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Short communication

HLA-DM mediates peptide exchange by interacting transiently and repeatedly with HLA-DR1

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ABSTRACT

The peptide editor HLA-DM (DM) catalyzes the exchange of peptides bound to MHC class II molecules within antigen presenting cells by generating a "peptide-receptive" MHC class II conformation ($MHC_{receptive}$) to which peptides readily bind and rapidly unbind. While recent work has uncovered the determinants of DM recognition and effector functions, the nature of $MHC_{receptive}$ and its interaction with DM remains unclear. Here, we show that DM induces but does not stabilize $MHC_{receptive}$ in the absence of peptides. We demonstrate that DM is out-competed by certain superantigens, and increasing solvent viscosity inhibits DM-induced peptide association. We suggest that DM mediates peptide exchange by interacting transiently and repeatedly with MHC class II molecules, continually generating $MHC_{receptive}$. The simultaneous presence of peptide and DM in the milieu is thus crucial for the efficient generation of specific peptide–MHC class II complexes over time.

1. Introduction

The antigen-specific activation of the CD4+ "T helper" arm of the adaptive immune response is intractably linked to the processing of antigens and the presentation of the peptides generated on MHC class II molecules on the surface of antigen presenting cells (APCs). MHC class II αβ heterodimers are synthesized with an invariant chain (Ii) derived peptide CLIP occupying the peptide binding groove. As this complex traffics to the endosomal compartments of the APC, the majority of Ii is cleaved, leaving CLIP bound to MHC class II (Blum and Cresswell, 1988; Cresswell, 1994; Peters et al., 1991). HLA-DM, a nonclassical HLA molecule, has been shown to play a critical role in mediating the exchange of CLIP for antigenic peptides in the low pH environment of the endosome (Denzin and Cresswell, 1995; Fung-Leung et al., 1996). DM interacts with various conformations of the molecule (Chou and Sadegh-Nasseri, 2000; Kropshofer et al., 1996, 1997; Sadegh-Nasseri et al., 2008; Ullrich et al., 1997; Weber et al., 1996) and is thought to stabilize empty MHC class II against denaturation at physiological temperatures (Kropshofer et al., 1997). Primarily however, DM is considered as a catalyst of peptide association and dissociation, and kinetic parameters for DM-mediated peptide binding have been described (Vogt et al., 1996). This characterization has prompted the speculation that soluble forms of DM could be added exogenously to catalyze speedy association and/or dissociation of specific peptides of interest in *in vitro* assays or even in *in vivo* settings. Recently, we have shown that structural changes, primarily around the P1 pocket of the MHC class II allele HLA-DR1 (DR1), could provide the basis for DM sensitivity (Chou and Sadegh-Nasseri, 2000), and conserved hydrogen bonds in the same region may be targeted by DM to effect peptide dissociation (Narayan et al., 2007). One of the emergent hypotheses from these and other studies (Rabinowitz et al., 1998; Zarutskie et al., 2001) was that DM could mediate both peptide association and dissociation by generating a "peptide-receptive" conformation of DR1 ($DR1_{receptive}$), to which peptides could readily bind and rapidly unbind (Natarajan et al., 1999a; Sadegh-Nasseri and McConnell, 1989; Vogt et al., 1996). Here, using soluble, recombinant DM and wild type and mutant DR1 molecules, along with variants of the Influenza Hemagglutinin derived peptide $HA_{306–318}$,

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we probe the interaction between DM and DR1. Several groups have reported the qualitative interaction between full-length DM and DR molecules in the context of cells, primarily by conventional biochemical assays (Denzin et al., 1996; Kropshofer et al., 1997; Weber et al., 2001; Zwart et al., 2005). We have targeted the soluble forms of these two molecules because of the possibility of use of soluble DM as an exogenously added catalyst of peptide exchange, but also because the usage of soluble isoforms has been used by us and other groups (Pashine et al., 2003) to accurately measure biophysical parameters of DM–DR interactions in solution. Here we characterize the central transitional conformation in the binding reaction, $DR1_{receptive}$, and in light of the results, we propose a "hit and run" model describing transient and repeated interactions between DM and DR1 in order to generate $DR1_{receptive}$ and mediate rapid binding of peptides.

2. Materials and methods

2.1. Production of proteins and peptide synthesis

Soluble recombinant DR1 and DM proteins were expressed and purified as originally described (Narayan et al., 2007). Baculovirus DNA (BaculoGold; PharMingen) and transfer vectors carrying the wild type or mutant genes were cotransfected into Sf9 insect cells to produce recombinant viruses and infect Hy5 cells; DR1 and DM proteins were purified from the culture supernatant using an anti-DR1 mAb (L243) or M2 (αFLAG) mAb sepharose resin (Sigma), respectively. Proteins were eluted at low pH or with 0.1 mg/mL FLAG peptide, and purified by gel filtration chromatography (Superdex 200 HR 10/30 column, Amersham Pharmacia). The peptides $HA_{306–318}$ (PKYVKQNTLKLAT), HA_{Y308A} (PKAVKQNTLKLAT) and $HA_{Anchorless}$ (PKAVKANGAKAAT) were synthesized and purified to >90% by reverse-phase preparative HPLC (Elim BioPharamceuticals). The irrelevant control peptide ETEC (IIYQIVDEKGKKK) is a peptide corresponding to amino acids 111–123 of CS6, a subunit of enterotoxigenic *Escherichia coli* pili protein (Natarajan et al., 1999b). Fluoresceinated peptides were similarly pre-synthesized, with the N-terminal addition of FAM and Glycine linker.

