PERIPHERAL BLOOD MICROCHIMERISM IN FEMALE RENAL RECIPIENTS FROM MALE DONORS

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Abstract - The relation between microchimerism and allograft tolerance is still a mystery. In this study we determined the presence of peripheral blood microchimerism (PBMC) in female renal transplant recipients from living male donors with second round polymerase chain reaction (PCR). Second round PCR was used to find Y chromocome products. The degree of PBMC in renal transplant recipients must be below the rate of 1.10^4 and second round PCR provides the detection of PBMC at the rate of 1.10^6. We divided our patients into two groups according to allograft function. Group 1 (16 patients) had normal allograft function. Group 2 (6 patients) had chronic allograft dysfunction. First PCR didn't show PBMC. Second round PCR with SRY primers of Y chromosome showed PBMC in 13.22 (59%) of patients. PMBC was positive in 10.16 (62%) of patients in group 1 and 3.6 (50%) of patients in group 2. There was acute rejection in 4.13 (30.7%) and 2.9 (22.2%) of patients with positive and negative PBMC, respectively. In our study, there was no significant correlation between the presence of PBMC and allograft function and the frequency or severity of rejection episodes.

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INTRODUCTION

Donor cells or genetic material can often be detected in solid organ transplant recipients. Such recipients exhibit peripheral blood microchimerism and in some patients the donor material is detected for long periods after transplantation (1). In some studies microchimerism induces the tolerance state and enhancement of microchimerism can prolong the allograft survival (1,2,3). Microchimerism refers to trace population of hematolymphopoietic cells of donor origin found in solid organ transplant recipients (2) which can be detected only with sensitive immunocytochemical techniques or with polymerase chain reaction (PCR). Microchimerism was first reported by Kashiwagi and co-workers in 1969 in long term surviving female liver recipients from male donors (4). It has now become clear that chimerism occurs not only in liver recipients but even in kidney, heart and lung recipients. Microchimerism is one of the mechanisms for induction of tolerance; we could have tolerance without microchimerism, in this way alternative mechanisms may cause tolerance (5). Alternative mechanisms include clonal deletion, clonal anergy, suppressor cells, veto cells and immune deviation that induces suppressive cytokines (5). Indeed, it is unclear whether microchimerism plays an active role in graft acceptance or is simply a consequence of maintenance of sufficient immunosuppression to avoid rejection (3,5,9). In this study we used second round PCR for detection of Y chromosome in peripheral blood of female renal recipients from living male donors and sought the relation between allograft function and the presence of peripheral blood microchimerism (PBMC).

MATERIALS AND METHODS

In Dr. Shariati Hospital twenty - five female renal recipients with male allografts who had a minimum of two years survival were selected. We divided our patients into two groups.

Group one included 18 renal recipients with normal allograft function (serum creatinine < 1.6 mg/dl). Mean serum creatinine was 1.09 mg/dl ± 0.27.

Group two included 7 renal recipients with chronic allograft dysfunction according to clinical impression and probably renal biopsy (serum creatinine > 1.6 mg/dl). Mean serum creatinine was 4.07 mg/dl ± 2.95. Mean age of patients was 35 ± 14 year and 32 ± 7 year in group one and two respectively.

Three patients were excluded (2 from group 1 and 1 from group 2) because of blood sampling error. Mean follow up period for group 1 and 2 were 58.8 and 66.1 months respectively and mean sampling time interval from transplantation time was 60.8 months. Blood sampling was performed between May and August 1999 in Dr. Shariati hospital and samples were sent to immunogenetic lab, Faculty of medicine. Microchimerism in peripheral blood was determined by PCR with Y-chromosome gene specific primers (SRY, DYZ-1).

Sex determining region of Y (SRY) with following primer sequences was selected (7,8).

5′CAGTGAAACGGAGAAAACAGT (SRY-1F)
5′CTTCCFACGAGGTCGATACTTATA (SRY-2R).

