Transplantational microchimerism – an introduction to a tolerance induction or an organ donor recipient’s epiphenomenon?

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ABSTRACT

Microchimerism is a presence of a small amount of cells, as well as genetic material that come from another organism. Scientists are trying to prove influence of transplantational microchimerism on different reactions that can be observed in a transplatology medicine. Conclusions are controversial and still there are no unambiguous premises regarding microchimerism. The immunological tolerance is a characteristic lack of the immunological system response to specific antigens. An achievement of full tolerance to transplanted organ can produce a possibility of discontinuation of immunosuppressive therapy and avoiding its side effects. The development of partial tolerance can allow for a reduction of immunosuppressive drugs dosage.

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longer organ donor recipient survival is associated with the problem of side effects development of chronic immunosuppressive therapy, such as infections, malignant tumours, metabolic disorders, direct toxic influence on exact systems and organs (for instance: neural, digestive or urinal system). That problem persuades to constant research of immunological tolerance for donor’s antigens. An achievement of full tolerance to transplanted organ can produce a possibility of discontinuation of immunosuppressive therapy and avoiding its side effects. The development of partial tolerance can allow for a reduction of immunosuppressive drugs dosage [1]. Present-day immunosuppressive drugs are able to prevent from acute rejection episodes, however they are not capable of protection from chronic organ dysfunction. There are some premises that achievement of tolerance can break that problem. The ability of transplant tolerance induction remains in a field of interest of many scientist and clinicians occupied with transplantology. There are high hopes concerned with research results that are connected with identification of biomarkers that can predict a development of tolerance, which enables identification of patients whom will be able to safely discontinue immunosuppressive treatment. Since many years scientist have been researching microchimerism as a biomarker of a tolerance to transplanted organ and antigens.

Microchimerism is a presence of a small amount of cells, as well as genetic material (less than 1%) that come from another organism [2]. Natural microchimerism, coming from foetal cell, which can pass through placenta during pregnancy, as well as microchimerism that can develop after blood transfusion or cells, tissue or organs transplantation, can be observed [3]. Since half of a century scientists are trying to prove influence of transplantational microchimerism on acute rejection episodes, graft versus host disease, transplanted organ acceptance or a development of immunological tolerance – totally different reactions that can be observed in a transplantology medicine. Conclusions are controversial and still there are no unambiguous premises regarding microchimerism.

Post-transplantational microchimerism is defined as a presence of cells and donor’s DNA at a recipient’s body, which can occur in places other that transplanted organ. The source of that can be lymphocytes or dendritic cells, so called passenger leukocytes that migrate from the transplanted organ. Some researches prove that this kind of cells can be detected after many years, using very sensitive molecular methods. Starzl who is one of pioneer in the field of organ transplantation, assumes that they are responsible for creation of post-transplantational microchimerism, which lengthens transplanted organ through weakening reactivity on alloantigens and the development of immunological tolerance.

There are a lot of research regarding significance of developing immunological tolerance on antigens and mechanisms leading to its creation [4]. The immunological tolerance is a characteristic lack of the immunological system response to specific antigens. During human ontogeny, negative selection (clonal detection) of lymphocytes T against own antigens takes place in thymus that is responsible for a central tolerance development to own antigens [5]. The transplantational tolerance is defined as a lack of immunological response to donor’s antigens. It is connected with persistent tolerance of transplanted organ with no need of immunosuppressive therapy. In experimental conditions, settling thymus by donor’s cells and activating clonal deletion of allo-reactive lymphocytes T can achieve immunological tolerance [1]. More than 50 years ago research was conducted with the use of animal models. Animals were injected with allogenes to their thymuses or with donor’s thymocytes, after removal of their own thymus. Neonatal tolerance can be achieved by administrating donor’s antigens during foetal or newborn period. Another major step forward was the first news about transplant tolerance induction, as a result of mixed chimerism made of donor’s haematopoietic cells, which were prior treated with strong nonmyeloablative scheme of immunosuppressive treatment [6, 7].

