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Case Report

Abnormal NK Cell Lymphocytosis Detected after Splenectomy: Association with Repeated Infections, Relapsing Neutropenia, and Persistent Polyclonal B-Cell Proliferation

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Abstract

We report the case of a boy with hereditary spherocytosis who presented with mild microcytic hypochromic anemia and recurrent leg ulcers that had been present since childhood. Chronic natural killer (NK) cell and B-cell lymphocytosis was detected 1 year after therapeutic splenectomy during investigation of recurrent episodes of neutropenia and persistent lymphocytosis. NK cells proved to be abnormal at immunophenotyping studies, and B-cells were polyclonal and displayed a normal immunophenotype. Genotypic analysis of T-cell receptor (TCR)-β and TCR-γ genes showed a germ-line pattern. The clinical course of this patient was characterized by multiple pulmonary infections and amygdalitis. We discuss the potential roles of persistent immune stimulation due to chronic hemolysis and severe leg ulcers and of splenectomy in the origin of NK cell lymphocytosis and the relationship between NK cells and recurrent infections, relapsing neutropenia, and polyclonal B-cell response. *Int J Hematol.* 2002;75:484-488.

Key words: NK cells; Neutropenia; B-cells; Spherocytosis; Splenectomy

1. Introduction

Hereditary spherocytosis is the most common congenital anemia due to red cell protein defects and selective retention of abnormal erythrocytes in the spleen [1]. From the clinical point of view, it is characterized by hemolytic episodes of varying severity, spherocytes on the blood film, increased red cell osmotic fragility, and a favorable clinical response to splenectomy. Leg ulcers have been reported in subjects with defects in spectrin protein. Various immunological abnormalities of multifactorial origin have been reported to occur in patients with hereditary spherocytosis, and hematological tumors have been occasionally described [2,3].

In healthy adults, approximately 15% of all circulating lymphocytes are NK cells. The function of NK cells includes cytolysis of tumor- or virus-infected cells and regulation of hematopoiesis [4]. The phenotypic features of the cells have been characterized in detail [5]. Lymphoproliferative disorders of NK cells constitute at least two different entities: (1) NK cell leukemia/lymphoma, which is characterized by an aggressive clinical course with multiorgan involvement and

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short survival, and (2) chronic NK cell lymphocytosis (CNKL), an indolent disease similar to T-cell large granular lymphocyte (LGL) leukemia, the main clinical features of which include cytopenia, vasculitis, fever, and arthritis [6-8].

Herein we describe a case of a CNKL with an abnormal immunophenotype associated with relapsing transient neutropenia, persistent polyclonal B-cell lymphocytosis, and repeated infections in a patient who had undergone splenectomy for hereditary spherocytosis.

2. Case Report

In 1983, an 8-year-old boy was referred to the Hospital S. João (Porto, Portugal). He presented with anemia (hemoglobin level, 9.4 g/dL) and an ulcer on the left leg. In January 1993, the diagnosis of hereditary spherocytosis due to β-spectrin deficiency was made. Family studies confirmed that the patient's twin brother also was affected.

The patient underwent therapeutic splenectomy in May 1995. The white blood cell (WBC) count immediately before splenectomy was 6.5×10^9/L with neutrophil and lymphocyte counts of 3.5×10^9/L and 1.9×10^9/L, respectively. Four days after surgery, fever developed, and the chest x-ray showed a left pleural effusion and lobar pneumonia, successfully treated with intravenous penicillin. The patient was discharged from the hospital 19 days later. At that time the hemoglobin level was 11.6 g/dL and the WBC count was 11.0×10^9/L (neutrophils, 5.2×10^9/L; lymphocytes, 4.0×10^9/L).

