COMMENTARY

Lost in translation? Microchimerism detection in experimental and clinical transplantation

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ABSTRACT

The importance of further elucidating the properties surrounding microchimerism in various experimental models and clinical transplantation are limited by current techniques and the sensitivity of available platforms. Development of reliable methods and use routine use of microchimerism detection in clinical practice could guide clinical decision making regarding rejection, stable function, and tolerance.

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The path toward immunologic tolerance in organ transplantation has the most promise via strategies that achieve either transient or durable chimerism. Both experimental and clinical protocols depend on preconditioning therapies including radiation and cytotoxic drugs followed by bone marrow transplantation to achieve detectable macrochimerism (defined as >1% donor chimerism) and often tolerance.^{1–3} We have been investigating vascularized composite allograft (VCA) facial transplantation in a well-established non-human primate (NHP) model and have observed transient macrochimerism without the use of preconditioning therapies when these grafts include vascularized bone marrow.⁴ We have also performed a clinical face transplant with T cell depletion and recently reported on the use insertion/deletion (In/Del) polymorphisms in a PCR assay to detect microchimerism (defined as levels of chimerism <1%).⁵ In contrast to both bone marrow and organ transplant models, the development of either macro- or micro-chimerism in VCA models did not prevent significant rejection responses in the absence of immunosuppression. Nonetheless, the immunologic consequence of microchimerism are largely unknown.

In efforts to better define the presence and consequences of microchimerism in a preclinical model, we applied the In/Del PCR technology used in our clinical face transplant patient to our preclinical model of

facial VCA transplantation in non-human primates. Our model utilizes Cynomolgus Macaques of Mauritian origin (MCM). The genetic closeness of this island population results in only 7 distinct MHC haplotypes that affect methods available to differentiate donor from recipient, and correspondingly chimerism. This is compared to the 2867 identified class I and class II MHC sequences that continues to grow in numbers, isolated from rhesus, cynomolgus, and pig-tailed macaques commonly used in research. Our group and others who work with the MCM model have generally used cross-reactive anti-human leukocyte antigen (HLA) antibodies to identify selective class I mismatching at the major histocompatibility complex (MHC). Post-transplant, anti-HLA antibodies are used to detect and follow for any development of chimerism.

The use of PCR techniques and next generation sequencing as an alternative to antibody chimerism detection methods in NHP could allow for more sensitive quantitative assays. Next generation sequencing, utilizing technology based in PCR, allows for not only the quantification of genes but also gives sequence information. This can be useful in detecting chimerism when a limited number of MHC haplotypes are concerned as in the MCM model that have been shuffled by recombination during the ~100 generations since the founders arrived on Mauritius. These techniques

applied to donor and recipient genetic differences are the basis for chimerism assays. When In/Del polymorphisms were used to screen 12 MCMs with varying haplotypes in our laboratory (unpublished data), amplification with the same few primers in all animals took place, not allowing for identification of specific primers to each haplotypes. As a result, a non-specific primer targeting a conserved sequence across all haplotypes based on MAFA alleles, which are HLA equivalents, should allow for the amplification and quantification of specific donor sequences to quantify chimerism. Sequencing was performed using the Ion Torrent system with the selected non-specific primer which amplified all Mafa-B (MCM class I B) major haplotypes MI-M7. Primers successfully amplified by standard PCR a region of Mafa-B with known variability in sequence respective to haplotype. The product of interest was confirmed in length via gel electrophoresis, and melting curves demonstrated that the region of interest was only amplified signifying single, specific products. When sequencing these different products after PCR, the number of alleles of each haplotype would be identified. The number of sequences for each corresponding haplotype should have allowed for quantification of chimerism. When known haplotype animals were tested sequencing found that appropriate PCR products were amplified for all animals; however, presumably due to differences in efficiency not all alleles of each haplotype amplified at the same rate. Thus, sequenced ratios did not represent starting DNA ratios. Haplotype specific primers could be constructed as a next step; however, equal and somewhat specific amplification of multiple primers across multiple haplotypes would be necessary for accurate quantification. While one primer may not work for all scenarios, differentiation between the most common haplotypes would be progress toward PCR-based chimerism assays. Some of the clinical and immunologic rationale to select MCM for transplantation studies also result in unique challenges in chimerism detection.

The use of PCR and Ion Torrent sequencing should ultimately be a more efficient way to detect microchimerism in primate models. Currently, the development of non-specific primers to amplify all alleles with high PCR efficiencies is a challenge. PCR efficiency of 100% demonstrates a change of 3.3 cycles in cycle threshold in each 10-fold dilution. If each haplotype has a maximized PCR reaction at 100% efficiency, we can accurately determine chimerism utilizing these ratios.

Moving forward, the design of the primer is critical and the basis to the success of chimerism detection using this method. Focus is now on optimizing PCR conditions to provide equal and optimized yields. We have considered combining primers to ensure all alleles are amplified. Multiple primers could be given the same barcode making it possible to identify differences between alleles, while knowing that they came from the same sample. However, all options should be explored with a non-specific primer set. Using one general non-specific primer to amplify all haplotypes would allow for a much more simplistic assay without the need to mix multiple primers before PCR.

The use of sensitive methods for detecting chimerism levels is of interest in solid organ transplantation. In kidney transplantation tolerance trials, various methods have been used to quantitate chimerism. In general, flow cytometry cell sorting is performed followed by short tandem repeat (STR) analysis. As compared to the use of In/Del polymorphisms, STR methods are inferior in terms of sensitivity based on PCR efficiency.⁶⁻⁸ Flow cytometry utilizing HLA specific antibodies is also a standard way of evaluating chimerism but is limited by antibody specificity, which can be affected by processing, and is still not as sensitive as In/Del evaluation.⁹ In the renal transplant protocols mentioned above, levels of chimerism reported ranged from 5–100%. While a more sensitive chimerism detection tool may not change clinical results, it could yield important data regarding cell subsets or long-term stable microchimerism. Microchimerism levels may also represent an immunological marker of clinical relevance.¹⁰⁻¹²

The use of this technology may be of particular interest in liver transplantation where cells of donor origin have been found in the recipient and can persist for many years. However, as with other transplanted organs the clinical or immunological significance of this chimerism is unclear. The liver is hypothesized to be a tolerogenic organ with spontaneous tolerance occurring at measurable levels.^{13,14} In/Del evaluations to quantify chimerism could be an interesting research tool in this population, especially when coupled with flow cytometric techniques to evaluate rare but persistent cells of donor origin.

Looking forward, significant promise exists for the use of next generation sequencing platforms in the evaluation of chimerism in transplantation. These platforms can provide a more sensitive and reliable

tool in experimental and clinical trials, as well as, standard organ transplant recipients. Microchimerism could be an immunologic marker that could guide clinical decisions regarding immunosuppression adjustments and predict outcomes of rejection, stable function, and most interestingly – tolerance.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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