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Childhood transient erythroblastopenia complicated by thrombocytopenia and neutropenia

Takashi Hanada, Kazutosi Koike, Ciaki Hirano, Tosiki Takeya, Tosiyuki Suzuki, Yasuko Matsunaga[1] & Hitoshi Takita

Department of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Ibaraki; and [1]Central Virus Diagnostic Laboratory, The National Institute of Health, Tokyo, Japan

We report on 4 children with transient erythroblastopenia complicated by thrombocytopenia and/or neutropenia. Bone marrow examination revealed severe erythroid hypoplasia with normal granulopoiesis and thrombopoiesis. Human parvovirus B19 infection was confirmed serologically in 2 children. An in vitro study using autologous bone marrow cells after recovery demonstrated IgG-mediated inhibition of erythropoiesis in 4 children. Additionally, antibodies directed against platelets and neutrophils were detected. These findings suggest that the IgG-mediated mechanism may be pathogenetic for the transient pancytopenia of these children.

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Transient erythroblastopenia of childhood (TEC) is a syndrome characterized by the disappearance of erythroblasts from bone marrow, followed by reticulocytopenia in the blood and development of normochromic normocytic anaemia (1, 2). Laboratory studies suggest that immune-mediated (IgG-, IgM- and T cell-mediated) inhibition of erythropoiesis may be pathogenetic for TEC in some patients (3–6). Recent reports demonstrated that human parvovirus B19 (HPV B19) infection is a cause of transient erythroblastopenia in some patients without underlying haemolytic disorders (7, 8).

In TEC, granulopoiesis and thrombopoiesis are usually unaffected and platelet and neutrophil counts are within the normal range. We describe 4 children with transient erythroblastopenia complicated by thrombocytopenia and/or neutropenia. The bone marrow of these patients revealed severe erythroid hypoplasia with normal granulopoiesis and thrombopoiesis. To elucidate the mechanisms of erythroblastopenia the effects of serum IgG and T cells on autologous erythroid colony formation were investigated. Additionally, the antibodies directed against platelets, neutrophils and HPV B19 were also examined.

Patients and methods

4 children with transient erythroblastopenia with thrombocytopenia and/or neutropenia were studied. The criteria for diagnosis of transient erythroblastopenia were: 1) normochromic normocytic ana-

TABLE 1
Haematological findings on admission

	Case 1	Case 2	Case 3	Case 4
Age, sex	2y, M	3m, M	10y, M	9y, M
Blood counts				
RBC ($\times 10^{12}$/l)	3.35	2.32	4.10	4.04
Hb (g/dl)	9.8	7.0	10.9	10.6
Hct (%)	29.0	20.8	33.4	32.5
Platelets ($\times 10^9$/l)	57	80	91	300
Neutrophils ($\times 10^9$/l)	0.50	0.39	0.55	0.12
Bone marrow				
Erythroid cells (%)	3.6	1.2	0.8	0.4
Myeloid cells (%)	71.6	89.6	68.8	47.2
Megakaryocytes	Normal	Increased	Normal	Increased
CFU-E (/2 $\times 10^5$ BM cells) (169.8±38.5)*	12.5	50.3	78.0	0
CFU-GM (/2 $\times 10^5$ BM cells) (122.9±25.9)*	67.7	103.3	102.8	358.3
PAIgG (ng/10^7 plts) (less than 45)*	96.5	75.5	111.5	78.4
NBIgG (channels) (less than 11.4)*	73.3	78.6	79.4	-9.2

( )*: Normal values.

emia with reticulocytopenia; 2) severe bone marrow erythroid hypoplasia with normal numbers of granulocytic and megakaryocytic cells; 3) spontaneous and complete recovery. Details of preparation of bone marrow cells and T cells from peripheral blood, as well as culture methods, have been described in a previous paper (9). For later use, T cells were frozen using a programmed freezing apparatus and preserved in liquid nitrogen. IgG fraction was purified from the serum by ammonium sulfate precipitation and DEAE Sephacel column chromatography as described by Mollison (10). Purified IgG was resolved at a concentration of 1000 mg/dl in RPMI 1640 medium. Serum, purified IgG and cryopreserved T cells on admission were co-cultured with autologous bone marrow cells after remission. The colony growth in the co-culture was compared with that of the control culture. The data were evaluated by Student's t-test. The platelet-associated IgG (PAIgG) was measured by a quantitative antiglobulin consumption technique described by Dixon et al (11). The neutrophil-binding IgG (NBIgG) in the serum was assayed using allogeneic neutrophils as described in a previous paper (12). Detection of HPV B19-antigen and HPV B19-specific IgM and IgG antibodies was performed using the enzyme-linked immunosorbent assay (13). (HPV B19 antigen was a kind gift from Dr. K. Fukada, Fukuoka Red Cross, Japan).

