EXTENDED REPORT

Correlations of Y chromosome microchimerism with disease activity in patients with SLE: analysis of preliminary data

M Mosca, M Curcio, S Lapi, G Valentini, S D'Angelo, G Rizzo, S Bombardieri

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See end of article for authors' affiliations

Correspondence to: Dr M Mosca, Department of Internal Medicine, Rheumatology Unit, University of Pisa, Via Roma 67, 56126 Pisa, Italy; marta@sssup.it

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Background: Recently it has been suggested that microchimerism may have a significant role in the aetiopathogenesis of some autoimmune diseases.
Objectives: To evaluate the incidence of microchimerism in systemic lupus erythematosus (SLE), to quantify the phenomenon, to evaluate changes of microchimerism during follow up, and to correlate these data with clinical and laboratory variables.
Methods: Patients were selected for the study on the basis of the following criteria: (*a*) pregnancy with at least one male offspring; (*b*) no history of abortion and blood transfusion. Microchimerism was detected using a competitive nested polymerase chain reaction for a specific Y chromosome sequence and an internal competitor designed ad hoc. Disease activity and organ involvement were also evaluated.
Results: Sixty samples from 22 patients with SLE and 24 healthy controls were examined. Microchimerism was seen in 11 (50%) patients and 12 (50%) controls. The mean number of male equivalent cells was 2.4 cells/100 000 (range 0.1–17) in patients with SLE and 2.5 (range 0.2–1.8) in healthy controls. No differences in the incidence of microchimerism or in the number of microchimeric cells were found between patients and healthy controls. Patients with a history of lupus nephritis had a higher mean number of fetal cells than patients with no such history. Disease activity did not appear to correlate with microchimerism.
Conclusions: The preliminary data suggest that microchimerism does not interfere with the disease course of SLE, although further analysis on larger groups will be necessary to confirm these observations.

The development of microchimerism after solid organ transplantation, blood transfusion, and pregnancy is a well recognised phenomenon.[1–4] The similarities between some autoimmune diseases and chronic graft versus host disease, which can develop after bone marrow transplant, have suggested that microchimeric cells of fetal or maternal origin may have a role in the pathogenesis of some autoimmune diseases.[5] Studies conducted in patients with scleroderma (SSc) have shown a significantly higher prevalence of microchimerism in patients (range 46–61.5%) than in healthy controls (range 4–50%).[5–9] Following these observations, the presence of microchimerism has been demonstrated in other autoimmune conditions, such as juvenile idiopathic inflammatory myopathies (IIM),[10 11] Sjögren's syndrome (SS),[12 13] primary biliary cirrhosis (PBC),[14] and systemic lupus erythematosus (SLE).[15] Together with the finding of fetal cells in maternal peripheral blood, the presence of cells in various maternal tissues has been demonstrated. Because the immunological alterations that characterise autoimmune diseases may differ greatly, one may suggest that chimeric cells "interfere" with the natural history of autoimmune diseases by different mechanisms.[16–20]

Examination of the correlation between microchimerism and the clinical and serological manifestations presented by a patient might shed light on the role of microchimerism in autoimmune conditions. To our knowledge no data are so far available on the presence of microchimerism in the peripheral blood of patients with SLE. Therefore, this study aimed at (*a*) evaluating the percentage of patients with SLE with microchimerism and quantifying the phenomenon; (*b*) correlating the data with the clinical and laboratory variables found at the time of the analysis; (*c*) evaluating changes in microchimerism over time (the presence/absence and quantity of male equivalent cells).

In this paper we present some preliminary data from our analysis.

PATIENTS AND METHODS

Patients

The study groups comprised 46 subjects: 22 patients with SLE being followed up at our units and 24 healthy controls selected on the basis of the following criteria: (*a*) a previous pregnancy with a male offspring; (*b*) no history of abortion and blood transfusions. Healthy subjects with no history of pregnancies or blood transfusion were also analysed as negative controls. The diagnosis of SLE was made on the basis of the 1996 revised criteria.[21] Clinical and epidemiological data on the patients with SLE were collected from their clinical charts and by direct interview and disease activity was evaluated with the European Consensus Lupus Activity Measurement (ECLAM) index.[22] Epidemiological and clinical data for the healthy controls were obtained by direct interview.

