Antibody detection in transplant patients

Xiaohai Zhang

Zhang X. Antibody detection in transplant patients. J Immunodiagnos. November-2017;1(1):4-5.

Organ transplantation is the most effective treatment for end stage organ failure. More than 500,000 patients have been transplanted with heart, lung, kidney, liver or small bowl allografts in the United States of America. There are more than 100,000 patients waiting for lifesaving organ transplants in the United States of America, mainly due to limited numbers of donors. It would be ideal if the allograft could function for a life time to fully utilize the limited resources. However, the transplanted organ is not a natural part of our body. In result, our immune system will attack the allograft as it recognizes it as foreign.

The adaptive immune system has two arms: cell mediated immune response, and antibody mediated immune response. In cell mediated immune response, cytotoxic T cells destroy foreign cells. In antibody mediate immune response, immunoglobulin that is produced by B cells neutralizes/destroys foreign antigens. Both cell and antibody mediated immune response depend on T helper cells. The specificity of immune response is controlled by the specificity of T cells. During T cell development, T cells which have high affinity with self-antigens, are deleted through a process called negative selection.

Major histocompatibility complex (MHC) is first identified in tumor transplant mouse models wherein tumors from an inbred strain failed to grow when they were transplanted into mice carrying different MHC alleles [1,2]. Human leukocyte antigen (HLA), a human version of MHC, is highly polymorphic. More than 10,000 alleles have been identified. In the setting of organ transplantation, the recipient's immune system detects the difference in HLA molecules between the recipient and donor, and mounts an attack against the graft that carries different HLA alleles. To prevent rejection of the allograft, it is necessary for transplant recipients to have their immune system suppressed. With immunosuppression regimen, we have successfully inhibited T cell mediated rejection, and improved short term outcome. Antibody mediated rejection, however, remains as a leading cause of allograft rejection and graft loss.

Antibodies injure cells through either complement dependent cytotoxicity or cell mediated cytotoxicity. Events of transfusion, previous transplant, and infections can stimulate the production of antibodies against HLA. To minimize the immunological risk, we match a donor with a patient that does not display, or displays less donor specific HLA antibodies. Detection of HLA antibodies also help diagnose antibody mediated rejection, which enables the treatment of rejection at an early stage. The complement dependent cytotoxicity assay is first used to detect donor specific antibodies in the field of organ transplantation [3]. In this assay, donor cells and recipient sera are mixed. If sera display donor specific antibodies and the concentration is high enough, the classical complement pathway can be activated, which may result in cell death. Later, the flow crossmatch technique is introduced to detect donor specific antibodies [4]. The amount of antibody binding on donor cells are detected by fluorescence labeled secondary anti-human antibody using flow cytometry. Though these two assays can detect the native form of antigens on the cell surface, they are not very sensitive. It is also not convenient to monitor antibody overtime since donor cells are needed every time.

Recently, solid phase beads based assays have been developed. The most popular platform is the Luminex single antigen test [5]. In this test, HLA antigens are conjugated to an array of polystyrene beads which are color coded. Each bead carries a unique HLA antigen. This array has about 100 beads covering HLA class I antigens and about 100 beads covering HLA class II antigens. The beads are mixed with sera and then incubated with the secondary fluorescence labeled anti-human IgG antibody. The amount of antibody binding on the beads are measured by the mean fluorescence intensity (MFI). The number of antigens conjugated on the beads is controlled by the manufacture to be consistent over different lots, which makes the assay relatively easy to be standardized. Importantly, this assay does not rely on donor cells, and can be used to monitor donor specific antibody more conveniently than complement dependent cytotoxicity and flow crossmatch antibody testing.

