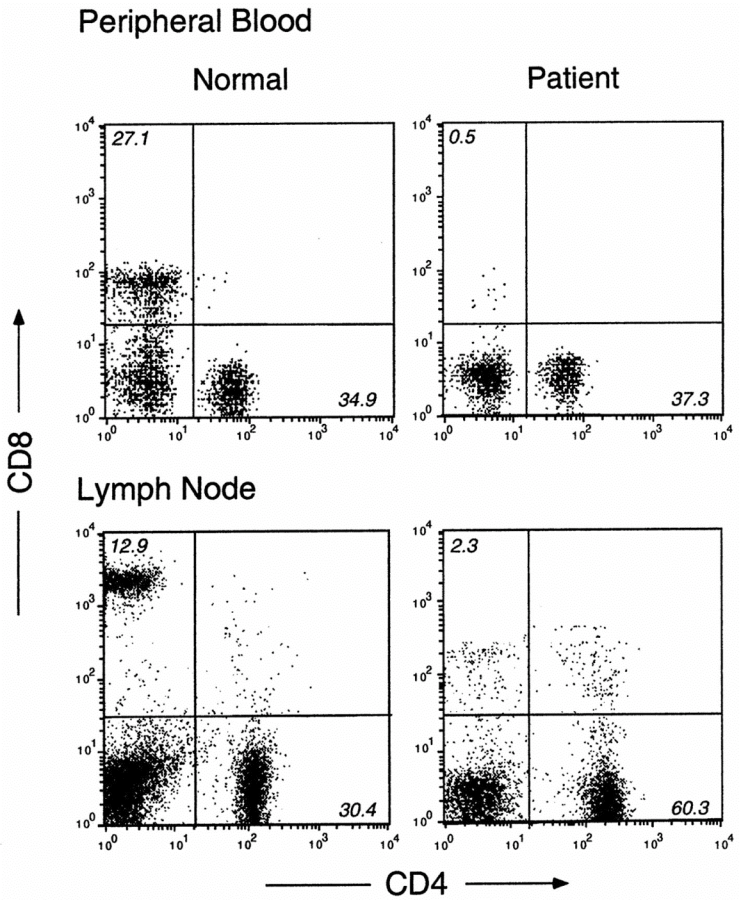

Fig. 1. Mononuclear cells from peripheral blood and a lymph node were obtained from the patient and a normal individual. Cells were stained with anti-CD4-PE and anti-CD8-FITC, and analyzed by flow cytometry.[2] Percentages of $CD4^+$ and $CD8^+$ T cells are indicated in italics.

Case S1, cont'd

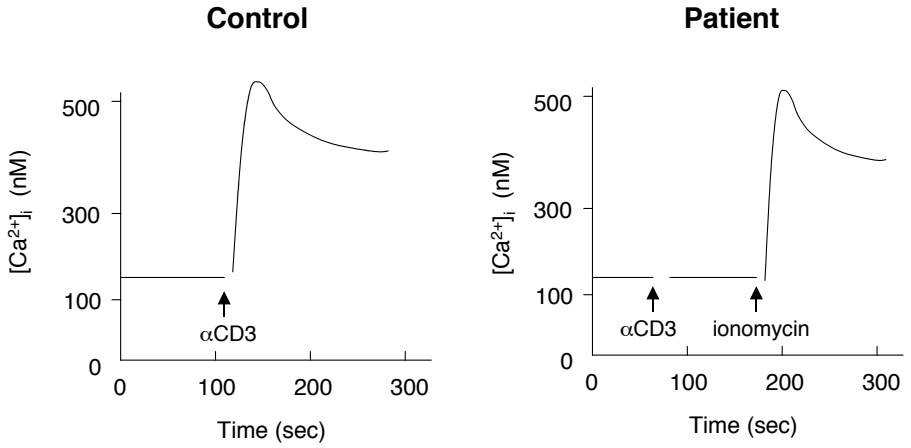

Fig. 2. Changes in cytosolic calcium after CD3 stimulation of lymphocytes from patient or normal control. Peripheral blood lymphocytes were isolated, loaded with a fluorescent calcium indicator dye, and stimulated with either an antibody against CD3 or the calcium ionophore, ionomycin. Ionomycin was added to ensure that the cells were viable and capable of manifesting changes in calcium independent of CD3. Cytosolic calcium was monitored using a spectrofluorometer.[3] Note lack of CD3-stimulated calcium fluxes in patient's lymphocytes.

[1]A fungal pneumonia that is seen commonly in AIDS and a few other conditions

[2]CD4-PE and CD8-FITC are mAbs conjugated with the fluorescent dyes, R-phycoerythrin and fluorescein isothiocyanate. When excited by the 488 nm laser in the flow cytometer, PE emits a red light and FITC emits a green light, which can be easily distinguished by photomultiplier detector tubes in the flow cytometer.

[3]An instrument that stimulates samples with light of specific wavelengths and detects light of different wavelengths. It is similar to the flow cytometer in that it detects fluorescent signals emitted from cells, except that it samples whole populations of cells, rather than single cells.

Questions for Case S1

(1) What is ZAP-70? What is its function in TCR signaling? Given the lack of a TCR-induced calcium response, what enzyme and signaling pathway is dependent on ZAP-70?

(2) The patient had no detectable circulating $CD8^+$ T-cells but did have circulating $CD4^+$ T-cells. What does this tell you about the requirement for ZAP-70 in lymphocyte development?

(3) Why did the patient develop *Pneumocystis carinii* pneumonia?

(4) Why did addition of phorbol myristate acetate and ionomycin to the patient's lymphocytes result in their proliferation?