CHEST Online Supplement

Implications for Human Leukocyte Antigen Antibodies After Lung Transplantation

A 10-Year Experience in 441 Patients

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e-Appendix 1.

Immunosuppression: Induction immunosuppression includes basiliximab (2 doses) and methylprednisolone. Maintenance immunosuppression includes prednisone, azathioprine and tacrolimus. For patients intolerant of azathioprine, mycophenolate mofetil is substituted. For patients intolerant of tacrolimus, cyclosporine is substituted.

Bronchoscopy and rejection: Bronchoscopy is performed at 1, 3, 6, 9, and 12 months after transplant and then annually or as clinically indicated. Acute rejection is determined by the severity of inflammation around vessels in samples from transbronchial biopsies, surgical lung biopsies and autopsy specimens. The severity of grading is determined using the ISHLT criteria. Acute rejection is treated with methylprednisolone for initial and minimal or mild rejection. Recurrent rejection is treated with antithymocyte globulin or alemtuzumab.

Details of HLA antibody determination: Recipients were routinely screened pretransplant and posttransplant to correspond to surveillance bronchoscopies (3, 6, 9 and 12 months postoperatively and then annually). Additional HLA antibody tests were performed with clinical decline. Solid phase technologies including flow cytometry and Luminex are used for human leukocyte antigen (HLA) antibody screening and identification. The flow cytometry HLA antibody screening (One Lambda, Inc. Canoga Park, CA) utilizes a pool of latex microspheres coated with purified HLA class I or class II antigens which are incubated with the patient's serum according to the manufacturer's protocol, stained with fluorescein isothiocyanate-conjugated with anti-human IgG, and analyzed using a flow cytometer. The class II microspheres fluoresce when excited at 488nm with an emission of 580nm, while the class I microspheres are non-fluorescent. Fine specificities of the antibodies are determined by flow cytometry using microspheres coated with single HLA antigens (One Lambda, Inc. Canoga Park, CA). After

 DOI: 10.1378/chest.12-0587

incubation with the patient's serum, R-Phycoerythrin (PE)-conjugated goat anti-human IgG is added to detect bound antibody. A flow analyzer is used to detect the fluorescent emission of the PE from each bead. Computer programs are used to analyze the reaction patterns and assign the panel reactive antibody (PRA) percentage and HLA-specificity.

Flow single antigen bead reagents are available for 64 of the most common HLA class I A and B antigens and 32 of the most common class II DR and DQ antigens. The Luminex method (One Lambda, Inc. Canoga Park, CA) employs a panel of color-coded beads, which are coated with purified HLA antigens. Luminex single antigen bead array assay covers 97 class I antigens and 91 class II antigens. The specificity of an antibody binding was determined by the Luminex detection of the fluorescent emissions from the fluorescence attached to the secondary antibody and from the color coded bead. Our center uses MFI=1000 as a standard cutoff for a positive antibody binding. The established positive MFI value for Luminex single antigen bead assay was based on comparisons with flow cytometric single antigen bead assay, correlations with flow crossmatch, and the recommendation of the reagent vendor. Our center requires a repeat sample to confirm all de nove HLA antibodies as well as donor specific HLA antibodies.

Beginning in January 2000, we utilized flow cytometry assays (FlowPRA Screening test and Flow HLA antibody identification, One Lambda, Canoga, CA) to determine HLA antibodies. Since 2005, we have utilized Luminex single antigen bead array as a primary technology to determine HLA antibody specificities because of its wider coverage of various HLA antigens and more objective determination of antibody specificities.

HLA screening protocol: Prior to transplant, all recipients were screened for pre-existing HLA antibodies. Potential lung donors were screened using a virtual crossmatch of donor antigens to recipient HLA antibodies and in some cases, an additional prospective crossmatch. At the time of transplant, all recipients undergo a retrospective crossmatch. After transplant, all recipients are screened routinely for the presence and specificity of HLA antibodies.

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