Role of microchimerism in the pathogenesis of oral lichen planus


Objective: Microchimerism of persistent fetal cells has been implicated in some cell-mediated autoimmune diseases. This study examines the hypothesis that fetal microchimerism plays a role in the pathogenesis of lichen planus (LP) affecting the oral cavity.

Study design: Mucosal biopsies of 12 women with oral LP (OLP) were tested for the presence of both male cells and male DNA originating from prior pregnancies or prior blood transfusions. Six male patients with OLP served as a control group. Biopsies were analyzed for the presence of Y-chromosome-positive cells by fluorescence in situ hybridization (FISH) with X- and Y-specific DNA probes. To confirm the FISH findings, we used fluorescent polymerase chain reaction (PCR) to identify Y-chromosome sequences in DNA extracted from mucosal lesions.

Results: Using FISH technology, all the 18 biopsy samples showed good hybridization results. In females, Y-chromosome-specific signals were not detected by FISH at any site of the lesions. PCR amplification demonstrated a single peak at the locus specific for the X-chromosome. Conclusion: Male DNA microchimerism was not present in any of the investigated lesions, suggesting that microchimerism because of persisting male fetal cells is unlikely to play a major role in the pathogenesis of OLP.

Key words: FISH – fluorescence PCR – microchimerism – oral lichen planus

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Introduction

Lichen planus (LP) is a chronic papulosquamous disease of unknown etiology, most commonly affecting middle-aged patients. Females have a three times higher prevalence than men. Patients with cutaneous LP show pruritic, faintly erythematous polygonal papules, preferentially on the flexural areas of arms and legs. The oral cavity is affected in approximately 60–70% of these patients. In up to 30%, oral lesions are the only manifestation of LP (1). Histologically, LP is characterized by infiltration of the lower levels of the epithelium by T lymphocytes causing basal cell destruction, apoptosis and secondary changes in epithelial thickness and maturation. These features, together with experimental evidence, are thought to represent a T-cell-mediated autoimmune response as the underlying mechanism for LP (2).

Numerous antigens may play a role in the pathogenesis of LP including drugs (e.g. β-blockers, calcium channel blockers) and topical agents (e.g. amalgam) (1).

Cutaneous and oral lesions resembling LP clinically and histologically are also found in chronic graft vs. host disease (GvHD). Hence, it has been suggested that, in some cases, the pathomechanism of LP may resemble that of GvHD (3).

Fetal microchimerism is defined by the persistence of fetal cells in the maternal circulation. The observation that fetal cells can persist for up to 27 years in the maternal circulation has led to the hypothesis that fetal microchimerism may trigger a fetus vs. host reaction and therefore may play a role in the pathogenesis of autoimmune disorders (4). In support of this hypothesis, studies of patients with systemic sclerosis revealed fetal cells both in lesional skin biopsies and peripheral blood samples (5,6).

Microchimerism has also been reported in a 9-year-old girl with an ulcerative acral variant of LP (7). However, two recent studies using an
enzymatic detection in situ hybridization technique failed to identify any evidence of microchimerism in biopsies of patients with sclerodermatous LP or oral LP (OLP) (8,9).

In the present study, we have used more sensitive methods of both fluorescence in situ hybridization (FISH) and fluorescence polymerase chain reaction (PCR) to investigate whether male fetal cells are present in OLP lesions.

Methods
The study population comprised 12 female patients with OLP seen at one of the following sites: Department of Oral and Dental Medicine, Department of Dermatology, both in Graz, Austria, or at the Department of Oral Medicine and Pathology at Guy’s Hospital, London, UK. Diagnosis of LP was made on clinical and histological grounds. Patients were enrolled in the study if mucosal striae or atrophy with striae were present. All patients had an incisional biopsy performed, and all of them met the standard criteria for diagnosis of OLP, showing a subepithelial lymphocytic infiltrate, lymphocytic infiltration of basal epidermis associated with apoptosis, colloid bodies, basal cell degeneration, and a fibrinoid or hyalinised basement membrane in the absence of dysplasia. Data on previous live births, abortions, or blood transfusion were recorded. Patients taking drugs known to be associated with lichenoid reactions were excluded. Six male patients with OLP were enrolled in the study and served as a control group. The study was conducted according to the guidelines of the local Ethics Committee.

