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EXPANSION OF LARGE GRANULAR LYMPHOCYTE SUBSETS IN WISKOTT-ALDRICH SYNDROME

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ABSTRACT

We describe a 9-year-old boy with Wiskott-Aldrich syndrome and IgM-rheumatoid factor-positive arthritis who presented expansion of two distinct subsets (one $CD8^{dim}$ and the other $CD8^{-}$) of large granular lymphocytes. Natural killer activity against the K-562 cell line was absent. An increased percentage of $CD5^{+}$ B cells was also observed.

Since patients with Wiskott-Aldrich syndrome are at risk of developing autoimmune disorders – conditions in which increased $CD5^{+}$ B cells have been observed – the high percentage of $CD5^{+}$ B cells together with the presence of IgM-rheumatoid factor and anti-platelet antibodies may represent an early manifestation of an autoimmune process. The possible relationship between $CD5^{+}$ B cells and large granular lymphocyte expansion is discussed.

Key words: LGL, Wiskott-Aldrich syndrome

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Wiskott-Aldrich syndrome (WAS) is an X-linked immunodeficiency characterized by thrombocytopenia with small-sized platelets, eczema and recurrent infections.[1] Immunological features include defective T-cell function, poor antibody response to polysaccharide antigens, elevated serum levels of IgA and IgE and low levels of IgM. The WAS gene has been identified[2] but its function is still unknown.

A high incidence of non-Hodgkin's lymphomas and autoimmune diseases, including Coombs'-positive hemolytic anemia, a juvenile rheumatoid arthritis-like syndrome and systemic arteritis, has been reported in affected children.[3]

Expansion of large granular lymphocytes (LGL) has been observed in several autoimmune conditions,[4] but it has never been reported in WAS so far.

We report the expansion of subsets of peripheral blood lymphocytes with morphologic characteristics of LGL in a 9-year-boy with WAS and IgM-rheumatoid factor (RF)-positive arthritis.

Materials and Methods

Case history

F.C., 9-year-old boy, was born after an uneventful pregnancy in May 1985. The child came to our observation in February 1986. Clinical examination revealed widespread petechiae and ecchymoses and extensive atopic dermatitis. Blood count showed 30×10^{9}/L small platelets (mean platelet volume = $3\mu^{3}$). Megakaryocytes were normally present on a bone marrow smear. Impairment of both humoral and cell-mediated immunity was present (see

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Results section). On the basis of clinical and laboratory data, a diagnosis of WAS was made. During follow-up the platelet count decreased progressively to 3×10^9/L; splenectomy performed at 5 years of age (July 1990) was followed by an increase in platelet number (80-140×10^9/L) and size. In February 1991, the patient developed arthritis of the right knee and serum IgM-RF was detected at 1/512 titer. Antiplatelet antibodies were present at a 1/198 titer, while antinuclear antibodies were not detectable. Treatment with nonsteroidal antinflammatory drugs led to clinical improvement, but positivity for IgM-RF persisted. Immunological re-evaluation performed at this time showed further derangement of humoral and cell-mediated immunity, which has not significantly changed since then(Table 1).

Table 1. Immunologic evaluation of the patient.

Years	*1986*	*1991*	*Normal values*
Lymphocytes ($\times10^9$/L)	3.4	2.9	
Platelets ($\times10^9$/L)	30	80-140	(250-350)
IgG (g/L)	10.62	7.29	
IgA (g/L)	1.47	0.68	
IgM (g/L)	0.68	0.14	
IgE (mg/L)	811	2400	
Isohemagglutinins	Absent		Absent
IgM-RF	n.d	1/528	
Antiplatelet antibodies	n.d	1/198	<1/168)
Antinuclear antibodies	n.d	Absent	
CD2 (%)	n.d.	73	(83±10)
CD3 (%)	77	43	(75±8)
CD4 (%)	31	12	(45±10
CD8 (%)	33	55	(28±9)
CD57 (%)	10	58	(18±12)
CD16 (%)	n.d.	4	(15±8)
CD19 (%)	8	10	(13±6)
CD5 (%)	n.d	53	(65±15)
TCR α/β (%)	n.d	42	(75±8)
TCR γ/δ (%)	n.d	0.3	(<9)
LGL (%)	n.d	56	(15±2)
PHA-induced lymphocyte proliferation (cpm $\times 10^{-3}$)	25	30	(60-107)
ConA-induced lymphocyte proliferation (cpm $\times 10^{-3}$)	10	18	(34-73)

n.d.= not done

Identification of the LGL

LGL were identified as slightly larger than normal lymphocytes with a relatively high cytoplasm nucleus ratio and weakly basophilic cytoplasm with azurophilic granules. Cytoplasm was positive for phosphatase and β-glucuronidase.