Standard, validated techniques were used for the laboratory analyses. Indirect immunofluorescence was used to detect the antinuclear and anti-dsDNA antibodies, with HEp-2 cells and rat liver cells as antigen sources for the antinuclear antibodies

Abbreviations: ECLAM, European Consensus Lupus Activity Measurement; IIM, idiopathic inflammatory myopathies; PBC, primary biliary cirrhosis; PCR, polymerase chain reaction; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; SSc, systemic sclerosis

Table 1 Primer sequences for the nested PCR

First step	
MIF-I	CAGGTGGAGCTGGAGCCGG
MIR-I	CAGGTGGAGCTGGAGCCGG
Second step	
MIF-II	AGGAAGGCCTTTTCTCGGCAG
MIR-II	CCGAAGAAACACTGAGAAGGATA

and *Crithidia luciliae* for the anti-dsDNA. Counterimmunoelectrophoresis was used to detect the anti-extractable nuclear antigen antibodies (anti-SSA/Ro, anti-SSB/La, anti-RNP, anti-Sm, anti-Scl-70, anti-Jo-1, anti-Ku). An enzyme linked immunosorbent assay (ELISA) was used to detect anticardiolipin antibodies.

Methods

Genomic DNA was isolated from EDTA anticoagulated blood under a biosafety hood to avoid any contamination. A specific Y chromosome sequence was detected by amplifying DNA in a nested polymerase chain reaction (PCR), using primers designed ad hoc by our laboratory (reported in table 1). Each PCR amplification step included positive (male DNA) and negative (no DNA) controls to detect PCR contamination. Specific Y chromosome DNA species were quantified by means of a competitive nested PCR using an internal standard and an automated DNA sequencer, according to Siebert *et al*.[23 24]

Statistical analysis

The Mann-Whitney U test was used to correlate the outcomes with the clinical, serological, and histological variables. For categorical variables the χ^2 test was used. Numerical variables were analysed using a correlation coefficient.

RESULTS

Forty six subjects, 22 patients with SLE and 24 healthy controls, were selected for the study on the basis of established criteria. Consecutive blood samples were analysed in 10 patients with SLE and a total of 60 blood samples were examined for the presence of Y chromosome microchimerism in the peripheral blood. Table 2 summarises the epidemiological data on the study subjects. No statistically significant differences were observed relative to the interval between the last male pregnancy and blood sampling for the research microchimerism.

Table 3 summarises the main clinical characteristics of the patients with SLE. In 14 patients with SLE pregnancy predated the onset of SLE (mean 8.1 years, min 1, max 23), while in eight patients pregnancy occurred a mean period of 6.8 years after the onset of the disease (min 2, max 11).

Y chromosome microchimerism was present in 12/24 (50%) healthy controls and 11/22 (50%) patients with SLE. Results were confirmed by direct sequencing of the amplification products, showing that no false positive results due to mispriming or aspecific amplification products were present.

Table 3 Main clinical characteristics of the patients with SLE

Variables	Results
Age at onset (years)	31 (15–47)
ECLAM at evaluation	2 (0–5)
Arthritis	82%
Raynaud's disease	59%
Photosensitivity	55%
Renal disease	50%
Non-haemolytic anaemia	50%
Leucopenia	45%
Serositis	41%
Malar rash	41%
Cutaneous vasculitis	36%
Thrombocytopenia	36%
Discoid rash	18%
Haemolytic anaemia	14%
Anti-dsDNA antibodies	68%
Anti-Ro/SSA antibodies	55%
Anticardiolipin antibodies	23%
Anti-RNP antibodies	18%

The mean number of male equivalent cells in positive samples was 2.4 cells/100 000 (range 0.1–17) in patients with SLE and 2.5 male equivalent cells/100 000 (range 0.2–1.8) in healthy controls. No statistically significant differences in the number of chimeric cells were seen between patients and controls.

Patients with renal disease (diagnosed by kidney biopsy in 10 patients, and on the basis of proteinuria with nephrotic syndrome and urinary sediment abnormalities in one patient) had a higher mean number of male equivalent cells than patients with no renal involvement (4.2 male equivalent cells *v* 0.89 male equivalent cells respectively; $p<0.05$).