Fish
Formalin-fixed paraffin-embedded mucosal tissue was used for in situ hybridization and fluorescent PCR of Y-chromosome DNA sequences to detect chimeric male fetal cells in the female patients’ mucosal biopsies. Four-micrometer thick paraffin-embedded tissue sections were mounted on positively charged slides. Tissue sections were subjected to an automated paraffin pretreatment instrument (VP 2000 Processor, Vysis Inc, Downers Grove, IL, USA) (10). After deparaffinization in xylene, the slides were transferred into two changes of 100% ethanol, followed by soaking in hydrochloroethanol. The slides were placed into the pretreatment reagent at 80% for 30 min and protease digested at 37°C for 10 min. The specimens were re-fixed in 10% neutral buffered formalin, rinsed in distilled water, and dehydrated in graded alcohol.

Pretreated specimen slides were placed on a Hybrite (Vysis Inc), held at 37°C, and 10 μl of the hybridization mixture (CEP X Spectrum-Orange and CEP Y Spectrum-Green, Vysis Inc) was applied to each section, cover-slipped and sealed with rubber cement (Fixogum, Marburg, Germany). The specimens and DNA probes were codenatured at 82°C for 5 min and hybridized at 37°C for 18 h. After hybridization, the rubber cement was removed and the slides soaked in ×2 saline sodium citrate (×2 SSC) with 0.1% NP 40 at room temperature until the cover-slips were removed. The slides were then immersed in ×2 SSC with 0.1% Nonidet 40 at 72°C for 2 min and then air dried in the dark. The nuclei were counter-stained with Dapi II (Vysis Inc) and viewed with a fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany) equipped with Dapi/green/orange/triple band pass, Dapi/green dual pass, Dapi/orange dual pass filter sets.

Fluorescence PCR
Five 5-μm paraffin-embedded tissue sections of each biopsy were placed into polypropylene tubes and the paraffin extracted with three washes in xylene and ethanol. After removal of the ethanol, the pellet was dried at 37°C. DNA was extracted by use of a QiAamp DNA mini kit (Quiagen, Valencia, CA, USA). The sequence of the first intron of X/Y homologous gene amelogenin (AMXY) was amplified (AMXY-F: CCCTGGGCTCTGTAAAGAATAGTG and AMXY-R: ATCAGAGCTTAAACTGGGAAGCTG). Additionally, primers for the sex-determining region SRY (SRY-F: TGGCGATTAAAGTCAAATTTGC and SRY-R: CCCC TAGTACCCTGACAATGTATT) (Oswel, Southampton, UK) located on chromosome Y were chosen for the detection of male DNA in female tissue. PCR amplification was performed in a total volume of 25 μl, containing approximately 50 ng extracted DNA, 200 μM dNTPs, 5–20 pmoles of each primer, 1 x Taq polymerase buffer (3 mM MgCl₂), and 1.5 U Taq polymerase (both Promega, Madison, WI, USA). After initial denaturation at 94°C for 5 min, hot start PCR was performed for 25 cycles at 94°C for 48 s, 60°C for 48 s, and 72°C for 1 min, and final extension was at 72°C for 5 min. The PCR products (3 μl) were mixed with 2.6 μl gel loading buffer and 0.4 μl Genescan-500 Rox (Applied Bio-systems Inc., Warrington, UK) containing the reference molecular-size standard. Electrophoresis was performed on a 373 DNA sequencer (Applied Bio-systems Inc.) using a 6% denaturing polyacrylamide gel. The amplification products were analyzed and their relative fluorescent intensities calculated using Genescan 672 software (Applied Bio-systems Inc.) as described by Perl et al. (11–13).

Serial dilution of male DNA in female DNA, using decreasing concentrations of male DNA in female DNA was performed. Male DNA was constantly detected using 0.1 ng of male DNA mixed with 100 ng of background female DNA on templates.

Results
OLP was diagnosed in all patients (12 females and 6 male controls) on clinical and histological grounds. The mean age of patients was 51.8 years (range: 27–71 years).

Biopsies in female patients were taken from the buccal mucosa in nine patients (75%), from the tongue in two (16.7%) and the lip in one patient (8.3%). Two (16.6%) of the 12 female patients had a history of previous blood transfusions, 10 (83.3%) previously gave birth to a son and five (41.6%) had a miscarriage or abortion, for which no information on the gender was available.

