

Monoclonal Blast Cell Proliferation in Transient Myeloproliferative Disorder*

T. Miyashita¹, S. Mizutani¹, M. Asada¹, J. Fujimoto², T. Inaba³, and T. Furukawa⁴

Introduction

Transient myeloproliferative disorder is a hematological condition observed during the neonatal period in patients with Down syndrome [1]. This disorder mimics congenital leukemia and the blast cells were often reported to show characteristics compatible with those of megakaryoblasts [2] or pluripotent stem cells [3]. Although this disorder usually resolves gradually without antileukemic treatment, in some patients, leukemia develops after periods of spontaneous remission [4], raising questions about the benign origin of this disorder.

It is therefore unclear if transient myeloproliferative disorder is a leukemia, preleukemia, or transient dysplasia of myelopoiesis. This important issue might be resolved by analysis of the clonality of the blast cells of this disorder. We therefore studied the clonality of blast cells in transient myeloproliferative disorder

using antigen receptor genes as well as X chromosome inactivation patterns.

Materials and Methods

Patient Samples. The patients studied included three newborn infants, all of whom had Down syndrome with standard trisomy 21 (Table 1). They all developed transient myeloproliferative disorder during the neonatal period with more blast cells in the peripheral blood than in the bone marrow, and were followed by spontaneous resolution without antileukemic treatment.

The follow-up period of these patients was from 72 days to 5 months. One out of three patients was alive at the time of this study (patient 3). The remaining two patients died while there was no evidence of leukemia. The clinical course of patient 2 was reported previously [5].

Cell Separation and Immunological Analysis. Heparinized peripheral blood was separated on a density gradient of Ficoll-Metrizoate (Lymphoprep, Nyegaard, Oslo, Norway). The interface of mononuclear cells was subjected to immunological and DNA analysis.

Reactivity of the blast cells with monoclonal antibodies against lineage-specific antibodies (Table 2) was assayed by an indirect immunofluorescence technique using a fluorescence-activated cell sorter as described before [6].

Southern Blot Analysis. High-molecular weight DNA was extracted from the mononuclear cells according to the

* This work was supported by a Grant-in-Aid from the Ministry of Health and Welfare as part of a comprehensive 10-year Strategy for Cancer Control; by a Grant for Pediatric Research 63-06 from the Ministry of Health and Welfare; by a Grant-in-Aid for Cancer Research; and by the Japanese Foundation for Multidisciplinary Treatment of Cancer.

¹ Department of Virology and

² Department of Pathology, the National Children's Medical Research Center, Tokyo, Japan.

³ Department of Hematology/Oncology, Saitama Children's Hospital, Saitama, Japan.

⁴ Department of Pediatrics, Dokkyo University Medical School, Tochigi, Japan.

Table 1. Clinical data of three patients with transient abnormal myelopoiesis

Patient no.	Sex	Age of sampling	WBC ($10^9/l$)	Hb (g/dl)	Platelet ($10^9/l$)	Blast (%)		Follow-up period	SR	Outcome
						PB	BM			
1	F	2d	50	16.8	254	47	8	72d	+	Died of SIDS*
2	F	15d	130	17.3	173	93	63	77d	+	Died of hepatic failure*
3	F	3d	12	13.5	16	24	12	5m	+	Alive and well

d: day; m: month; SR: spontaneous remission; SIDS: sudden infant death syndrome; Hb, Hemoglobin; PB, peripheral blood; BM, bone marrow
* No evidence of leukemia at the time of death.

method described previously [6]. For the antigen receptor gene analysis, IgJH, C β , J γ , C δ , and J δ 2 probes were employed, with 6 μ g of *Bam*HI-digested DNA samples. Clonal analysis with the X-linked phosphoglycerate kinase (PGK) probe [7] was performed as described by Vogelstein et al. [8].

Results

Immunological Analysis of Blast Cells. All of the samples of blast cells that we studied were positive for platelet associated antigens (CD41, CD42b, or KOR-P77 [9]). Two out of three samples also showed positivity for CD7 antigen. None of three samples expressed lymphoid lineage-associated antigens such as CD3, CD10 or CD19 (Table 2).

Antigen Receptor Gene Configuration of Blast Cells. None of the three samples of blast cells showed any rearrangements of the IgH or TCR loci using JH, TCR β , γ , and δ probes (data not shown).

