existence of mammalian pinealofugal projections adds an anatomical entry to a growing list of features, primarily biochemical (*7, 15*), establishing that the mammalian pinealocyte in the sensory cell line evolved from the photosensitive pinealocyte of poikilotherms (*1*).

These findings also challenge current concepts of how the mammalian pineal sends messages. Substantial evidence supports the general belief that it communicates exclusively by releasing melatonin into the blood (*4*). Our evidence leads to the speculation that pinealocyte processes may deliver chemical messages directly to specific target sites in the central nervous system.

An obvious question to address is whether any function of the mammalian pineal gland might depend on central pinealocyte projections. The answer might lie in the body of reports on effects of the administration of melatonin. Many of these studies have been ignored because they use doses of melatonin that are considered high, on the basis of the physiological concentration of the compound in blood (0.1 n*M*). However, the concentration of melatonin outside a pinealocyte process might be several orders of magnitude higher than the normal blood concentration because melatonin within the pineal gland can be 10,000 n*M* and because as a highly lipophilic compound it can readily cross the plasma membrane. Along the same lines, it seems reasonable that other pineal products could be released from these processes. Taurine, for example, is a transmitter substance that is rapidly released from pineal cells through an adrenergic–cyclic AMP–regulated mechanism (*16*).

This development makes the mammalian pinealocyte a candidate for a role as neuron. It will now be important to characterize mammalian pinealocyte processes, to determine if and where they make functional contacts, and to establish their contents and whether they have a physiological function. A more complete understanding of the physiological role of the mammalian pineal gland could result as investigators try to uncover a functional relationship between the pineal organ and areas of the brain receiving pineal projections. Perhaps these connections transmit information about daily rhythmicity.

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9. With the antiserum used in this study, we have demonstrated S-antigen–like immunoreactivity in retinal photoreceptors and in typical photoreceptors of fish and amphibia, in modified pineal photoreceptors of sauropsids, and in pinealocytes of mammals including humans. [H.-W. Korf *et al.*, in *Pineal and Retinal Relationships*, D. C. Klein and P. O'Brien Eds. (Academic Press, New York, in press)]. Immunoreactivity was also found in invertebrate photoreceptors (T. van Veen *et al.*, unpublished results).
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High Titers of Autoantibodies to Topoisomerase I (Scl-70) in Sera from Scleroderma Patients

J. H. SHERO, B. BORDWELL, N. F. ROTHFIELD, W. C. EARNSHAW*

Patients with rheumatic diseases often have circulating autoantibodies to nuclear components. The clinical significance of the antibodies is controversial, although in some cases they are valuable in the diagnosis of the disease. This report presents results of a study of Scl-70, an autoantigen recognized by sera of many patients with the most severe form of progressive systemic sclerosis. It was possible to show, by three independent criteria, that Scl-70 is the abundant nuclear enzyme DNA topoisomerase I. Therefore, antibody probes of high titer and high affinity are now available for the study of this important nuclear enzyme.

RHEUMATIC DISEASES SUCH AS SYSTEMIC lupus erythematosus and scleroderma are often associated with the presence of antinuclear autoantibodies (*1*). With few exceptions (*2*) the cellular identity of these antigens is unknown. We have, therefore, recently begun the detailed characterization of the major chromosomal autoantigens recognized by sera of scleroderma patients (*3, 4*).

Scl-70, a nuclear autoantigen reported to have a molecular weight of 70,000 (70K) (*5, 6*), is recognized by sera from certain scleroderma patients (particularly those with diffuse disease). However, when we used the immunoblotting method to characterize human Scl-70 (Fig. 1), the antigen was found to be significantly larger than previously estimated (*5, 7*). We observed a single

J. H. Shero and W. C. Earnshaw, Department of Cell Biology and Anatomy, Johns Hopkins School of Medicine, Baltimore, MD 21205.
B. Bordwell and N. F. Rothfield, Division of Rheumatic Diseases, Department of Medicine, University of Connecticut Health Sciences Center, Farmington 06032.

*To whom correspondence should be addressed.

