USE OF RECOMBINANT GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR IN THE BRAZIL RADIATION ACCIDENT

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Summary 8 patients with bone marrow failure after a caesium-137 radiation accident were treated with recombinant human granulocyte-macrophage colony stimulating factor (rHuGM-CSF). The 7 who were evaluable had prompt increases in granulocytes and bone marrow cellularity. 2 patients died of radiation toxicity and haemorrhage and 2 of bacterial sepsis acquired before the start of rHuGM-CSF treatment. 4 patients survive, including 2 who were treated early and never became infected. This therapeutic approach to radiation-induced granulocytopenia may therefore be useful after radiation and nuclear accidents.

Introduction

IONISING radiation causes a dose-dependent inhibition of bone marrow function with corresponding decreases in granulocytes and platelets.[1-3] This is most pronounced when exposure is acute, at high dose rate, and involves a total body field. With acute total body doses of 1–2 Gy, bone marrow suppression is modest and spontaneous recovery is likely. Doses of 2–5 Gy depress bone marrow function more severely; recovery is possible but infection and bleeding are common and may be fatal. Doses in excess of 5 Gy cause severe bone marrow depression; some individuals recover but many die of intercurrent infection or bleeding. With doses over 15 Gy, particularly in accidents, the likely cause of death is damage to other organs such as gastrointestinal tract, lung, and central nervous system rather than bone marrow suppression.

The probability of recovery after acute total body exposure to 2–5 Gy represents a balance between the rate of haematopoietic recovery and the risk of severe infection or haemorrhage. Such doses are unlikely to kill all the haematopoietic stem cells, and it should be possible to increase survival of exposed individuals by increasing the rate of bone marrow recovery.

Several haematopoietic growth factors have been identified and molecularly cloned.[4-6] One, granulocyte-macrophage colony stimulating factor (GM-CSF), stimulates growth of myeloid progenitor cells in the bone marrow and increases granulocytes.[7-9] When treated with GM-CSF, irradiated rodents and subhuman primates recover bone marrow function and granulocytes more rapidly than controls.[5,10] This agent has already been used clinically in marrow failure of other types.[11-19] We therefore decided to use recombinant human GM-CSF (rHuGM-CSF) in victims of a radiation accident in Brazil.

Methods

Accident

On Sept 13, 1987 two persons discovered an abandoned ^{137}Cs radiotherapy unit in a deserted clinic in Goiania, Brazil. Details of the accident have been reported[20,21] and are summarised in figs 1 and 2. The unit, a 'Cesaspan 8300' was loaded with 28 g ^{137}Cs as $CsCl_2$ powder in 1971. At the time of the accident the source contained 19·26 g of $CsCl_2$ equivalent to 1375 Ci with a specific activity of 15·11 Ci per g. The ^{137}Cs was within a tungsten and steel capsule surrounded by lead shielding. The accident was discovered on Sept 29. Between Sept 13 and Sept 29, 249 persons were exposed to radiation (fig 1). 120 had light surface or clothing contamination and were rapidly decontaminated. 129 required greater attention: 79 had more serious skin or external exposure and were treated as outpatients; and 50 others received higher external or internal doses of whom 20 were admitted to hospital. Bone marrow failure developed in 14 persons; 10 of these and 4 others without bone marrow failure were transferred to a specialised unit in Rio de Janeiro.

Exposure was variable; some individuals were exposed acutely and others daily over the course of two weeks. Details of radiation exposure in the 10 persons discussed in this report are given in fig 2 and the table. Median duration of exposure was 14 days (range 1–14). In most instances radiation exposure was predominantly external; some individuals also received internal radiation from ^{137}Cs contaminated food. ^{137}Cs is a K analogue and its distribution is largely intracellular.[22]

