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Dysplastic Changes in Idiopathic Thrombocytopenic Purpura and the Effect of Corticosteroids to Increase Dysplasia and Cause Hyperdiploid Macropolycytes

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This study evaluates the dysplastic hematological changes in nine patients with idiopathic thrombocytopenic purpura (ITP) in 11 attacks, before and after corticosteroid treatment. The pretreatment blood smears of patients with ITP, displayed more neutrophils with bizarre nuclei ($P < 0.001$), Döhle or Döhle-like inclusions ($P < 0.01$), irregular distribution of granules ($P < 0.05$), hypo-agranulation ($P < 0.05$), pseudo-Pelger-Huet-like cells ($P < 0.01$), and nuclei with chromatine clumping ($P < 0.01$) than the normal children. The eosinophils of ITP patients were also dysplastic, before treatment. The pretreatment diameter of the neutrophils and the percentage of macropolycytes were greater than those of the patients with viral infections and normal group ($P < 0.05$ for all). The percentage of neutrophils with bizarre nuclei and nuclei with chromatine clumping and the diameter of neutrophils and macropolycyte percentage increased with corticosteroid therapy ($P < 0.01$, < 0.01, < 0.01, and < 0.05, respectively). The neutrophil diameter, percentage of macropolycytes, and number of neutrophils with bizarre nuclei decreased within 1–4 weeks after the therapy was stopped. In the neutrophils of two patients, diploidy and hyperdiploidy were established before and on the last day of therapy, respectively, and diploidy reversed after therapy was stopped. In conclusion, ITP patients display dysplastic findings in both neutrophils and eosinophils before treatment and corticosteroids cause transient significant increase in some of the dysplastic changes in neutrophils. Am. J. Hematol. 65:99–104, 2000.

Key words: dysplasia; neutrophils; eosinophils; ITP; corticosteroids; hyperdiploidy

INTRODUCTION

Hematologic dysplastic changes are encountered in various disorders other than myelodysplastic syndrome (MDS) [1]. In our clinics, we have observed that the patients who receive corticosteroid therapy because of ITP display dysplastic changes and macropolycytes during corticosteroid therapy. In order to confirm this observation, we evaluated the blood smears of nine patients with ITP. We also observed that dysplastic changes and macropolycytes were already present in patients with ITP before treatment and that they increased after corticosteroid therapy. In addition, we evaluated the neutrophils of the two, in three attacks, with respect to polyploidy and cell size, before, during, and after treatment.

METHODS

Patients

Blood smears of nine children (5 F, 4 M; mean age, 8.5 years; range, 6 months–15 years) with ITP were available. Seven of them had acute ITP and developed eight thrombocytopenic attacks. Two had chronic ITP (YP, EP), and neither displayed any laboratory or clinical sign of collagen tissue disease during follow-up. The bone marrow obtained from all of the patients displayed in-

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creased or normal megakaryotcyte production. The attacks in both groups (n = 11) were treated with methyl prednisolone (MP) for seven days (30 mg/kg/day × 3 days and subsequently 20 mg/kg/day × 4 days) by oral route [2,3], except a chronic ITP patient (YP) for whom MP was continued for 8 months, tapering the dose down to 0.1 mg/kg/day. None of the patients displayed any clinical or laboratory evidence of infection while being evaluated.

The blood smears of the patients were evaluated before and on the last day (7^{th} day) of the treatment. For a chronic ITP patient (YP), additionally all available blood smears taken each month while the corticosteroid dose was being tapered down, were evaluated. Additionally, the smears after the cessation of the therapy were examined for only seven patients, within weeks 1–4, during which time no one had thrombocytopenia. Written consent was signed by the parents.

Control Groups

Three control groups were developed within the same age group. Control group 1 consisted of 8 children whose blood smears had been taken when they admitted because of viral infections (rubella, mumps, measles, Epstein-Barr virus infection, fifth disease); control group 2 consisted of 8 children with bacterial infection (maxillary sinusitis, cellulitis, tonsillitis, otitis media, urinary tract infection); and control group 3 consisted of 8 normal children. The patients in both the control and ITP group were admitted to our hospital from different regions of Ankara and the country.