The cycling schedule consisted of denaturation at 94° c for 1 munute, annealing at 65° c for 2 minutes and extension at 72°c for 2 minutes; 25 cycles were performed.

Following the first PCR, the second PCR was performed under the same condition. The other target was the DYZ-1 region. The first primer sequences were:

5′ AATTTGAGCATTCGTGTCCATTCT and 5′ AATGCCCTTGAATTAAATGGACT.

The cycling was scheduled at 60° c for 30 seconds and extension at 72° for 1 minute; 30 cycles were performed.

Following the first PCR, the nested PCR was performed with second primers with sequences:

5′ CGAGGTCCARRCCATTACCGT and 5′ CGGAATGGAATGCAACGCAA.

PCR reaction mixtures were electrophoresed on 3% agarose gels and stained with ethidium bromide.

The presence of specific PCR products for Y - chromosome was determined under UV illumination.

RESULTS

The degree of PMBC in renal transplant recipients is less than 1.10^4 and standard PCR is not sensitive to detect donor chimeric cells in renal transplant recipients. Second round PCR raises the sensivity to 1.10^6 to detect PBMC (4,6,7). So in our study first PCR with SRY primers failed to detect microchimerism but second round PCR with SRY demonstrated PBMC in 13.22 (59%) of patients.

PBMC was positive in 10.16 (62%) of patients in group 1 and 3.6 (50%) of patients in group 2 (Table 1).

Unfortunately, study with DYZ - 1 primers was unreliable and in repeated tests we had different results. The sensitivity of second PCR for detection of microchimerism was 1 in 100000 cell ratio. The sensitivity of test was determined by amplification of DNA extracted from a serial dilution of male lymphocytes diluted with female lymphocytes.

Acute rejection episodes had developed in 6.22 (27.2%). There were acute rejection episodes in 4.13 (30.7%) and 2.9 (22.2%) of patients with positive and negative PBMC, respectively.

In this study, there was no significant correlation between the presence of PBMC and allograft function and the frequency or severity of rejection episodes.

Table 1

Variable	Group1 (n = 16)	Group 2 (n = 6)
Age (year)	35 ± 34.3	66.1 ± 7
Mean sampling time interval since transplant (month)	58.8 ± 34.3	66.1 ± 34.1
Serum creatinine (mg/dl)	1.09 ± 0.27	4.07 ± 2.95
Positive PBMC	10 (62.5%)	3 (50%)
Acute rejection episode	3 (18.7%)	3 (50%)

DISCUSSION

A role for microchimerism in induction of allograft acceptance has been reported but the role of PBMC is indeed a hot debated matter, (1,2,3,8). In some studies, the prescence of long term microchimerism didn't indicate tolerance. In this way, alternative mechanisms may cause tolerance and microchimerism might be only an epiphenomenon of organ transplantation (3,5,7,8).

It has been proposed that following organ transplantation, passenger leukocytes of donor origin home to recipient's tissues and are replaced in the graft by similar cells of the recipient (5,9). The rapid disappearance of these donor leukocytes from successfully transplanted organs was thought to reflect their selective destruction by recipient immune system. Allograft acceptance was therefore explained by immune elimination of passenger leukocytes in combination with a panoply of other factors including the appearance of veto, suppressor or other immune-regulatory cells, changes in cytokine profile and production of antibodies and idiotype networks. One of the mechanisms for tolerance is the ability of donor leukocytes to migrate to and persist in lymphoid organs. Interaction between coexisting donor and recipient immune cells causes reciprocal clonal expansion, followed by peripheral clonal deletion (5,9).

We examined the presence of SRY region of Y-chromosome in female renal recipients with male allografts. In order to raise the sensitivity of test we performed second PCR following first PCR. We didn't show significant correlation between the PBMC and allograft function and the frequency or severity of rejection episodes. This study shows that the presence of microchimerism does not indicate tolerance.

Futher studies in a large recipients population are necessary to determine the causal relationship between the PBMC and allograft function.

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