Lack of evidence for leukocyte maternal microchimerism in primary biliary cirrhosis

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INTRODUCTION

Molecular biological techniques have shown that fetal cells can be detected in maternal peripheral blood in most pregnancies[1-3]. On this observation, the radical hypothesis of autoimmune disease, proposed in 1996, may be caused by fetal cells that are retained after pregnancies[4]. In systemic sclerosis, it was reported that fetal antimaternal graft-versus-host reactions might be involved in the pathogenesis of systemic sclerosis in some women[5,6]. Thus, the hypothesis that microchimerism might cause the primary biliary cirrhosis (PBC) is very fascinating[7,8], because it may explain clinical similarities between PBC and chronic graft-versus-host disease (GVHD) in liver after bone marrow transplantation. Some previous reports hypothesized that fetal microchimerism may also play a role in the pathogenesis of PBC[9-11]. However, they concluded that fetal microchimerism was not important in the development of PBC.

Although there is a possibility that PBC may be developed by maternal microchimerism, not fetal microchimerism in female patients, maternal microchimerism has not been considered until now. Thus, we produced one hypothesis that maternal microchimerism might cause PBC.

To test the hypothesis, we investigated 3 male PBC patients by fluorescence in situ hybridization (FISH) on paraffin-embedded tissues.

MATERIALS AND METHODS

Patients

Three male patients with PBC were studied after giving informed consent. They were diagnosed at Kyoto Prefectural University of Medicine by liver biopsy and the positivity of serum antimitochondrial antibody (AMA). All patients were compatible with the feature of PBC. None of these patients had scleroderma. They had no experience of blood transfusion.

Methods

We reported tissue-FISH method previously[12-14]. Sections from paraffin-embedded tissues were placed on silane-coated glass slides. The slides were deparaffinized immediately in 2 rinses of 1 000 g/L xylene for 10 min each. Each slide was rehydrated in an ethanol series for 5 min. The slides were then treated with 0.2 mol/L HCl for 20 min, followed by 2×SSC (0.3 mol/L sodium chloride and 0.03 mol/L sodium citrate) for 20 min at 80 °C, treated with 0.05 mg/mL proteinase K in TEN (0.05 mol/L Tris-HCl, pH 7.8, 0.01 mol/L EDTA, and 0.01 mol/L sodium chloride) for 10 min at 37 °C, and placed in 40 g/L formaldehyde in PBS for 10 min. Both FISH probes and target DNA were denatured simultaneously for 10 min at 90 °C, and the slides were incubated overnight at 42 °C. The slides were deparaffinized immediately in 2×SSC (0.3 mol/L sodium chloride and 0.03 mol/L sodium citrate) for 20 min at 80 °C, treated with 0.05 mg/mL proteinase K in TEN (0.05 mol/L Tris-HCl, pH 7.8, 0.01 mol/L EDTA, and 0.01 mol/L sodium chloride) for 10 min at 37 °C, and placed in 40 g/L formaldehyde in PBS for 10 min. Both FISH probes and target DNA were denatured simultaneously for 10 min at 90 °C, and the slides were incubated overnight at 42 °C.

RESULTS

It was easy to recognize the architecture of liver tissues by...
fluorescence microscope BX40-RF (Olympus, Tokyo, Japan). The lymphocytes aggregated at the portal zone (Figure 1). We assessed only non-overlapping nuclei having XY or XX signals. Almost infiltrating lymphocytes at septa revealed the XY chromosome pattern (Figure 2). The frequency of nuclei having XY in 3 patients is 96%, 98%, and 97%, respectively. (P<0.01).

**DISCUSSION**

The hypothesis that fetal microchimerism causing PBC is fascinating. However, this hypothesis does not explain why some cases with PBC were fetal microchimerism negative and why even males and nulliparous women were suffering from PBC. On the other hand, the hypothesis that maternal microchimerism causes PBC is more fascinating, because it resolves the above questions. Every one has the risk of maternal microchimerism, because it has been already established that maternal cells persist for a long-term in immunocompetent offspring[15]. However, this hypothesis could not be resolved by polymerase chain reaction (PCR), because PCR can only detect the Y-chromosome specific DNA.

To clear whether infiltrating lymphocytes derived from either maternal or fetal cells in male PBC patients, we performed tissue-FISH. Tissue-FISH is able to distinguish infiltrating lymphocytes from hepatocytes, because this method preserves the architecture of liver tissues and the shapes of cells. Tissue-FISH revealed that almost infiltrating lymphocytes were male cells in the liver sample of male patients. These results showed that maternal microchimerism did not play an important role in introducing and maintaining primary biliary cirrhosis.

In conclusion, we were unable to provide the evidence of maternal microchimerism in PBC patients. Although PBC shows the similar histological features with GVHD, PBC may be different from GVHD in etiology.

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