Microscopic Evaluation of the Size and Morphology of Neutrophils

The diameter of each neutrophil was measured using a micrometer (magnification ×100–1,000) by light microscopy. A neutrophil with a diameter more than 14 μm was considered to be a "macropolycyte" [4,5].

In each blood smear, 150–600 neutrophils were measured; each nucleus found to be dysplastic was sketched individually.

Evaluation of the Dysmorphic Findings

The neutrophils of the patients were evaluated for bizarre nuclei (large, strange, irregularly or asymmetrically lobulated nuclei or nuclei with projections in the shape of clubs, hooks, tags, or the presence of microlobes), nuclei with chromatine clumping, hypo- or agranularity, hypergranularity, irregular distribution of granules, pseudo-Pelger-Huet-like cells, nucleocytoplasmic asynchrony, presence of Döhle or Döhle-like inclusions, and cytoplasmic vacuoles in the cytoplasm. The individual lobes which were not clearly separated and the lobules which grow out from a single point were not considered as a distinct lobe, as Lindenhaum et al.'s [6] definition of a distinct lobe was applied. The neutrophils with two distinct lobes, each of which has the aforementioned characteristics, were considered as "pseudo-Pelger-Huet-like neutrophils" [7].

The amount of dysplastic neutrophils were expressed as percentage of total neutrophils.

In addition, the eosinophils were evaluated as to the presence of three or more nuclei, nucleic projections or microlobe, cytoplasmic vacuoles, and irregular distribution of the cytoplasmic granules.

Flow Cytometric Evaluation

The DNA index, in relation to cell size, was evaluated by flow cytometry in two patients (İY, YP) for three attacks, before and on the last day (7^{th} day) of the treatment, and 7–11 days after the cessation of therapy.

Leukocyte suspensions were prepared, and the DNA index was evaluated as previously recommended [8,9].

Statistical Evaluation

For evaluation of the elevations in diameter, macropolycyte percentage and dysplastic findings of the neutrophils, the values attained before and on the last day (7^{th} day) of the treatment were compared by the Wilcoxon two-tailed test. For evaluation of the difference between the post-treatment values and the values of the control groups, the Mann–Whitney *U* test was used.

RESULTS

All of the patients developed neutrophilia and a left shift on the last day of treatment. The most prominent changes were observed to involve the neutrophils.

Neutrophil Diameter and Percentage of Macropolycytes

The pretreatment diameter of the neutrophils and the percentage of macropolycytes were significantly greater than that of the patients in the control group 1 and group 3 but not in group 2. The post-treatment mean diameter and macropolycyte percentage were significantly greater than the pretreatment values (Table I).

The diameter of the neutrophils and percentage of macropolycytes were observed to decrease in six of seven patients who could be followed up during the subsequent 1–4 weeks after the cessation of the therapy. The patient 5, who had otitis media while being evaluated, displayed a slight increase in these values (Fig. 1a,b).

For the chronic ITP patient, YP, the diameter and macropolycyte percentage were observed to decrease as the dose decreased and to increase with the duration of therapy over eight months.

Dysplastic Appearance of the Neutrophils

It was found that the pretreatment blood smears of the patients with ITP, displayed significantly more neutrophils with bizarre nuclei ($P < 0.001$), Döhle or Döhle-like inclusions ($P < 0.01$), irregular distribution of granules

TABLE I. Size and Dysmorphic Findings of the Neutrophils in the Children With ITP and Control Groups

