Flow Cytometry for Crossmatch Evaluation in Renal Transplantation

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Dear Sir,

I read with interest the article by Stefoni et al. [1] published in your journal. One must applaud these authors on the magnitude of work required of such an endeavor. Promotion of long-term organ graft survival is the goal of every transplantation team, and so the development of new sensitive techniques for detecting donor-specific presensitization in potential recipients is paramount. Flow cytometric analyses can be performed rapidly on large numbers of cells (5,000-10,000 cells within a fraction of a minute) and so provide objective evaluations of patient serum antibodies to HLA-specificities of donor target cells. This technology is a major advancement over the labor-intensive and subjective visual appraisal of 200 cells on the light or fluorescent microscope used routinely, as these authors have clearly shown in their study.

The questions we ask are more important, however, than the ‘tools’ we use to answer them. Stefoni et al. [1] apparently have only asked if they can better detect weak positive reactions (false-negative crossmatches) using flow cytometry rather than light microscopy. Indeed they could, but they were only asking whether the flow cytometer is more capable of detecting cell death than a technologist using a microscope to visualize the incorporation of a vital dye (eosin-Y). They succeeded in improving the sensitivity of the ‘reading’ of the assay results, with subsequent improvement in the clinical outcome within the first month post-transplantation, but missed the opportunity to capitalize on the additional and established methods of increasing assay sensitivity.

Successful early graft function and survival is a benefit of improved pretransplantation crossmatching [2-9] which is accomplished most frequently by enhancing sensitivity of the crossmatch assay. The use of extended incubation periods [10], cell washes after the cell-serum sensitization step (to remove serum-borne anticomplementary factors) before adding complement [10] or 2nd antibody (antiglobulin) [4,11-15] each increases the sensitivity of the basic lymphocytotoxicity assay with subsequent improvement in the early post-transplantation period. The antiglobulin technique in particular has been successful in improving long-term graft survival, apparently by detecting not only low titered antibodies but also non-complement-binding IgG molecules. These antibodies are the initiators of the antibody-dependent cellular cytotoxicity which occurs when killer cells recognize and kill antibody-bound target cells [16-18]. Such antibody is not detected by complement-dependent cytotoxicity testing but is readily discovered with a 2nd (antiglobulin) antibody. Furthermore, the use of flow cytometric analysis with any of these techniques further...
enhances our ability of finding antibody (incompatibility) in an otherwise ‘compatible’ (antibody-negative) crossmatch due to the ability of the flow cytometer to detect only a few molecules of antibody on a cell membrane [2, 3, 6-9]. Indeed, Garovoy [9] has estimated that flow cytometric analysis can provide a level of sensitivity 50 times greater than that of microscopic assessment.

Stefoni et al. [1] may find, in addition to their flow-cytometric analysis, that using a fluorescein-conjugated F(ab')2 antihuman immunoglobulin to detect cell-bound antibody with isolated T cells (one-color fluorescent assay), or in conjunction with phycoerythrin-conjugated anti-CD3 and unseparated (B and T) lymphocytes (two-color assay), even greater improvement in graft survival is achieved. Furthermore, this methodology circumvents the use of the frequently troublesome biological reagent, rabbit complement. Finally, the inclusion of autocrossmatches would facilitate the final interpretation of allogeneic serum crossmatch results in cases when the host has formed antilymphocyte antibodies [19-21].

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Improved Crossmatching by Flow Cytometric Analysis