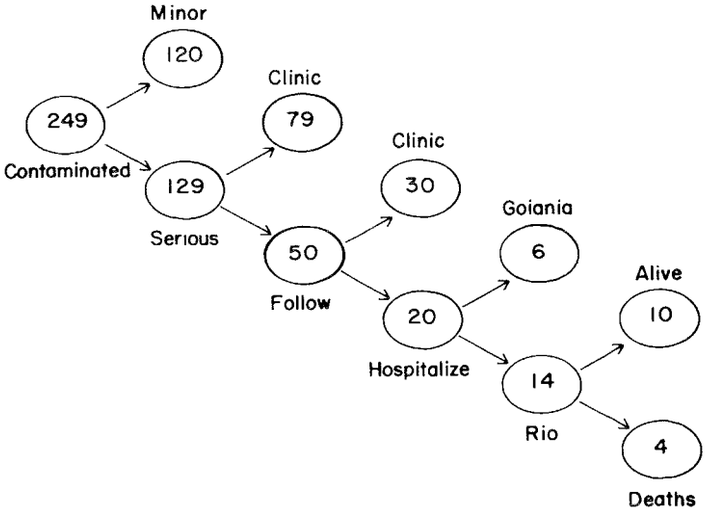

Fig 1—Management of accident victims.

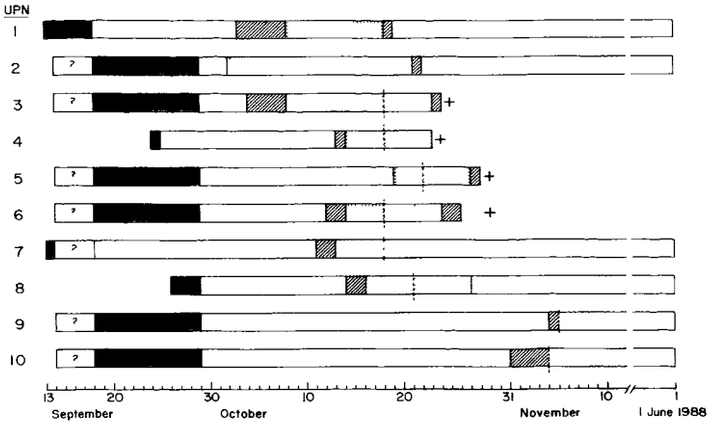

Fig 2—History of radiation exposure.

UPN = unique patient number; dark bar = days when ⩾ opportunity for exposure; ? = possibility of exposure; open bar = time; diagonal hatching = granulocytes $< 0\,5 \times 10^9/l$; stippling = granulocytes $< 0{\cdot}1 \times 10^9/l$; dashed line = start of rHuGM-CSF therapy; + = death. ^{137}Cs source was removed from the clinic on Sept 13, 1987, by patients 1 and 7 and transferred to patient 2 on Sept 18 (or possibly Sept 14).

RESULTS OF THERAPY WITH GM-CSF

No	Sex	Age	Estimated dose (range)*	Days from exposure to therapy	Granulocytes $<1\times10^9$/l to therapy (d)	Granulocytes ($\times10^9$/l)†		Outcome
						Pretreatment	Maximum	
3	F	37	6·0 (5·1–6·8)	30	10	0	0·6	Died, haemorrhage‡
4	F	6	6·0 (5·1–7 3)	24	4	0	NE	Died, haemorrhage§
5	M	22	4·0 (3 2–4·8)	34	3	0·2	0·5	Died, infection¶
6	M	18	5·3 (4·4–6·5)	30	4	<0·1	23·1	Died, infection
7	M	19	2·5 (1·7–3·4)	35	5	<0·1	19·7	Alive
8	F	57	4·3 (3·0–5·0)	25	5	0	21·4	Alive
9	M	42	4·4 (3·5–5·5)	40	1	0·7	9·9	Alive
10	M	21	3·0 (2·0–4·0)	47	4	0·5	7·3	Alive

*Integrated sum of external and internal doses. Calculations based on neutrophil and lymphocyte kinetics, dicentric chromosomes, and physical measurements of ^{137}Cs incorporation.
†Includes myelocytes, metamyelocytes, and bands.
‡This patient also had cerebral oedema and pre-existing hepatic cirrhosis.
§Diffuse pulmonary haemorrhage was immediate cause of death.
¶Bronchopneumonia.