		Before treatment	After treatment	Control 1 (viral)[c]	Control 2 (bacterial)[d]	Control 3 (normal)
Diameter (μ)	Median	12.33	13.32	10.34	12.42	9.75
	SD[a]	1.26	1.01	1.12	2.71	1.59
	Variance (SD^2)	1.59	1.02	1.25	7.34	2.53
Macropolycyte (%)[b]	Median	13.84	28.20	3.00	19.50	7.00
	SD	7.94	14.30	2.87	25.02	6.34
	Variance (SD^2)	63.04	204.49	8.24	626.00	40.20
Bizarre nucleus (%)	Median	42.00	56.00	15.00	41.00	17.00
	SD	10.62	15.08	8.38	10.75	4.96
	Variance (SD^2)	112.78	227.41	70.22	115.56	24.60
Döhle (%)	Median	2.00	2.00	2.00	0.00	0.00
	SD	1.27	1.55	3.55	1.64	0.00
	Variance (SD^2)	1.61	2.40	12.60	2.69	0.00
Irregular distribution of granules (%)	Median	15.00	11.00	36.00	29.00	2.50
	SD	13.55	13.78	10.99	19.46	4.23
	Variance (SD^2)	183.60	189.89	120.78	378.69	17.89
Hypo-agranulation (%)	Median	54.00	78.00	23.00	39.00	18.00
	SD	27.42	33.04	26.33	13.55	19.41
	Variance (SD^2)	751.86	1091.64	693.27	183.6	376.75
Chromatine clumping (%)	Median	61.00	77.50	82.00	59.00	15.00
	SD	28.56	31.32	11.64	32.27	11.30
	Variance (SD^2)	815.67	980.94	135.49	1041.35	127.69
Pseudo-Pelger-Huet-like cells (%)	Median	13.00	9.00	26.00	11.50	3.50
	SD	9.51	8.99	13.20	10.58	2.66
	Variance (SD^2)	90.44	80.82	174.24	111.94	7.08

[a]SD, standard deviation.
[b]% implies the percentage of neutrophils.
[c]Patients with viral infections.
[d]Patients with bacterial infections.

($P < 0.05$), hypo- or agranulation ($P < 0.05$), chromatine clumping ($P < 0.01$), pseudo-Pelger-Huet-like neutrophils ($P < 0.01$) than those of the control group 3. The pretreatment values of bizarre nuclei and irregular distribution of granules were also significantly different from those of control group 1 although all of the dysplastic criteria were statistically similar to those in control group 2 (Table I).

It is interesting that the percentage of dysmorphic neutrophils with bizarre nuclei and chromatine clumping increased considerably after corticocorticosteroid therapy (Table I, Table II, Fig. 2).

After the cessation of the treatment, it was observed in seven patients who could be followed up during the subsequent 1–4 weeks that the effect of steroids on dysplastic changes decreased in time (Fig. 1c).

Eosinophils

Although the percentage of eosinophils did not differ after the therapy, it was striking that both before treatment and on the last day of treatment, the eosinophils were dysplastic (Fig. 2). The percentage of eosinophils with bizarre nuclei did not differ after the treatment (0.82 ± 0.12 vs 0.64 ± 0.15, $P > 0.05$).

Flow Cytometric Findings

The DNA index of İY and YP, in relation to cell size, evaluated by flow cytometry, revealed diploidy of the neutrophils before treatment and hyperdiploidy on the last day of treatment (7^{th} day). The DNA index, evaluated also 7–11 days after the termination of therapy revealed that diploid pattern reappeared (Fig. 3a–c). It was also determined by flow cytometry that the large cells of İY and YP corresponded to the hyperdiploid neutrophils.

Myeloperoxidase evaluated for one patient (YP) during steroid therapy was found normal (14.4%).

DISCUSSION

This study presents the dysplastic hematological changes in patients with ITP and the increase in some of these changes after corticosteroid therapy.

The diameter of the neutrophils and the percentage of macropolycytes in ITP patients were higher than normal values before treatment, and the corticocorticosteroid therapy caused increase in these parameters, compared to pretreatment findings. The drug effect is reversed after the therapy was stopped.

Macropolycytes are encountered in bacterial [4], viral infections [5], neutropenia [10], acquired granulomatous disease [11], myelokathexis, megaloblastic anemia, growth factor (GCSF, GMCSF) administration or treatment with chemotherapy, chronic myeloid leukemia [5], and hereditary [12].

