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LETTERS AND CORRESPONDENCE

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Lenograstim and Filgrastim Effects on Neutrophil Motility in Patients Undergoing Chemotherapy: Evaluation by Computer-Assisted Image Analysis

To the Editor: The effects of Chinese hamster ovary-derived glycosylated rhG-CSF (Lenograstim) and *Escherichia coli*-derived non-glycosylated rhG-CSF (Filgrastim) on random migration and chemotaxis of circulating neutrophils from patients with non-Hodgkin's Lymphoma undergoing a modified Promice-CytaBom regimen [1] were evaluated, far from any direct effect of cytostatic drugs (administered on days 1 and 8). rhG-CSF was administered, in order to avoid a delay of the next course, from days 22 to 26 (5 μg/kg/day subcutaneously). The tests were performed immediately before rhG-CSF administration and 48 hr after rhG-CSF interruption. Nine patients received Lenograstim (F:M 5:4; mean age 47.5 years); nine patients received Filgrastim (F:M 5:4; mean age 52 years). A very sensitive computer-assisted image processing technique was applied to the micropore filter method in the Boyden chamber [2]. It calculates the distance traveled by the cells and builds the interpolating curve describing the cell kinetics throughout the filter. May-Grünwald-Giemsa stained smears from capillary puncture were also performed.

Neither Lenograstim nor Filgrastim induced any change in random migration, which was and remained in the normal range before and after the administration.

With respect to chemotaxis, Lenograstim-induced neutrophils displayed normal values (133.5 ± 10.3 μm) as compared with normal range (120–160 μm) without differences with basal values (137.9 ± 11.1 μm). The kinetics (typically characterized by a "peak" of cell accumulation beyond the first plane) was and remained normal.

Filgrastim-induced neutrophils displayed a defective chemotaxis (107.5 ± 16.3 μm) as compared with values before administration (131.6 ± 17.7 μm); $P = 0.008$. Moreover, the typical chemotactic "peak" was replaced by a Gaussian pattern, just as under random conditions (Fig. 1, upper panels).

According to the literature, two hypotheses may be drawn to explain this

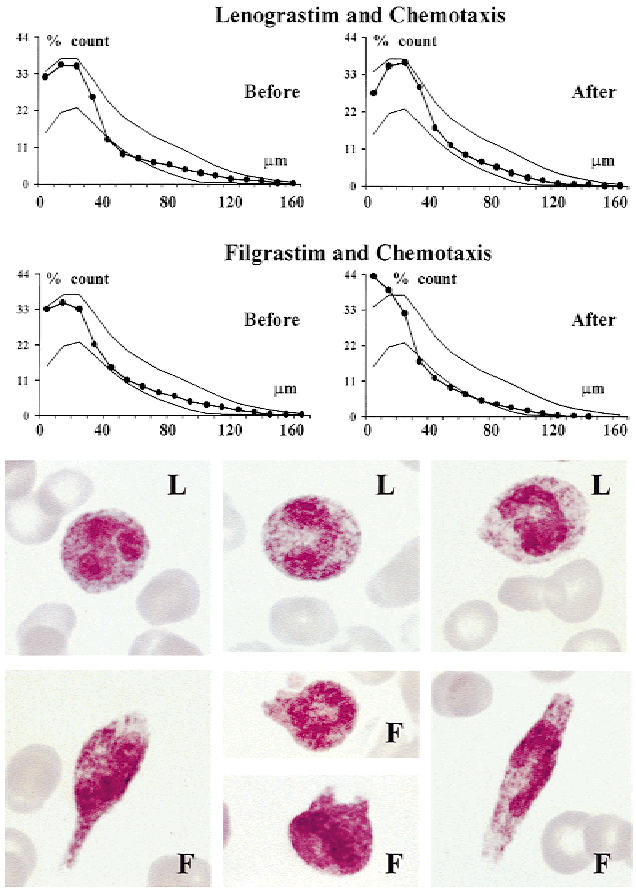

Fig. 1. (Upper panels). Continuous curve with circles: plots of the curves which interpolate the average cell counts throughout micropore filters during Chemotaxis. Continuous curves: kinetics of migration (±2 SD) in normal donors, characterized by a peculiar "peak" beyond the first plane. The distance traveled by neutrophils (137.9 ± 11.1 μm vs. 133.5 ± 10.3 μm before and after Lenograstim, *n* = 9, *P* = n.s.; 131.6 ± 17.7 μm vs. 107.5 ± 16.3 μm before and after Filgrastim, *n* = 9, *P* = 0.008), is calculated by the algorithm after (i) decimal logarithm transformation of counts (ordinate); (ii) square transformation of depth (abscissa); (iii) calculation of the regression line; (iv) square root of the interception values on the abscissa, obtained when the logarithm value decreases by 2 units. (Lower panels) May-Grünwald-Giemsa staining of blood smears prepared 48 hr after the last dose of Lenograstim and Filgrastim. "L" refers to examples of Lenograstim-induced neutrophils: the cells appear larger than normal and display the presence of abundant toxic granules. "F" refers to examples of Filgrastim-induced neutrophils: the cells appear strongly polarized (hand mirror-shaped or cigar-shaped) or display membrane blebs or one or more pseudopodia.

different effect: (i) Filgrastim increases surface expression of β_2 integrin, which mediates the neutrophil adhesive interactions and transmigration, with consequent reduction of the chemotactic response [3], while Lenograstim seems not to interfere with β_2 integrin expression [4]. (ii) According to classical studies demonstrating that leukocytes with disassembled microtubules lose their directional movement whereas they still move at random, our data may indicate that an imperfect cytoskeleton assembly might sustain the reduced motility in Filgrastim-induced neutrophils. Moreover, membrane deformability strictly depends on a well-assembled cytoskeleton and correlates with neutrophil maturation. Recently a marked contrast between blood neutrophil count and skin localization after Filgrastim administration was demonstrated and attributed to the structural immaturity of the circulating neutrophils caused by the accelerated entry into the blood [5].