Results

Haematological findings on admission (Table 1): 3 patients (cases 1, 2 and 4) had had upper respiratory infection 2 wk prior to admission. None had a history of previous transfusion with red cells or other blood products. All patients studied fulfilled the criteria for diagnosis of transient erythroblastopenia. All the patients showed reticulocytopenia (0%) and normocellular bone marrow with erythroid hypoplasia. Ferrokinetic study revealed serum iron of 123–168 μg/dl, total iron binding capacity of 201–358 μg/dl, transferrin saturation of 25–96%, serum ferritin of 91–1600 ng/ml and plasma iron disappearance time (T1/2) of 145–330 min. In addition, all the patients were neutropenic and 3 patients (all except case 4) were thrombocytopenic. Reticulocytes began to increase 7–12 d after admission and then anaemia disappeared. Platelets increased to more than 100×10^9/l from 6–8 d and neutrophils to more than 1.5×10^9/l from 7–16 d after remission. All the patients showed negative direct Coombs' test. PAIgG and NBIgG were significantly increased in 3 patients with

TABLE 2
Effects of serum, serum IgG and T cells on autologous erythroid colony formation (CFU-E)

Co-cultured with	Case 1	Case 2	Case 3	Case 4
Control	216.3 ± 10.3	174.8 ± 20.5	310.0 ± 17.2	110.3 ± 12.1
On admission	(8 March 1985)	(21 May 1985)	(19 Dec. 1986)	(4 Dec. 1986)
Serum (10%)	117.7 ± 15.8**	71.0 ± 9.8**	58.0 ± 10.7**	0.5 ± 0.9**
(5%)	159.0 ± 15.1*	92.5 ± 19.9**	144.0 ± 9.2**	2.3 ± 0.8**
IgG (10%)	115.3 ± 8.4**	66.3 ± 13.1**	114.8 ± 10.0**	26.5 ± 7.4**
(5%)	148.0 ± 8.5**	89.3 ± 9.0**	181.5 ± 14.9**	37.5 ± 2.1**
T cells (2×10^5)	233.3 ± 29.7	173.3 ± 13.3	349.8 ± 25.9	99.8 ± 3.8
(1×10^5)	205.3 ± 17.2	167.3 ± 15.4	ND	ND
After recovery	(18 March 1985)	(31 May 1985)	(7 Jan. 1987)	(7 Jan. 1987)
Serum (10%)	202.5 ± 23.3	165.3 ± 17.3	349.0 ± 16.9	118.0 ± 8.5
T cells (2×10^5)	199.8 ± 12.3	169.8 ± 17.8	320.5 ± 15.6	102.5 ± 10.2

ND: not done. Numbers represent the mean ± SD of quadruplicate cultures. 2×10^5 autologous bone marrow cells after recovery were co-cultured with various concentrations of serum or purified IgG, and with various numbers of cryopreserved T cells. Difference from control (co-cultured with none) significant, ** $p < 0.001$, * $p < 0.01$.

both thrombocytopenia and neutropenia (cases 1–3). In 1 patient with neutropenia but without thrombocytopenia (case 4), PAIgG was increased whereas NBIgG was within the normal range.

Effects of serum, purified IgG and T cells on autologous CFU-E (Table 2)

The addition of serum or purified IgG on admission significantly inhibited CFU-E growth of autologous bone marrow cells in all cases. The inhibitory activities of serum and purified IgG disappeared after remission. There was no case in which the addition of cryopreserved T cells on admission or after remission inhibited autologous CFU-E growth.

HPV B19 antigen and antibody

On admission the HPV B19 antigen was detected in case 4 and the HPV B19-IgM antibody in case 3. After remission the IgM and IgG antibodies against HPV B19 were found in cases 3 and 4. No HPV B19-specific antibody was detected in cases 1 and 2.

Discussion

Previous studies have demonstrated the presence of an IgG and T-cell inhibitor of erythropoiesis in vitro, not only in adults with chronic pure red cell aplasia (PRCA) but also in children with TEC (2, 4, 9, 14). Recently, HPV B19 infection was reported to be a cause of transient erythroblastopenia in patients with haemolytic disorders (aplastic crisis) (13). HPV B19 is directly cytotoxic for erythroid progenitor cells and inhibits erythropoiesis (15). Infrequently, HPV B19 inhibits haematopoiesis of 3 cell lineages and causes transient pancytopenia (7, 16).

In the present study the inhibitory effect of the serum on autologous CFU-E growth in vitro was demonstrable only in the sera drawn at the time of diagnosis of erythroblastopenia, but not in sera drawn after remission. The diagnosis of HPV B19 infection was established serologically in 2 patients (cases 3 and 4) and HPV B19 antigen was detected in the serum on admission in case 4. However, the inhibitory effect of the serum was due to its IgG in all cases. These findings suggest that inhibitory IgG may be pathogenetic for transient erythroblastopenia of these children. HPV B19 may inhibit erythropoiesis not only by direct action on progenitor cells but also by elaborating inhibitory IgG.

Granulopoiesis and thrombopoiesis in TEC are known to be uninvolved and both platelet and neutrophil counts remain normal. Increased levels of PAIgG and NBIgG were found in 3

patients with both thrombocytopenia and neutropenia. In one patient with neutropenia but without thrombocytopenia (case 4), NBIgG was normal whereas PAIgG was increased. The anti-neutrophil IgG might be combined with the neutrophils, although neutrophil-associated IgG (direct test) was not measured. Also, the platelet count may remain normal because of compensated thrombolysis. These findings suggest the presence of IgG-mediated mechanisms for both thrombocytopenia and neutropenia in the present cases. TEC is an acute form of PRCA and sometimes may be complicated by acute (transient) immune thrombocytopenia and/or neutropenia.

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Correspondence to:
Takashi Hanada, M.D.
Department of Pediatrics
Institute of Clinical Medicine
University of Tsukuba
1-1-1 Tennodai, Tsukuba
Ibaraki 305
Japan