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Modification of DNA patterns in plasma and nucleated blood cells from systemic sclerosis patients

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ABSTRACT

Objective

To analyze the DNA patterns extracted from plasma and nucleated blood cells (lymphocytes) in systemic sclerosis (SSc) with a new MFC DNA extracting kit.

Methods

Ten SSc patients and 9 healthy controls were studied. Heparin containing blood samples were separated into plasma and buffy coat fractions and subjected to DNA extraction. The DNA pattern was revealed by 0.4% agarose electrophoresis and analyzed in a Gel - blot Programme file (UVP Product).

Results

In control samples the DNA pattern observed in plasma extract was differ - ent from that of the buffy coat. For the plasma a series of peaks ranging from 2-23 Kb were present, and for the buffy coat we usually observed 2 to 3 princi - ple bands, respectively, at around 33 Kb and 0.5 Kb. For SSc patients the DNA patterns that resulted from the plasma and buffy coat were totally dif - ferent from the control samples, with some exceptions.

Conclusion

We observed that SSc samples contain a distinctively different DNA pattern compared to healthy controls. Further studies are needed to establish whether or not this DNA pattern might be con - sidered peculiar to SSc, and whether or not the method is a useful tool for path - ogenic studies of the disease and for diagnostic purposes.

Introduction

It is known that the plasma of patients affected by cancer contains higher DNA levels than healthy individuals. It is also known that DNA mutation can be detected in the plasma or serum of cancer patients and that altered patterns of circulating DNA can be also found in these sera, probably due to the modification of the DNA structure, size and form (for a review, see ref. 1). Higher serum and plasma DNA levels have also been found in systemic lupus erythematosus and rheumatoid arthritis (1), but no information is available in the literature regarding the serum DNA pattern in these and other autoimmune disorders. It is conceivable that in chronic inflammatory diseases immune reactive cells such as nucleated white blood cells/lymphocytes might be involved in the remodeling of their gene operation system, either actively or imposed by pathogens. We hypothesize that the first sign of this change could be the alteration of their DNA pattern, which should be possible to detect both in serum and in the DNA extracted from circulating immunocompetent cells with the use of appropriate technology.

Systemic sclerosis (SSc) is an autoimmune condition characterized by an inflammatory response, with lymphocyte infiltration in different organs and tissues (2). As in SSc a higher prevalence of chromosomal alterations have been found (3, 4) compared to other autoimmune disorders, we analyzed samples extracted from the plasma and peripheral lymphocytes of SSc patients with the purpose of verifying whether it is possible to detect altered DNA patterns in this condition and whether the DNA pattern could be typical of SSc. In order to test this hypothesis we used a new DNA extraction procedure (5).

Materials and methods

Ten SSc patients, 7 with diffuse (dSSc) and 3 with limited scleroderma (lSSc) (6) ranging in age between 29 and 71 years, and 9 control subjects from 26 to 78 years of age were enrolled in the study (Tables I and II). Out of 7 dSSc patients, 6 had anti-Scl70 antibodies (aScl70) and one had anti-centromeric antibodies (ACA), while out of 3 lSSc patients 2 were ACA positive and one was aScl70 positive. At the time of the study all patients were treated with prostacycline infusions and vasodilators, while 2 patients received low corticosteroid therapy.

The procedure for the DNA extraction from plasma and nucleated white blood cells was carried out according to the method described in the Patent Pending (5). The reagents supplied as a MFC DNA extraction kit were kindly provided by the Patent Pending owner for a clinical trial. Briefly, the DNA extraction method was carried out as follows. 5 ml blood was collected in a heparin