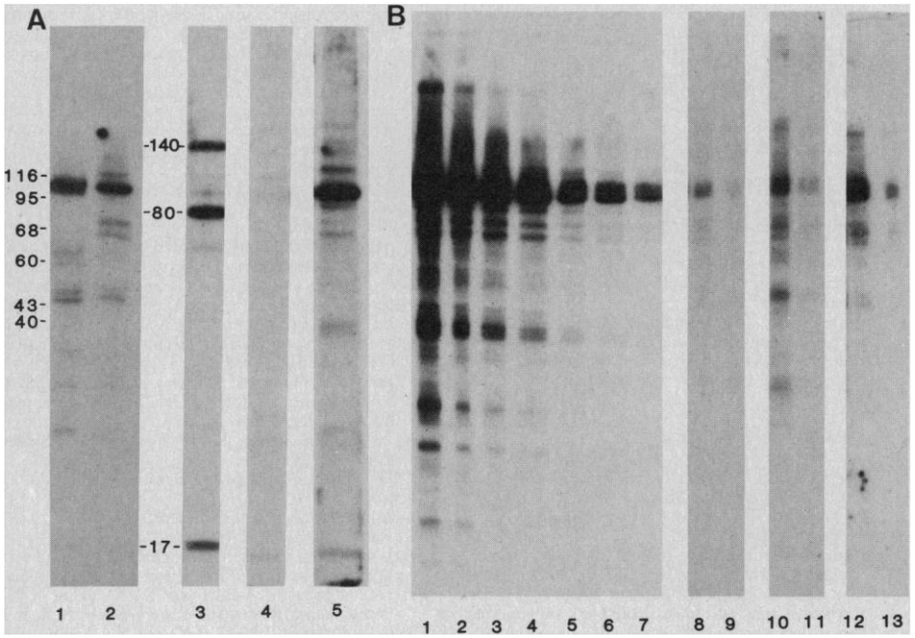

Fig. 1. Characterization of human cellular and chromosomal antigens recognized by anti–Scl-70 positive sera. (A) Detection of Scl-70 in boiled HeLa cells with serum SC (1:5000) (lane 1) and crude mitotic cell extracts (lane 2), and with serum JJ (1:200) in purified mitotic chromosomes (lane 5). Lanes 3 and 4 represent immunoblots of purified chromosomes with anti-centromere serum (lane 3; serum GS, 1:500) and patient serum that did not bind to chromosomal antigens in immunoblots (lane 4; serum G-270, 1:200). Relative mobilities of the three centromere antigens are noted between lanes 2 and 3. (B) Immunoblot analysis of HeLa nuclear proteins by means of serial dilution of the following anti–Scl-70 sera: SC (lanes 1–9) 1:2,000, 1:4,000, 1:8,000, 1:16,000, 1:32,000, 1:64,000, 1:128,000, 1:125,000, and 1:625,000; F-268 (lanes 10 and 11) 1:125,000 and 1:625,000; E-807 (lanes 12 and 13) 1:125,000 and 1:625,000, respectively. For the boiled cell preparation, 3×10^6 HeLa cells were centrifuged and resuspended in 1 ml of boiling sample buffer in the presence of SDS (*19*). Crude mitotic chromosomes were isolated from 1.5×10^7 HeLa cells as described (*20*). Purified mitotic chromosomes were obtained by the polyamine procedure (*19*). Crude interphase nuclei were obtained as described for crude mitotic chromosomes, with omission of the colcemid block. SDS-PAGE in 10 percent gels (*19*), electroblotting to nitrocellulose (*21*), and immunodetection with serum and ^{125}I-labeled protein A (*3*) were as described. Marker proteins were: β-galactosidase [116 kilodaltons (kD)], phosphorylase B (95 kD), bovine serum albumin (68 kD), catalase (60 kD), actin (43 kD), and aldolase (40 kD).

