Original citation:

Permanent WRAP URL:
http://wrap.warwick.ac.uk/94714

Copyright and reuse:
The Warwick Research Archive Portal (WRAP) makes this work by researchers of the University of Warwick available open access under the following conditions. Copyright © and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable the material made available in WRAP has been checked for eligibility before being made available.

Copies of full items can be used for personal research or study, educational, or not-for-profit purposes without prior permission or charge. Provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

Publisher’s statement:
© 2018, Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International http://creativecommons.org/licenses/by-nc-nd/4.0/

A note on versions:
The version presented here may differ from the published version or, version of record, if you wish to cite this item you are advised to consult the publisher’s version. Please see the ‘permanent WRAP URL’ above for details on accessing the published version and note that access may require a subscription.

For more information, please contact the WRAP Team at: wrap@warwick.ac.uk
Direct quantitative measurement of the kinetics of HLA-specific antibody interactions with isolated HLA proteins.

Sunil Daga1,2,9, Harry Moyse3, David Briggs4, David Lowe5, Neil Evans3, James Jones5, Rico Buchli7, Curtis McMurtrey6, Arend Mulder8, William Hildebrand6, Frans Claas8, Robert Higgins1,9, Daniel A Mitchell8*, Daniel Zehnder1,9*,

*Equal contributions

Institutions

1. Renal Department, UHCW NHS Trust, Coventry, UK
2. Renal Department, St. James’s University Hospital, Leeds, UK
3. School of Engineering, University of Warwick, Coventry, UK
4. H&I department, NHSBT, Birmingham, UK
5. H&I department, Royal Liverpool University Hospital, Liverpool, UK
6. University of Oklahoma Health Science Center, Oklahoma City, OK, United States.
7. Pure Protein LLC, Oklahoma City, OK, United States.
8. Immunohaematology and Blood Transfusion, Leiden University Medical Center, 2333 ZA Leiden, the Netherlands
9. Warwick Medical School, University of Warwick, Coventry UK

Corresponding author: Dr Sunil Daga sunildaga@nhs.net
Abstract

HLA specific antibodies vary in their pathogenicity and this is likely to be the net effect of constant chain usage, quantity, specificity, and affinity. Here we have measured the affinity of human monoclonal antibodies for a range of HLA proteins. Purified antibodies and ligands allowed dynamic interactions to be measured directly by surface plasmon resonance. Physiochemical differences between pairs of ligands were quantified using electrostatic mismatch and hydrophobic mismatch scores.

All antibodies were characterized by fast on-rates and slow off rates but with a wide range of association rates ($k_{on}$, 3.63-24.25x10⁵ per mol per second) and dissociation rates ($k_{off}$, 0.99-10.93x10⁻³ per second). Dissociation constants ($K_D$) ranged from 5.9 x10⁻¹⁰M to 3.0 x10⁻⁸M. SN320G6 has approximately a twenty-fold greater affinity for HLA A2 compared with SN607D8, but has a similar affinity for HLA-A2 and B57. In contrast, SN607D8 has greater than a two-fold greater affinity for HLA-A2 compared with A68. Similarly, WK1D12 has about a three-fold greater affinity for HLA-B27 compared with B7. The higher affinity interactions correlate with the specificity of stimulating antigen. This is the first study to directly measure the binding kinetics and affinity constants for human alloantibodies against HLA.

Key words: HLA-specific antibodies, affinity, surface plasmon resonance, binding kinetics, epitope, eplet.