No statistically significant correlations were found between the presence of microchimerism and the number of fetal cells, the disease activity measured by ECLAM (fig 1), or other manifestations presented by the patients before the evaluation.

In 10 patients with SLE consecutive samples were examined (four samples in one patient, three samples in two patients, and two samples in seven patients); the interval between samples ranged from 6 to 36 months. In three patients changes in the presence of microchimerism were seen (table 4): in two patients (Nos 1 and 2) disease activity remained low at the different evaluations, whereas in the third patient (No 3) a change in disease activity was seen, with an increase of activity corresponding with the absence of microchimerism. The first two patients were not receiving treatment at the time of the evaluation, whereas the third patient was receiving low dose steroids at the time of the first sample, high dose steroids at the time of the second sampling, and cyclosporin at the time of the last sampling.

Table 2 Epidemiological characteristics of the patients with SLE and healthy controls

	Patients with SLE (22)	Controls (24)	p Value
No of pregnancies (mean)	1.7 (1–2)	1.7 (1–4)	NS*
No of male births	1.5 (1–3)	1.5 (1–2)	NS
Age at first pregnancy	29 (19–37)	31 (24–39)	NS
Age at examination	44 (29–60)	48 (34–65)	NS
Interval between last son and examination (months)	187 (2–420)	212 (36–408)	NS

*NS, not significant

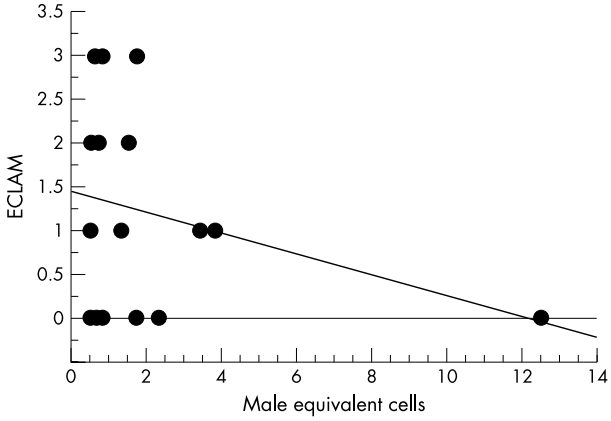

Figure 1 Correlation between the number of chimeric cells and the ECLAM index in patients with SLE.

Table 4 Characteristics of patients with SLE presenting changes of microchimerism in repeated samples

Patient	First sample	Second sample	Third sample
1	Negative ECLAM 0 No treatment	Positive (2.3 cells) ECLAM 0 No treatment	–
2	Positive (3.4 cells) ECLAM 1 No treatment	Negative ECLAM 0 No treatment	–
3	Positive (1.5 cells) ECLAM 2 Low dose steroids	Negative ECLAM 4 Pulse steroids	Positive (3.8 cells) ECLAM 1 Cyclosporin A

DISCUSSION

Microchimerism is a condition characterised by the presence in an individual (recipient) of a small amount of genetically distinct cells originating from a "donor". It is well known that microchimerism can develop after pregnancy, blood transfusions, and solid organ transplantation. However, the immunological significance of this phenomenon remains to be clarified.[1–4]

In 1998 Nelson *et al* tested the hypothesis that microchimerism may play a part in the pathogenesis of SSc. These authors found a higher incidence of microchimerism in patients with SSc than in healthy controls (26% *v* 59%) and showed that among subjects who were positive for Y chromosome microchimerism, the number of male cells was significantly higher in patients with SSc than in healthy controls (a mean of 0.38 male cells per 16 ml whole blood in healthy controls *v* a mean of 11.1 male cell DNA equivalents per 16 ml whole blood of patients with SSc).[5] In the same year Artlett *et al* found Y chromosome sequences in the peripheral blood mononuclear cells of 32/69 (46%) patients with SSc compared with 1/25 (4%) healthy controls. In the same paper the authors reported also the presence of Y-specific sequences in the skin lesions of patients with SSc (11/19 (58%) patients), thus providing further support for the hypothesis that fetal cells persisting in the mother may have a role in the aetiopathogenesis of the disease.[6] After these initial reports, many other authors evaluated the presence of fetal microchimerism in patients with SSc and obtained similar results (table 5).[7–9]