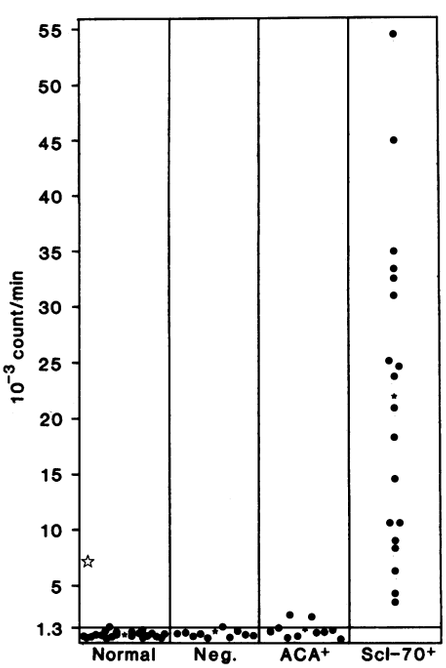

Fig. 2. Binding of sera (1:500) to purified bovine topoisomerase I in a solid-phase radioimmunoassay. Each point represents the average of three determinations. Binding of rabbit anti–topoisomerase I was 7203 count/min (☆). The mean ± standard deviation for each category (★) was as follows: normal human sera (667 ± 284 count/min, $n = 21$); patient sera that did not bind to chromosomal antigens in immunoblots (Neg.; 966 ± 429 count/min, $n = 10$); anti-centromere positive sera (ACA$^+$; 1128 ± 713 count/min, $n = 10$); anti–Scl-70–positive sera (Scl-70$^+$; 21,900 ± 16,055 count/min, $n = 19$). The line at 1337 is 2 standard deviations above the mean for the normal human controls. Stored sera came from patients (with scleroderma, Raynaud's phenomenon, or both, and other manifestations of rheumatic disease) of the Division of Rheumatic Diseases, University of Connecticut Health Center. All available anti–Scl-70–positive sera (classified by Ouchterlony double diffusion test) were tested. Control sera were chosen at random from the collection. Purified bovine topoisomerase I (*9*) was dried onto the wells of an Immulon 2 (Dynatech, Alexandria, VA) microtiter plate (0.14 μg per well in 30 m*M* Imadazole, *p*H 7.2). The plates were then processed as for detection of antigens on nitrocellulose blots (*3*).

major antigen of molecular weight 100K in boiled whole cells, crude mitotic cell extracts, and highly purified mitotic chromosomes but not in control immunoblots probed with antibody to the centromere or with nonbinding patient control sera (Fig. 1A). This 100K antigen was also detected in nuclei isolated from human spleen (*8*).

Precipitin analysis was used to examine the relationship between the 100K antigen and Scl-70. Extracts containing soluble 100K antigen were prepared by treatment of isolated nuclei from HeLa cells with 1.0*M* NaCl and compared with a commercially prepared bovine thymus nuclear extract (Alpha Antigens, Columbia, MO) used in clinical laboratories as a standard for Scl-70 (*6*). A reaction of immunological identity was obtained in Ouchterlony double diffusion analysis with a prototype patient serum specific for Scl-70 (*8*). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting revealed a single 100K antigen in the HeLa extract and a single 73K antigen in the bovine extract. Therefore, the molecular weight of human Scl-70 is 100,000.

Antibodies to Scl-70 (anti–Scl-70) were present at extremely high titers in many patient sera; positive binding was observed on immunoblots of HeLa nuclear proteins at a dilution of 1:625,000 (Fig. 1B). In contrast, the maximum dilution at which serum SC (Fig. 1B) was positive by Ouchterlony double diffusion was 1:16 (*8*). Therefore, immunoblotting or solid phase immunoassays (see below) are substantially more sensitive for detection of anti–Scl-70 than the Ouchterlony test.

The two size classes of Scl-70 resemble two classes of DNA topoisomerase I previously isolated from human cells (*9, 10*). Topoisomerase I was originally isolated from tissues as a nicking-closing enzyme of molecular weight 67K (*11, 12*), but is present in cells as a 100K polypeptide from which the smaller species is apparently derived by proteolysis (*9*). The existence of similar size classes of Scl-70 and topoisomerase I led us to further investigate the relationship between the two.