The production of giant neutrophils under GCSF

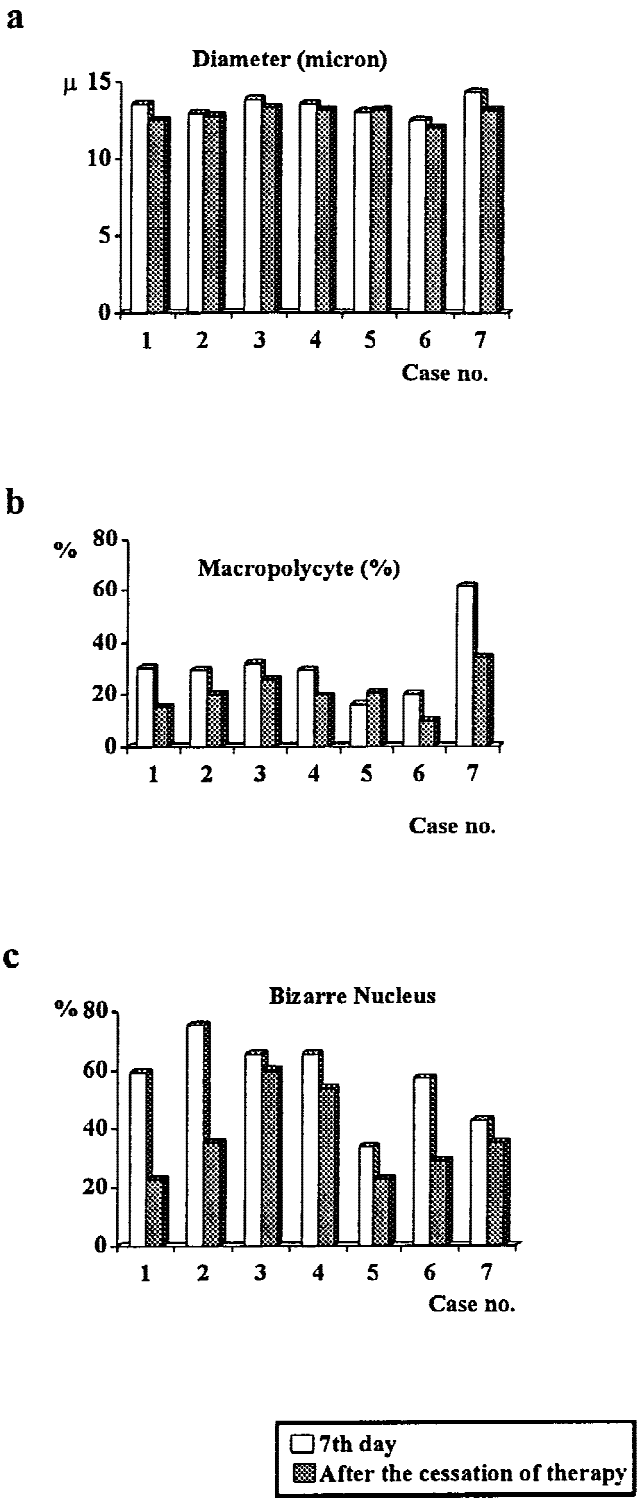

Fig. 1. Changes in diameter (a), macropolycyte percentage (b), and bizarre nuclei (c) of the neutrophils during therapy (7th day) and 1–4 weeks after the cessation of therapy.

stimulation of the bone marrow was reported to be analogous to the descriptions of "stress erythropoiesis" where the appearance of large erythroid cells is observed [13]. Tuncer et al. [14] demonstrated that corticosteroids

TABLE II. *P* Values Obtained After Comparison of Pretreatment Values with Posttreatment and Control Group Values*

	After treatment	Control 1 (viral)	Control 2 (bacterial)	Control 3 (normal)
Diameter (μ)	0.0099[b]	0.0050[b]	0.8364	0.0039[b]
Macropolycyte (%)	0.0033[b]	0.0117[c]	0.4324	0.0351[c]
Bizarre nucleus (%)	0.0080[b]	0.0013[b]	1.0000	0.0004[a]
Döhle (%)	0.5147	0.6401	0.2667	0.0012[b]
Irregular distribution of granules (%)	0.9528	0.0110[c]	0.1360	0.0223[c]
Hypo-agranulation (%)	0.3590	0.1463	0.3415	0.0351[c]
Chromatine clumping (%)	0.0218[c]	0.1071	0.5600	0.0067[b]
Pseudo-Pelger-Huet-like cells (%)	0.1731	0.1469	0.6197	0.0022[b]

*For values not marked by a, b, or c, $P > 0.05$.
[a]$P < 0.001$.
[b]$P < 0.01$.
[c]$P < 0.05$.

caused elevation in GCSF and GMCSF levels in AML and ALL patients. Our findings suggest that corticosteroids may have caused increments of macropolycytes by increasing the levels of GCSF and GMCSF.