containing tube and analyzed within 3 hrs; after centrifugation at 3000 rpm for 5 min, the plasma and buffy coat (BC) fractions were carefully harvested. 0.4 ml of each sample was added the same volume of Tris buffer saline pH 7.8 (Sol. A) in a 2 ml Eppendorf tube; the tubes were boiled for 5 min and then centrifuged in a MSE Micro Centrifuge at 13000 rpm for 5 min. Then 1/10 vol of Sol. B (10% SDS in Sol. A) was added to the supernatant; after centrifugation at 13,000 rpm for 3 min, 1/5 vol of Sol. C (2M KCl) was added to the sample which was then incubated at 25°C for 10 min. The tubes were centrifuged again at 13,000 rpm for 20 min and the supernatants (30-60 l) were ready for further analysis in agarose (0.4%) containing ethydium bromide as usual. The DNA pattern was documented by an UVP ImageStore 7500 and subsequent analysis in an Uvp GelBase-GelBlot Programme. Statistical analysis was carried out using the method described by Snedecor and Cochran (7).

Results

The typical DNA patterns obtained from control subjects and SSc patients are shown in Fig. 1 (A and B). The pattern was divided into 4 arbitrary peaks (I, II,III and IV) with apparent molecular weights (MW) of 33, 4-23, 2 and 0.5 Kb, respectively.

The results are summarized in Tables I and II. Two distinctive aspects are noticeable among the control group (Table I) and the SSc patients (Table II). The common features of the SSc samples were: 1) in plasma, the lack of a lower molecular weight band (peak III) around 2 kb (see Fig. 1 B, plasma); 2) in buffy-coat, a remarkable alteration of the DNA pattern showing only one wide band (peak IV) with a molecular weight of around 0.5 Kb (see Fig. 1, B BC). This latter phenomenon was found in up to 60% of tested patients. Twenty percent of patients exhibited a minor modification in DNA motility, and to the same extent, 20% showed a pattern similar to the control subjects. By observing the combined data obtained from plasma and BC, it is evident that the DNA patterns were completely altered with respect to the control groups. No differences were found between patients carrying aScl70 antibodies, and patients with ACA antibodies.

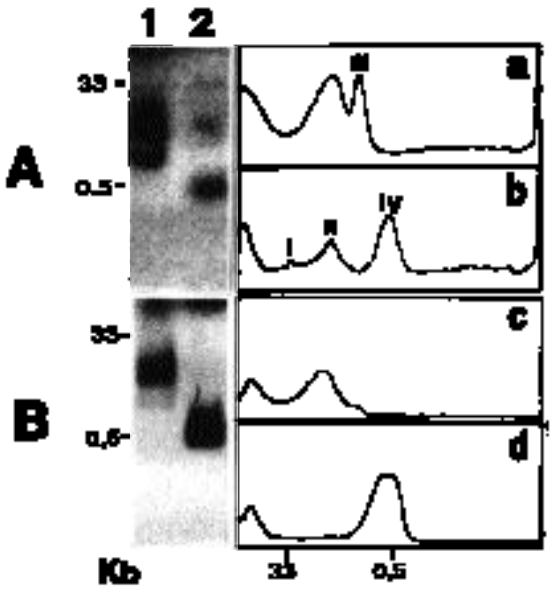

Fig. 1. Typical DNA patterns in plasma and nucleated blood cells in control (**A**) and SSc patients (**B**). For agarose gel electrophoresis,line 1 = plasma and line 2 = buffy coat. **a**, **b**, **c**, and **d** show the curves scanned using theUVP GelBase-GelBlot Programme respectively for A line 1,A line 2,B line 1,and B line 2. Peaks I, II, III, IV indicate the molecular sizes of 33, 4-23, 2 and 0.5 Kb, respectively.

Table I. DNA patterns from plasma and nucleated white blood cells in 9 control subjects.