A complete chimerism is a condition when all of the haematopoietic cells come from donor’s bone marrow. Inducing a complete chimerism is connected with high risk of death, stemming from high dosage of ionizing radiation, essential to create enough space for donor’s cells. According to experimental research it results as a development of total tolerance to transplantation alloantigens of the same donor. This method is not used to achieve chimerism and tolerance at solid organ recipients who have healthy haematopoietic system. A mixed chimerism is coexistence of donor’s and recipient’s cells in one body. It can be created by application of donor’s bone marrow cells to an adequately prepared recipient’s body (by lower dosage radiation). After transplantation of a solid organ that comes from the same donor, a partial tolerance for transplanted donor’s antigens can be observed [8,9]. However, immunological alloreactivity is kept and it is necessary to take immunosuppressive therapy. In 1998 a clinical research started, with a presence of patients suffering from end stage renal disease, as a result of a multiple myeloma, at whom simultaneous renal and bone marrow transplantation was conducted (after myeloablative therapy) that were harvested from the same donor. At all of the patients, temporary, mixed chimerism could be observed. At 4 patients it was possible to discontinue immunosuppressive therapy. The mixed chimerism disappeared after a while, still, the renal function was stable. It is very important observation that a stable level of chimerism is not
necessary to sustain tolerance. Nowadays, experimental research explores tolerance that is induced by protocols leading to create partial and temporary chimerism, not complete and persistent.

During a process of creating mixed chimerism and a tolerance to alloreactive lymphocytes $T$ in a recipient’s thymus clonal deletion of lymphocytes take place. There are some proofs, that that process takes place also in a peripheral tissues, which induces so called peripheral tolerance. A lot of different immunoregulatory mechanisms were found, that are responsible for development of peripheral tolerance. They were presented on the animal models as well as human. Among them there are: alloreactive lymphocytes $T$ anergy that can be achieved by blocking the costimulation, suppression caused by dominant suppressive lymphocytes, as well as covering the alloantigens on the surface of cell by antigens (mechanism of an immunological facilitation). However, blocking the costimulation does not induce a development of a complete transplantational tolerance at human species [10]. It seems that peripheral immunoregulatory processes are responsible for interplaying of microchimerism on the development of tolerance to transplanted organs.

According to Starzl [1,11], permanent contact with donor’s cells in peripheral nodes is a necessary condition to maintain immunological tolerance. Coexistence of mutual immunological response induces reciprocal clonal depletion and a gradual development of a tolerance. That theory can explain reversible nature phenomenon of rejection episodes and rare GVHD occurrence and the lesser significance of full HLA compatibility as long as solid organ transplantation is concerned.

In 1992, for the first time, peripheral microchimerism was detected and described [5]. A small amount of donor’s leucocytes could have been observed in a kidney or liver recipient’s blood for almost 30 years. A chromosome $Y$ identification method (if an organ transplantation was harvested from a male donor and transplanted to a female recipient) or donor specific HLA antigens.

Starzl’s team described a transplantational organ chimerism for the first time in 60’s. At women with transplanted liver that was harvested from male donor $100$ days post transplantation a presence of Kupffer’s cells with female karyotype were detected. Shortly, it was proved that all of the transplanted organs become chimeric structures after a while. As opposed to described above chimerism phenomenon, post transplantation microchimerism comes into being spontaneously and does not require proper preparation neither of recipient nor a donor. It is known that ischaemia reperfusion injury of a transplanted organ is connected with haemodynamic disturbances as well as persisting immunological reaction intensifies realising of different cells and its fragments to a recipient blood. It was proven that some part of them is captured and phagocytosed by monocytes and some part is incorporated to dendritic cells. Next, they can be detected in macrophages located in a spleen or lymph nodes. It was proven that at animals after heart transplantation, decreasing amount of donor’s cells that can be detected in a recipient’s blood, the number of copies of SRY fragments is increasing. That means that tissue microchimerism is developing. There are a lot of experimental research that show that acute rejection episode of a transplanted organ and inflammation have an influence on increasing microchimerism as well as in a blood and in a peripheral tissues. Is it possible to determine quantitative the level of microchimerism that is significant to predict an acute rejection episode? It is unknown how high level of donor’s cell and free DNA is maintaining in a recipient’s blood during acute rejection episode, as long as a dynamics of phagocytosis and enzymatic disintegration of such genetic material is concerned. Immunosuppressive therapy is decreasing the level of microchimerism, which is still present in recipient’s blood and tissues. As for now, in none of clinical research in which patients with peripheral microchimerism participated, immunosuppressive therapy was discontinued. However, long term observations of organs with good function that can be observed at patients who wilfully discontinued immunosuppressive therapy at different period post transplantation, are promising and still are encouraging to follow-up microchimerism in a field of transplatology in a context of tolerance development [12].