The patient's clinical and analytic picture remained stable until May 1996, when moderate neutropenia (1.8×10^9/L) and lymphocytosis (8.3×10^9/L) were noticed for the first time. The peripheral blood smear showed increased numbers of LGL. Immunophenotypic studies performed in June 1996 revealed increased numbers of circulating NK cells (25% lymphocytes, 1173×10^6/L; normal range, 200-400 $\times 10^6$/L), B-cells (21%, 985×10^6/L; normal range, 200 to 400 $\times 10^6$/L), and T-cells (54%, 2530×10^6/L; normal range, 1100-1700 $\times 10^6$/L). An abnormal NK cell population partially expressing low levels of surface CD3 (ε chain) in the absence of T-cell receptor (TCR) αβ and γδ chains was detected. Within T-cells, a low CD4/CD8 ratio (1.1; normal range, 1.2-1.9) was observed, as were increased numbers of $CD8^+$ (24%, 1102×10^6/L; normal range, 500-900 $\times 10^6$/L) and $CD4^+$ T-cells (25%, 1163×10^6/L; normal range, 700-1100 $\times 10^6$/L). The bone marrow film showed 31% lymphocytes, 29% of which were NK cells. The myeloid/erythroid ratio was normal, and myelomonocytic cells showed a normal maturation pattern. Serum immunoglobulin levels revealed polyclonal IgA (462 mg/d; normal range, 78-312 mg/dL) and IgG (1893 mg/dL; normal range, 650-1500 mg/dL) hypergammaglobulinemia. Results of tests for antineutrophil antibodies were negative, as were those for rheumatoid factor, antinuclear, anti–double-stranded DNA, anti-centromere, anti-ribonucleoprotein, and anti–parietal cell antibodies. No serologic evidence of infection with hepatitis B, human T-cell lymphotropic type I and II, or Epstein-Barr viruses was found. Abdominal ultrasonography did not reveal organomegaly. Further investigations were not performed because the patient had no symptoms and both neutrophil and lymphocyte counts spontaneously returned to normal values.

Two additional episodes of transient neutropenia and absolute lymphocytosis were observed in September 1996 and February 1997. In January 1998 a more detailed analysis of the peripheral blood lymphocytes was performed, and NK cells were further characterized with 4-color flow cytometry and a whole-blood stain-lyse-and-wash method as previously described in detail [5]. At that time peripheral blood counts were as follows: WBC 11.7×10^9/L (neutrophils, 5.2×10^9/L; lymphocytes, 4.6×10^9/L); hemoglobin, 14.8 g/dL; and platelets, 325×10^9/L. Lymphocytes consisted of 44% of $CD3^+/TCR^+$ T-cells (2008×10^6/L), 38% of $TCR^-/CD56^+/CD16^+$ NK cells (1734×10^6/L), and 22% of $CD19^+$ B-lymphocytes (1004×10^6/L).

The majority (85%) of peripheral blood NK cells were phenotypically aberrant, whereas the remaining NK cells had an immunophenotype similar to that we have previously described for the majority of NK cells that circulate in normal blood [5] (Figure 1). Abnormal NK cells displayed variable expression of surface CD3 (24% of $CD3^{+dim}$ cells) in the absence of reactivity for TCRαβ and TCRγδ, decreased levels of CD7, very high and homogeneous expression of CD57, and decreased expression of CD11b and CD38. In common with normal circulating NK cells, these cells coexpressed CD2, CD16, CD56, CD94, and high levels of CD11a and CD45RA. Moreover, they were partially positive for $CD5^{+dim}$ (56%) and $CD8^{+dim}$ (49%).

B-cells displayed a normal mature phenotype ($CD19^+/CD20^+/CD22^+/CD10^-/CD5^{-/+}/CD38^{+/-}$) with a normal κ/λ ratio (κ light chains 62%, λ light chains 38%). T-cells showed a normal immunophenotype, with a CD4/CD8 ratio of 1.7 and both normal $CD8^+$ (15%, 684×10^6/L) and $CD4^+$ (26%, 1186×10^6/L) lymphocyte counts.

Chromosome studies, carried out after short-term culture of bone marrow cells, revealed a normal 46,XY karyotype. TCR genotypic analysis by Southern blot with Eco RI and Hind 3 restriction enzymes showed a germ-line pattern for both Cβ and γ genes.

Since 1998, the clinical course was characterized by multiple pulmonary infections and recurrent amygdalitis. NK cell and B-cell lymphocytosis persisted, and an inverse correlation was found between absolute lymphocyte and neutrophil counts (Figure 2). In the last 7 months, there was a tendency to both an increase in neutrophil count and a decrease in absolute numbers of NK cells, although abnormal NK cells persisted in the blood.

3. Discussion

We describe a case of CNKL with an abnormal phenotype associated with relapsing transient neutropenia, persistent polyclonal B-cell lymphocytosis, and repeated infections in a patient who had undergone splenectomy for hereditary spherocytosis.