Dosimetry

Integrated dose estimates were based primarily on biological dosimetry.[23] The variables included analyses of dicentric chromosomes and kinetics of granulocyte and lymphocyte decreases.[24–26] Internal contamination estimates were based on total body counting and on urinary and faecal excretion of ^{137}Cs. External contamination estimates were based on surface counting. Multiple determinations of both biological and physical dose were made in Brazil and in reference laboratories in the United States, West Germany, and the United Kingdom. The integrated doses in the table must be regarded as preliminary, since they are based largely on chromosome analyses with a chronic exposure model.

Medical Treatment

The 14 most severely affected individuals were flown to Hospital Marcilio Dias in Rio de Janeiro for treatment. 10 had bone marrow failure, 9 with granulocytes $\leq0\cdot5\times10^9$/l and 1 with granulocytes $0\cdot7\times10^9$/l. 3 persons with bone marrow failure also had radiation related burns as did 4 others. This report focuses on the 8 patients with bone marrow failure who received rHuGM-CSF.

Patients were treated in a specialised medical unit with extensive radiation protection precautions. For prevention of infection they were isolated in one-bed or two-bed rooms and were given oral cotrimoxazole or norfloxacin. Acyclovir was given to prevent activation of latent herpes virus infection. Persons with temperature >38·5°C and granulocytopenia were treated with systemic antibiotics (cefoperazone, imipenem, and/or piperacillin). Those with fever for >48–72 h received vancomycin and/or amphotericin B. Central lines were used for venous access. Red blood cell transfusions were given to maintain haemoglobin ≤10 g/dl. Platelet transfusions, usually from volunteer unrelated donors, were obtained by platelet pheresis (Fenwall 2992, Baxter Healthcare, Morton Grave, IL) and were given to maintain platelets $>20\times10^9$/l. Blood products were irradiated with 15 Gy to prevent engraftment and graft-versus-host disease.

6 patients received metoclopramide and cimetidine for symptoms of presumed radiation-related gastroenteritis. 7 were given Prussian blue to increase gastrointestinal tract excretion of ^{137}Cs.[27]

The accident victims were assigned special patient numbers by date of exposure. Those who received rHuGM-CSF are nos 3–10.

rHuGM-CSF

The rHuGM-CSF used in this study was supplied by Immunex Corp (Seattle, USA) and Behringwerke AG (Marburg, FRG). It was isolated from a cDNA library constructed by use of mRNA from the HUT102 human T-cell line and inserted and expressed in yeast.[9] Purified rHuGM-CSF had a specific activity of 5×10^7 colony-forming units per mg protein. It was given as a 24 h continuous intravenous infusion at a dose of 500 μg/m² per day in 0·9% NaCl with 0·9% albumin. Treatment was continued until granulocytes were $>2\times10^9$/l for 3 days. The dose was then decreased to half for 3 days, to a quarter for 3 additional days, and then discontinued. Day 0 was designated as the first day of treatment.

Laboratory Studies

Complete blood count with differential, platelets, haemoglobin, and reticulocytes was done daily together with measurement of Na, K, urea nitrogen, and glucose. Other measurements, including creatinine and liver function tests, were done twice a week. Radioactive contamination of clinical samples limited laboratory investigations. Patients had bone marrow aspiration and biopsy before receiving rHuGM-CSF and weekly thereafter when possible. Necropsies were performed in those who died but microscopic studies were omitted because of ^{137}Cs contamination of specimens.

Results

Bone Marrow Failure

Of the 10 individuals with bone marrow failure 2 (nos 1 and 2) recovered before day 35 and were not considered for rHuGM-CSF therapy. Their estimated integrated doses were 6·2 (range 5·2–7·6) Gy and 7·1 Gy (5·0–8·5). Details of exposure and subsequent granulocytopenia in the 8 rHuGM-CSF recipients are given in the table and figs 2 and 3. Median interval from initial exposure to granulocytes $<1\cdot0\times10^9$/l was 23 days (range 11–41) and to granulocytes $<0\cdot5$ was 20 days (15–30). Haemoglobin and platelets were relatively less depressed; this was accounted for only in part by transfusions. Reticulocytes were $<10\times10^9$/l in all persons tested. Bone marrow aspirates and biopsy specimens showed <10% cellularity. 2 patients (nos 9 and 10) had severe granulocytopenia but only modestly decreased platelets.