Up to now, there are no satisfying answers to many questions, moreover observations were conducted on too little or heterogeneous group of patients, to prove statistical significance. Most of the present conclusions that try to determine the nature and the significance of the microchimerism in transplatology come from single-centre, retrospective observations in which the correlation of microchimerism was estimated in respect of single clinical parameters, for instance acute rejection episodes.

Limitations of the most, known methods to mark microchimerism are of great importance. For instance, as far as the method of marking SRY sequence on the $Y$ chromosome is concerned, the limitations are: necessity of exclusion of men, patients who have had blood transfusions, after transplantation and women who have male descendants [13]. Methods that are used to detect DNA on a cellular level are little peculiar and sensitive. Among them there is a flow cytometry that enables to differ cells one from each other by suspending cells in a stream of fluid and passing them by an electronic detection apparatus. Southern blotting or fluorescence in situ hybridization (FISH) is used to detect and localize the presence or absence of specific DNA sequences.

Currently, the most common methods to detect
donor DNA on the molecular level are the one with PCR reaction. As for now, the most popular among them is the method of identification SRY region [14]. It is applicable only in case of an organ donation from male to female. The method is sensitive and peculiar, however it is very important to avoid contamination of genetic material.

Fairly often polymorphism of HLA antigens is used. It is possible to detect donor specific alleles, the most frequent in DRB1 locus. The limitation of that method is the necessity of occurrence of at least one HLA DR mismatch, that is conflicting the intention of HLA class II compliance between donor and recipient. More and more frequently detecting donor’s DNA method in a recipient’s full blood that is based on polymorphic microsatellite repeats is used [15]. Short tandem repeats due to its attributes are widely used in DNA identifications in blood and tissue. STR (short tandem repeats) is creating small tandem blocks that are spread in a genome. Eukaryotes, in the form of simple nucleotide repeats that are built from 1 to 6 pair bonds, make up 20 to 30% of whole genome, they are characterized by high polymorphism (90%) and they show in genome evenly, every 6-10 kpz. They are used in the judiciary (contentious paternity) and forensics (genetic material identification). Commonly analysed in forensic loci are: pancreatic phospholipase A2 HUMPLA2A1(AAT) cytochrome P-450 HUMCYARO(AAT) and locus D1S80. The method that is taking advantage of high polymorphic microsatellite repeats is giving an opportunity to include to the examination all of pairs donor – recipient [13, 16, 17]. That DNA sequences do not have their own locus within chromosome Y and they are not the genes of the major histocompatibility complex. It is not necessary to eliminate male recipients, pairs of the same sex to mark their polymorphism.

Undoubtedly, in order to research on practical significance of microchimerism credibly, it is necessary to standardize methods of marking it and finding such methods that will allow to examine all of the pairs donor-recipient.

The significance of microchimerism phenomenon in a tolerance development is still not confirmed. There is a research, which was conducted for 20 years: peripheral microchimerism was observed at 15 kidneys recipients whose kidney function was proper (immunosuppressive therapy included azathioprine and prednisone). DRB1 gene polymorphism and SRY marking were used to analysis. After 20 years, a peripheral microchimerism was detected at one recipient, presence of donor’s DRB1 alleles in the donor’s skin and kidney biopsy material (4 patients). Low sensitivity of that method was underlined in the research comments. In days of acute rejection episodes were the main reason of graft loss, Lo, Zhang et al, were estimating the correlation between microchimerism present in the urine and the frequency of acute rejection episodes [18]. SRY sequence was detected in the urine of 14 out of 17 female recipients of male kidney graft and its level was increasing rapidly during acute rejection episodes, whereas it was decreasing after an effective treatment. There are also another outcomes regarding microchimerism. According to Mosca et al. microchimerism is detected less frequently at patients with better kidney function up to 2 years post transplantation [19].