Abstract word count: 192

Article word count: 2,937
1. Introduction

HLA specific antibodies are a relative barrier to solid organ transplantation. Higher levels, which typically precipitate a cytotoxic crossmatch, can cause hyperacute rejection [1]. Lower levels may be tolerated or be associated with acute rejection and/or poor outcome [2][3][4][5][6]. Post-transplantation, the appearance of donor HLA-specific antibodies (DSA) is often, but not always, associated with acute or chronic rejection and transplant glomerulopathy [7][3][8][9][10]. The ability of an HLA-specific antibody or antibody mixture (e.g. serum) to activate the classical pathway of the complement system appears to be related antibody pathogenicity, although this functionality is not necessarily proportional to measured antibody level [11][12][13][14]. Antigen specificity (or the degree of expression of the target antigen) is a further important criterion related to pathogenicity as, for example, transplantation can be successful in the presence of cytotoxic positive crossmatches due to HLA Class II antibodies [15].

The specificity of HLA antibodies is defined by reaction patterns against different alleles sharing specific amino acid residues [16]. These are generally considered to be the nominal epitopes allowing binding of the antibody and tend to be reduced to the minimal number of required residues, sometimes to a single amino acid. It has been suggested that the critical interactions for binding involve those residues within a 3 Å diameter patch, termed an eplet [17][18]. However, the exact conformation and orientation of the interaction has yet to be shown for any HLA protein-anti-HLA antibody complex [19]. The footprint of the antibody complementarity determining regions (CDRs) can be up to 900 Å² in surface area [20][21], and therefore a larger number of amino acid residues within the HLA protein, perhaps up to 25, will likely be involved in determining the nature of the interaction, imparting both specificity and affinity. Importantly, the reactivity of HLA proteins with antibody can vary even when sharing the critical epitope/eplet. It is speculated that the difference in reactivity is due to a difference in release of binding energy which is proportional to the total surface area of interaction [22][23]. The immunogenicity of the HLA antigen, defined as its ability to evoke/stimulate a de novo antibody response can also vary and is believed to be related to electrostatic and hydrophobic properties of amino acid residues within polymorphic regions of the HLA protein [24][25][26].

Current solid phase single antigen bead assays measure mean fluorescence intensity (MFI) and this readout is often used to estimate amounts of antibodies, although this has not been validated. High value MFIs will have components relating to both higher absolute antibody concentrations and to stronger antibody binding (affinity). Resolving the distinction between concentration and affinity is important because affinity measurements are likely to provide a greater insight into antibody function. During the immune response, the affinity of antibodies typically increases by affinity maturation. This is generated via somatic hypermutation of immunoglobulin gene segments, leading to clonally selected variations in amino acid sequences that favor
increased binding to target antigens [27]. Only one study has previously determined the affinity of HLA antibodies, and that was of mouse monoclonal antibodies against human HLA-A2 protein expressed on human B-cell lines. That study shows marked differences in affinity between F_{ab} fragments from two separate monoclonal antibodies (determined to be in the micromolar and nano-molar range, respectively), which were due to difference in the dissociation rates [28]. The authors used a saturation binding technique with radio-labeled antibodies. Our study is the first to directly measure the affinities of human allo-antibodies against HLA.

Through the use of real time biosensor techniques (by surface plasmon resonance), we have been able to measure binding kinetics and the affinity constants of antibodies against different HLA proteins that share epitopes/eplets but differ in adjacent residues. This approach was used to determine quantitatively the importance of epitope configurations in relation to affinity. Here, we used this technique to measure the affinity of clinically relevant anti-HLA antibodies.

2. Material and Methods

2.1. Biotinylated soluble recombinant HLA proteins.

Biotinylated soluble recombinant HLA proteins (sHLA) were provided by Pure Protein LLC (Oklahoma City, OK, USA) [29]. Products of the following genes were studied; HLA-A*01:01, A*02:01, A*68:01, B*57:01, B*07:02, and B*27:05. We will refer to these proteins as HLA-A1, A2, A68, B57, B7, and B27, respectively, for simplicity. The concentration of the purified biotinylated molecules was determined using the Micro BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL) using bovine gamma globulin (Sigma; Poole, UK) as a protein standard.

