Malignant Myeloid Transformation in Congenital Forms of Neutropenia

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Abstract

Background: Granulocyte colony-stimulating factor has had a major impact on the management of severe chronic neutropenia – a collective term referring to congenital, idiopathic, or cyclic neutropenia. Almost all patients respond to G-CSF with increased neutrophils, reduced infections, and improved survival. Some responders with congenital neutropenia (termed Kostmann's syndrome herein) and Shwachman-Diamond syndrome have developed myelodysplastic syndrome and acute myeloid leukemia, which raises the question of the role of G-CSF in pathogenesis. The issue is complicated because both disorders have a propensity for MDS or AML as part of their natural history.

Objective and Methods: To address this, the Severe Chronic Neutropenia International Registry used its large database of chronic neutropenia patients treated with G-CSF to determine the incidence of malignant myeloid transformation in the two disorders, and its relationship to treatment and to other patient characteristics.

Results: As of January 2001, of the 383 patients with congenital forms of neutropenia in the Registry, 48 had MDS or AML (crude rate, about 12.5%). No statistically significant relationships were found between age at onset of MDS or AML and patient gender, G-CSF dose, or duration of G-CSF therapy. What was observed, however, was the multistep acquisition of aberrant cellular genetic changes in marrow cells from Kostmann's syndrome patients who transformed, including activating ras oncogene mutations, clonal cytogenetic abnormalities, and G-CSF receptor mutations. The latter in murine models produces a hyperproliferative response to G-CSF, confers resistance to apoptosis, and enhances cell survival.

Conclusions: Since Kostmann's syndrome and Shwachman-Diamond syndrome are inherited forms of bone marrow failure, G-CSF may accelerate the propensity for MDS/AML in the genetically altered stem and progenitor cells, especially in those with G-CSF receptor and ras mutations (82% and 50% of Kostmann's syndrome patients who transform, respectively). Alternatively, and equally plausible, G-CSF may simply be an innocent bystander that corrects neutropenia, prolongs patient survival, and allows time for the malignant predisposition to declare itself. Only careful long-term follow-up of the cohort of patients receiving G-CSF will provide the answer.

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Kostmann's syndrome, a subtype of congenital neutropenia, is characterized by the onset of profound neutropenia in early childhood, recurrent life-threatening infections, and a typical maturation arrest of bone marrow myeloid precursors at the promyelocyte-myelocyte stage of differentiation [1]. It is now apparent that most patients with this form of congenital neutropenia have heterozygous mutations in the gene encoding neutrophil elastase [2]. Shwachman-Diamond syndrome is an additional subtype of congenital neutropenia inherited in an autosomal recessive manner [1]. Essential for the diagnosis are neutropenia of variable severity often with other cytopenias, and exocrine pancreatic dysfunction; supportive features of SDS are short stature, skeletal abnormalities, and abnormal liver function tests [3]. The SDS locus maps to the centromeric region of chromosome 7 (7p12-q11) [4]. SDS marrow cells show abnormally increased apoptosis mediated through the fas pathway [5], and SDS patients also have a serious generalized marrow dysfunction with abnormal bone marrow stroma in terms of its ability to support and maintain hematopoiesis [6].

Historically, it was recognized that patients with both types of neutropenia have an inordinately high predisposition to the spontaneous development of myelodysplastic syndrome and/or acute myeloid leukemia. In Kostmann's syndrome there were three case reports of patients who developed AML prior to the use of hematopoietic growth factors [7–9], and a more recent case of a patient who was diagnosed with acute leukemia prior to starting G-CSF [10]. Because most patients with KS died early in life from bacterial sepsis or pneumonia in the pre-cytokine era, the true risk of patients with KS developing MDS/AML was not clearly defined. However, there were 128 cases of KS reported through 1989, which is the first year that G-CSF was available for general use, leading to a crude estimated risk of leukemia of 2%. Of the approximately 200 cases reported through 2000 of patients who did not receive G-CSF, 4 had leukemia (2%) [1]. In SDS, neutropenia is usually less severe than in KS and most patients survive the childhood years. The crude rate for MDS or acute leukemia in patients with SDS was 8% in one case series (7 of 88 patients) [3] and 33% in a smaller case series (7 of 21) [11]. It was 5% in approximately 200 SDS cases reported in the literature prior to 1990, and 10% in more than 300 patients who had not received G-CSF reported through 2000 [1]. This predisposition to hematologic malignancy is confined to congenital neutropenia and SDS and has not been observed in other severe chronic neutropenias including glycogen storage disease 1b, cyclic neutropenia, or idiopathic neutropenia.