Sera positive for anti–Scl-70 were able to bind to purified bovine topoisomerase I in a solid phase radioimmunoassay (Fig. 2). The signal obtained with anti–Scl-70–positive sera was substantially greater than that obtained with normal sera (32-fold), anti-centromere–positive sera (19-fold), and patient sera that were negative by immunoblotting (23-fold), and was significantly above that obtained with an experimentally prepared rabbit antibody to topoisomerase I (3-fold). The high serum titers of anti–Scl-70 detected by immunoblotting were confirmed by

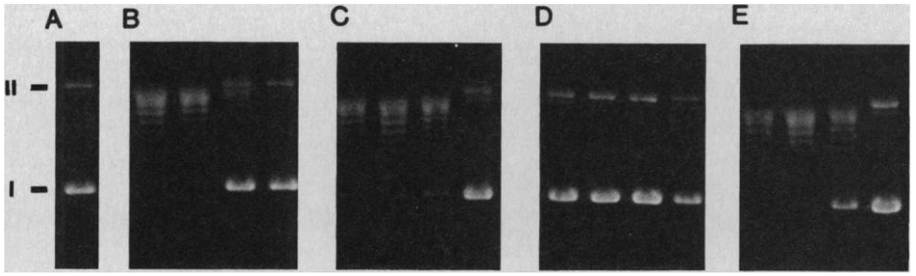

Fig. 3. Specific inhibition of topoisomerase I activity in crude chromosomal extracts by anti–Scl-70 sera. (A) Topoisomer distribution of the input pBR322 DNA. Positions of form I (supercoiled) and form II (open circle) topoisomers are indicated. Form I_{rel} (relaxed) comigrates with form II DNA in this gel. Panels B to E show the topoisomer distribution after incubation of the DNA with dilute extracts preincubated as follows: (B) no antibody, (C) normal human serum, (D) anti–Scl-70 serum, and (E) anti-centromere serum. Successive lanes in each panel represent treatment of the DNA with serial fivefold dilutions of extract. Topoisomerase I extracts were prepared from crude isolates of mitotic chromosomes (*22*). Inhibition of the enzyme activity was with a 50-fold dilution of serum (*23*). The topoisomerase I relaxation assay (with 1 μl of extract), electrophoresis, and photography were as previously described (*9*).

radioimmunoassay. For example, serum SC (Fig. 1B) was 13-fold higher than a normal control in the radioimmunoassay at the highest dilution tested (1:200,000).

Anti–Scl-70 autoantibodies quantitatively absorbed topoisomerase I activity from crude extracts. Extracts prepared by solubilizing proteins from mitotic chromosomes with 1*M* NaCl had high levels of topoisomerase I activity (Fig. 3). This activity was abolished when anti–Scl-70–positive sera were added to the extracts and immune complexes subsequently removed by adsorption to fixed *Staphylococcus aureus* (Fig. 3D), Anti–Scl-70–positive sera from three patients gave similar results. Normal sera and patient sera that were negative for anti–Scl-70 by immunoblotting had no effect on the enzyme activity (Fig. 3, C and E). Low levels of adenosine triphosphate–dependent DNA-relaxing activity (presumably due to topoisomerase II) remained in extracts absorbed with anti–Scl-70.

Anti–Scl-70 and anti–topoisomerase I were able to bind to an identical ladder of partial peptides after digestion of crude chromosomal and nuclear extracts with α-chymotrypsin in the presence of 0.1 percent SDS (*13*). Extracts were digested for 30 minutes with varying amounts of α-chymotrypsin, separated by SDS-PAGE, and analyzed by immunoblotting. The immunoblots with anti–Scl-70 and rabbit antibody to highly purified bovine topoisomerase I were virtually identical, while that with human anti-centromere–positive serum was completely different from the other two (Fig. 4). As a control, the anti-centromere antibodies were eluted from the nitrocellulose with 3*M* NH_4-thiocyanate (*4*), and the strip was reprobed with anti–Scl-70–positive serum. This revealed the characteristic topoisomerase I fragment pattern (Fig. 4D).

The interactions of topoisomerases I and II in vivo are complex, and may reflect homeostatic control of the level of chromosomal supercoiling (*14*). Both *Escherichia coli* and yeast lacking topoisomerase I are viable (*10, 15, 16*), presumably due to compensatory action of topoisomerase II. Double mutants lacking both enzymes are inviable (*10, 15*) and may give rise to subtle changes in nuclear structure (*15*). Thus, a combined genetic and immunochemical effort may be required to ultimately define the role of the enzyme in vivo. The high titer and high affinity anti–Scl-70 autoantibodies should supplement currently available experimental antibodies to topoisomerase I in establishment of such an approach. For example, autoimmune sera are useful for cloning complementary DNA's in bacterial expression systems (*17*), and human Scl-70 has recently been cloned and characterized (*18*). The immunological and complementary DNA reagents now available will be useful for further characterization of the synthesis, distribution, and function of topoisomerase I during the mammalian cell cycle.

