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CLINICAL SIGNIFICANCE OF HLA-DR$^+$, CD19$^+$, CD10$^+$ IMMATURE B-CELL PHENOTYPE AND CD34$^+$ CELL DETECTION IN BONE MARROW LYMPHOCYTES FROM CHILDREN AFFECTED WITH IMMUNE THROMBOCYTOPENIC PURPURA

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ABSTRACT

In children with immune thrombocytopenic purpura (ITP), bone marrow lymphocytes can express the *common acute lymphoblastic leukemia* antigen (CALLA) pattern with no evidence of leukemia or lymphoma. Bone marrow lymphocytes from 23 children and 20 adults affected with ITP were studied to determine the incidence and the clinical impact of lymphocytes with the immature B-cell phenotype and CD34$^+$ cell expression. In this investigation we identified a group consisting of 52% of the children who showed the immature B phenotype, while the remaining 48%, similarly to adult ITP displayed an increase of T-cell antigens. CD34 was positive in 53% of children, but it was present in only half of the patients with the immature B phenotype and it was always absent in adults. IgH genes disclosed a germline configuration in all six patients in the immature B phenotype group. No difference was found in the two groups of children in terms of age, presentation of the disease or final outcome. Finally, no patient in either children's group has developed an acute lymphoproliferative disorder.

Key words: thrombocytopenia, idiopathic thrombocytopenic purpura, B-cell, CALLA, CD34 antigen, IgH gene rearrangement

It has been reported that in children with immune thrombocytopenic purpura (ITP) bone marrow lymphocytes express the common acute lymphoblastic leukemia antigen (CALLA), together with HLA-DR and CD19 antigens, without any evidence of leukemia or lymphoma.[1-3] Similarly, an increase in bone marrow CALLA lymphoid cells has been described in ALL patients off therapy, even several months after the withdrawal of therapy.[4]

Moreover, the progenitor cell antigen CD34, which is normally expressed on 1% of bone marrow cells,[5] has also been found in high percentages in acute leukemia blasts.[6]

We studied the immunophenotype of bone marrow lymphocytes using a panel of monoclonal antibodies, i.e. HLA-DR, CD19 and CD10, which identify *early* B-cells and CD34, in patients with ITP to determine the incidence and the clinical impact of this phenotype on the outcome of these patients.

Materials and Methods

Patients

Twenty-three consecutive children, whose mean age was 9.6 years (range 6 months-14.2), and twenty adults affected with ITP entered this study. The diagnosis of ITP was made according to established criteria.[7] In particular, all patients showed cutaneous hemorrhages of varying degree.

Bone marrow flow cytometry analysis

Ficoll-Hypaque separated bone marrow mononuclear cells were stained using either the directly fluorescinated isothiocyanate (FITC) monoclonal antibodies (MoAbs) CD2 and CD10 (Ylem, Avezzano, Italy), CD19 and HLA-DR (Becton-Dickinson, Milan, Italy) or the purified MoAbs CD34 (Techno-Genetics, Milan, Italy), and CD65 (kindly provided by Dr. Knapp, Vienna, Austria), subsequently labelled with a FITC conjugated goat anti-mouse immunoglobulin (IgG + IgM) (Ylem, Avezzano, Italy). CD34 was tested in 15 children and 17 adults. All fresh samples were analyzed on an Epics-Profile II flow cytometer (Coulter Electronics, Inc., Hialeah, FL, USA) and the lymphocyte gate was set. Each MoAb was considered positive if it stained > 20% of lymphocytes.

Viral findings

Antibodies to Herpes virus type 1 and 2, CMV, EBV (virus capsidic antigen), german measles virus, B and C hepatitis virus, mycoplasma, varicella zooster and toxoplasma were tested.

DNA analysis

Ten micrograms of cryopreserved bone marrow cell DNA were digested with ECO-RI, HIND III and BAM-HI restriction endonucleases, fractionated by electrophoresis on 0.8 agarose gel, blotted on nitrocellulose paper and hybridized according to the Southern blot method. The configuration at the IgH locus was analyzed using as probe a 6.6 Kb fragment containing a major portion of the heavy chain joining (JH) segments.

Statistical analysis

The SAS/STAT software package (release 6.06 from the SAS Institute Inc., 1993) was used for the simple statistical calcula-

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Marker	Adults		Children			
			Group 1		Group 2	
	# of samples	%	# of samples	%	# of samples	%
CD10*	20	5.1±4.6	12	35.5±13.5	11	8.4±5.8
CD19*	20	6.1±4.0	12	1.3±10.4	11	6.1±5.0
HLA-DR*	20	13.6±9.0	12	37.8±9.4	11	11.5±7.5
CD2	20	45.5±11.6	12	27.2±14.0	11	36.2±11.1
CD65	20	5.9±2.9	12	6.3±1.1	11	3.8±3.0
CD34°	20	5.9±2.9	12	6.3±1.1	7	26.4±21.6

Table 1. Flow-cytometry analysis of immature B-cell immunophenotype and CD34 surface marker among adults and two groups of children with ITP.