Cell markers and lymphocyte function

Expression of cell surface markers on PBMC was studied by FACS analysis. One and two-color analyses were performed using a panel of fluorescein (FITC)-conjugated or phycoerythrin (PE)-conjugated monoclonal antibodies recognizing different cluster differentiation antigens (CD3, CD4, CD8, CD2, CD57, CD16, CD19, CD5, TCR-αβ, TCR-γδ). Fifty microliters of heparinized blood were incubated with specific monoclonal antibodies for 30 min at 4°C.

After two washings, red cells were lysed and the remaining cells were evaluated for fluorescence. Gates were selected on lymphoid cells, including large lymphocytes. Samples were analyzed on a log-scale using electronic compensation standardized with samples stained with FITC- or PE- alone to correct for background fluorescence in two-colour analysis. Non specific staining was found using FITC- and PE-conjugated non-immune mouse IgG.

Lymphocyte proliferation following stimulation with PHA or ConA, and NK activity using the K-562 cell line as target were assessed according to the standard techniques.

Results

Immunological changes during follow-up

The results of immunological evaluation performed when the patient was admitted to our Department in 1986 are shown in Table 1. Immunological re-evaluation repeated in 1991 showed a progressive derangement of humoral and cell-mediated immunity: IgG, IgA and IgM decreased while IgE increased; both the percentage and absolute number of $CD4^+$ and $CD3^+$ cells decreased while those of $CD57^+$ and $CD8^+$ cells increased causing an inversion of the CD4/CD8 ratio. Mitogen-induced lymphocyte proliferation was persistently decreased and isohemagglutinins were absent. The sum of the percentages of $CD4^+$ plus $CD8^+$ cells was much

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greater than the percentage of $CD3^+$ cells (Table 1); expansion of LGL was demonstrated, with LGL accounting for 56% of lymphocytes.

Two-color fluorescent analysis

Two-color fluorescent analysis of the patient's PBMC showed that all $CD4^+$ cells were $CD3^+$, but only about half of the $CD8^+$ were $CD3^+$ (Figure 1 panels A and B); by contrast, in the age-matched normal control nearly all $CD4^+$ and $CD8^+$ cells were also $CD3^+$ (Figure 1 panels G and H). Furthermore, two subsets of $CD8^+$ cells were found in the patient as well as in the control (Figure 1 panels B and H): a $CD8^{high}$ subset co-expressing the CD3 antigen, and a $CD8^{dim}$ subset that did not express CD3. The fraction of the former subset ($CD8^{high}$ $CD3^+$) was comparable in the patient and in the control, while the latter subset ($CD8^{dim}$ $CD3^-$) appeared to be greatly expanded in the patient (19.4% vs 2.3%). Both the $CD8^{high}$ and the $CD8^{dim}$ subsets co-expressed the CD2 antigen (data not shown). Furthermore, the portion of $CD8^-$ $CD3^-$ cells was also increased in the patient as compared to the control (38.9% vs 18.1%).

Expansion of NK-associated antigen CD57 was remarkably increased in the patient as compared to the control (Figure 1 panels C and I). Three subsets of $CD57^+$ cells could be identified in the patient on the basis of CD8 co-expression: $CD8^{high}$ $CD57^+$, $CD8^{dim}$ $CD57^+$, $CD8^-$ $CD57^+$. When co-expression of CD3 and CD57 was analyzed, a higher percentage of $CD57^+$ cells was observed among both $CD3^+$ and $CD3^-$ cells in the patient than in the control (38.6% vs 5.6% and 27.3% vs 1.6%, respectively) (Figure 1 panels D and L). In contrast, expression of the CD16 NK antigen was not increased in the patient (4%) with respect to the control (6%). An increased percentage of $CD3^-$ $CD5^-$ cells was observed (46.6% in the patient vs 17.7% in the control), while the percentage of $CD3^-$ $CD5^+$ cells was comparable in both patient and control (Figure 1 panels E and M). Fifty-six per cent of the patient's peripheral lymphocytes had azurophilic granules, which accounted for an absolute number of $1624/mm^3$. NK activity against the K-562 cell line was absent (data not shown). Seventy-five percent of the patient's B cells (CD19) coexpressed the CD5 marker as compared to 20% of control B cells (Figure 1 panels F and N).

