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Chromosome Studies in Scleroderma with Consideration of Anticentromere Antibody Status and Assessment of Possible in vitro Clastogenic Activity

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The constitutional karyotype and frequency of sporadic chromosome abnormalities in peripheral blood leukocytes from 30 scleroderma patients and 15 normal controls were studied. Fifteen of the scleroderma patients were positive for the anticentromere antibody (ACA) and 15 were negative. The constitutional karyotype of all patients and controls were normal. No statistically significant difference in sporadic chromosome abnormalities was detected among the two groups of scleroderma patients compared with the control group. The possibility of clastogenic activity in serum from scleroderma patients was investigated by culturing lymphocytes from three normal individuals in medium enriched with serum from either a normal control, an ACA-negative scleroderma patient or an ACA-positive scleroderma patient. There was no statistically significant difference in the frequency of sporadic chromosomal abnormalities among the cells in these experiments. The results of this study suggest that, contrary to previously reported studies, the frequency of sporadic chromosome abnormalities is not increased significantly in scleroderma patients. In addition, although the anticentromere antibody is reactive with chromosomal material, patients with this antibody do not have increased chromosome breakage or aneuploidy, and the antibody does not induce chromosomal changes in vitro. (Received December 16, 1985.)

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There has not been any consistent constitutional chromosome abnormality associated with scleroderma. However, some investigators have reported a high frequency of random chromosome breakage and excessive aneuploidy in lymphocytes from patients with scleroderma (systemic sclerosis) (1–7). It has been suggested that these chromosomal abnormalities occur about three times more frequently among metaphases from scleroderma patients than from normal controls (8), and this is reported to occur in about 95% of scleroderma patients. The chromosome abnormalities reportedly were not accounted for by the age of patients, radiation exposure or drug ingestion (9). Thus, they are presumed to be associated with the patients disease. In one experiment, lymphocytes of healthy donors cultured in medium enriched with serum from a scleroderma patient were reported to exhibit excessive chromosome breakage (10). Thus, it has been postulated that there might be a clastogen in the sera of patients with scleroderma that may induce chromosome damage.

The anticentromere antibody (ACA) has been found in the sera of some patients with scleroderma (11, 12). This antibody is reactive with a DNA-histone protein antigen in the kinetochore region of the centromere area of chromosomes (12, 13). The ACA is found mainly in patients with the CREST (calcinosis cutis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) variant of scleroderma (14–17) which generally

has a slowly progressive and relatively benign clinical course. The significance of ACA to the etiology or pathogenesis of the CREST syndrome is as yet unknown.

The influence of the ACA on chromosome segregation in cell division and on the frequency of sporadic chromosome abnormalities is not known. We undertook this study to establish the frequency of sporadic chromosome abnormalities in 15 ACA-positive scleroderma patients, 15 ACA-negative scleroderma patients and 15 ACA-negative normal controls. To determine if the ACA was associated with a serum clastogen and could induce chromosomal damage in lymphocyte cultures in vitro, we cultured cells from three ACA-negative normal patients grown in media enriched with serum from either ACA-positive or ACA-negative scleroderma patients and examined them for the presence of chromosome abnormality.

METHODS AND MATERIALS

Anticentromere antibody test

Anticentromere antibody screening was carried out on all the individuals studied using a previously described technique (17). Briefly, sera was diluted 1 : 40 in veronal buffer and incubated on a HEp 2 cell line substrate (Antibodies, Inc.). Fluorescent conjugated goat anti-human IgG (Coppel Labs.) was then incubated on the substrate and slides were examined using a Leitz Ortholux II epilumination fluorescence miscroscope. The ACA was recognised by its characteristic staining patterns in the different phases of mitosis. The selectivity of staining for the centromere region was confirmed on metaphases of human osteogenic sarcoma cells.

Chromosome analysis

The cytogenetic investigators were not told either the clinical diagnosis or the ACA status of any of the patients until the study was completed. Chromosome analysis was done on 15 ACA-positive scleroderma patients, 15 ACA-negative scleroderma patients, and 15 ACA-negative normal volunteers using the standard procedure for culturing peripheral blood and analysis using GTG-banding (18). This method involved 10 ml of heparinized peripheral blood. Lymphocyte cultures with phytohemagglutinin stimulation were incubated for 72 hours. Cells were harvested with 2.5 μg/ml actinomycin D and 0.05 μg/ml Colcemid for 45 min. Cells were then treated with hypotonic 0.075 M KCL and fixed in methanol-glacial acetic acid (3 : 1). Air dried slide preparations were stained for GTG-banding with Giemsa stain and trypsinization. Thirty metaphases were analysed and photographed from each individual: all abnormalities in structure or number of chromosomes were noted. The results of the chromosome abnormalities in the two groups of scleroderma patients were compared with each other and with the control group.

Experiment with different sera

Leukocytes from each of three normal volunteers were cultured in three different ways. In one instance, their cells were grown in media enriched with 10% serum from a normal ACA-negative individual. In another instance, their cells were cultured in 10% sera from an ACA-negative scleroderma patient. In the third instance, their cells were grown in 10% serum from an ACA-positive scleroderma patient. All cultures were incubated and harvested simultaneously in the same manner as described above. The cytogenetic investigators were not aware of the source of the cells and the culture procedure until all their studies were completed.