Although it has been assumed that macropolycytes are

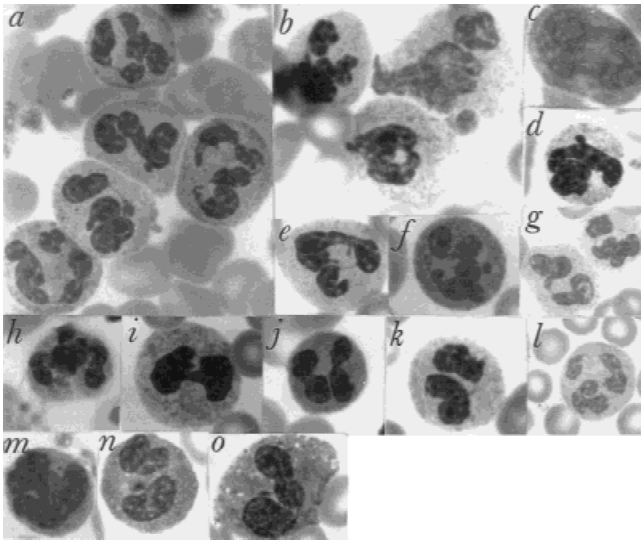

Fig. 2. Dysplastic changes in neutrophils and eosinophils of ITP patients before and after steroid treatment. (a) Macropolycytes; (b,c) neutrophils with bizarre nuclei and (d,e) abnormal projections; (f) microlobes; (b,d) striking chromatine clumping; (e,g,l) hypogranulation; (f,i) hypergranulation; (d,h,i) irregular distribution of granules; (i–k) pseudo-Pelger-Huet-like cells; (d,l) cytoplasmic vacuole; (b,m) Döhle or Döhle-like inclusions; (m) nucleocytoplasmic asyncrony; (n) eosinophils with three lobes and a nuclear projection; (o) and cytoplasmic vacuoles and a nuclear projection (×100–1,000).