G-CSF = granulocyte colony-stimulating factor
MDS = myelodysplastic syndrome
AML = acute myeloid leukemia

SDS = Shwachman-Diamond syndrome
KS = Kostmann's syndrome

In 1987, clinical trials began to test the utility of recombinant human G-CSF therapy for chronic neutropenia. The efficacy was demonstrated immediately as more than 90% of the total group of patients with idiopathic, cyclic, and congenital forms of neutropenia showed a selective, sustained increase in neutrophil numbers [12]. This set the stage for long-term administration of G-CSF for these disorders. Shortly after G-CSF was acknowledged as first-line therapy for chronic neutropenia, reports began to emerge on the development of MDS/AML in patients with KS and SDS who were receiving it. These cases were compiled by Kalra et al. [13], who reported 13 patients on G-CSF (12 with Kostmann's syndrome or congenital neutropenia, and 1 with SDS) who had overt transformation, and a 14th patient with SDS who developed a clonal cytogenetic abnormality of marrow cells but without MDS/AML. A literature survey of all reported KS patients who received G-CSF identified 23 of 101 in whom MDS and/or AML occurred (a 23% crude risk). Among the SDS reports, 4 of 12 patients who received G-CSF developed leukemia [1].

Patients and Methods

In response to the obvious need to obtain detailed data on these and other patients receiving long-term G-CSF therapy, the Severe Chronic Neutropenia International Registry was established in 1994 to continue monitoring the clinical course, treatment, and disease outcomes of neutropenic patients. Clinical trial data from 1987–1994 were retrospectively added to the data of newly diagnosed patients from 1994 onward. Patient data were submitted internationally to the data coordinating centers at the University of Washington, Seattle, and the Medizinsche Hochschule, Hannover, Germany. As of 1 January 2001, the following numbers of patients were enrolled in the SCNIR: 383 with congenital forms of neutropenia including 26 with SDS, 160 with cyclic neutropenia, and 288 with idiopathic neutropenia.

Results

Malignant myeloid transformation from the SCNIR database

In 1996 and 1997, the SCNIR tabled its first report on patients with congenital neutropenia on G-CSF therapy who developed MDS/AML [14]. Of 249 patients at risk, 23 underwent malignant transformation for a crude rate of about 9%. Since that report, there has been progressive enrollment of new patients from the United States, Europe and Australia; there has also been, in parallel, an annual accrual of new patients with KS and SDS on G-CSF who evolved into MDS/AML. As of 1 January 2001, there were 48 patients with MDS/AML of the 383 patients with congenital forms of neutropenia in the SCNIR (crude rate, about 12.5%). Two of 26 who transformed had SDS and 46 of 357 had congenital neutropenia. There are no cohort data prior to the use of G-CSF. A comparison of literature reports with the SCNIR data of congenital neutropenia patients for whom G-CSF was not used suggests a significantly increased risk of transformation with G-CSF (4 of 200 cases without G-CSF vs. 46 of 357 with G-CSF, $P = 0.01$). The number of SDS cases in the SCNIR is relatively low, and comparison with the literature is less meaningful (34 of 324 cases without G-CSF vs. 2 of 26 with G-CSF, P = NS).

To determine if the incidence of MDS/AML in the SCNIR patients was related to G-CSF dosage or duration of therapy, or to patient demographics, a detailed analysis was conducted on data received up to 31 December 1998 on 352 patients with congenital neutropenia treated with G-CSF [14]. Of these, 31 developed MDS/AML for a crude rate of transformation of nearly 9%. For each yearly treatment interval, the annual rate of MDS/AML development was less than 2%. No statistically significant relationships were found between age at onset of MDS/AML and patient gender, G-CSF dose, or duration of treatment. There were nine additional patients whose bone marrow studies showed cytogenetic clonal changes but the patients had not transformed to MDS/AML. Thus, the data did not support a cause-and-effect relationship between the development of MDS/AML and G-CSF therapy or other descriptors. However, a direct contribution of G-CSF in the pathogenesis of the malignancy could not be excluded (see below).

Aberrant cellular genetic changes

Conversion to MDS/AML in KS patients receiving G-CSF is associated with one or more cellular genetic abnormalities that provide insight into the pathobiology of the transformation and may be useful in identifying patients at high risk. The cellular genetic changes are acquired after starting G-CSF and occur singly or in combinations. Remarkably, the abnormalities have predictably similar characteristics in most patients and underscore a fairly specific multistep pathogenesis in the evolution into MDS/AML [Table 1]. As summarized, almost all patients with congenital neutropenia (but not SDS) inherited a heterozygous mutation in the gene encoding neutrophil elastase [2]. At varying time points after starting G-CSF therapy, about one-half of the congenital neutropenia patients who transform acquire the same activating ras oncogene mutation – namely, a GGT (glycine) to GAT (aspartic acid) substitution at codon 12 [13]. Almost all patients who transform also show an acquired cytogenetic clonal alteration in bone marrow cells, usually -7 or 7q-, but also +21 [13,15]. Occasionally both chromosome abnormalities occur in the same cell. Most patients also develop one or more G-CSF receptor point mutations. These nonsense mutations result in the truncation of the C-terminal cytoplasmic region, a subdomain that is crucial for G-CSF–induced maturation [16–18]. The acquired mutation is directly operative in the conversion to MDS/AML. In murine

Table 1. Cellular genetic changes characterizing the stepwise evolution of MDS/AML in patients with congenital neutropenia taking G-CSF who transform

Abnormality	Patients affected *
Neutrophil elastase mutation [2]	90%
ras oncogene mutation [13]	50%
-7 or 7q-, +21, other [1,15]	> 90%
G-CSF-R mutation[16–20,26,27]	82%
MDS/AML	Likely 100%

* The percentage of patients affected with each cellular genetic abnormality was compiled from unpublished research data in the SCNIR, and from published sources as indicated.