Peripheral blood expansion of polyclonal, oligoclonal, or monoclonal T-cell or NK cell LGL has been described in association with several pathologic conditions. It has been suggested that LGL proliferations arise as a consequence of chronic immune stimulation [9-11]. LGL lymphocytosis also has been described among patients who have undergone splenectomy, although clonality was not investigated in most

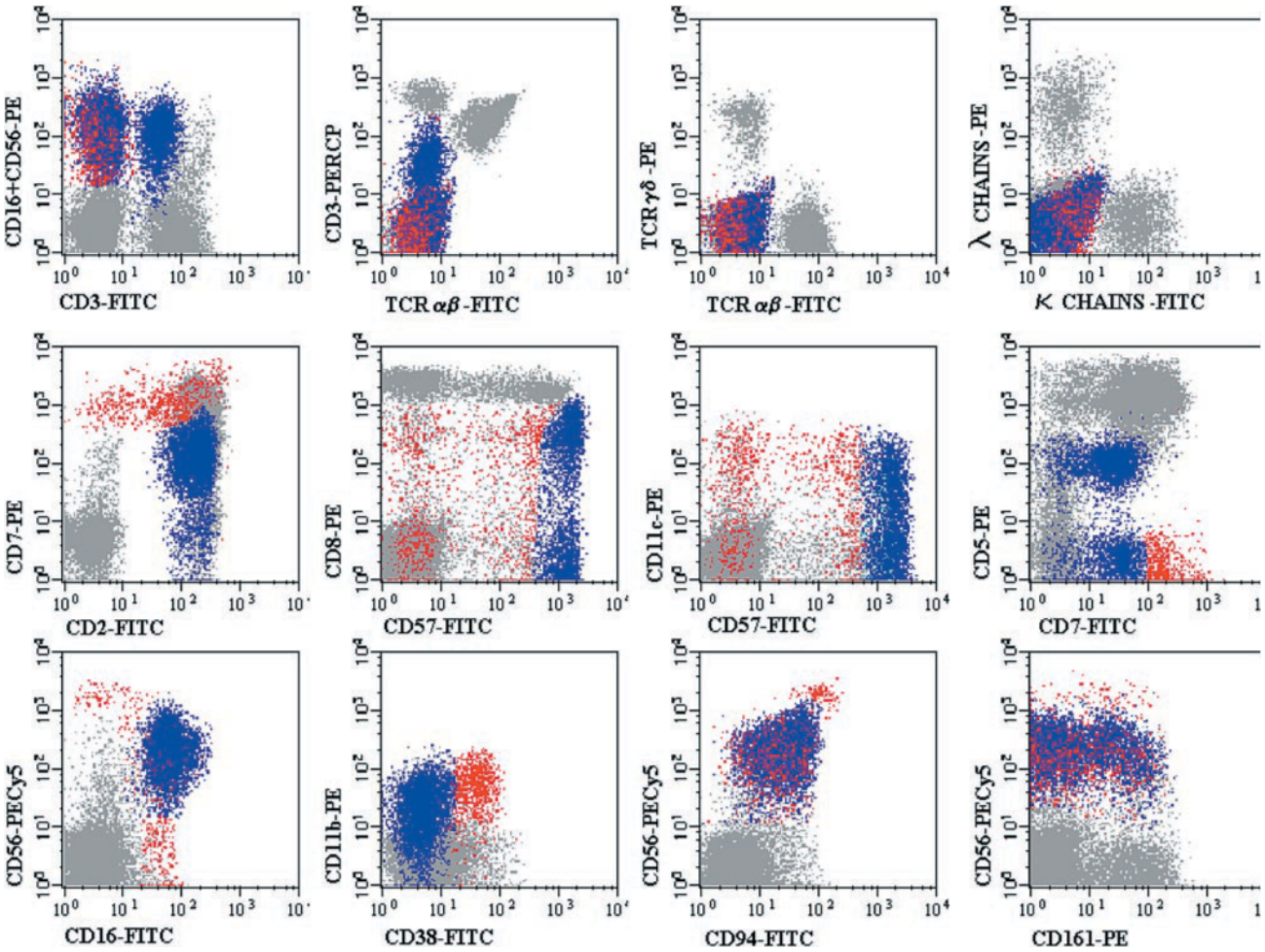

Figure 1. Dot plots illustrating the main immunophenotyping differences between abnormal (blue dots) and normal residual (red dots) NK cells. Gray dots represent T-cells and B-cells. The immunophenotype of NK cells was characterized with 4-color flow cytometry, by combining allophycocyanin (APC)-conjugated anti-CD3 and phycoerythrin–cyanine-5 (PECy5)-conjugated anti-CD56 with a large panel of fluorescein isothiocyanate (FITC) and PE-conjugated monoclonal antibodies directed against other T-cell– and NK cell–associated antigens, as previously described in detail [5]. Abnormal and normal NK cells were distinguished on the basis of knowledge of the specific immunophenotypic patterns observed in normal peripheral blood NK cells with the same panel of monoclonal antibodies [5]. The immunophenotype of the abnormal NK cells was as follows: $CD2^{+}$ (homogeneous), $CD3^{-/+dim}$ (24%), $TCR\alpha\beta^{-}$, $TCR\gamma\delta^{-}$, $CD4^{-}$, $CD5^{+/-dim}$ (56%), $CD7^{+dim}$ (heterogeneous, decreased), $CD8^{+/-dim}$ (49%), $CD11a^{+bright}$ (homogeneous), $CD11b^{+dim}$ (heterogeneous, decreased), $CD11c^{+/-}$ (56%), $CD16^{+}$ (homogeneous), $CD25^{-}$, $CD38^{-}$, $CD45RA^{+bright}$ (homogeneous), $CD45RO^{-}$, $CD56^{+}$ (homogeneous), $CD57^{+bright}$ (homogeneous), $CD94^{+}$ (homogeneous), $CD161^{+/-dim}$ (33%), $CD122^{+dim}$, $HLA\text{-}DR^{-}$, $NKB1^{-}$.