2.2. Human monoclonal HLA-specific antibodies.

Human monoclonal HLA-specific antibodies (mAbs) were secreted by human hybridomas produced by Epstein-Barr virus (EBV) transformation of B-lymphocytes from HLA antibody positive multi-parous women (sensitised during pregnancy), followed by fusion and sub-cloning of antibody producing EBV lines (Table 1). SN607D8 and SN203G6 were derived from the same multiparous donor (HLA type A*24 A*29, B*07, B*44, C*07, C*16) sensitized during pregnancy (child’s HLA type A*02, A*29, B*44, B*57). WK1D12 was derived from a female donor (HLA type A*01, B*08, C*07) sensitized during pregnancy (child’s HLA type A*01, A*11, B*08, B*27, C*01, C*07).
Antibody-containing supernatants were dialyzed (8,000 MWCO Spectra/Por® Dialysis) against PBS overnight. The supernatants were further processed by affinity chromatography using HLA protein immobilized on Sepharose beads [29] and ion-exchange chromatography using Q-Sepharose. HLA-A2 (from A*02:01) and B7 (from B*07:02) were separately coupled to cyanogen bromide activated Sepharose and the resulting columns were used to enrich mAbs SN203G6 and SN607D8, and WK1D12 respectively. Overall this approach yielded highly purified monoclonal IgG and avoided contamination with residual bovine IgG and other serum components from the hybridoma culture medium. The homogeneity of the purified IgG was confirmed via SDS-PAGE using 4-12% gradient polyacrylamide gels. The concentration of purified antibodies was determined using Lowry’s assay [30]. CDC defined antibody specificities were confirmed in a single antigen bead assay (One Lambda, Canoga Park, CA) as per manufacturer’s instructions. Dose response curves from antibody doubling dilution series were used to calculate effective concentrations that gave 50% of maximal MFI signal (EC-50). Hill-type EC-50 values were calculated using curve fitting performed in MATLAB.

2.3. Surface Plasmon Resonance.

Sensorgrams were obtained using the Bio-Rad Proteon XPR36 biosensor platform [13] [14]. 2.5 µg/ml of biotinylated HLA protein was immobilized on neutravidin-coated sensor chips (ProteOn™ NLC Sensor Chip #1765021) with a flow rate of 25 µl/min over 300 seconds. Purified monoclonal HLA-specific antibodies were flowed over the chip at a rate of 25 µl/min (slowest rate for the equipment to allow maximum binding) for 960 seconds to obtain equilibrium phase (where the sensorgram of binding phase plateaus) and at 37°C in the association phase to represent physiological binding temperatures. Following this, the running buffer (PBS containing 0.05% Tween 20) was passed over for 960 seconds at a flow rate of 25 µl/min for the dissociation phase. Interactions of all three HLA-specific antibodies were studied in duplicate over a range of six different concentrations (3.125 to 100 nM) against the same surface concentration of immobilized HLA protein.

The data obtained from the sensorgrams were modelled using an implementation of differential evolution [31] on MATLAB software (Mathworks®) with the simulation tool FACSIMILE (MCPA Software) [32]. Together these allow for curve fitting and simultaneous determination of kinetic association ($k_{on}$) rates, dissociation ($k_{off}$) rates and the Dissociation Constant $K_D$ (as the ratio of the dissociation $k_{off}$, and association $k_{on}$, rate constants).

2.4. Molecular differences between the allele pairs.

The PyMOL Molecular Graphics System, Version 1.1 Schrödinger, LLC was used to model the crystallographic structures of HLA-A2 (PDB ID: 3MRK), A68 (PDB ID: 4HWZ), B27 (PDB ID: 10GT identifier), B57 (PDB ID: 2YPK), and B7 (PDB ID: 3VCL). These structural diagrams were used to show
molecular differences between each allele pair shown. We considered the area within a 15 Å radius around the designated epitope on the HLA molecule examined (as compared to the whole protein), since this corresponds to the maximum radius of the antibody recognition site comprising the cognate antibody paratope [18]. Within this putative contact area on the HLA molecule the number of amino acid differences, electrostatic mismatch score (EMS), and hydrophobic mismatch score (HMS) [26] [24], were estimated to quantify physiochemical differences between pairs of HLA proteins. Two sets of comparisons were made, including and excluding differences located within the peptide binding groove.