RESULTS

Demography

The mean age of the 15 ACA-negative scleroderma patients was 43.3 years and ranged from 14 to 80 years: this group consisted of 9 females and 6 males. The mean age of the 15 ACA-positive scleroderma patients was 57.3 years, ranged from 38 to 66 years, and comprised 14 females and 1 male. The mean age of the 15 ACA-negative normal controls was 38 years and ranged from 21 to 61 years: this group contained 14 females and 1 male.

The duration of scleroderma in the ACA-negative group ranged from 3 months to 32 years with a mean of 12 years, while in the ACA-positive scleroderma group the disease duration range was from 7 months to 40 years with a mean of 19 years. Patients with ACA-negative scleroderma generally had more extensive cutaneous sclerosis and more systemic involvement than patients that were ACA-positive. They were also receiving more systemic medications, including d-penicillamine and corticosteroids. Radiation exposure was similar in both groups of scleroderma patients.

Chromosome analysis

None of the patients had a constitutional chromosome abnormality. The results of chromosome analysis for the 30 scleroderma patients and 15 controls are shown in Table I. The same number of cells (30) were studied from each patient. Thus, a total of 450 metaphases were examined from each group. The frequency of cells and patients with minor chromosome abnormalities such as chromatid and chromosome gaps were similar among the three groups. There was nearly twice as many cells with major anomalies such as deletions, translocations, acentrics, and markers in the two scleroderma groups than in the control group. However, over 96% of cells were normal in each group. Thus, these differences between the groups were not statistically significant. There was no apparent consistent breakpoint of either minor or major abnormalities among the three groups.

The frequency of cells with autosomal aneuploidy and the number of patients affected was similar among the three groups. The same trend seems to apply to the gain of X chromosomes. Interestingly, the ACA-positive scleroderma patients tended to have the most loss of X chromosomes, but this did not prove to be statistically significant.

Experiment with different sera

The chromosome results for the cultures from 3 normal individuals grown in normal serum, ACA-negative serum, and ACA-positive serum are shown in Table II. Thirty metaphases were analysed for each culture for each normal individual. Thus, a total of 90 metaphases per patient were analysed. The frequency and types of chromosome abnormalities were similar among the three groups and the small differences proved not to be statistically significant.

Table I. *Summary of chromosome abnormalities from 30 metaphases from each of 15 ACA-negative normal controls, 15 ACA-negative and 15 ACA-positive scleroderma patients*

*ct = chromatid anomalies; cs = chromosome anomalies; del = deletions; t = translocations; ace = acentric fragments; mar = markers; +autosome and −autosome = gain or loss of 1 or more autosomes; +X and −X = gain or loss of X chromosomes

Type of Abnormality*	Controls		Scleroderma ACA-negative		Scleroderma ACA-positive	
	Cells	Pats	Cells	Pats	Cells	Pats
ct, cs	52	14	46	12	39	13
del, t, ace, mar	7	6	15	8	17	11
+autosome	4	3	2	2	6	4
−autosome	25	12	41	14	28	13
+X	8	6	3	2	7	6
−X	5	3	6	3	30	8

Table II. *Chromosome abnormalities from metaphases from three normal individuals each cultured in three different sera*

*ct = chromatid anomalies; cs = chromosome anomalies; del = deletions; t = translocations; ace = acentric fragments; mar = markers; +autosome and − autosome = gain or loss of 1 or more autosomes; +X and −X = gain or loss of X chromosomes

Type of abnormality*	Normal serum (cells)	Scleroderma ACA-negative serum (cells)	Scleroderma ACA-positive serum (cells)
ct, cs	16	19	21
del, t, ace, mar	0	0	2
+autosome	0	0	0
−autosome	3	2	2
+X	0	0	0
−X	1	0	0

DISCUSSION

There are previous reports of chromosome studies that suggest that among cells from scleroderma patients, there is an increased frequency of sporadic chromosome breakage and aneuploidy (9, 19). None of these reported studies were blind investigations however. In both the ACA-negative and ACA-positive group, we found that the frequency of sporadic translocations, deletions, acentric fragments and marker chromosomes was low, but the frequency was more than twice the control group. We also found that there tended to be more loss of X chromosomes among the ACA-positive scleroderma patients than among the controls. However, these results did not prove to be significantly different considering the numbers of cells studied in each group and metaphases examined. Thus, we were unable to demonstrate a significant increase in either chromosome breakage or aneuploidy among the participants of this study.

It has been suggested that patients with scleroderma may possess a serum clastogen that might cause chromosome breakage. However, there was no evidence of increased chromosome breakage in our experiment exposing cells from a normal individual to serum from scleroderma patients that were either ACA-negative or ACA-positive. Thus the ACA in scleroderma does not appear to have clastogenic properties in vitro.

The influence of the ACA on the frequency of chromosome breakage and aneuploidy is not yet known. The results of this study would suggest that the frequency of either structural chromosome abnormalities or aneuploidy may not be affected by its presence. The frequency of chromosome abnormalities among our ACA-positive scleroderma patients was very similar to the ACA-negative scleroderma patients and ACA-negative control group.

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