Pt. no.	Age/ Sex	Skin subset §	Dis dur. years	DNA patterns* Plasma I	II	III	IV	Buffy coat I	II	III	IV
1	30 F	-	-	-	+	+	-	+	-	-	+
2	33 F	-	-	-	+	+	-	+	+	-	+
3	35 M	-	-	-	+	+	-	+	-	-	+
4	41 M	-	-	-	+	+	-	+	-	-	+
5	43 F	-	-	-	+	+	-	+	+	-	+
6	44 M	-	-	-	+	+	-	+	+	-	+
7	50 M	-	-	-	+	+	-	+	-	-	+
8	62 F	-	-	-	+	+	-	+	+	+	+
9	78 F	-	-	-	+	-	-	+	+	+	-

Mean ± SD 42.5 ± 6.24 for M (4)
49.2 ± 20.4 for F (5)
46.22 ± 15.3 for M + F

§Skin subset: D= diffuse; L= limited.
*DNA patterns: I = 33 Kb; II = 4-23 Kb; III = 1-2 Kb; IV = 0.5 Kb.

Discussion

Increased levels of circulating DNA have been demonstrated in some autoimmune disorders (8), but their clinical and pathological significance is unknown. In addition, the pattern of DNA in these conditions was not evaluated while it has been demonstrated that patients with cancer not only have higher circulating DNA levels, but often show peculiar patterns of circulating DNA (for a review, see ref. 1).

Our study demonstrates that, by using an MFC DNA extraction kit (5), it is possible to find a remarkable modification of circulating DNA patterns in SSc patients. We can also confirm that these alterations were not drug-related as our patients were not taking any potential clastogenic compound at the time of

Table II. DNA patterns from plasma and nucleated white blood cells in 10 SSc patients.

Pt. no.	Age/ Sex	Skin subset §	Dis dur. years	DNA patterns* Plasma I	Plasma II	Plasma III	Plasma IV	Buffy coat I	Buffy coat II	Buffy coat III	Buffy coat IV
1	29 M	D	3	-	+	-	-	-	-	-	+
2	46 F	D	3	-	+	-	-	-	-	-	+
3	50 F	L	5	-	+	-	-	-	-	-	+
4	60 F	D	9	-	+	-	-	-	-	-	+
5	60 F	D	7	-	+	-	-	+	-	-	+
6	60 F	D	8	-	+	-	-	-	+	+	+
7	61 F	L	39	-	+	-	-	-	+	+	+
8	67 F	L	8	-	+	-	-	-	-	-	+
9	68 F	D	5	-	+	-	-	-	-	-	+
10	71 F	D	25	-	+	+	-	+	+	-	+
Mean ± SD	29.0 ± 0 M (1)		3.0 ± 0 M (1)								
	60.33 ± 8.13 F (9)		12.11 ± 11.9 F (9)								
	57.27 ± 12.5		11.2 ± 11.6								
	M + F (10)		M+F (10)								

§ Skin subset: D= diffuse; L= limited

* DNA patterns: I = 33 Kb; II = 4-23 Kb; III = 1-2 Kb; IV = 0.5 Kb..

the study. According to recent studies performed in cancer patients, it can be hypothesized that the modification of the DNA pattern may be due to DNA mutations, or alterations (1) that can also occur in patients affected by an autoimmune disorder such as SSc. In fact, chromosomal damage can be seen early in the disease, both by cytogenetic study (3) and by FISH analysis (4). It was suggested that the chromosomal abnormalities and DNA mutations frequently seen in SSc, may be due to the presence of circulating clastogenic factors that might be endogenous or exogenous or both (9). An alternative explanation takes into account the possibility that lymphocytes respond to the chemical or pathological challenge by synthesizing episoms/extra-chromosomal DNA (eDNA) (10-15) whose form, structure and size could be characteristic of different diseases depending upon the pattern of immune response that characterizes each pathological condition. Our findings constitute an unprecedented scientific and clinical endeavor and, if the results shown in this pilot study are confirmed in a larger group of scleroderma patients and in subjects with other autoimmune disorders, it will be possible to hypothesize that, by using a simple and feasible method, the study of DNA patterns in serum and in peripheral lymphocytes might represent a useful tool for gathering new insights into the physiopathology of autoimmune disorders as well as for diagnostic purposes.

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