HLA-targeted cell sorting of microchimeric cells opens the way to phenotypical and functional characterization

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Microchimerism refers to the presence of less than 1% of non-host cells in a person. Our group developed a reliable method for separating viable microchimeric cells from the host environment. Optimal separation of microchimeric cells at proportions as low as 0.01% could be established with two monoclonal antibodies directed against different HLA antigens, one targeting the microchimeric cells and the other the host cells. Purity of separated cell populations was validated by HLA-allele-specific and Y-chromosome directed real-time qPCR assays. The methodology was used successfully to separate microchimeric maternal cells from child umbilical cord mononuclear cells after pregnancy.

Cell sorting with HLA monoclonal antibodies targeting allelic differences enables reliable microchimeric cell detection and separation in blood specimens. With this approach, maximal enrichment of potentially viable microchimeric cells from a background cell population is reached, which opens the way to phenotypical and functional characterization of microchimeric cells.

The low frequency of microchimeric cells in peripheral blood samples necessitates a technical approach of detection that is reproducible and sensitive. Due to their highly polymorphic character, the human leukocyte antigens (HLA) represent an attractive and widely applicable target for microchimerism detection.¹⁻³ The oligonucleotide primers that are suitable for PCR-based HLA typing of transplanted patients can be applied for microchimerism

detection. However, once chimeric cells have been processed for qPCR purposes, further functional studies are precluded. The technique we described makes use of flow cytometric cell sorting based on phenotypic HLA differences, and yields potentially viable, highly enriched microchimeric cell populations.⁴

From a large set of 120 in-house developed human HLA specific monoclonal antibodies we selected ten antibodies to study microchimerism in pregnancy. With this set of antibodies, distinction between mother and child can be made based on HLA class I differences in over 90% of pregnancies of Caucasian women.⁵ Purity after cell separation was verified by qPCR analysis, whereby HLA class I and II alleles and the Y-chromosome were targeted. Separation of microchimeric cells at proportions as low as 0.01% could be established. As single HLA monoclonal antibody labeling of cell populations in a frequency of <0.4% leads to less sufficient separation, double HLA monoclonal antibody labeling is crucial for optimal separation of low-frequency microchimeric cells.

Chimerism between mother and child is seen following cell transfer during pregnancy and after delivery.⁶⁻⁸ In umbilical cord samples we (unpublished observations) and others⁹ have encountered by PCR a relatively high degree of maternal microchimerism. We applied HLA-targeted FACS sorting in the pregnancy setting for separating microchimeric maternal cells from child umbilical cord. Practically, the method is applicable when the HLA type of the minor cell population is known, e.g., in case of quantitative studies on microchimerism in pregnancy and

Key words: microchimerism, HLA, FACS, cell sorting, monoclonal antibodies

Submitted: 12/02/11

Accepted: 12/20/11

<http://dx.doi.org/10.4161/chim.2.4.19133>

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Addendum to: Drabbels JJ, van de Keur C, Kemps BM, Mulder A, Scherjon SA, Claas FH, et al. HLA-targeted flow cytometric sorting of blood cells allows separation of pure and viable microchimeric cell populations. *Blood* 2011; 118:149–55; PMID:21931111; <http://dx.doi.org/10.1182/blood-2011-06-362053.vcv>

transplantation. For the detection of fetal chimerism in maternal blood obtained before the delivery, when the HLA type of the child is still unknown, one could target HLA antigens from both paternal HLA haplotypes. This approach would be most useful in first pregnancies because in next pregnancies persistent chimeric cells from previous pregnancies may interfere.

In human pregnancy, the functional relationship of cells in the context of both maternal and fetal microchimerism is still under debate. Recently, it was shown that maternal cells can cross the placenta and localize in lymph nodes of the fetus, where they can induce development of T cells that regulate maternal immunity to fetal alloantigens.¹⁰ A similar mechanism may be involved in the diminished alloreactivity observed in patients directed against the non-inherited maternal HLA antigen (NIMA). Both with respect to HLA antibody induction,¹¹ kidney graft survival¹² and hematopoietic stem cell transplantation,¹³ a beneficial effect of maternal HLA mismatches was observed. On the other hand, survival after hematopoietic stem cell transplantation is improved in case of mother to child transplantations.^{14,15} This beneficial effect is presumed to be the result of previous exposure of the maternal immune system to fetal antigens during pregnancy, which would lead to development of persisting immunity throughout life of the mother against paternal antigens of the child. Further studies are needed to characterize the maternal microchimeric cells.

Fetal microchimerism has been associated with both beneficial and adverse effects in women.¹⁶⁻¹⁸ Some fetal cells in the mother have progenitor cell-like features. These can persist throughout life and may differentiate into different cell types in maternal organs including blood, skin, central nerve system, liver, kidney and brain.¹⁹⁻²² On the other hand, fetal microchimerism in the mother was found to be associated with autoimmune disease later in life.²³⁻²⁵

Microchimerism can be found after solid organ transplantation as a result of migration of passenger hematopoietic cells from the donor graft into the peripheral circulation and tissues of the recipient. Reports are contradictory with respect to

association between microchimerism and graft outcome: some show that microchimerism is associated with graft acceptance²⁶ and lower incidence of rejection^{27,28} while others did not show an effect on graft acceptance.^{29,30} Further research on isolated microchimeric cells is helpful in clarifying functional mechanisms.

Although the technique of HLA-targeted cell sorting is too laborious for use as a screening tool for microchimerism on larger panels of samples, the methodology is an excellent tool for mechanistic studies related to the occurrence of microchimerism as it enables phenotypical and functional characterization of microchimeric cells.

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