Flow cytometric assay for the Detection of Alloreactive T Cells as a Potential Tool for Immune Monitoring

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Both the direct and the indirect allorecognition pathways play a role in graft rejection. We and others have hypothesized that the presence of alloreactive primed memory T cells before and after transplantation, may increase the risk of a poor outcome following transplantation. Using the Miltenyi catch reagent, our laboratory has optimized a flow cytometric assay to identify cytokines secreting memory T cells in healthy individual's fresh whole blood cells (WBCs), and in separated PBMC's, in response to super antigen as well as pathogen's specific antigens, such as Tetanus Toxoid and PPD.

We used this method to assess our ability to determine and quantify alo-specific T cell responses due to both the direct and indirect recognition, in a one way MLR stimulation assay. Our data show that within 13-17 hours post re-stimulation, we are able to identify and quantify IFN-g secreting cells in MLR cultures following re-stimulation via the direct as well as the indirect-recognition pathways. We currently are applying this method to monitoring post transplantation kidney patients. We find that following transplantation and treatment with immunosuppresive drugs, patients' lymphocytes exhibit reduced ability to secrete IFN-g in response to super antigen or pre-immunized specific antigens. In addition, we have observed that when a patient has high numbers of IFN-g secreting lymphocytes immediately before transplantation, this patient may exhibit an early episode of acute rejection immediately following transplantation.

Our data clearly show that with our flow cytometric assay we can detect pre-immunized, memory T cells. The percents of detection in our hands are comparable to similar experiments done by other investigators, using the ELISPOT technique.

We find that the use of WBC simplifies the assay, and is also more physiological relevant, since the assay is performed in the presence of immunosuppressive agents. Moreover, cells can be phenotyped, enriched for, and further studied for function and other markers of immune activation.