Concerning cell morphology, only 8 ± 3% of Lenograstim-induced neutrophils displayed major morphological modifications, while 27 ± 4% of Filgrastim-induced neutrophils appeared strongly polarized (hand mirror-shaped or cigar-shaped) and with blebs or pseudopodia (16 ± 6%) (Fig. 1, lower panels). This might be the morphological expression of structural defects responsible for the motility disorder.

Our data show that Lenograstim seems to respect neutrophil motility more than Filgrastim. We do not know if glycosylation, which makes the first factor more similar to the natural one, plays any role. In any event, this difference should be taken into account, in order to preserve as well as possible all the functions of the induced neutrophils.

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Positive Predictive Values of Imaging Studies Used Before Accessory Splenectomy

To the Editor: A patient had recurrent ITP, and I asked the research question "which imaging technique is best for locating an accessory spleen?" I searched the world's medical literature using Medline to identify published cases where imaging was used to locate an accessory spleen before a successful accessory splenectomy. Only twenty-six cases were reported with surgical confirmation of the accessory spleen (Table I). A ^{99}technetium scan was used most frequently with a 95% positive predictive value. Three other imaging techniques were reported sparingly, however these imaging techniques accurately predicted accessory splenic tissue. In conclusion, when an accessory spleen is suspected in recurrent ITP, a ^{99}technetium scan is an accurate way to detect functioning splenic tissue. When the scan is negative but a high suspicion remains, or for better anatomic localization, a second study may be helpful.

TABLE I. Positive Predictive Values of Imaging Studies Used Before Accessory Splenectomy (AS)

Before AS	Used: Image present	Used: Image absent	Not used	Positive predictive value (%)
^{111}indium	2	0	24	100
^{99}technetium	19	1	6	95
Computed tomography scan	2	0	24	100
SPECT[a]	1	0	25	100

[a]Single-photon emission computed tomography.

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Symptomatic Presentation of a Sickle Cell Heterozygote: An Evaluation of Genetic Factors

To the Editor: Sickle hemoglobin (HbS) is caused by a point mutation (A→T) in the β-globin gene. The clinical manifestations of sickle cell homozygotes are extremely variable, whereas sickle cell heterozygotes are generally believed to be asymptomatic and lead a normal life.

We report a 20-year-old female sickle cell heterozygote with a history of pallor, jaundice, swelling of joints, frequent episodes of painful crisis (predominantly involving bones, abdomen, and muscles), and attacks of fainting who was hospitalized twice for her painful crisis. She had never been transfused. On physical examination, the patient was afrebrile with no lymphadenopathy or hepatosplenomegaly. Cardiovascular, respiratory, and nervous systems were normal. Her Hb was 12.6 g/dl, MCH, 26.3 pg; MCV, 80 fl; MCHC, 32.7%; HbS, 41.6%; HbA_2, 3.3%; and HbF, 0.4%. G6PD levels were normal. Her sickle status was confirmed by PCR and digestion with *Dde*I restriction enzyme. The β-globin gene cluster analysis revealed that the β^S gene was linked to the Arab–Indian haplotype (#31). Scanning the entire β-globin gene (−238 nt from "cap site" at 5′ end to 76 nt 3′ to the poly A tail) by DGGE showed no other mutations except the β^S mutation. α-Genotyping showed a single additional α gene ($\alpha\alpha\alpha^{3.7}/\alpha\alpha$).

It has been well established that a number of linked and unlinked genetic factors like β-globin gene cluster haplotypes, elevated HbF levels, presence of other mutations, like HbS-Oman or HbS-Antilles, in the β-globin gene cluster and α-thalassemia when associated with the sickle cell gene can modify the expression of the sickle cell syndrome [1,2]. The co-inheritance of α-thalassemia with sickle cell anemia and the consequent reduction of α-chains often result in milder clinical manifestations [3]. On the other hand, excess α-globin genes could increase the severity of sickle cell anemia [4]. Kinetic studies have shown that α-chains have lesser affinity for β^S chains as compared to β^A chains and that the formation of HbS is significantly lower in association with α-thalassemia which decreases the intracorpuscular gelation [5].

Molecular analysis of the β-globin gene cluster did not explain the clinical severity seen in this sickle heterozygote. The presence of excess α-genes ($\alpha\alpha\alpha^{3.7}/\alpha\alpha$) appears to have resulted in the unusual severe clinical presentation. The excess α-globin chains due to α gene triplication might have led to greater HbS production (41.6%), resulting in increased gelation, sickling and complications. Increased HbS levels (35–45%) in sickle cell heterozygotes with a tendency towards crisis and hypoxia have been reported earlier [6]. Thus, an in-depth study on clinical and molecular analysis in a large number of sickle cell heterozygotes would be interesting.

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