of these cases [2,12,13]. Unlike evaluation of T-cell LGL proliferations, in which clonality can be assessed with molecular analysis of the TCR genes and TCR variable region repertoire studies [9], assessment of clonality remains a challenge in NK cell disorders. Thus demonstration of an aberrant immunophenotype may be of a great help in the detection of clonal expansion of NK cells. In this case, the expanded NK cell population expressed low levels of surface CD3 ε chain and had other features that are not usually present in the majority of normal circulating NK cells, such as expression of CD5, low reactivity for CD7, and strong positivity for CD57. The fact that NK cells have a natural ability to synthesize various subunits of the CD3 molecule—NK cells express the ζ and η signal-transducing components of the CD3 molecule as a part of the low-affinity IgG Fc receptor, and cytoplasmic expression of the CD3 γ, δ, and ε chains has be reported in fetuses [14,15]—could probably explain the expression of low levels of surface CD3 by the abnormal NK cell population herein described.

Several hypotheses can be considered to explain the association of recurrent infections, fluctuating neutropenia, abnormal NK cell lymphocytosis, and polyclonal B-cell lymphocytosis in this patient. The possibility of an underlying congenital immunodeficiency is improbable because the patient's twin brother did not have recurrent infections and did not have neutropenia or lymphocytosis. Thus these abnormalities were probably acquired defects. Although the abnormal NK cell population was detected in the peripheral