SCNIR = Severe Chronic Neutropenia International Registry

models, the mutation results in impaired ligand internalization, defective receptor down-modulation, and enhanced growth signaling that produces an exaggerated hyperproliferative effect in response to G-CSF [19–22]. This also confers resistance to apoptosis and enhances cell survival [23], which favors clonal expansion *in vivo* [20]. The clinical interplay between G-CSF and the receptor mutation was confirmed by the report of a patient with congenital neutropenia on G-CSF who developed the receptor mutation and AML [24]. When G-CSF was stopped, the blast count in blood and in marrow fell to undetectable levels on two occasions without chemotherapy, although the mutant receptor was persistently detectable during the remissions.

Do all patients with the G-CSF receptor mutation develop MDS/AML? The likely answer is yes, although there are some congenital neutropenia patients taking G-CSF who have acquired the receptor mutation but who have not yet developed MDS/AML [25]. Obviously, these patients are at high risk and are being monitored closely.

Discussion

Since there is no definitive evidence that the dose of G-CSF or the duration of G-CSF therapy is directly related to malignant transformation, G-CSF may simply be an innocent bystander that corrects the neutropenia, prolongs patient survival, and allows time for the malignant predisposition to declare itself. Alternatively, G-CSF may accelerate the propensity for MDS/AML in the genetically altered stem and progenitor cells in congenital neutropenia and in SDS. G-CSF may rescue malignant clones that would otherwise be destined for apoptosis. An axiom of oncogenesis is that rapidly dividing cells are more susceptible to mutational events. Since therapeutic G-CSF provides a powerful proliferative signal for marrow cells, it is a reasonable hypothesis that KS and SDS marrow progenitors acquire new mutations. From the evidence cited herein, acquisition of a G-CSF receptor mutation in the face of therapeutic G-CSF in KS can provide the hyper-responsive replicative scenario that can relentlessly evolve into MDS/AML. Finally, is recombinant human G-CSF a carcinogen? This would seem highly unlikely. As a physiologic regulator of hematopoiesis, it would be unexpected for G-CSF to break molecular bonds and cause DNA damage, even when used in therapeutic dosages. It is noteworthy that MDS/AML has not been seen in other SCN patients on long-term G-CSF therapy.

G-CSF has a clear therapeutic role in the management of severe chronic neutropenia. The role of G-CSF in hematopoietic malignant transformation is less clear, but suspect, at least in KS and SDS. Only careful long-term follow-up of the cohort of patients receiving this treatment will provide the answer.

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I am opposed to socialism because it dreams ingenuously of good, truth, beauty, and equal rights

Nietzsche, 19th century German philosopher who rejected the accepted absolute moral values of Christianity

Capsule

MCH and weight gain

The past several years have seen the discovery and characterization of an increasingly large number of factors involved in various aspects of metabolic regulation. One such peptide, melanin-concentrating hormone (MCH), has repeatedly been linked to increased food intake and reduced energy utilization. However, some previously published studies appear to contradict this model, creating some confusion as to MCH's true physiologic function. This past month's *International Journal of Obesity* presents the findings of Nigel Levens' group, which demonstrate a significant and sustained increase in food intake and body weight in two strains of rats subjected to acute and chronic ventricular infusion with MCH. This work bolsters existing evidence that MCH regulates specific orexigenic pathways, and therefore may prove to be a valuable target for anti-obesity therapeutics.

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Capsule

Defensins strategies

The innate immune system responds rapidly to pathogens, either by obstruction and direct killing of microbes or by activation of the adaptive arm of the immune system. Defensins have feet in both camps, and two studies add insight into how they work. Although chemokines play a role in the inhibition of human immunodeficiency virus type 1 (HIV-1) replication by CD8 T cells, the full identity of the factors involved has been elusive. Zhang et al. (*Science* 2002;298:995) used mass spectrometry and protein chip technology to examine culture supernatants from CD8 cells isolated from patients who are long-term non-progressors to AIDS. On the basis of amino acid sequencing and antibody recognition, they identified a set of defensins that only appeared upon T cell activation. Antibodies to these molecules blocked viral inhibition by CD8, and commercial preparations of alpha-defensins 1 and 2 inhibited different HIV-1 isolates. Biragyn et al. (p. 1025) find that one defensin, beta defensin 2, could activate dendritic cells by binding Toll-like receptor-4 (TLR-4), a cell-surface pattern-recognition protein hitherto considered to be limited to recognizing pathogen-derived molecules, such as the Gram-negative bacterial endotoxin, lipopolysaccharide. In dendritic cells, the interaction of beta defensin 2 with TLR-4 induced much the same cellular activation program as lipopolysaccharide, with the stimulation of co-stimulatory molecule and pro-inflammatory cytokine expression.