Discussion

The immunologic abnormalities seen in our patient are consistent with WAS: IgM levels were low and IgE levels high; isohemagglutinins were absent and progressive derangement in the number and function of T lymphocytes was observed. A decrease of $CD3^+$ and $CD4^+$ cells and an increase of $CD8^+$ and $CD57^+$ cells (both in terms of percentages and absolute numbers) was present and found to be associated with the appearance of two distinct subsets, one $CD8^{+dim}$ and the other $CD8^-$, which are likely to be $CD2^+$, $CD3^-$, $CD4^-$, $CD5^-$, $CD57^+$, $CD16^-$. The attribution of these two subsets to an NK lineage is supported by the absence of the CD5 marker. In addition, increased expression of the CD57 antigen was also observed on $CD3^+$ $CD8^{high}$ cells. In our patient the percentage of $CD57^+$ cells and of LGL was 58% and 56%, respectively; it is thus likely that these three subsets account for the increased percentage of LGL found in the patient. In spite of increased CD57 expression, no evidence of NK activity was found in the patient. This is in keeping with the observation that $CD57^+$ $CD16^+$ cells have a greater NK activity than $CD57^+$ CD16- cells.[5]

Expansion of LGL can be associated with T-γ lymphocytosis or T-γ lymphoproliferative disorder, T chronic lymphocytic leukemia, and lymphoproliferative disorder of granular lymphocytes.[6] Immunologic analysis has demonstrated that LGL expansions consistently show a mature, homogeneous post-thymic phenotype, probably representing a clonal expansion; most of these cells have a $CD3^+$, $CD8^+$ phenotype, often associated with CD57 expression. In rare cases, a $CD3^+$, $CD4^+$ phenotype or an apparently non-T-cell phenotype ($CD2^+$, $CD3^-$, $CD4^-$, $CD8^-$, $CD16^+$) has been reported.[7]

Our patient suffered from WAS, a disease with an increased risk of immune-mediated disorders and lymphoma;[1] however, an overt neoplastic process seems unlikely here because

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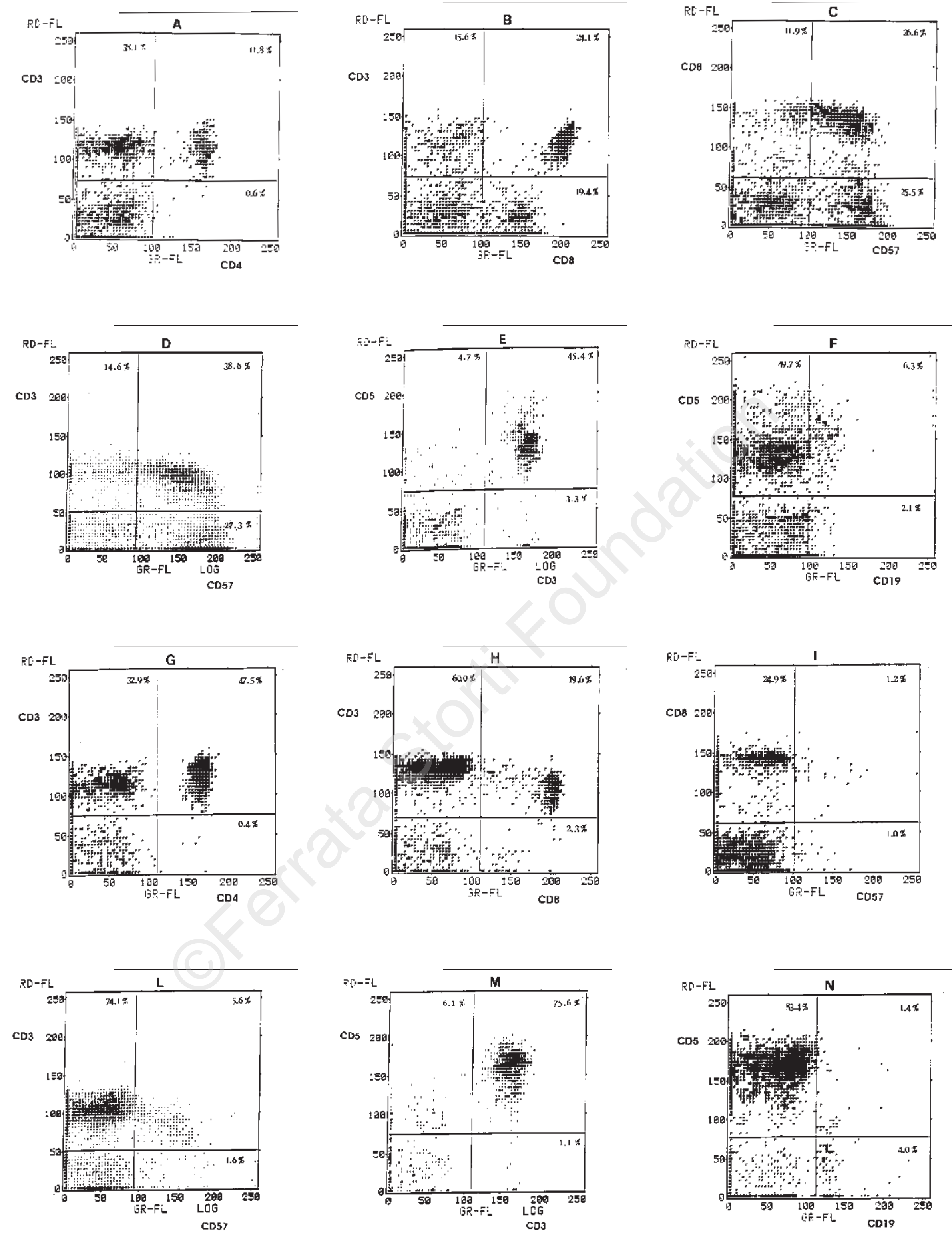