rHuGM-CSF

8 individuals had granulocyte counts $\leq0\cdot5\times10^9$/l and no evidence of spontaneous haematopoietic recovery within 35 days of the onset of the accident, when the drug becaame available. They received rHuGM-CSF at a median interval from initial exposure of 32 days (range 24–48). Serial granulocyte counts are shown in fig 3a. Excluded from this figure is one patient (no 4), who died on day 6 without evidence of response. In these initial cases 2 persons with resistant klebsiella infections acquired before rHuGM-CSF treatment died despite increasing granulocytes, therefore it was decided to treat the next 2 patients (nos 9 and 10) before infection developed; granulocytes were $0\cdot5\times10^9$/l and

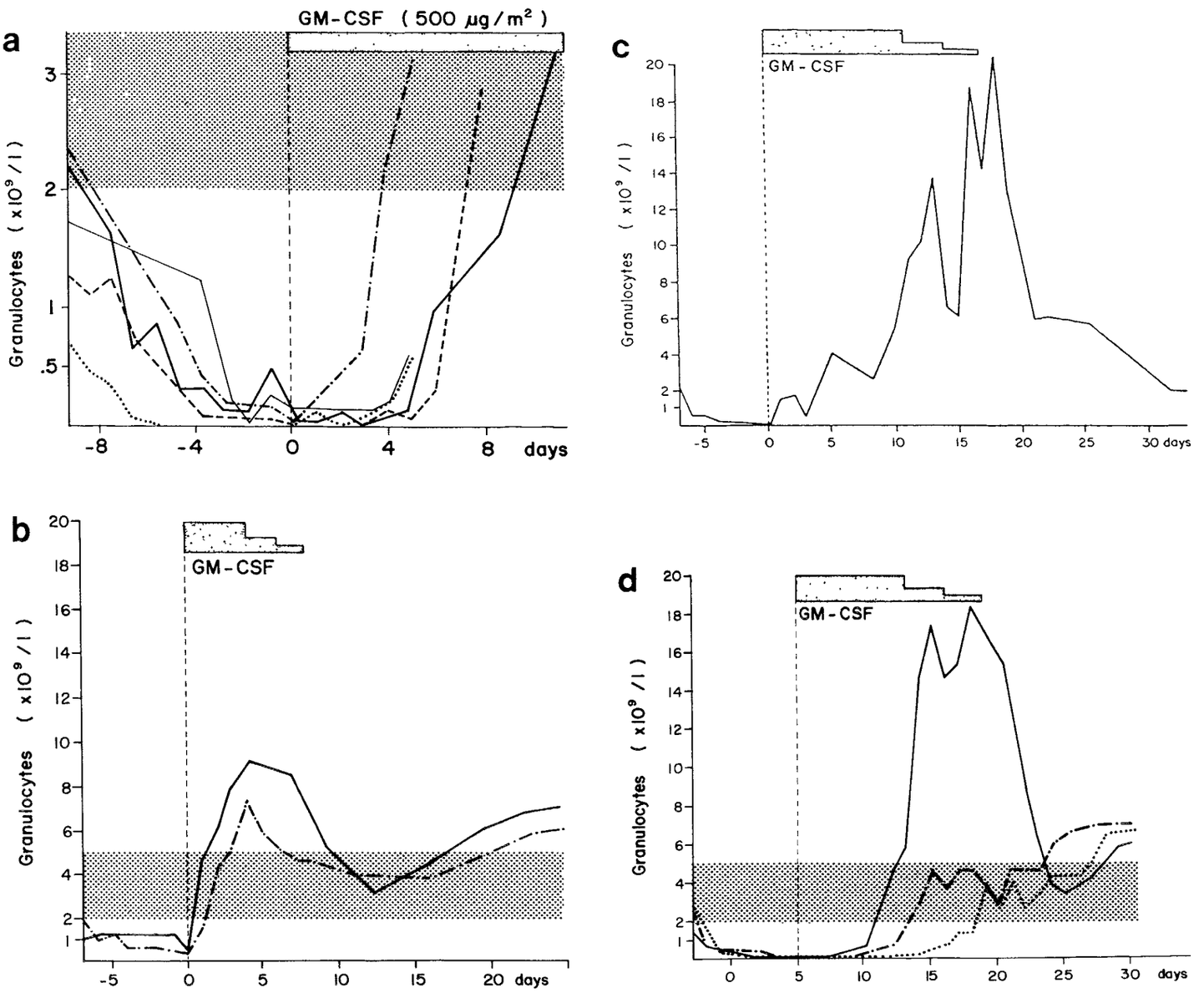

Fig 3—Responses to rHuGM-CSF.

Curves adjusted such that day 0 is start of therapy:
(a) · · · = patient 3; —— = patient 5; – – – = patient 6; – · – = patient 7; ——— = patient 8.
(b) —— = patient 9; – · – = patient 10. Different scale from (a). Initial dose of rHuGM-CSF 500 µg/m² per day.
(c) Patient 8. Initial dose 500 µg/m² per day.
(d) Granulocyte recovery in patients 1 (– · –) and 2 (· · ·) (no rHuGM-CSF) and in patient 8 (——). Curves adjusted to granulocytes $< 0{\cdot}5 \times 10^9$/l on day 0. Initial dose of rHuGM-CSF (patient 8) 500 µg/m² per day.

$0{\cdot}7 \times 10^9$/l respectively. Fig 3b shows their response to rHuGM-CSF. Both had substantial increases in granulocytes within 12 h of beginning treatment. Decreases in rHuGM-CSF dose were followed by a rapid decrease in granulocytes, then recovery (fig 3c). Serial bone marrow aspirates and biopsies reflected changes in granulocyte levels. Kinetics of granulocyte recovery differed strikingly between treated and untreated individuals. Fig 3d compares the kinetics of recovery in the 2 individuals with spontaneous recovery (nos 1 and 2) and a representative patient (no 4) treated with rHuGM-CSF (no 4). rHuGM-CSF did not seem to affect red-blood-cell or platelet recovery.

Outcome