Three 4-μm thick paraffin-embedded tissue sections originating from biopsies with a diameter of 5 mm were investigated. A total number of 450 nuclei (150 per section) were screened for Y chromosomes. The DNA equivalent was approximately 50 ng per sample.

All biopsies from male control patients were positive for Y-chromosome-specific DNA by in situ hybridization and fluorescence PCR (Figs 1 and 2). In contrast, no Y-chromosome-specific DNA could be detected by in situ hybridization.
and fluorescence PCR in the mucosal biopsies of the female patients (Fig. 3).

The six OLP samples originating from male patients were also studied for the presence of female microchimerism. In none of these samples, intact nuclei with two X chromosomes were detected. 

**Discussion**

The hypothesis that microchimerism might contribute to some cases of LP derives from several lines of evidence. First, following bone marrow transplantation, there is an iatrogenic chimerism. Graft cells may mount a cell-mediated immune reaction against the host giving rise to chronic GvHD, and in a fraction of cases, chronic GvHD has striking clinical and histological similarities to LP (14). Second, microchimerism has been demonstrated to result from fetomaternal cell transfer during pregnancy. Microchimerism was reported as a possible cause of systemic sclerosis in a prospective and blinded qualitative comparison of female patients and healthy women (5). Male DNA was detected only in female patients who had given birth to sons. Additionally, women with systemic sclerosis had significantly higher levels of male DNA than healthy women.

LP does not show the characteristic profile of a classical autoimmune disease. LP has a histological and immunological appearance similar to GvHD and has putatively been associated with several immune-mediated conditions including primary biliary cirrhosis and postviral chronic hepatitis (2,15). Thus, the hypothesis that persisting fetal cells may cause a graft vs. host immune reaction seems to be a plausible explanation for the pathogenesis of LP.

However, in the present study, Y-chromosome-specific DNA indicating microchimeric male cells was not found in any of the OLP lesions of female patients, who had given birth to sons, had a history of previous miscarriage or blood transfusion. This negative result seems to be genuine, because both the tests used in the present study are of high sensitivity. In our female patients, male DNA was not detected by fluorescence PCR up to such low levels as 0.1 ng in the background of 100 ng of female DNA. Importantly, Artlett et al. (5) successfully identified Y-chromosome sequences in skin biopsy specimens from women with systemic sclerosis using a nested PCR technique. Although the nested
PCR used by Artlett et al. is more sensitive than fluorescence PCR, it is more prone to false-positive results (16). It might be possible that a positive PCR result was missed in the present study because of the lower sensitivity of the fluorescence PCR used. However, in the present study, PCR has been complemented by fluorescence in situ hybridization to detect signals from single cells. Yet, using both the tests, microchimerism was not discovered in any of the investigated lesions.

Our negative results are in agreement with two recent studies, investigating the role of microchimerism in cutaneous LP and OLP, respectively (8,9). Both used an enzymatic in situ hybridization method and were unable to detect microchimerism in LP lesions.

Another possible explanation for these negative results might be the small sample size of the biopsies analyzed. We used 4-μm punch biopsies, which were approximately of the same size as those used by Lombardi et al. (9). In contrast to our negative results, Asplund et al. analyzed 16-μm serial sections of human basal cell cancer samples. Using an X-inactivation assay, they were able to detect genetic mosaicism in one of 18 patients (17). Therefore, the assessment of microchimerism might be negative in small skin biopsy samples.

Microchimerism has been identified in a dizygotic twin with a rare ulcerative acral variant of LP by Vabres et al. (7). The authors also reported that the twins had the same heterogenous HLA-DQA1*0501 allele. This allele is very similar to the DQA1*0505 allele. This allele is very similar to the twins had the same heterogenous HLA-DQA1*0501 allele, which has been shown to be associated with the persistence of microchimerism in patients suffering from scleroderma (18). Increased frequencies of HLA DR1 and DQ1 alleles have been found in cutaneous LP (19–21), whereas in OLP, an increased frequency of DR6 and DR9 alleles has been observed (22,23). HLA genotyping, however, was not performed in the present study.

It can not be excluded that microchimerism may contribute to the pathogenesis of certain cutaneous variants of LP. The rather poorly defined clinical and histological features comprising LP appear to be the result of a cell-mediated reaction against basal keratinocytes. It seems possible that many different causes might trigger this final common pathway, and microchimerism may be one, albeit an uncommon one.

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References