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LETTER TO THE EDITOR

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PCR confirmation of microchimerism and diagnosis of Graft versus Host Disease after liver transplantation

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Sir: Graft versus host disease (GVHD) is a well known complication of bone marrow transplantation (BMT) that is becoming increasingly recognized after solid organ transplantation [6]. It is suggested that donor's whole blood cells are introduced into the host by transplantation of hepatic lymphoid tissue. The clinical manifestations are skin rash, diarrhea and fever. The target organs (skin, gut, bone marrow) show increased expression of MHC class I and II molecules that are recognized by donor $CD4^+$ and $CD8^+$ cells. During the past decade, some 20 well documented cases following liver transplantation have been described. In most of these reports, the presence of donor cells in the recipient's peripheral blood or tissues was detected by tissue typing, but PCR technology to prove GVHD can also be applied [7]. This letter details the clinical course of GVHD in a liver transplant patient and focuses on the diagnotic power of PCR.

A 63-year old Spanish man, blood group A, rhesus negative, underwent elective orthotopic liver transplantation (OLT) for Child's class C alcoholic cirrhosis. As the liver graft had become available soon after he was placed on the waiting list, HLA typing was performed postoperatively (A2, A32, B51, Bx, DR4 (DRB1*04), DR8 (DRB1*0801), DR53 (DRB4*01)). Screening for circulating anti-erythrocitic antibodies was negative. The donor was a 23-year old Caucasian man, blood group A, rhesus positive, with A28, A11, B51, B52, DR11, DR14 HLA antigens. During the transplantation, 3 units of PRC, blood group A, rhesus negative, were transfused. Postoperatively, cyclosporin A (CyA) (60 mg iv. bid) and methylprednisolone (125 mg iv. bid) were initiated as immunosuppressive therapy. On postoperative day 3, the patient developed an ARDS related to Influenza B. He left ICU 9 days later and was discharged from the hospital on post operative day 22. Immunosuppressive therapy consisted then of CyA, 200 mg bid; prednisone, 20 mg/d; and azathioprine, 50 mg bid orally. The patient was readmitted on post operative day 35 because of fatigue, diarrhea, fever, and an erythematous maculo-papular skin rash. The next day he became drowsy with symptoms suggestive of meningitis. A thorough infectious investigation was however negative. On day 37 he developed pancytopenia (WBC = $1.6 \times 10^3/mm^3$, haemoglobin = 77 g/l, platelets = 70 G/l). On biopsy, the bone marrow was asthenic. On day 38, the rash extended to the axilla, the trunk, and the extremities; bullae appeared transiently before turning into erosions. A skin biopsy was performed: histology was consistent with GVHD, demonstrating total epidermal necrosis with subepidermal blister formation. A discrete perivascular lymphocytic infiltrate was also noted. Deposition of granular IgG and C3 was found by direct immunofluorescence at the dermal-epidermal junction. Additional skin biopsies were then performed for microchimerism detection. Immunosuppressive therapy was not changed until day 41, when CyA was discontinued and a single 1200 mg dose of cyclophosphamide was administered. On day 45 the patient died of multiple organ failure and bleeding from GI and respiratory mucosae, although his liver function tests had remained normal. Autopsy was not permitted.

Serological tissue typing was carried out on peripheral blood by the microlymphocytotoxicity test, using well standardized alloantisera obtained from the Collaborative Transplant Study (CTS, Heidelberg, Germany) and from commercially available kits (HLA-A/B lymphotyping trays, Biotest AG, Dreieich, Germany). HLA DNA typing was performed by PCR-SSP [8] and by PCR-SSO [3]. The recipient's HLA antigens, as determined retrospectively by DNA typing only, were A*02, A*32, B*51, Bx, DRB1*04, DRB1*0801, DRB4*01. The donor's HLA antigens, as determined prospectively by serology (class I) and by PCR-SSP (class II) were A28, A11, B51, B52, DR11 (DRB1*11), DR14 (DRB1*14), DRB3*02. Skin biopsy DNA was obtained by proteinase K treatment, phenol/chloroform extraction and ethanol precipitation. HLA-DRB3 locus specific PCR was performed using primers DRBAMP-52 (5'CCCAGCACGTTTCTTGGAGCT) and DRBAMP-B (5'CCGCTGCACTGTGAAGCTCT), using 1 µl-aliquots of the DNA extracted from skin biopsy. DNA samples from DR52-negative blood donors (i.e. donors without a DRB3 locus) were used as controls, including samples that shared DRB1*04 and DRB1*0801 alleles with the recipient. In addition, the donor/recipient origin of the biopsy was determined by PCR amplification of 33.6 and YNZ22 minisatellite DNA regions [11].