4 of 8 persons who received rHuGM-CSF survive (nos 7–10). The 4 who died were colonised with gram-negative bacteria before the start of rHuGM-CSF treatment. In patients 3 and 4 the cause of death was diffuse haemorrhage. Patients 5 and 6 died of bacterial sepsis unresponsive to antibiotics and vasopressors. The klebsiella isolated from these individuals was resistant to all antibiotics tested except polymyxin B. Patients 9 and 10 were treated with rHuGM-CSF before they became infected and then remained free of infection.

Side-effects of rHuGM-CSF treatment were mild in most instances. Patient 9 had temperatures of 38°C episodically on the first 2 days of treatment without evidence of infection; these resolved on continued therapy. In patients 5, 6, and 7 respiratory failure and/or pulmonary oedema developed during therapy. All 3 had bacterial sepsis. Although these episodes were ascribed to infection, we cannot exclude an effect of rHuGM-CSF. Of the 2 individuals with spontaneous haematological recovery, both survived; the first (patient 1) required amputation of a forearm because of severe radiation burns.

Discussion

Exposure to acute, high-dose total body radiation suppresses normal bone marrow function. The resultant granulocytopenia is associated with an increased risk of infection. Prophylactic antibiotics, protected environments (like laminar air flow), and dietary modifications decrease infection. Prophylactic antibiotics, protected environments (eg, laminar air flow), and dietary modifications decrease recovery of granulocyte production.

A second approach is to increase granulocyte levels by transfusions. Unfortunately prophylactic or therapeutic granulocyte transfusions have shown no convincing benefit[28-31]—probably became only small numbers can be given and their survival is brief.

Restoration of normal granulocyte production is the most direct approach, and can be accomplished by transplantation of haematopoietic stem cells.[32-34] This technique is complex. In many instances the dose of radiation will not be sufficiently immunosuppressive to permit sustained engraftment. A histocompatible donor must be identified. There are other risks—the need for post-transplant immunosuppression, graft-versus-host disease, cytomegalovirus reactivation with resultant interstitial pneumonitis—but transplantation is the only therapeutic option if no residual haematopoietic stem cells remain or if the time required for recovery exceeds the limits of supportive care.