With the wide use of the single antigen test, several limitations of this assays have been identified. First, this assay can be interfered by other components in sera, such as activated products of complement components. An abundant antibody binding on the beads can stimulate the classical complement pathway. The activated complement components may mask the beads and result in the negative reaction [6]. Several methods have been used to remove this inhibition [7]. Second, the HLA antigens conjugated on the beads are derived from purified recombinant proteins. During the manufacture process, the native conformation of HLA antigens may be adversely altered. It has been suggested that some HLA antigens on beads are denatured [8]. Antibodies which bind these denatured antigens do not bind the native form of HLA antigens on the cell. In addition, some denatured beads tend to display non-specific binding, which results in a high background in the assay. Third, even though the amount of antibody binding on the beads in the test is expressed as numbers of mean fluorescence intensity (MFI), it is important to note, however, this test is not a quantitative test. According to the United States Food and Drug Administration (FDA), a quantitative test needs to have the coefficient of variation (CV) less than 20%. Because of many variables in the test procedure, the CV is likely to be about 50% [9]. Fourth, the mean fluorescence intensity (MFI) is not always linear correlated with the concentration of antibodies in sera. This assay is saturated at mean fluorescence intensity (MFI) at about 15,000 -20,000. When the antibody binding reaches the saturated level, sera need to be diluted first. Last, the density of HLA antigens on the beads is generally much higher than that on the surface of the cell. HLA expression also varies among alleles and individuals. Thus, the mean fluorescence intensity (MFI) of the beads cannot be directly translated into the amount of antibody binding on donor cells. Knowing these limitations is required to interpret single antigen test results correctly.

Methods of HLA antibodies testing are still evolving. The current single antigen array only covers about 100 alleles for HLA class I and class II,

HLA and Immunogenetics Laboratory, Comprehensive Transplant Center, Cedars- Sinai Health System, 8723 Alden Drive, SSB 197, Los Angeles, CA 90048, USA

Correspondence: Zhang X, HLA and Immunogenetics Laboratory, Comprehensive Transplant Center, Cedars-Sinai Health Systems, HLA and Immunogenetics Lab-SSB 197, 8723 Alden Dr., Los Angeles, CA 90048, USA. E-mail: Xiaohai.Zhang@cshs.org

Received: November 01, 2017, Accepted: November 02, 2017, Published: 07 November, 2017

respectively. There are many alleles which are not included in the array. The current single antigen testing may not provide sufficient information if a donor carries relative rare alleles. A single antigen test including more alleles will be helpful in those cases. Besides HLA, it is suggested that antibodies against non-HLA antigen may also cause antibody mediated rejection [10]. It is not uncommon that patients with allograft dysfunction and histological characteristics of antibody mediated rejection on biopsy, do not have donor HLA specific antibodies. Identification and detection of non-HLA antibodies will also help diagnose antibody mediated rejection and stratify the risk of antibody mediated rejection.

REFERENCES

1. Gorer A. The genetic and antigenic basis of tumour transplantation. J Pathol 1937;44:691.
2. Snell D. Methods for the study of histocompatibility genes. J Genet 1948;49:87-108.
3. Patel R, Terasaki I. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 1969;280:735-39.
4. Ogura K, Terasaki I, Johnson C et al. The significance of a positive flow cytometry crossmatch test in primary kidney transplantation. Transplantation 1993;56:294-98.
5. Reinsmoen L. Impact of solid phase antibody testing on organ allocation in the United States. Clin Transpl; 2013:393-98.
6. Schnaidt M, Weinstock C, Jurisic M et al. HLA antibody specification using single-antigen beads--a technical solution for the prozone effect. Transplantation 2011;92:510-5.
7. Zhang X, Reinsmoen L. Comprehensive assessment for serum treatment for single antigen test for detection of HLA antibodies. Hum Immunol 2017:S0198-8859(17)30492-95.
8. Sullivan C, Gebel M, Bray A. Understanding solid-phase HLA antibody assays and the value of MFI. Hum Immunol 2017;78:471.
9. Reed F, Rao P, Zhang Z et al. Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA-drilling down on key sources of variation. Am J Transplant 2013;13:3050-51.
10. Zhang X, Reinsmoen L. Impact of Non-Human Leukocyte Antigen-Specific Antibodies in Kidney and Heart Transplantation. Front Immunol 2017;8:434.