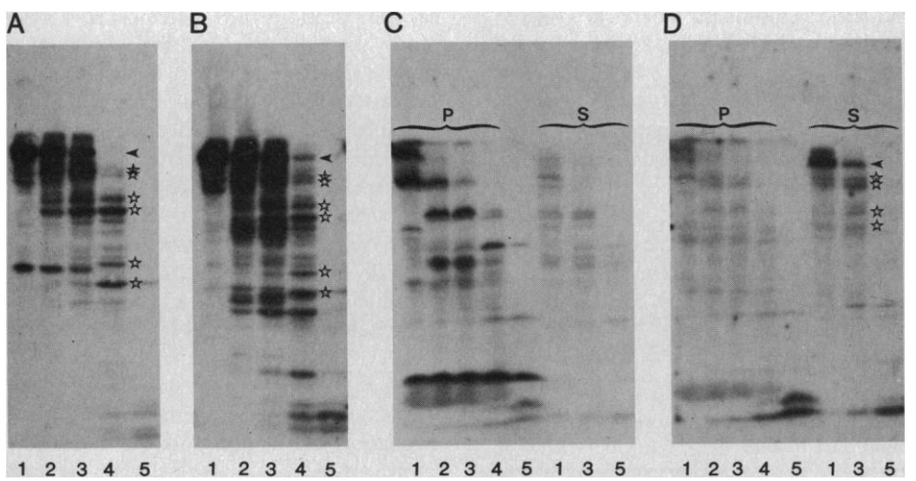

Fig. 4. Anti–topoisomerase I and anti–Scl-70 bind to common partial peptides generated by chymotryptic digestion of crude nuclear extracts. HeLa nuclei were resuspended in buffer S [0.4*M* NaCl, 5 m*M* tris (*p*H 7.4), 0.375 m*M* spermidine, 2 mMKCl] for 20 minutes at 4°C; insoluble material was removed by centrifugation at 16,500*g* for 10 minutes, and both pellet and supernatant were placed in sample buffer that contained SDS (*13*). Samples were incubated at 37°C with increasing concentrations of α-chymotrypsin for 30 minutes to obtain partial peptides (*13*). (Lanes 1) No enzyme; (lanes 2 to 5) α-chymotrypsin at 4 μg/ml, 20 μg/ml, 100 μg/ml, and 500 μg/ml, respectively. Digests were separated by SDS-PAGE in 15 percent gels and were immunoblotted (*3, 13*) with rabbit anti–topoisomerase I (A), human anti–Scl-70–positive serum (B and D), and human anti-centromere serum (C). Stars indicate proteolytic fragments recognized by antisera. The arrowheads show the position of undegraded antigen (100 kD). In the control experiment (C and D) both the salt-insoluble (P) and salt-extracted (S) fractions are shown. (A and B show only the S fractions). The centromere antigens occur exclusively in the salt-insoluble fraction (*3*). (D) The same nitrocellulose strip after elution of the anti-centromere antibody and reincubation with anti–Scl-70 serum. Scl-70 was only detected in the soluble fraction (D).

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The Effects of Ivermectin on Transmission of *Onchocerca volvulus*

E. W. CUPP, M. J. BERNARDO, A. E. KISZEWSKI, R. C. COLLINS, H. R. TAYLOR, M. A. AZIZ, B. M. GREENE

Ivermectin, given to onchocerciasis patients as a single oral dose of 200 micrograms per kilogram of body weight, substantially reduced the uptake of *Onchocerca volvulus* microfilariae by *Simulium yahense*, an efficient black fly vector of the parasite in the tropical rain forests of West Africa. Three months after treatment, patients given ivermectin infected flies at a significantly lower rate than those who had received diethylcarbamazine or placebo, thereby reducing the number of developing larvae in the vector population. This diminished rate of infectiousness was also evident 6 months after treatment. These results strongly suggest that ivermectin could be effective in interrupting transmission of *Onchocerca volvulus* for epidemiologically important periods of time.