X Chromosome Inactivation Analysis of Blast Cells. 94%, 99%, and 82% of the peripheral mononuclear cells were blast cells after the density gradient cell separation in patients 1, 2, and 3 respectively. After an additional digestion by restriction enzyme *Hpa*II, DNA of the blast cells of the three patients showed complete loss of either the 1.05 kb or the 0.9 kb band, suggesting monoclonal expansion of the blast cells (Fig. 1, lanes 2, 6, and 8). In contrast, the mononuclear cells of patient 1 after spontaneous remission showed the retention of both of the alleles (Fig. 1, lane 4).

Discussion

Our initial study into the lineage and clonality of blast cells using the antigen receptor genes IgJH and TCR β , γ , and δ showed no rearrangements of these loci in the blasts of any of the three patients. As

Table 2. Peripheral blood immunophenotyping

Antigens	Patient 1 (% of cells)	Patient 2 (% of cells)	Patient 3 (% of cells)
CD 3	-	NT	NT
CD 4	23.5	-	NT
CD 7	52.4	81.8	NT
CD 8	-	NT	NT
CD 10 (J5)	-	-	-
CD 13 (MY7)	-	-	NT
CD 14 (My4)	-	-	NT
CD 19 (B4)	-	-	-
CD 20 (B1)	-	NT	NT
CD 25	-	-	NT
CD 41	81.6	NT	19.3
CD 42b	73.2	NT	NT
KOR-P77	59.2	48.8	NT

Peripheral blood mononuclear cells were immunophenotyped by the method described in the materials and methods. Percentage of positive cells in fluorescent antibody screening is shown. *NT*: not tested; -, <15%.

all the patients demonstrate more than 82% of blast cells in the mononuclear cell fraction, our negative findings for clonal rearrangement cannot be attributed to the low sensitivity of this assay. Furthermore, as almost all of the leukemias with lymphoid characteristics show antigen receptor gene rearrangement [10], our results suggest that the proliferating blast cells are of nonlymphoid origin.

In order to study the clonality of blast cells, we employed the X chromosome-

linked polymorphic gene, *PGK*. In patient 1, mononuclear cells were compared during blastic phase and remission phase, which was achieved without therapy, for sensitivity to *HpaII* restriction enzyme digestion. As the results show, the absence at blastic phase, but the presence at remission phase of the allele identified at 1.05 kb indicates that mononuclear cells at blastic phase are of single clonal origin. Although we could not examine the samples at remission phase in patients 2 and

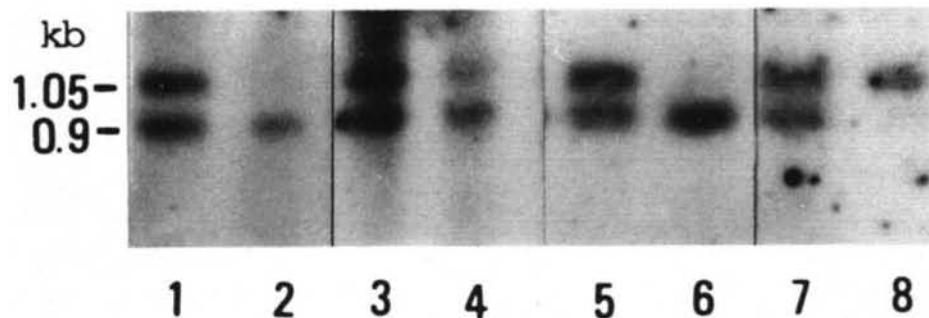


Fig. 1. PGK analysis of X chromosome inactivation patterns in three patients. An autoradiograph of a Southern hybridization experiment is shown, wherein a PGK gene probe was hybridized to DNA from mononuclear cells from patient 1 (initial presentation and after remission, lanes 1 and 2 and lanes 3 and 4, respectively) and mononuclear cells from patients 2 and 3 (initial presentation,

lanes 5 and 6 and lanes 7 and 8, respectively). In lanes 1, 3, 5, and 7 the DNA has been digested with restriction enzymes (*BstXI*, *PstI*) that reveal two polymorphic PGK alleles (1.05-kb and 0.9-kb bands). In lanes 2, 4, 6, and 8 DNA has been digested with these enzymes and also with *HpaII*, an enzyme that distinguishes active from inactive alleles through methylation differences.

3, the complete absence of one of two alleles indicates a monoclonal proliferation of blast cells. Altogether our results strongly suggest that monoclonal expansion of a progenitor cell with nonlymphoid characteristics occurs in this disorder with Down syndrome. Megakaryoblastic features characterized by expression of CD41 (patients 1 and 3), CD42b (patient 1), or KOR-P77 (patients 1 and 2) are of interest in the light of the recent recognition that acute leukemia in Down syndrome is much more frequently of a megakaryoblastic nature than previously recognized [11].