3. Results

3.1. Determination of binding kinetics for human monoclonal HLA-specific antibody-HLA protein interactions.

The sensograms illustrating the interactions between human HLA-mAbs and immobilized HLA-proteins are distinct, specific and reproducible (Figure 1). Differences in signal (defined by response units, RU) and kinetics ($k_{on}$ and $k_{off}$ values) were observed for different antigen-antibody interactions (data given in Table 2). Consistent with the known specificities of these antibodies (Table 1) SN607D8 showed binding to HLA-A2 and A68 and not to HLA-A1; SN230G6 bound to HLA-A2 and B57 and not to HLA A1; WK1D12 bound to HLA-B7 and B27 and not to HLA-A1.

Modelling of the sensorgram data was optimised using MATLAB together with the simulation tool FACSIMILE, with the kinetic parameters determined across both phases using the full dynamic data. The quality of the fit of the model to the data to provide the kinetic constants was assessed visually (Figure 2) and quantitatively within MATLAB/FACSIMILE via the residual sum of squares. The fitting process yielded fits with low residual sum of squares in which typically the estimated SPR signal remained within the sensorgram signal throughout the experiment (Figure 2). The binding kinetics modelled varied greatly between the three antibodies against the HLA proteins which they are known to recognize (Table-2). All are characterized by fast on-rates and slow off rates but there is a wide range in the magnitude of these parameters for the reactions tested. The association rates ($k_{on}$) varied between 3.63 and 24.25 $\times 10^5$ per mol per second and the dissociation rates ($k_{off}$) varied between 0.99 and 10.93 $\times 10^{-3}$ per second. The dissociation constant ($K_D$) values obtained were in the range of 0.1-10 nM (Table 2).
SN230G6 has an affinity (inverse of $K_D$) for both HLA-A2 and B57 around an order of magnitude greater than that of SN607D8 for HLA-A2 or A68; against just HLA-A2, the former exhibits a twenty-fold greater affinity compared with the latter. Figure 1 shows that SN230G6 has a faster on-rate, and reaches a higher equilibrium level than SN607D8. The greater affinity of SN230G6 for HLA-A2 is also seen with a dose-response experiment via bead assay: SN607D8 requires about a ten-fold greater concentration to reach half-maximal binding (EC-50) compared with SN230G6 (Table 2). EC-50 analysis was not able to distinguish any difference in the binding of these two antibodies to HLA-A2 compared to either A68 or B57 (SN607D8 and SN230G6, respectively). In contrast, the biosensor assay showed that SN607D8 has approximately 2.5 times the affinity for HLA-A2 compared with A68. SN230G6, on the other hand, has similar affinities for HLA-A2 and B57 (1.33-fold difference). We also found that WK1D12 has about a three-fold greater affinity for HLA-B27 compared to B7. Thus, in the two cases where the stimulating HLA for the antibody is known, the affinity is greater for that allele compared to a different allele but one which shares the critical binding motif.

3.2. Influence of molecular difference on binding affinities.

Figure 3 shows the structural differences between the HLA pairs within a radius of 15Å about the notional epitope or eplet that associates with the reactivity of each antibody. This corresponds to the binding footprint as described by Duquesnoy et al. [18]. Within this area (900Å$^2$) we counted the number of amino acid residue differences, and the EMS and HMS for each pair (data given in Table 3). All the designated epitopes/eplets lie adjacent to the peptide-binding groove and it is not certain how, or indeed if substitutions within the groove could contribute to the binding of these alloantibodies. Therefore, we have calculated the differences between the HLA pairs with and without the internal binding groove residues. These results are given in Table 3. It is immediately obvious that none of these physical differences correlate with the affinity differences between any of the HLA pairs and their respective antibodies. The largest number of amino acid differences of the antigen pairs examined is seen between HLA-A2, and B57, this is unsurprising as antigens encoded by different HLA gene loci would be reasonably expected to show increased variation. However, the difference in SN230G6 binding to these individual antigens is lowest. SN607D8 and WK1D12 exhibit similar differences in affinity for their tested ligands but there is a two-fold difference in the $\Delta$HMS and $\Delta$EMS values.
4. Discussion