Since radiation results in a fractional cell kill, some stem cells are likely to survive even very high doses. This was our rationale for trying rHuGM-CSF.

Evaluation of the effectiveness of rHuGM-CSF treatment requires knowledge of the radiation dose. This analysis was complex. Exposure was episodic and difficult to determine accurately. In some instances it occurred over several hours; in other instances persons were exposed intermittently over one to two weeks. Dose rate also varied considerably, depending on distance from the source, and could not be precisely determined. Nor could we judge uniformity of exposure or potential shielding. We therefore opted for biological dosimetry, analysing such variables as dicentric chromosomes and interval from first exposure to granulocytes $< 1{\cdot}0$ and $< 0{\cdot}5 \times 10^9$/l. Most data regarding theses effects are derived from acute, high-dose external radiation exposure—something that happened to few if any persons in this study. We used a chronic exposure model in our calculations; an acute model would result in dose estimates about 50% less.

Did the granulocyte recovery result from rHuGM-CSF treatment? Three observations suggest that it did—namely, the rapid rise in granulocytes within 12 h in several individuals, the decline in granulocytes after dose attenuation or discontinuation, and the different patterns of recovery in treated and untreated persons.

A second question is whether rHuGM-CSF saved the lives of those who survived. It is unanswerable. We know that risk of infection is correlated with the level and duration of granulocytopenia; and since rHuGM-CSF increases granulocyte counts and shortens the period of granulocytopenia we could reasonably expect fewer infections. Consequently, if enough patients are treated, survival is likely to be increased unless the therapy is toxic.

A third question is whether these observations are relevant to other radiation accidents. If we accept that rHuGM-CSF treatment is likely to decrease the risk of fatal infections, then it should increase survival after radiation up to doses that destroy all stem cells—in other words, it will increase the operational 50% lethal dose of radiation.

A final question is whether treatment with GM-CSF could have adverse consequences. Haematopoietic stem cells are capable of self-replication and/or differentiation and maturation. Normally these functions are balanced such that the stem cell pool is maintained while adequate numbers of mature cells are produced. It is possible that treatment with rHuGM-CSF might perturb this balance resulting in stem cell depletion. This might be particularly true if the pool of residual stem cells was considerably reduced. Treatment with GM-CSF induces differentiation of granulocyte progenitors in vitro. However, late bone-marrow failure has not been reported in AIDS, cancer, or transplant patients receiving GM-CSF. Although follow-up is only ten months we have not observed delayed bone marrow failure in any of the GM-CSF-treated survivors of the Brazil accident.

Five haematopoietic growth factors have been identified in man. Might some of the others be useful after irradiation? Interleukin 3 (multi-CSF) acts on less mature stem cells than those stimulated by GM-CSF. M-CSF, G-CSF, and erythropoietin act on more mature stem cells. Factors active on the most immature stem cells would increase red blood cells and platelets as well as granulocytes, but transfusions of red blood cells and platelets are adequate alternatives. Conversely, stimulation of relatively mature stem cells might be advantageous since the residual less mature stem cells would be transiently protected from an increased drive towards differentiation and maturation. These approaches, and the possibility of combination therapy, are best explored in laboratory animals, as in the timing of growth factor therapy. Our data suggest that GM-CSF should be started without delay in accident victims who are expected to become granulocytopenic.