On the other hand there is research conducted by Tajik, Nikbin et al. [20]. They have been observing a presence of microchimerism at 13 out of 20 female kidney graft recipients whose kidney came from male donor. They say that there is positive correlation between microchimerism and low frequency of acute rejection episodes. Few years later [21] the same group of researches proved that recipients whose kidneys were harvested from a living donor have higher frequency of microchimerism detection. Crispim et al. [22] confirmed that there is an influence of a donor type on the microchimerism detection frequency. There is no statistically significant correlation between microchimerism and other analysed parameters such as: recipient’s and donor’s age, blood transfusions, labours, creatinine serum concentration, BMI or a frequency of acute rejection episodes. Yao-Wen Fu et al. [23] examined a group of 126 women who received a kidney graft from a male donor. SRY sequence and DYZ1 gene that are coming from chromosome Y were detected in plasma of 97 women. According to analysis, in group with microchimerism the mean time of graft survival was longer, graft function was better and acute rejection episodes were less frequent in a comparison to a control group. The dynamics of microchimerism phenomenon was also analysed: the frequency of its detection was higher as the time went by. There is a question: is microchimerism present since the moment of ischaemia reperfusion injury or is it a consequence of incorrect graft function and it develops after a while?

Vlachojannis et al. [24] was marking microchimerism using quick and sensitive method qRT-PCR (polymorphism of genes on the chromosome Y) in the 17 women’s urine and blood. Analysis of graft function, donor’s age, time of dialyses, type of kidney replacement therapy, type of immunosuppressive therapy revealed no correlation neither in group with present microchimerism nor in the control group. They paid attention to an aspect of quantitative estimation of microchimerism; the number of copies of marked gen was three times higher at patients with graft dysfunction. For the first time the conclusion that there might be possible correlation between microchimerism as a predictor of the late kidney graft loss was raised. On the basis of conclusions above, there is no straight answer to the question if there is a correlation between microchimerism and acute rejection episodes or other incorrect processes that affect the kidney graft function. There are no conclusions regarding that phenomenon at other organ recipients. However, there is higher frequency of microchimerism
detection at liver donors (liver is transmitting more antigen presenting cells). Ueda et al. [25] presented a changing nature of microchimerism at liver recipients whose graft came from a living donor: during early period post liver transplantation the level of donor’s cells was high, then it was undetectable, then after dozen or so weeks again it was present in the blood and tissues. The source of donor’s cells in the early period post transplantation was mature passenger cells, whereas later it were donor’s maternal cells that are present in the liver tissue and are essential for the tolerance development. There is also another research referring to the significance of maternal cells in the tolerance development [26]. Schlitt and Pichlmayr [27] were exploring the dynamics of microchimerism in the blood and skin of the heart transplant recipients. On the contrary to the Pujal et al. [28] research results, they revealed no correlation between peripheral microchimerism and heart rejection episodes. The same group, as one of the first ones, revealed the decreasing of microchimerism to the undetectable level soon after removing the transplanted liver [29]. Elwood et al. [30] observed the dynamics of microchimerism phenomenon at group of 17 kidney recipients, 7 liver graft recipients and 6 patients who received simultaneously pancreas and kidney. They indicate that there is a high changeability of HLA DR gene detection in the patients’ blood as months go by... Acute rejection episodes were independent from the microchimerism dynamics. As for now, it seems it is impossible to determine the numerical value of microchimerism phenomenon that might have significance for prognosis of developing some side effects post solid organ transplantation [31].

Interesting conclusions are raised by Sahota et al who carried out meta-analysis of 37 papers that have been published between 1991 and 1997 concerning influence of microchimerism on the organ rejections [32]. Microchimerism significantly increased the risk of kidney rejection (up to 12 months post transplantation) and heart (3 and 6 months), whereas it decreased the risk of liver and lungs rejection (12 months post transplantation). Microchimerism was detected in all of the works that were included in the meta-analysis.

Lately, longer kidney graft survival was proven at the patients with higher level of microchimerism, which was created prior transplantation after donor’s bone marrow cells transfusion in a comparison to a group of patients without bone marrow cells infusion. The significance of immunoregulatory microchimerism cells was depicted, through blocking the response of recipient’s lymphocytes to donor’s antigens (donor-specific mixed lymphocyte reaction). Transfusing donor’s bone marrow cells prior kidney transplantation is not a routine procedure, however the results of that method regarding kidney graft survival rate are very promising. Higher frequency of microchimerism and its higher concentration at recipients after bone marrow cells infusion was proven in the comparison to a control group. Nevertheless, the results of clinical parameters analyses are different according to various authors.

**CONCLUSION**

To sum up, getting to know the real biological role of microchimerism may contribute to more effective monitoring of patients’ immunological status and immunosuppressive therapy dosage. On the other hand, microchimerism may turn out to be only an epiphenomenon connected with organ transplantation.

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