This paper describes the first use of a real time, label free biosensor technique to characterize biophysical properties of human, monoclonal, HLA-specific antibodies. We were able to show distinct $K_D$ values for different HLA antibody/HLA allele combinations, in the order of 0.1nM to 10.0nM. We have shown that the signal obtained displays the expected HLA specificity and we can discern a distinct signal for low nanomolar concentrations of these human monoclonal HLA-specific antibodies. The dissociation constants obtained for mAbs in this study are similar to previously reported values for target ligands [33] [34] [28]. SPR has been used to detect and study the binding interactions of dsDNA-specific antibodies in patients with SLE [35] while a recent study of ninety-nine patients showed the avidity of dsDNA antibodies correlates with disease activity of lupus nephritis [36]. It is therefore reasonable to assume that the characteristics of the HLA antibodies we describe here relate to their pathogenicity and are therefore likely to be of clinical relevance. Further to this, a given antibody may differ in its pathogenicity depending on its target allele because its affinity will vary accordingly. Duquesnoy et al [22] have shown that the same monoclonal antibody can be associated with different degrees of complement-dependent cytotoxicity depending on the HLA allele expressed on the target cell. Those authors speculated that this involved target affinity differences and here we prove that such affinity differences do exist.

The two human antibodies (SN230G6 and SN607D8) which bind to HLA-A2 were derived from the same multiparous woman. SN607D8 is clearly the consequence of HLA-A2 immunization whereas SN230G6 could be the result of HLA-A2 or B57 immunization, or possibly by both as they were co-expressed by the sensitizing individual. The affinity of SN230G6 for HLA-A2 (epitope 62GE) is 20-fold greater than of SN607D8 for HLA-A2 (epitope 142TKH). Kushihata et al [37], measured the CDC activity of these mAbs and showed a ten times higher CDC reactivity by SN230G6 compared to SN607D8 against the same target cell. We showed a similar difference of their EC-50 values in a single antigen bead assay (Table 2). This EC-50 analysis also demonstrates very starkly that the amount of serum antibody that binds to each bead is not a simple reflection of its serum concentration; it is also affinity dependent. The affinity difference for HLA-A2 is related to differences in kinetic rates; the association rate is faster and the dissociation rate slower with SN230G6. The variation in affinity of a single mAb, SN607D8, for the two serologically related antigens, HLA-A2 and A68, could be due to interaction with different residues in the immediate vicinity of the distinguishing epitope which in turn may alter the spatial orientation and steric accessibility of the epitope from one antigen to the other. Thus, both the best fit and surface area involved in the overall interaction may determine the stability of binding [19]. Although the difference in dissociation constant is relatively small (within one order of magnitude), the kinetic parameters (association and dissociation rates) differed more widely. Therefore, for comparisons, dissociation constants alone may not give a full picture. Close inspection of our data reveals that while the on-rates for binding of one antibody to different HLAs may be similar, the overall affinity may
be more dependent on the dissociation rate, and this may be markedly slower for the target HLA that corresponds to the immunizing antigen (Figure 4). These are useful observations as current solid phase assays do not distinguish these differences in binding characteristics which may be important in determining biological reactivity of the HLA-specific antibodies and their downstream effects. Thus, the higher affinity associated with a stimulating antigen suggests that a repeat antigen mismatch transplant might impart a greater risk than if only the epitope were repeated in an antibody incompatible transplant.

