Conformational Variants of HLA-I Antigens on Luminex Single Antigen Beads

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We read with great interest the report by Jucaud et al¹ describing the use of monoclonal antibodies for determining the relative proportions of HLA-I conformational variants displayed by Luminex Single Antigen Beads (LSAB). They confirmed previous results showing that (1) LSAB display significant amounts of HLA-I that are not associated with beta-2-microglobulin (β 2m) and/or peptide, as opposed with native and functional peptide-associated β 2m-associated heavy-chains (pepA- β 2aHC),² (2) iBeads are largely devoid of β 2m-free heavy-chains (β 2fHC) but have lower pepA- β 2aHC levels,^{2,3} (3) resting cells only express pepA- β 2aHC,⁴ explaining why antidenatured HLA antibodies (anti-dHLA) rarely provide positive crossmatches and do not impair graft survival.^{2,3,5} Their findings are interesting but several issues could be discussed.

First, the TFL-006 monoclonal antibody generated by the authors does not seem very efficient at staining the β 2fHC that are present on LSAB. Indeed, TFL-006 provided lower mean fluorescence intensities (MFI) than HC-10 on acid-treated beads (that display only β 2fHC, letter Figure 1), this later showing, as expected, comparable MFI with W6/32 on nontreated LSAB. In line with this, in patients' sera, we described anti-dHLA directed against all the beads which are not recognized by TFL-006.³ Therefore, assessing the fraction of β 2fHC on HLA-I beads by comparing TFL-006 and W6/32 MFI and stating that HC-10 reactivity on iBeads is mainly due to peptide-free β 2m-associated heavy-chains

FIGURE 1. Reactivity of 3 monoclonal antibodies on nontreated and acid-treated HLA-I LSAB. Nontreated and acid-treated LSAB were incubated with W6/32, anti- β 2m (both from Thermo Fisher Scientific, Rockford, IL) and HC-10 (kindly provided by One Lambda) at 10 μ g/mL, then were washed and incubated with 200-fold diluted PE-labeled Goat F(ab')₂ fragment anti-mouse IgG (H + L) (Beckman Coulter, Marseille, France). After acid treatment, all beads reached MFI < 1000 with W6/32 and 3 beads still had MFI $\geq$ 1000 with anti- β 2m.

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(pepF- β 2aHC) solely on the basis that TFL-006 displayed lower MFI than HC-10 should be considered with caution.

Second, the “alkali-treatment” of LSAB described by the authors is performed with concentrated guanidinium chloride, a chaotropic agent, and is much harsher than a denaturation induced by a mild pH change. After this treatment, the MFI of TFL-006 became higher than with acid-treated LSAB, but remained lower than HC-10 MFI that did not reach the same level observed on acid-treated beads. These results indicate that TFL-006 could recognize an isoform of β 2fHC that is not denatured the same way as the one present on LSAB, and underline that the kind of LSAB denaturing treatment could greatly impact the denaturation process in ways that are not understood yet.

Finally, the authors proposed that our previous results showing that anti-dHLA can still stain iBeads corresponded to antibodies directed against pepF- β 2aHC.^{3,5} To us, this is highly unlikely because acid-induced denaturation is known to release β 2m from HLA molecules, leading to an almost complete loss of β 2aHC. Moreover, we clearly demonstrated that reactivities resembling those of anti-dHLA can correspond to anti-native HLA (anti-nHLA) antibodies recognizing peculiar epitopes displayed by pepA- β 2aHC that are conserved on β 2fHC, and/or mixtures of anti-nHLA and anti-dHLA antibodies recognizing the same antigens.⁴

In conclusion, Jucaud et al¹ brought additional data about denatured HLA-I molecules borne by LSAB. Their results underline the difficulty of correctly interpreting anti-dHLA patterns using strategies relying on bead-specific cutoffs based on monoclonal antibodies reactivity or chemical treatments of LSAB. As suggested by the authors, the transplant community could plead in favor of the resurgence of iBeads production because this assay proved efficient in eliminating the anti-dHLA detection and showed promising results.^{2,3,5}

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The Authors' Reply

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We thank Visentin and coauthors¹ for pointing out that our findings² are in line with their earlier observations. Because the current clinical objective is to detect the antibodies (Abs) binding to peptide-associated β 2-microglobulin-associated HLA-I heavy chains (pepA- β 2aHC), the native HLA-I conformation expressed on the surface of resting cells, the presence of other conformational variants on single antigen beads (SAB) prevents the distinction between clinically relevant and irrelevant HLA Abs, in pre- and posttransplant patients, when the mean fluorescence intensities is below a certain threshold depending on the specific bead. The indiscriminate usage of the term anti-denatured (dn) HLA Abs adds additional concern. El-Awar et al³ have referred peptide-free β 2-microglobulin-free HLA-I heavy chains (pepF- β 2fHC) as natural antibodies or “anti-dnHLA Abs.” To distinguish pepA- β 2aHC from pepF- β 2fHC, the

regular beads are subjected to acid or alkali or heat treatments, which not only releases β 2-microglobulin and a peptide from the heavy chain (HC) but also alters the physical configuration of the HC, depending on the treatments. Failure to recognize this alteration has created ambiguity and misperception, as in a recent publication.⁴ Opposite conclusions were inferred from the impact of anti-dnHLA Abs using different denaturation methods. Visentin et al⁵ have used “acid-treated beads” to conclude that anti-dnHLA Abs do not impair graft survival,¹ whereas Cai et al⁶ used the “heat-treated (90°C) beads” to state that “complement-fixing antibodies against denatured HLA and MICA antigens are associated with antibody mediated rejection.” The β 2fHC are also expressed naturally (“open conformers”⁷) on activated lymphocytes,⁸ and serve as ligands for leukocyte receptors.⁹ Therefore, the naturally occurring (or expressed) β 2fHC cannot be referred to as dnHLA. Perhaps beads with only β 2fHC may specifically identify clinically relevant β 2fHC Abs.

It is true that monoclonal antibodies TFL-006 provided lower mean fluorescence intensities than monoclonal antibodies HC-10 on acid-treated beads (displaying only pepF- β 2fHC). Obviously, the acid denaturation (acid-dn) affected the epitope recognition by TFL-006 more strongly than the one recognized by HC-10. The following reasons support the above contention:

- TFL-006, raised by immunizing the folded β 2m-free HLA-E^{R107} HC,¹⁰ recognizes a conformational epitope on β 2fHC. In

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