HUMAN ONCHOCERCIASIS IS ENdemic in parts of sub-Saharan Africa, the Arabian Peninsula, southern Mexico, Guatemala, Venezuela, Brazil, Colombia, and Ecuador. An estimated 20 to 40 million people suffer from this parasitic disease, which is caused by the filarial nematode *Onchocerca volvulus* (*1*). The adult worms reside in subcutaneous nodules or occasionally lie free in the subcutis (*2*). Females, which may live more than a decade, produce thousands of embryonic forms, the microfilariae. This stage of the parasite is also subcutaneous and actively migrates through the skin where, in concert with the host's immune response, it precipitates intense pruritus with papular rash, depigmentation, atrophy, and a variety of eye lesions. Microfilariae are relatively long-lived and may survive in the skin for up to 30 months (*3*).

The severest form of the disease occurs in the African savannah, where the parasite is transmitted by the bites of blood-sucking female flies belonging to the *Simulium damnosum* species-complex (*4*). The immature stages of the fly live in aquatic habitats; hence parasite transmission occurs in or near these riverine settings. For this reason and because chronic infection with the parasite often results in ocular lesions leading to impaired vision and irreversible blindness, human onchocerciasis has come to be called "river blindness."

The disease has traditionally been controlled by the application of insecticides to infested streams and rivers as a means of killing the larval stage of the black fly vector. A vector control program has been initiated by the World Health Organization in the savannah region of West Africa in an attempt to lower annual transmission rates of the parasite to a level that significantly reduces the risk of blindness (*5*). This program encompasses over 700,000 km², embraces seven countries, and is designed to protect over 10 million people (*1*). The results are encouraging, but the development of insecticide resistance (*6*) and reinfestation of the program area loom as potential problems of considerable magnitude (*7*).

Chemotherapy of human onchocerciasis has been limited to the use of diethylcarbamazine (DEC) for the microfilarial stage of the parasite and suramin as a macrofilaricide (*8, 9*). However, both drugs produce frequent side effects and sometimes major clinical complications (*10*). Ivermectin, a novel semisynthetic drug exhibiting a wide range of pharmacological activity against parasitic nematodes and arthropods of veterinary importance (*11*), has also been shown to be efficacious against the microfilarial stage of *O. volvulus* (*12*). When compared with DEC in a recent double-blind, placebo-controlled trial, ivermectin was shown to be more clinically acceptable as well as more effective in reducing microfilarial skin populations as determined by skin biopsies ("skin snips") (*13*).

We report here the results of several field experiments that complement this clinical evaluation and demonstrate that ivermectin treatment reduced transmission of the parasite for periods of time that could be epidemiologically important [this effect is short-

Table 1. Uptake of microfilariae (mf) (expressed as geometric means) by *Simulium yahense* from patients receiving placebo, DEC, or ivermectin at 3 and 6 months after treatment.

Treatment group	*n*	Mean mf per mg of skin	No. of flies dissected	Mean mf* per fly ($\bar{x}$)	Thoracic mf per fly ($\bar{x}$)	Flies with thoracic mf (%)
			At 3 months			
Placebo	4	40.77	28	34.80	14.10	96.4
DEC	5	12.28	59	4.95†	1.99	57.6
Ivermectin	3	0.54	25	0.32†‡	0.10	12.0
			At 6 months			
Placebo	4	49.63	48	10.52	3.06	75.0
DEC	5	20.05	56	4.26§	2.11	53.6
Ivermectin	3	8.60	34	2.28†‖	0.56	32.4

*Means compared by means of the *t* test with Bonferroni equation. †Significantly different from placebo at $P < 0.001$. ‡Significantly different from DEC at $P < 0.001$. §Significantly different from placebo at $P < 0.008$. ‖Not significantly different from DEC.

E. W. Cupp, M. J. Bernardo, A. E. Kiszewski, Department of Entomology, Cornell University, Ithaca, NY 14853.
R. C. Collins, 14155 N. Wentz Road, Marana, AZ 85238.
H. R. Taylor, International Center for Epidemiologic and Preventive Ophthamology, Wilmer Ophthamologic Institute, Baltimore, MD 21205.
M. A. Aziz, Merck, Sharp, and Dohme Research Laboratories, Post Office Box 2000, Rahway, NJ 07065.
B. M. Greene, Department of Medicine, Case Western Reserve University, University Hospitals of Cleveland, Cleveland, OH 44106.