The high degree of sequence homology between alleles (and between the products of different loci) results in cross-reactivity due to shared immunogenic epitopes and eplets. We have shown that despite sharing a critical sequence, which confers specificity, substitutions in other parts of the target molecule can result in large affinity differences. It was not obvious which of the mismatched residues or their physiochemical properties accounted for these differences and this is most likely because we do not know the orientation of binding or the exact interface surfaces. This is also illustrated by the similar affinity of SN230G6 for HLA-A2 and B57, products of different loci, which implies that there might be an almost identical antibody-accessible surface on these two molecules. We are unable to resolve whether or not non-exposed residues in the peptide binding groove contributed to the binding kinetics of any of the antibodies studied, either directly or by virtue of the peptides presented. Although SN607D8 binds differently to HLA-A2 and A68, the only differences between these alleles within 15A of the presumed critical eplet (142TKH) are within the peptide binding groove. Taken together, this suggests that the model by which antibody binding is precisely centered around an eplet [18] may not be correct, at least for these antibodies. Ultimately these issues are likely to be resolved by structural imaging experiments.

In conclusion, the binding assay described here possesses advantages over current assays since it captures a broader, real-time picture of the binding events and levels, compared with simpler end-point assays such as Luminex single antigen bead assay, ELISA and flow cytometry. There are, of course difficulties with SPR, the requirement for highly purified antibodies because the sensor chip surface is very sensitive to non-specific binding. By purifying the antibodies, we have been able to directly measure the binding kinetics of HLA antibodies and these measurements are likely to be critical in the understanding of the pathogenicity of these antibodies. Another exciting opportunity lies in the ability of SPR experiments to obtain thermodynamic data (such as free energy released) for these interactions, yielding further parameters of potential physiological value plus deepening our understanding of antibody function and biophysics. Our experiments have also underlined the limitations of our knowledge of how alloantibodies bind to their HLA targets. Although we can now show whether or not a particular antibody binds differently to its known ligands and measure the magnitude of any variation, we do not yet know completely the molecular basis underlying such differences.
Acknowledgement: The research was funded by CLRN-NIHR West-Midlands (South) and Coventry Renal Research Fund, UHCW NHS Trust.
References


17 M. Marrari, R.J. Duquesnoy, Correlations between Terasaki’s HLA class II epitopes and HLAMatchmaker-defined eplets on HLA-DR and -DQ antigens, Tissue Antigens. 74 (2009) 134–146.


24 D.H. Mallon, J.A. Bradley, P.J. Winn, C.J. Taylor, V. Kosmoliaptsis, Three-Dimensional Structural Modelling and Calculation of Electrostatic Potentials of HLA Bw4 and Bw6 Epitopes to Explain the Molecular Basis for Alloantibody Binding: Toward Predicting HLA Antigenicity and Immunogenicity, Transplantation.99 (2015) 385-90


### Table 1: Characteristics of IgG human monoclonal HLA-specific antibodies. Epitope designations use the nomenclature from the HLA epitope registry (www.epregistry.br) and are based on reaction pattern rather than a known binding specificity. *Corresponding to the alleles from which the proteins were derived.

<table>
<thead>
<tr>
<th>mAb</th>
<th>Immunizing HLA</th>
<th>Bead assay-defined HLA specificities*</th>
<th>Epitope designation</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN607D8</td>
<td>A2</td>
<td>A<em>02:01/03/06, A</em>68:01/02, A*69:01</td>
<td>142TKH</td>
<td>IgG1, κ</td>
</tr>
<tr>
<td>SN203G6</td>
<td>A2 and/or B57</td>
<td>A<em>02:01/03/06, B</em>57:01/03, B*58:01</td>
<td>62GE</td>
<td>IgG1, λ</td>
</tr>
<tr>
<td>WK1D12</td>
<td>B27</td>
<td>B<em>27:05/08, B</em>07:02, B<em>13:02, B</em>40:01/02/06, B*81:01</td>
<td>163EW</td>
<td>IgG1, κ</td>
</tr>
</tbody>
</table>