GVHD is common after bone marrow transplantation, but infrequent following solid organ transplantation. Histocompatibility dif-

ferences between the donor and the recipient, immunocompetent cells in the graft, and the inability of the host to reject the graft are usually observed [1]. In BMT, the recognition of allogeneic histocompatibility antigens on haematopoietic precursor and epithelial cells by the immunocompetent transfused lymphocytes seems to be the main trigger. An antibody-mediated reaction in patients receiving an ABO-mismatched graft is another well documented cause of GVHD, causing haemolytic anaemia [10]. Cellularly mediated GVHD generally involves the skin and the gastro-intestinal tract. A maculo-papular rash frequently progresses over the whole body, leading to an erythroderma within a month after transplantation, with bullae formation in severe cases [9]. In the reported patient, these typical symptoms were clearly present. In solid organ transplantation, lymphoid tissue can be transferred from the donor to the immunosuppressed recipient. For example, donor livers still contain some 10^9–10^{10} mononuclear cells, mostly lymphocytes, after perfusion with a cold storage solution. Thus, as immunocompetent cells remain in the organ and as the host is immunosuppressed, an environment is created that allows the donor cells to proliferate and mount an immune response [15]. For instance, donor-derived long-term multilineage haematopoiesis has been reported in a liver transplant recipient due to stem cells with pluripotent function in the allograft [2]. In our case, the clinical pattern of GVHD was confirmed by the demonstration of donor DNA in the patient's skin biopsy. Using PCR-SSP technique, we were able to amplify recipient MHC class II alleles (DRB1*04/DRB1*080/DRB4*01) as well as, at a lower level, donor MHC class II alleles (DRB1*11/DRB1*14 and DRB3*02). We also took advantage of the absence of DRB3 (DR52) alleles in the recipient's HLA haplotypes and the unique presence of HLA DRB3*02 (commonly associated with DRB1*11 and DRB1*14 alleles) in the donor to confirm a weak amplification signal from the biopsy DNA, that was absent in DR52-negative controls. Furthermore, using minisatellite analysis, amplification profiles on the loci 33.6 and YNZ22 revealed a low level (1–5 %) of donor-specific alleles in the patient's sample. Thus, both donor and recipient DNA were found in the skin biopsies suggesting the presence of chimerism. In this patient, the PCR diagnosis was reached late in the development of the disease, and the treatment failed. Had this analysis been performed earlier, treatment may have been initiated more prompty, potentially resulting in a better prognosis.

In allogeneic BMT, HLA mismatch is the most important determinant of the severity of GVHD, and the risk correlates with increasing recipient age, increasing donor parity and donor-recipient sex mismatch. In liver transplantation however, the immunocompetence of the recipient and the quantity of alloreactive cells transplanted with the graft seem to be more important factors [4]. In addition, some authors have suggested the possible role of minor antigens as well [5]. In our observation, HLA chimerism was present in the skin, but no search was carried out in the bone marrow and the peripheral blood. Other reports suggest, however, that systemic migration of donor graft immunocompetent cells occurs very often after liver transplantation, resulting in systemic chimerism [12]. So far, this chimerism has generally been considered the cause of GVHD, and its demonstration is used as the cornerstone of the diagnosis. Recently however, it has also been suggested that chimerism induces a tolerance mechanism in solid organ transplantation [14] and that it may just be a marker of graft acceptance [16]. We feel, therefore, that chimerism can no longer be seen as a gold standard criterion for the diagnosis or as the sole cause of GVHD. As GVHD can occur only when chimerism is present, it is necessary to understand why chimerism is clinically unobtrusive in most cases: the induction and perpetuation of graft acceptance, i.e. non-responsiveness, seems to be the balance between the alloactivation of graft versus the host, and that of the host versus graft rejection [13].

In conclusion, GVHD following liver transplantation often leads to multiple organ failure and death. PCR examination in the target tissues, as soon as the diagnosis is suspected clinically, may allow earlier treatment and thus improve the poor prognosis of this complication.

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