There are several possible explanations for blast cell proliferation in this disorder. Firstly, blast cells might proliferate in response to an increase in production of growth factors such as IL-3 and/or IL-6 [12]. Secondly, hemopoietic dysregulation, including that caused by defective immunological surveillance during the neonatal period, might be responsible for this disorder. Thirdly, transient myeloproliferative disorder could be a pre-leukemic state. Since in the former two interpretations polyclonal proliferation of blast cells is expected, our findings strongly support the third possibility. This idea is also supported by other reports that this is due to the result of a spontaneous resolution of a malignant clone [4, 13]. Alternatively this condition might be heterogeneous, some cases being benign reactive conditions with polyclonal myeloproliferation, while others are actually preleukemic conditions. Studies on more patients with a longer survival period will clarify whether there is any heterogeneity in clonality among patients with this disorder.

Acknowledgements. We are indebted to Drs. J. Singer-Sam, P. Leder, T. Rabbits, and T. W. Mak for providing the DNA probes, PGK, IgJH (λ CH 28-6), TCR γ , and TCR β and δ respectively. We also thank Dr. S. Nakazawa for immunophenotyping data from patient 1.

References

1. Nagao T, Lampkin BC, Hug G (1970) A neonate with Down's syndrome and transient abnormal myelopoiesis: serial blood and bone marrow studies. *Blood* 36:443-447
2. Koike T, Aoki S, Maruyama S, Narita M, Ishizuka T, Imanaka H, Adachi T, Maeda H, Shibata A (1987) Cell surface phenotyping of megakaryoblasts. *Blood* 69:957-962
3. Suda J, Eguchi M, Akiyama Y, Iwama Y, Furukawa T, Sato Y, Miura Y, Suda T, Saito M (1987) Differentiation of blast cells from a Down's syndrome patient with transient myeloproliferative disorder. *Blood* 69:508-513
4. Morgan R, Hecht F, Cleary ML, Sklar J, Link MP (1985) Leukemia with Down's syndrome: translocation between chromosomes 1 and 19 in acute myelomonocytic leukemia following transient congenital myeloproliferative syndrome. *Blood* 66:1466-1471
5. Nakagawa T, Nishida H, Arai T, Yamada T, Fukuda M, Sukamoto S (1988) Hyperviscosity syndrome with transient abnormal myelopoiesis in Down syndrome. *J Pediatr* 112:58-61
6. Nakamura K, Sasaki M, Fujimoto J, Enomoto Y, Kaneko Y, Ozaki M, Miyashita T, Tsunematsu Y, Hata J, Kobayashi N, Mizutani S (1990) Molecular diversity of precursor B acute lymphoblastic leukemias identified by the immunoglobulin heavy chain gene organization. *Leukemia* 4:106-111
7. Keith DH, Singer-Sam J, Riggs AD (1986) Active X chromosome DNA is unmethylated at eight CCGG sites clustered in a guanine-plus-cytosine-rich island at the 5' end of the gene for phosphoglycerate kinase. *Mol Cell Biol* 6:4122-4128
8. Vogelstein B, Fearon ER, Hamilton SR, Feinberg AP (1985) Use of restriction fragment length polymorphisms to determine the clonal origin of human tumors. *Science* 227:642-646
9. Nakazawa S, Sugita K (1987) Establishment and characterization of a murine anti-platelet monoclonal antibody and its usefulness for the identification of megakaryocyte-lineage cells. *Keio J Med* 36:53-58
10. Asou N, Hattori T, Matsuoka M, Kawano F, Takatsuki K (1989) Rearrangements of

- T-cell antigen receptor δ chain gene in hematologic neoplasms. *Blood* 74:2707-2712
11. Zipursky A, Peeters M, Poon A (1986) Megakaryoblastic leukemia and Down's syndrome - a review. In: McCoy EE, Epstein CJ (eds) *Oncology and immunology of Down syndrome*. Liss, New York, pp 33-56
 12. Ikebuchi K, Wong GG, Clark SC, Ihle JN, Hirai Y, Ogawa M (1987) Interleukin 6 enhancement of interleukin 3-dependent proliferation of multipotential hematopoietic progenitors. *Proc Natl Acad Sci USA* 84:9035-9040
 13. Lazarus KH, Heerema NA, Palmer CG, Baehner RL (1981) The myeloproliferative reaction in a child with Down syndrome: cytological and chromosomal evidence for a transient leukemia. *Am J Hematol* 11:417-422