**Children's group 1 vs Adults and Children's group 2 (p = 0.0001); °Children (both groups) vs Adults (p = 0.02).*

tions, including the Wilcoxon rank sum test and Pearson's correlation coefficient.

Results

Two groups of children affected with ITP were defined on the basis of immunophenotype (Table 1). The first group, which included 52.1% of the children, showed an increase of HLA-DR, CD19 and CD10 cells, while bone marrow lymphocytes from the second group and from the adults expressed mainly T-cell antigens. The CD34 antigen was positive in 53% of the children, but it was present in only half of those with the immature B phenotype; none of the adults displayed positivity for this antigen.

A possible correlation between the main clinical and hematological parameters and the two groups of children was investigated. As shown in Table 2, no differences were found in terms of age, absolute peripheral lymphocyte count, platelet count, bone marrow lymphocytosis, number of previous fever episodes or positivity to IgM and IgG isotype viral antibodies. In particular, in two patients (one belonging to each group) affected with German measles and infectious mononucleosis, respectively, positivity for IgM antibodies to the corresponding viruses was detected.

Five out of the 12 patients in the first group were treated with 6-methylprednisolone (PDN), 5 with high-dose immunoglobulin (HD-Ig) ± PDN and 2 received no therapy. In the second group, 6 received PDN, 2 HD-Ig ± PDN and 3 no treatment. Response to therapy was similar in both groups (data not shown). All the patients who received no therapy spontaneously recovered from thrombocytopenia. Two children in the first group failed to respond to therapy and one relapsed. Three patients in the second group did not respond and one relapsed. However, both relapsing patients fully recovered after several months of low-dose PDN. Time to recovery from thrombocytopenia (data not shown) was quite similar in both groups. Of the 5 children who did not respond to therapy, two successfully underwent splenectomy, while the third one continued to maintain stable thrombocytopenia. Finally, no patient in either group developed an acute lymphoproliferative disorder after two years of follow-up.

Discussion

Our experience, carried out on 23 consecutive children affected with ITP, confirmed that the incidence of the immature B phenotype, in accordance with other authors,[1] is about 50% of the examined cases.

Table 2. Statistical evaluation of several variables in the two groups of children with ITP. p values were not significant in any comparison.

Variable	Children			
	# of samples	Group 1	# of samples	Group 1
Mean age (months)	12	53.7±40.9	11	72.7±46.7
Peripheral lymphocytes (x10⁹/L)	12	3,524±516	11	3,434±1,859
Platelets (x10⁹/L)	12	14,416±9,519	11	17,800±10,778
Bone marrow lymphocytes (%)	12	32.6±2.6	11	28.1±1.1
Fever (%)	12	41.6	11	36.6
Positivity (%) to viral antibody (IgM)	12	8.3	11	9.0
Positivity (%) to viral antibody (IgG)	12	72.7	11	66.6

The CD10 antigen, together with HLA-DR and CD19, normally present on the lymphoid cells of fetal bone marrow, persists in considerable amounts in the bone marrow of normal children, decreases with advancing age and disappears almost entirely in adults.[8,9]

In our study only about half of the ITP children showed a significant increase of the CALLA on their lymphocytes, and no relationship was found between patient age and positivity for the immature B-cell phenotype (p=0.7).

Moreover, no difference between the two group of children was noticed with regard to the main clinical and hematological and viral findings,or with regard to final disease outcome. Finally, no patients in the HLA-DR, CD19, CD10 group have developed lymphoproliferative malignancies after a follow-up of over two years, as expected from the germline configuration of the IgH genes observed in all the patients in the immature B phenotype group tested.

Furthermore, positivity to the CD34 cell antigen in our experience was observed in half of the cases examined in both children's groups.

All these data suggest that a mechanism involving immunostimulation of the lymphoid population probably underlies the increased proportion of lymphocytes expressing the immature B phenotype, as demonstrated in regenerating bone marrow cured of ALL[4] and after autologous bone marrow transplantation in acute myeloid leukemia.[10]

We cannot exclude that an unknown viral or drug injury might be the cause which promotes an immunological response leading to the expression of the immature B phenotype and CD34 expansion. Alternatively, this phenomenon could be the result of the regeneration of the stem cell compartment after transient damage involving the platelet compartment.

In conclusion, the immature B phenotype and CD34 expression observed in children with ITP bone marrow could be the consequence of an immunological response to the cause which determined the disease, or the regeneration of the stem cell compartment following transient damage. However, further studies are necessary to understand why the immature B-phenotype is not always present in all ITP cases.

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