2.2. Peptide association, dissociation and receptive conformation assays

Purified $DR1_{wt}$ (1 μM) was incubated in the absence or presence of 0.2 μM DM with 50 μM fluoresceinated peptides for various times in solutions of varying amounts of sucrose or glycerol in 0.15 M citrate phosphate buffer, pH 5.5 (CP) at 37 °C. After removal of free peptides by a Sephadex G-50 spin column equilibrated with PBS pH 7.4, fluorescence emission of the DR–FITC–peptide complexes was measured at 514 nm with an excitation at 492 nm on an LS-50B spectrofluorimeter (PerkinElmer) at room temperature. In case of the "pre-incubation" experiments, $DR1_{wt}$ (1 μM) was pre-incubated with DM (0.2 μM) for 5 min at 37 °C, and then the association experiment was carried out as before. When required, excess αFLAG MAb resin was added to all groups for 20 min and quickly centrifuged to spin out any DM in the solution. Equal volumes of the supernatant were then used for association experiments. The dissociation experiments in the presence of SAg was essentially performed as described previously (Chou and Sadegh-Nasseri, 2000); here, 7 μM SAg (SEA, SEB or SEH, Toxin Technologies) was added at the start of the dissociation reaction. The mutant SEA molecule was kindly donated by Dr. Robert G. Ulrich, US Army Medical Research Institute of Infectious Diseases at Ft. Detrick. For $DR1_{receptive}$ lifetime assays, DR1 variants were incubated with non-fluorescent HA_{Y308A} peptides in CP at 37 °C to yield maximal loading. Excess unbound peptides were removed by a spin, and samples were incubated at 37 °C with no competitor peptide in the presence or absence of DM (DR:DM = 5:1), for required lengths of time. At the end of the incubations, the pH of the solution was changed to 7.5 by the addition of appropriate molar concentrations of phosphate buffer pH 8.0 to inactivate DM, and the samples were pulsed with an excess (50 μM) of fluoresceinated peptide for 15 min ($DR1_{\beta H81N}$ or $DR1_{\beta G86Y}$) or 60 min ($DR1_{wt}$). After one more spin through a Sephadex G-50 spin column to remove excess fluorescent peptide, fluorescence was measured as above. All the raw dissociation data were fitted into single exponential dissociation equations as follows:

$$Y = Y_0 + A_1 e^{-x/t1}$$

All curves were normalized, with the arbitrary value of 1.0 assigned to the fluorescence obtained at the start of measured dissociation curves.

2.3. Preparation of co-solvent buffers at various viscosities

Solutions of varying concentrations of sucrose (Fisher) or glycerol (Sigma) in CP pH 5.5 were run through an Ubbelohde viscometer to determine viscosity of each sample. From the %w/v vs. viscosity standard curves, co-solvent buffers of specified viscosities were produced and used.

2.4. Real-time binding experiments

2.4 μM $DR1_{wt}$ complexed to HA_{Y308A} was produced and isolated, and then incubated at 37 °C with or without 1 μM DM or 7 μM superantigen (SEA, SEB or TSST-1, Toxin Technologies, for various experiments) or both for 20 min in de-gassed CP pH 6.0. These solutions were injected on a peptide-decorated CM5 chip in a BIAcore 2000 machine at a flow rate of 4 μL/min, followed by wash buffer (injection buffer + 0.01% Tween20) flowed at 5 μL/min. To monitor any RU change induced by presence of EDTA, 2.4 μM $DR1_{wt}$/HA_{Y308A} with or without 1 μM DM was incubated with 5 mM EDTA and then injected on a $HA_{Anchorless}$ surface. The experiment with the control peptide, ETEC was performed with a similar protocol. The peptide bound chip itself was prepared as described previously (Narayan et al., 2007).

3. Results and discussion

3.1. DM is out-competed by DR1β81His binding superantigens

Using various soluble DR1 mutants, we have demonstrated the importance of the P1 pocket of DR1 for DM recognition and effector functions (Narayan et al., 2007). To characterize the interaction between DM and DR1 without genetic manipulation, we measured DM-mediated peptide exchange in the presence of certain "superantigens" (SAg), bacterial toxins (Proft and Fraser, 2003) that bind DR1β81His, a conserved residue near the P1 pocket that forms a short strong hydrogen bond with peptide (McFarland et al., 2001; Stern et al., 1994). We tested the ability of one such toxin, Staphylococcal Enterotoxin A (SEA) to out-compete DM. SEA binds DR1 via a high (nM) affinity site, coordinating a zinc with β81His, as well as a weaker (μM affinity) zinc independent site on the α chain of DR1 (Thibodeau et al., 1997).

In a real-time binding experiment utilizing surface plasmon resonance (SPR) measured in a BIAcore machine, we injected a solution of DR1 and DM on a chip decorated with $HA_{306–318}$ or its synthetic variant $HA_{Anchorless}$ (see Section 2). As expected, DR1 aided by DM bound both peptides immobilized on the chip (Fig. 1a, blue). The pre-incubation of DR1 with SEA abrogated peptide binding (black), suggesting that the binding of β81His by SEA either physically blocked the peptide binding groove of DR1, or prevented the