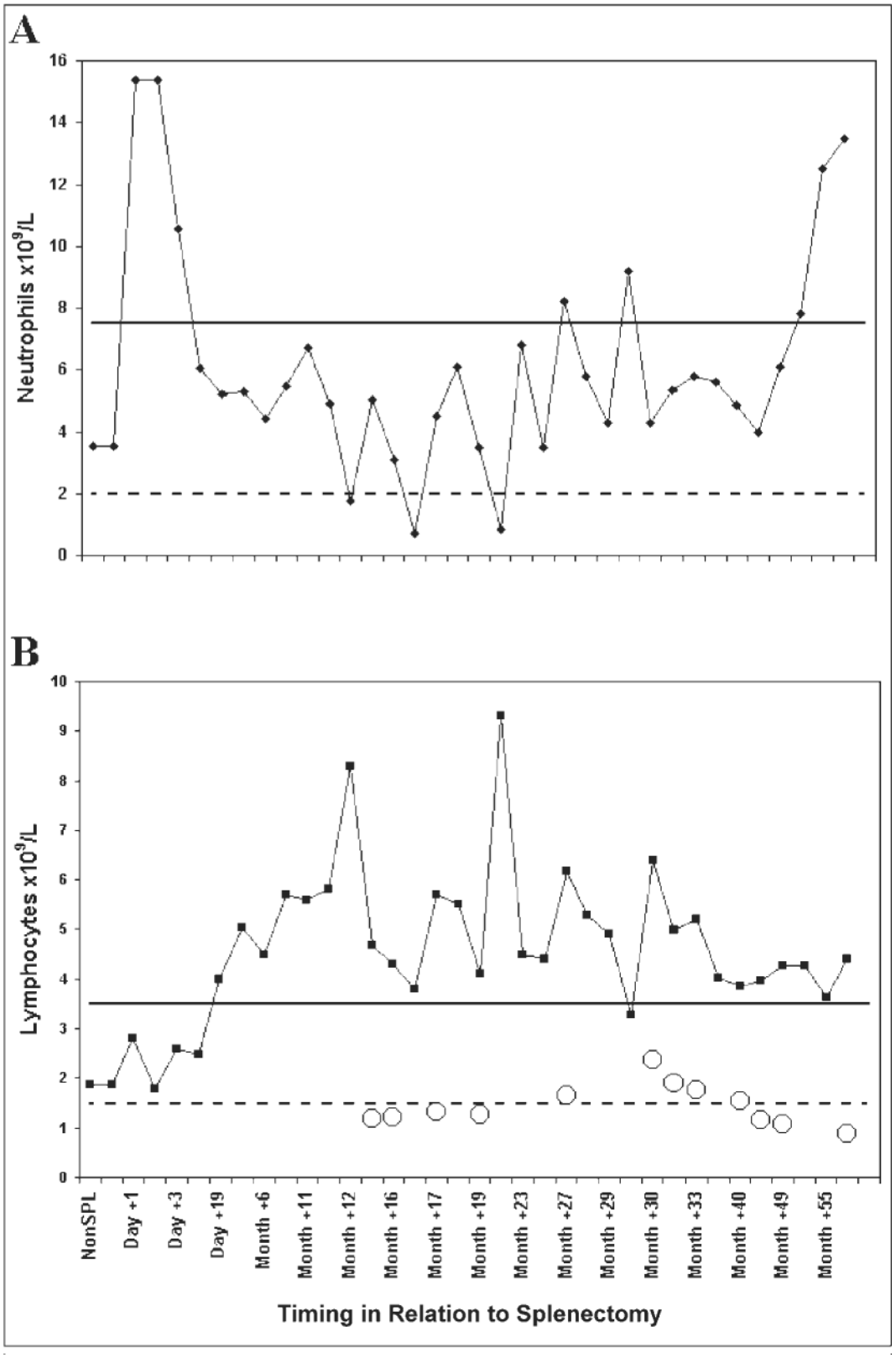

Figure 2. Neutrophil (A) and lymphocyte (B) counts as well as absolute numbers of peripheral blood NK cells (B, ○) prior to splenectomy and at different times thereafter. Solid lines and dashed lines indicate upper and lower limits of normal neutrophil (A) and lymphocyte (B) counts.

blood only 1 year after splenectomy, once immunophenotypic studies were performed for the first time, lymphocytosis developed very soon after surgery (ie, on day 19) and it is virtually impossible to know whether abnormal NK cells were already present at the time of or even before splenectomy. In this case, it could be hypothesized that the NK cell lymphocytosis arose as a consequence of the chronic immune stimulation throughout the life history of this patient owing to chronic hemolysis and severe leg ulcers and that splenectomy favored the circulation of these abnormal cells owing to a disturbance in lymphocyte homing. An alternative explanation is that the asplenic state favored the appearance of the abnormal NK cells as a consequence of deficient clearance of senescent or abnormal lymphocytes. It also can be argued that the primary defect in this patient is chronic fluctuating neutropenia and that both NK and B-cell lymphocytosis were secondary to infection. The fact that NK cells display an abnormal immunophenotype and that these abnormal NK cells were detected in the peripheral blood in the absence of infectious episodes would argue against this hypothesis. The facts that neutropenia has been previously associated with CNKL and that it was previously demonstrated that LGL in general have a regulatory effect on hematopoiesis mediated both by cell-to-cell adhesion and through secretion a variety of cytokines, such as interferon-γ and tumor necrosis factor [4], would suggest that a close relationship exists between the abnormal NK cell population and the episodes of transient neutropenia. The inverse relationship between the neutrophil and lymphocyte counts would support this hypothesis. Autoimmune phenomena and B-cell dysfunction also are frequently observed in patients with lymphoproliferative disorders of LGL [16], and NK cells produce B-cell growth factors able to both sustain proliferation of activated B-cells and induce B-cells to initiate immunoglobulin secretion [17,18]. This finding suggests a possible relationship between NK cell lymphocytosis and the polyclonal expansion of peripheral blood B-cells.

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