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Anti-HLA-E mAb 3D12 mimics MEM-E/02 in binding to HLA-B and HLA-C alleles: Web-tools validate the immunogenic epitopes of HLA-E recognized by the antibodies

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ABSTRACT

HLA-E shares several peptide sequences with HLA-class Ia molecules. Therefore, anti-HLA-E antibodies that recognize the shared sequences may bind to HLA-class Ia alleles. This hypothesis was validated with a murine anti-HLA-E monoclonal antibody (mAb) MEM-E/02, which reacted with microbeads coated with several HLA-B and HLA-C antigens. In this report, the hypothesis was reexamined with another mAb 3D12, considered to be specific for HLA-E. The antibody binding is evaluated by measuring mean fluorescence index [MFI] with Luminex Multiplex Flow-Cytometric technology. The peptide-inhibition experiments are carried out with synthetic shared peptides, most prevalent to HLA-E and HLA-Ia alleles. The results showed that mAb 3D12 simulated MEM-E/02 in recognizing several HLA-B and HLA-C antigens. Both 3D12 and MEM-E/02 did not bind to HLA-A, HLA-F and HLA-G molecules. As observed with MEM-E/02, binding of 3D12 to HLA-E is inhibited by the peptides sequences ^{115}QFAYDGKDY^{123} and ^{137}DTAAQI^{142}. Decrease in binding of mAb 3D12 to HLA class Ia, after heat treatment of antigen coated microbeads, supports the contention that the epitope may be located at the outside of the "thermodynamically stable" α-helix conformations of HLA-E. Several sequence and structure-based web-tools were employed to validate the discontinuous epitopes recognized by the mAbs. The scores obtained by these web-tools distinguished the shared peptide sequences that inhibited the mAb binding to HLA-E. Furthermore, ElliPro web tool points out that both mAbs recognize the conformational discontinuous epitopes (the shared inhibitory peptide sequences) in the secondary structure of the HLA-E molecule. The study favors the contention that the domain of the shared inhibitory peptide sequences may be the most immunogenic site of HLA-E molecule. It also postulates and clarifies that amino acid substitution on or near the binding domains may account for the lack of cross reactivity of 3D12 and MEM-E/02 with HLA-A, HLA-F and HLA-G molecules.

1. Introduction

HLA-E (low polymorphic and highly conserved non-classical HLA class Ib molecules) is ubiquitously transcribed in all human tissues (Wei and Orr, 1990), over expressed and shed into circulation upon inflammation (Coupel et al., 2007; Lin et al., 2009) and neoplastic transformation (Malmberg et al., 2002; Derre et al., 2006; Bianchini et al., 2006; Kren et al., 2010). HLA-E shares several peptide sequences with HLA-Class Ia molecules[1] (Ravindranath et al., 2010a), with exception of two peptide sequences ^{65}RSARDTA^{71} and ^{143}SEQKSNDASE^{152} (Ravindranath et al., 2010b). Since HLA-E shares several peptide sequences with HLA class Ia molecules, it is hypothesized that the monoclonal antibodies raised against HLA-E may cross react with HLA-Ia molecules, provided they recognize the shared peptide sequences. In support of the hypothesis, we have shown that anti-HLA-E mAb, MEM-E/02, considered specific for HLA-E (Menier et al., 2003; Lo Monaco et al., 2008, 2010), bound to several HLA-Ia molecules (HLA-B &-C) coated on to microbeads (Ravindranath et al., 2010a). The binding of MEM-E/02 to β2m-free HLA-E and to HLA-Ia molecules was inhibited dosimetrically by the shared epitope sequences ^{115}QFAYDGKDY^{123} and ^{137}DTAAQI^{142}, but not by the peptide sequence (^{126}LNEDRSWTA^{135}) in between the two epitopes (see Fig. 4D in Ravindranath et al., 2010a). Instead, the middle sequence enhanced the binding, suggesting that the epitope recognized by MEM-E/02 may be discontinuous. Examination of the 3D structure and location of the peptides revealed

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[1] ^{47}PRAPWMEQE^{55}, ^{59}EYWDRETR^{65}, ^{90}AGSHTLQW^{97}, ^{108}RFLRGYE^{123}, ^{115}QFAYDGKDY^{123}, ^{117}AYDGKDY^{123}, ^{126}LNEDLRSWTA^{135}, ^{137}DTAAQIS^{143}, ^{137}DTAAQI^{142}, ^{157}RAYLED^{162}, ^{163}TCVEWL^{168}, ^{182}EPPKTHVT^{190}.

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that the sequence ^{115}QFAYDGKDY^{123} at the terminal region α2-helix is exposed in β2m-free soluble HLA-E and masked by β2m in intact HLA-E. HLA-E antibodies binding to HLA-E specific sequences could be HLA-E-specific, but those binding to shared sequences are capable of cross-reacting with class Ia molecules. Thus, murine anti-HLA-E mAbs (MEM-E/02, MEM-E/6, MEM-E/07 and MEM-E/08) developed against such soluble recombinant HLA-E heavy chains cross-reacted with several HLA-Ia molecules (Ravindranath et al., 2010a). The HLA-Ia alleles recognized by these mAbs are remarkably identical.

The biological significance of the finding is elucidated when the pattern of HLA-Ia reactivity of the human sera (anti-HLA-E positive) closely paralleled to that of mAb MEM E/02 and when the binding of the human sera to HLA-E and HLA-Ia alleles were inhibited by the shared epitope sequences (^{115}QFAYDGKDY^{123} and ^{137}DTAAQI^{142}). These observations suggested that these sequences may be the most immunogenic site in HLA-E (Ravindranath et al., 2010c).