*Corresponding to the alleles from which the proteins were derived.
Table 2: Difference in kinetic rates and dissociation constants for mAbs interaction with different alleles. Each shown $k_{on}$, $k_{off}$, and $K_D$ value is from two separate binding experiments. The original sensitizing antigens are shown in bold. ND = not done. EC-50 values were calculated using dose response curve fitting performed in MATLAB.

<table>
<thead>
<tr>
<th>mAb</th>
<th>Target HLA</th>
<th>Bead assay</th>
<th>Sensorgram analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EC-50</td>
<td>$k_{on}$ (M$^{-1}$s$^{-1}$)</td>
</tr>
<tr>
<td>SN607D8</td>
<td>A2</td>
<td>2</td>
<td>4.6x10$^5$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8</td>
<td>4.05x10$^5$</td>
</tr>
<tr>
<td></td>
<td>A68</td>
<td>1.6</td>
<td>3.23x10$^5$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.05x10$^5$</td>
</tr>
<tr>
<td>SN230G6</td>
<td>A2</td>
<td>0.18</td>
<td>23.2x10$^5$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.83</td>
<td>19.6x10$^5$</td>
</tr>
<tr>
<td></td>
<td>B57</td>
<td>0.15</td>
<td>28.7x10$^5$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>19.8x10$^5$</td>
</tr>
<tr>
<td>WK1D12</td>
<td>B27</td>
<td>ND</td>
<td>9.8x10$^5$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.1x10$^5$</td>
</tr>
<tr>
<td></td>
<td>B7</td>
<td>ND</td>
<td>14.2x10$^5$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.8x10$^5$</td>
</tr>
<tr>
<td>mAb</td>
<td>HLA pairs</td>
<td>All</td>
<td>Exposed</td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
<td>-----</td>
<td>---------</td>
</tr>
<tr>
<td>SN607D8</td>
<td>A2, A68</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>SN230G6</td>
<td>A2, B57</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>WK1D12</td>
<td>B27, B7</td>
<td>15</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 3: Molecular and physiochemical differences between alleles in the region described by a 15 Å radius around the presumed epitope. *’All’ includes those amino acid residues within the peptide binding groove and exposed excludes these when comparing between the two antigens listed on each row of the table.
**Figure 1**: Binding kinetics of three of human mAbs to HLA. a. SN607D8; b. SN230G6; c. WK1D12. The sensorgrams show antibody binding at six concentrations as shown in the top right panel. Note the scale of the y axes is the same for and b (max 300 RU) and different for c (max 600 RU).

**Figure 2**: Data fitting examples (solid black lines) shown (SN607D8 binding to HLA-A2 (a) SN230G6 to HLA-B57 (b) and WK1D12 to HLA-B27 (c).

**Figure 3**: Structural differences between HLA pairs, each recognized by the same antibody (a. SN607D8; b. SN230G6; c. WK1D12). Red positions indicate the amino acid residues that associate with the specificity of the antibody and define the notional epitope. On the left-hand ribbon diagrams, yellow shows the area within a radius of 15Å from each such epitope and suggest a binding footprint of the antibody. Yellow residues on the right marks the amino acid differences within this footprint for each HLA pair.

**Figure 4**: Relationships between association (on) and dissociation rates (off) for the three mAbs and their target ligands, as follows: △ SN607D8+HLA A2; ▲ SN607D8+HLA A68; □ WK1D12+HLA B7; ■ WK1D12+HLA B27; ● SN230G6 + HLA A2 and HLA B57. Where the target HLA and the HLA present during immunization are the same the symbols in the figure are filled (HLA A2 and B57 reactions with SN230G6 are identified). Each of the duplicate results are shown.