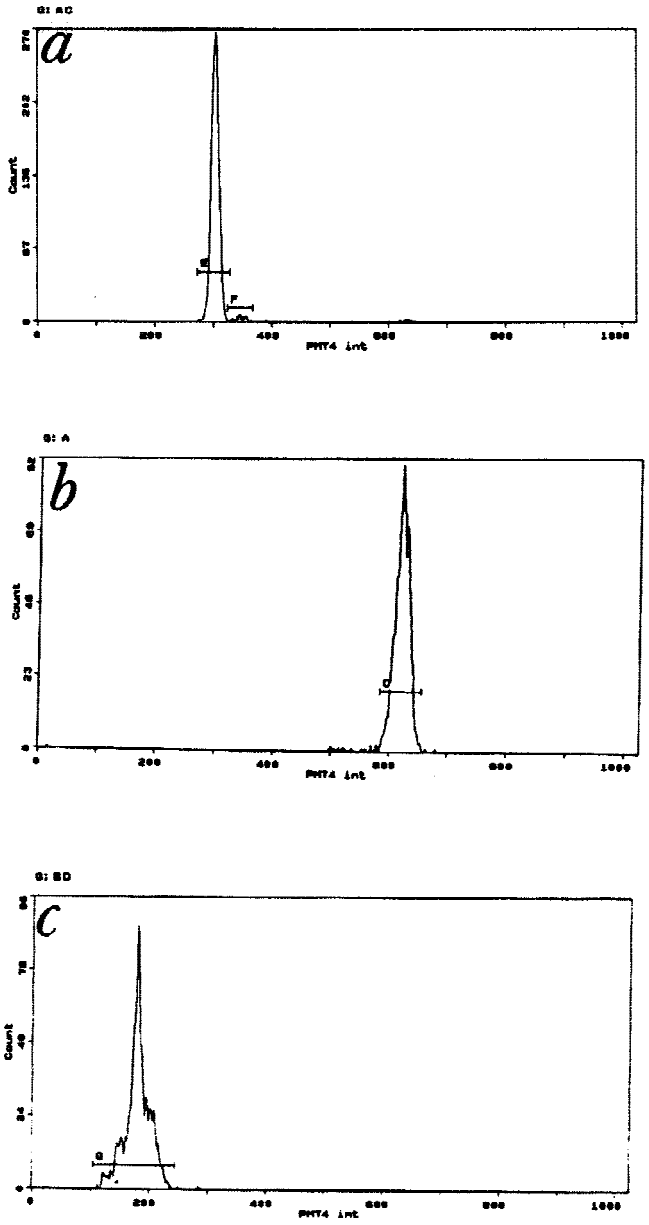

Fig. 3. Flow cytometric appearance of diploidy before therapy (a), hyperdiploidy during therapy (b), and diploidy 7 days after the cessation of therapy (c).

tetraploid cells, the ploidy of these cells has, to our knowledge, been studied in few cases [10,11,13,15]. Here, we demonstrated that corticosteroids also cause hyperdiploid macropolycytes, and after the cessation of the therapy, diploidy is reversed.

Myeloperoxidase (MPO) activity of one patient was found normal unlike it is in the large neutrophils encountered in AIDS [7] and after GCSF treatment [13].

Our study demonstrates that patients with ITP display the aforementioned dysplastic changes in neutrophils (Table I). Therapy with corticosteroids gives rise to a significant increase in neutrophils with bizarre nuclei and nuclei with chromatine clumping which are already encountered in several disorders [1,5,7,16–23]. The dysplastic features have existed in the eosinophils also.

At evaluation, none of the acute or chronic ITP patients had any sign of infection documented by physical or laboratory examination. However, silent or abortive preceding viral infections cannot be excluded. On the other hand, the presented significant discrepancies of the examined parameters between the patients with viral infection and pretreatment values of ITP patients show that the morphologic changes in ITP before treatment are due to reasons other than any viral infection. The patients with ITP and the control group admitted to our hospital from different regions of the country and Ankara. Therefore, there is little probability of a toxic product exposure to give rise to the same kind of morphological abnormalities in all of the patients.

ITP is a consequence of both increased immune-mediated destruction and in some cases, diminished marrow platelet production [24,25]. Parker et al. [24] have evaluated seven immunotherapy resistant chronic ITP patients, some of whom displayed dysmorphic megakaryocytes in their bone marrow. They have suggested that an intrinsic megakaryocyte proliferative defect results in deficient platelet production in the bone marrow of chronic ITP patients and this defect may be at the level of the common erythroid-megakaryocytic stem cell and may progress to overt myelodysplasia or be preleukemic in nature [24]. Here we propose that this intrinsic defect may be (1) before or at the level of CFU-GEMM and (2) the antiplatelet antibodies may be effective at this stage and this defect may cause myelodysplastic changes in all of the ITP patients whether the disease has acute or chronic course. Whether this intrinsic proliferation defect is dependent or independent on decreased, increased, or normal platelet production requires in vivo studies of platelet turnover.

The myelodysplastic features encountered in children with juvenile rheumatoid arthritis is another example that reflects that the bone marrow and peripheral blood respond to autoimmune stress by myelodysplasia [26], probably via some cytokines [27].

Acquired pseudo-Pelger-Huet anomaly is reported to be due to an arrest or dysplasia of nuclear chromatin synthesis [28]. Thus pseudo-Pelger-Huet-like cells may reflect a defect in chromatine synthesis, both in ITP and after corticosteroid therapy.

The presence of dysplastic changes in viral or bacterial infections suggests that infections may cause dysplastic changes by increasing the level of endogenous corticosteroids and hence growth factors.

As far as we know, the dysplastic features in neutrophils and eosinophils which patients with ITP present at admission and the effect of corticosteroids to increase

some dysplastic changes in neutrophils have not been reported before.

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