Anti-HLA-E mAb (mAb 3D12), generated by immunization of HLA-B27 transgenic mice with recombinant HLA-E purified from AEH cells (*Lymphoblastoid cell line LCL 721.221 cells transfected with HLA-E gene*), is also considered to be specific for HLA-E (Lee et al., 1998). In order to reconfirm the hypothesis. This investigation examines whether mAb 3D12 recognizes shared peptides sequences or HLA-E specific peptide sequences, using shared peptide sequences as inhibitors. The affinities of 3D12 and MEM-E/02 for HLA-Class Ia and Ib alleles were compared.

Several sequence and structure based web-tools (http://tools.immuneeptiope.org/tools) are used to predict that the epitope sequences recognized by the mAbs. These tools also allow visualization of the epitopes in 3D-structure of HLA-E. In the present study, these tools are utilized to validate the experimental findings on the discontinuous epitopes of anti-HLA-E mAbs, derived from the peptide inhibition experiments.

2. Materials and methods

2.1. Monoclonal anti-HLA-E antibodies

This investigation includes four murine mAbs (MEM-E/02, MEM-E/06, MEM-E/07, MEM-E/08; Affinity Bioreagents (ABR, Golden, Colorado) and mAb 3D12 (eBioScience, Cat. No.: 14-9953-82, Lot # E032916, www.ebioscience.com). The specificity of mAb 3D12 was tested for cross-reactivity with other HLA class Ia alleles.

2.2. Immunoassay with single antigen beads

To simultaneously detect the binding of minimal quantities of murine mAbs to HLA-E, HLA-F, HLA-G, HLA-A, HLA-B and HLA-C alleles, multibead multiplex immunoassay is used, as described earlier (Ravindranath et al., 2010a,c). The single antigen beads (HLA class Ia) are obtained from One Lambda Inc. (Canoga Park, CA, USA). The HLA-class Ib molecules are coated on microbeads at One Lambda. Data generated with Luminex Multiplex Flow Cytometry (LABScan 100) are analyzed using computer software, as reported earlier (Ravindranath et al., 2010a). The HLA-Ia microbeads supplied by One Lambda (Canoga Park, CA, USA) have inbuilt negative control beads (Human serum albumin coated) and positive control beads (human IgG-coated). For HLA-E, we have separately included positive and negative control beads. Each experiment is done in duplicate. For each analysis, a minimum of 100 beads are counted. The Trimmed Mean is obtained by trimming a percent off the high and low ends of a distribution of fluorescence intensity and finding the mean of the remaining distribution. Isotype control is run simultaneously. Trimmed mean cut-off MFI is 500. To express the values of anti-HLA antibodies at different dilutions, the sample specific fluorescent value (trimmed MFI) for each bead are normalized and used for analysis.

Four different 'Trimmed means' of MFI are obtained. They are (1) for MFI obtained with mAbs against HLA class I alleles, (2) for MFI of the negative control beads (used for each mAb), (3) for MFI of PE-conjugated 2nd antibody only, tested on HLA-Ia coated beads. (4) For MFI of negative control beads (for PE-conjugated 2nd antibody). From these four different Trimmed Mean MFIs, the normalized trimmed MFI is calculated as follows:

$$[\text{MFI of}(1) - \text{MFI of}(2)] - [\text{MFI of}(3) - \text{MFI of}(4)].$$

For heat treatment, the beads are incubated in the assay buffer at 37 °C for 30 min and exposed to freshly diluted antibodies at room temperature. Origin Graphics Software® is used to plot the data. Basic statistical analyses are carried out with Excel software.

2.3. Immune epitope database (IEDB) analysis resource: antibody epitope prediction (AEP)

Two systems of web-tools are utilized: (1) to determine the frequency of the linear sequence and (2) to predict discontinuous and conformational epitopes, based on 3D protein structure. Recently, a new structure-based tool for the prediction of antibody epitopes is proposed, called ElliPro (Ponomarenko et al., 2008). Essentially, ElliPro is based on the geometrical properties of the protein structure and its usefulness in predicting the protein–antibody interaction. Each method has its own limitations. Parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity of polypeptides chains have been correlated with the location of the epitopes. AEP tutorial recommends that when computing the score for a given residue *i*, the amino acids in an interval of the chosen length, centered around residue *i*, are considered. In other words, for a window (sequence) size n, the $i - (n-1)/2$ neighboring residues on each side of residue *i* were used to compute the score for residue *i*. Unless specified, the score for residue *i* is the average of the scale values for these amino acids. In general, a window size of 5–7 is appropriate for finding regions that may potentially be antigenic. We have applied as many methods as possible to ascertain and compare the immunogenicity scores of the peptides inhibited the binding of MEM mAbs between species. The web-tool system # 1 is supported by several methods that include:

(1) Chou and Fasman beta turn prediction which predicts beta turns in protein secondary structures (Chou and Fasman, 1978).
(2) Karplus and Schulz flexibility scale based on a flexibility scale is similar to classical calculation, except that the center is the first amino acid of the six amino acids window length, and there are three scales for describing flexibility instead of a single one (Karplus and Schulz, 1985).
(3) Kolaskar and Tongaonkar antigenicity scale makes use of physicochemical properties of amino acid residues and their frequencies of occurrence in experimentally known segmental epitopes was developed to predict antigenic determinants on proteins. Application of this method to a large number of proteins has shown by the authors that the method can predict antigenic determinants with about 75% accuracy which is better than most of the known methods (Kolaskar and Tongaonkar, 1990).
(4) Parker hydrophilicity prediction (Parker et al., 1986): in this method, hydrophilic scale based on peptide retention times during high-performance liquid chromatography (HPLC) on a reversed-phase column was constructed. A window of seven residues was used for analyzing epitope region. The corresponding value of the scale was introduced for each of the seven