On the basis of these epidemiological data it has been suggested that microchimerism may have a role in the pathogenesis of some autoimmune diseases, particularly SSc, although its development in healthy subjects raises also the possibility that it is only an epiphenomenon with no clinical significance.[16–20]

Table 5 Analyses of microchimerism in studies of SSc patients reported in the literature

Author	No of patients	SSc (%)	Controls (%)
Nelson (1998)[5]	23 Controls, 17 SSc	59	26
Artlett (1998)[6]	25 Controls, 69 SSc	46	4
Murata (1999)[25]	12 Controls, 13 SSc	61.5	50
Evans (1999)[26]	48 Controls, 20 SSc	60	33
Lambert (2000)[27]	19 Controls, 12 SSc	42	47
Artlett (2000)[28]	63 Controls, 64 SSc	65	28

Some authors have evaluated the presence of microchimerism in other autoimmune conditions such as juvenile IIM,[10 11] SS,[12 13] and PBC.[14] Maternal microchimerism has been found in both the peripheral blood and muscle biopsies of juvenile IIM in two different studies. In contrast, negative results have been obtained in PBC by Invernizzi *et al*,[14] and a similar low incidence of microchimerism was recently reported in SS by Toda *et al* and Carlucci *et al*.[12 13] Many explanations have been proposed for these discrepancies, such as differences in the pathogenesis of the diseases, patient selection criteria, and the methods used for the detection of microchimerism.

Although the occurrence of microchimerism has been thoroughly studied in SSc, SS, and PBC, few data have been reported so far on microchimerism in patients with SLE. Recently, the presence of fetal cells in the tissues of a patient with SLE was described, suggesting that microchimerism may participate in the pathogenesis of some of the organ manifestations in this condition as well.[15]

In the present work we have studied microchimerism in patients with SLE by (*a*) evaluating its presence and the number of fetal equivalent cells; (*b*) by correlating its presence (and any changes over time) with disease activity and clinical manifestations.

Although our study was limited to a small number of patients, we did observe that (*a*) the incidence of microchimerism was high both in patients and controls; (*b*) there were no differences in the incidence of microchimerism or in the number of equivalent fetal cells between patients and healthy controls; (*c*) there were no correlations between disease activity (ECLAM) and microchimerism; (*d*) patients with renal disease had a higher mean number of male equivalent cells than patients with no renal involvement (4.2 equivalent cells *v* 0.89 equivalent cells respectively; $p<0.05$); (*e*) changes in microchimerism were present.

Our findings on the incidence of microchimerism and the levels of fetal equivalent cells are in agreement with previously published data. Direct sequencing showed that there were no false positive results. Furthermore, patients were selected on the basis of strict criteria, excluding those with a history of abortion or blood transfusion to avoid any confusion about the origin of Y chromosome bearing cells.

The observation of a higher number of fetal equivalent cells in subjects with renal disease may be of some interest; however, it must be reiterated that the number of patients in our study was small and that this analysis was carried out on peripheral blood and not at the time of onset of renal disease. Further analysis both on renal biopsy specimens and peripheral blood may provide more information on this issue.

Finally, changes in the presence of microchimerism were seen in 3/10 patients with repeated samples, although it was not possible to correlate these changes with clinical manifestations or disease activity.

In conclusion, our results suggest that microchimerism does not have a role in the disease course of SLE; however, in view of the relatively small number of patients examined thus

far and their low level of disease activity (as shown by the ECLAM scores) further analysis on larger groups seems necessary to better evaluate the evolution of microchimerism over time and to correlate this phenomenon with the clinical and serological variables of this autoimmune disease.

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Authors' affiliations

M Mosca, S Bombardieri, Rheumatology Unit, Department of Internal Medicine, University of Pisa, Italy
M Curcio, S Lapi, G Rizzo , Laboratory of Immunogenetics, Ospedale S Chiara, Pisa, Italy
G Valentini, S D'Angelo, Rheumatology Unit, Second University of Napoli, Italy

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