We thank the physicians, nurses, and technicians of the Naval Hospital Marcilio Dias for their invaluable contribution. Dr Carlos Eduardo Brandao and Dr Alexander Olivera evaluated the patients in Goiania and referred them for treatment. The support of agencies of the Federal Government of Brazil, including Ministry of the Navy, the National Committee for Nuclear Energy, the Ministry of Health, and Nuclebras is greatly appreciated. We thank Dr Armand Hammer and the Armand Hammer Center for Advanced Studies in Nuclear Energy and Health for generous financial support. American and European companies made generous gifts of drugs, medical supplies, and equipment: they included Behringwerke AG (FRG), Baxter-Healthcare (USA), Eli Lilley (USA), Lederle Laboratories (USA), Miles Pharmaceuticals (USA), Roerig Pharmaceuticals (USA), Burron Medical (USA), and Darol Corporation (USA). Ms Tommy Tierney and Mr Emmanuel Maidenberg (USA) helped with communication links and support. Dr Giorgi Selidovkin (USSR) helped with the dose estimates. Dr Roland Mertlesman and F. Herrmann (FRG) assisted in obtaining rHuGM-CSF. Ms Linda Rodman typed the manuscript. R. P. G. is the Wald Foundation Scholar in Biomedical Communications.

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DOES Scl-70 MODULATE COLLAGEN PRODUCTION IN SYSTEMIC SCLEROSIS?

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Summary Scl-70, an autoantigen in systemic sclerosis, may accelerate collagen gene transcription by virtue of its activity as a topoisomerase I (topo I), a DNA template-modifying enzyme. A survey of sequences corresponding to all or part of the known topo I binding sequence $A^{G}_{A}ACTTAGA^{G}_{A}AAA^{TTT}_{AAA}$ in four fibrillar collagen genes (three of them dermal) and sixteen non-collagen genes showed a striking preponderance of the tetramer 5′-CTTA-3′, comprising the core of this binding sequence, at the exon-intron junctions of the fibrillar collagen genes (59% compared with 16% in the control group). In addition, a non-random clustering of three potential topo I binding sites was seen within 350 base-pairs of 5′ flanking DNA in the dermal collagen gene α_2(I), and a fourth site occurred in the promoter region of the α_1(III) gene. The findings suggest that a selective vulnerability to the action of Scl-70/topo I is built into the structure of the dermal collagen genes.

Introduction

OVEREXPRESSION of collagen gene DNA is probably wholly or partly responsible for the excessive production of collagen in systemic sclerosis.[1,2] Both the levels of procollagen mRNA[1] and the rate of collagen gene transcription[2] are increased in proportion to the increase in dermal collagen in fibroblasts from scleroderma patients.[1,2] Approximately 25% of systemic sclerosis patients, whose illness takes an especially progressive course, produce autoantibodies directed against Scl-70,[3] recently shown to be a DNA topoisomerase I (topo I).[4] The occurrence of these antibodies suggests that anomalous changes in the quantity or level of activation of Scl-70/topo I may influence the pathogenesis of systemic sclerosis. Such can be the case only if there is a structural basis for a selective effect of this enzyme on the dermal collagen genes. Levels of production of other cellular proteins in scleroderma fibroblasts, including actin and fibronectin, are normal.[5,6]

The involvement of topo I in selective gene activation in eukaryotes has been well substantiated. The activation of genes by experimental manipulation is accompanied by an increase in the number of topo I-induced single-strand breaks both within the transcribed portion of the activated genes and in the 5′-flanking DNA.[7] Topo I from human and other eukaryotic cells demonstrates a high affinity for the hexadecameric sequence $A^{G}_{A}ACTTAGA^{G}_{A}AAA^{TTT}_{AAA}$.[8] The core of this hexadecamer, the CTT trimer, is most consistently involved in high-affinity recognition and binding by the enzyme.[9] Therefore the nicking and unwinding activity associated with gene activation is likely to involve topo I binding to the CTT core sequence plus an unknown number of nucleotides to either side. Selective vulnerability to the action of Scl-70/topo I in a particular gene family may be the consequence of an unusual abundance or distribution of sequences containing the CTT core.

To determine whether such a mechanism contributes to excessive collagen production in systemic sclerosis, the number of potential Scl-70/topo I binding sites in the fibrillar collagen genes was compared with sixteen randomly selected non-collagen genes. The fibrillar collagen gene family includes the three dermal collagen genes α_1(I), α_2(I), and α_1(III), and the cartilage collagen gene α_1(II).

Methods

Collagen and control gene sequences were searched with the Bionet Resource program QUEST, and by visual search. Analysis

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