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Figure 1. Two-color FACS analysis of co-expression of different CD antigens in patient's (panels A, B, C, D, E, F) and age-matched normal control 's (panels G, H, I, L, M, N) PBMC using FITC- and PE-conjugated monoclonal antibodies. CD4 vs CD3 (panels A, G); CD8 vs CD3 (panels B, H); CD57 vs CD8 (panels C, I); CD57 vs CD3 (panels D, L); CD3 vs CD5 (panels E, M); CD19 vs CD5 (panels F, N).

of the expansion of distinct subsets with the morphological characteristics of LGL and because of the indolent clinical course of the disease, which suggest a reactive rather than a neoplastic origin for the LGL expansion. Furthermore, no evidence of clonality was demonstrated by means of TCR β genes, at least not within the $CD3^+$ $CD8^+$ $CD57^+$ subset (data not shown).

During follow-up our patient complained of recurrent episodes of migratory arthritis that resolved spontaneously; at the age of 6 years he developed arthritis of the right knee, associated with the presence of IgM-RF. In adults, rheumatoid arthritis is frequently associated with LGL expansion; furthermore, LGL expansion has been observed in some RF-positive patients without signs of clinical rheumatoid arthritis, suggesting a role for these cells in the development of autoimmunity.

To our knowledge, no expansion of either $CD8^{dim}$ or $CD8^-$ subsets with the morphologic characteristics of LGL has been reported in WAS so far. Expansion of $CD8^+$ $CD57^+$ cells has been previously described in WAS, but no evidence of LGL morphology was reported.[8] Our patient was splenectomized 7 months prior to evidence of LGL expansion. It is well known that LGL expansion and abnormalities of T-cell subsets may follow splenectomy.[8-10] On the other hand, expansion of $CD8^+$ $CD57^+$ cells has also been observed in non-splenectomized WAS patients,[8] making the possible contributory role of splenectomy in LGL expansion questionable.

Our patient also had an increased percentage of $CD5^+$ B cells (75% vs 20% in the age-matched control). The observation that $CD5^+$ B cells can produce polyreactive antibodies predominantly of an IgM isotype has suggested their involvement in autoimmune processes;[11,12] indeed, elevated percentages of $CD5^+$ B cells have been detected in some autoimmune disorders.

The association between WAS and autoimmune diseases is well known and the presence of IgM-RF and antiplatelet antibodies, together with the increased percentage of circulating $CD5^+$ B cells in our patient could be interpreted as an early expression of an autoimmune process.

Whether the expansion of LGL subsets and of $CD5^+$ B cells is the natural evolution of genetically determined immune dysfunctions associated with WAS, with splenectomy playing only a possible secondary role, and whether the appearance of these LGL is the early expression of an underlying autoimmune process remain to be established and warrant further investigation in non transplanted long-term survivors, both splenectomized and non splenectomized, affected with WAS.

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