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Review

Plasticity of empty major histocompatibility complex class I molecules determines peptide-selector function

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ABSTRACT

Major histocompatibility complex class I (MHC I) proteins provide protection from intracellular pathogens and cancer via each of a cell's MHC I molecules binding and presenting a peptide to cytotoxic T lymphocytes. MHC I genes are highly polymorphic and can have significant diversity, with polymorphisms predominantly localised in the peptide-binding groove where they can change peptide-binding specificity. However, polymorphic residues may also determine other functional properties, such as how dependent MHC I alleles are on the peptide-loading complex for optimal acquisition of peptide cargo. We describe how differences in the peptide-binding properties of two MHC I alleles correlates with altered conformational flexibility in the peptide-empty state. We hypothesise that plasticity is an intrinsic property encoded by the protein sequence, and that co-ordinated movements of the membrane-proximal and membrane-distal domains collectively determines how dependent MHC I are on the peptide-loading complex for efficient assembly with high affinity peptides.

1. MHC I alleles differ in dependence upon tapasin and the peptide-loading complex

Peptides presented at the cell surface by MHC I molecules help to protect the host from intracellular pathogens and tumours. These peptides are generated in the cytoplasm by the proteasome and other proteases, and are translocated by the transporter associated with antigen processing (TAP) into the endoplasmic reticulum (ER) where MHC I peptide-loading occurs. A TAP-associated peptide-loading complex (PLC), of which the cofactor tapasin is a key constituent, ensures MHC I molecules efficiently load with peptides. Once assembled, peptide-MHC I complexes pass through the secretory pathway to populate the cell surface. Here the peptides are presented to cytotoxic T lymphocytes which discriminate between healthy and abnormal cells. Which peptides are selected for presentation by MHC I is therefore of fundamental importance for modulating the immune response.

MHC I peptide selection is thought to occur in two stages (Lewis and Elliott, 1998), with empty MHC I molecules initially binding whatever peptides are likely to be the most abundant in the ER. This cargo is unlikely to bind with high affinity as few peptides transported by TAP will match the peptide-binding specificities of the MHC I alleles present. Although this initial peptide cargo fails to elicit an effective immune response because it does not sufficiently stabilise MHC I (Lewis and Elliott, 1998), a low affinity cargo will prevent the rapid and irreversible inactivation that MHC I undergoes in the absence of peptide. MHC I molecules subsequently exchange this sub-optimal cargo for high affinity peptides in a process catalysed by tapasin and the PLC. Tapasin facilitates MHC peptide-loading in multiple ways: tethering unloaded or poorly loaded MHC I to the TAP peptide portal (Sadasivan et al., 1996); stabilising TAP and consequently increasing the rate of peptide translocation (Grandea et al., 2000; Lehner et al., 1998); synergistically strengthening the interactions between components of the PLC (van Hateren et al., 2010); perhaps most importantly tapasin improves the rate and the extent of peptide loading and the discrimination that occurs between peptides (Williams et al., 2002; Wearsch and Cresswell, 2007). This peptide selection function results in MHC I becoming loaded with those peptides that bind with high affinity, prolonging cell surface expression (Howarth et al., 2004).

A survey of the assembly properties of common North American HLA B alleles revealed a hierarchy in which the intrinsic ability to select and assemble with optimal peptides is inversely

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correlated against the extent that tapasin enhances MHC I peptide loading (Rizvi et al., 2014). This observation of an inverse correlation is consistent with earlier studies showing human MHC I alleles require tapasin and the PLC to different extents for efficient peptide-loading (Greenwood et al., 1994; Peh et al., 1998). Like their human counterparts, the MHC I alleles of rodents differ in tapasin dependence: with murine H2-K^b molecules being more reliant on tapasin than H2-D^b molecules (Garbi et al., 2000). Additionally rat RT1-A^u molecules can gain an optimal peptide repertoire independently of the PLC when RT1-A^a molecules are over-expressed, illustrating that RT1-A^u possess significant intrinsic ability to self-select and assemble with high affinity peptides (Ford et al., 2004).

Various arguments have been proposed to explain why diversity in assembly pathways exists. As components of the PLC are frequently inhibited by viral subversion proteins or their expression down-regulated following oncogenic transformation, there is an obvious advantage if MHC I alleles are able to load peptides efficiently without the assistance of tapasin and the PLC. An alternative and not mutually exclusive argument is that tapasin-dependent MHC I loading allows the evolutionary appearance of new peptide-binding domains that accommodate new pathogenic peptides, because tapasin may ameliorate the potentially disruptive effect that changes in the peptide-binding domain might entail (van Hateren et al., 2013).

Understanding why MHC I alleles differ in this way has been intensively researched, particularly the HLA B*44:02 and B*44:05 alleles (B4402 and B4405 hereafter) which differ only at position 116 (B4402: Asp116, B4405: Tyr116) and may define extreme ends of this phenotype. While B4402 relies upon tapasin and the PLC to assemble with high affinity peptides, B4405 efficiently self-selects high affinity peptides (Zernich et al., 2004). There is no obvious structural correlate of this difference in function, but short molecular dynamics (MD) simulations have suggested that B4402 and B4405 differ in their conformational flexibility in the peptide-empty state (Sieker et al., 2007; Garstka et al., 2011; Ostermeir et al., 2015), despite near identical static structures of the peptide bound state solved by X-ray diffraction (Zernich et al., 2004).

Our comparison of the peptide-binding properties of chicken MHC I alleles (van Hateren et al., 2013), separated from a common ancestor of humans by ~350 million years, has shown that these differences in MHC I peptide-binding properties and assembly pathways are an evolutionarily ancient property. We found that the chicken MHC I molecules BF2*15:01 and BF2*19:01 (BF2*15 and BF2*19 hereafter), which differ by only eight amino acids and bind a similar peptide repertoire, differ in their peptide-binding properties (van Hateren et al., 2013), and thus show similarities to human alleles (Rizvi et al., 2014). We found BF2*15 was similar to B4405 and certain other alleles that have greater intrinsic ability to select and assemble with high affinity peptides than alleles such as BF2*19, B4402 and other "poor self-assembling" molecules. Consistent with the correlation described in humans (Rizvi et al., 2014), BF2*19 receives greater enhancement from tapasin than BF2*15. Lastly we have since shown that BF2*15 discriminates between high and low affinity peptides to a greater extent than BF2*19 (Fig. 1a), reminiscent of the greater intrinsic ability of good self-assembling molecules to select high affinity peptide (Williams et al., 2002).

2. Variable protein plasticity may explain the different peptide-selector function

In the absence of a structural correlate which may account for the variable tapasin dependence of MHC I alleles, we compared the dynamic behaviour of the ER luminal domains of BF2*15 and

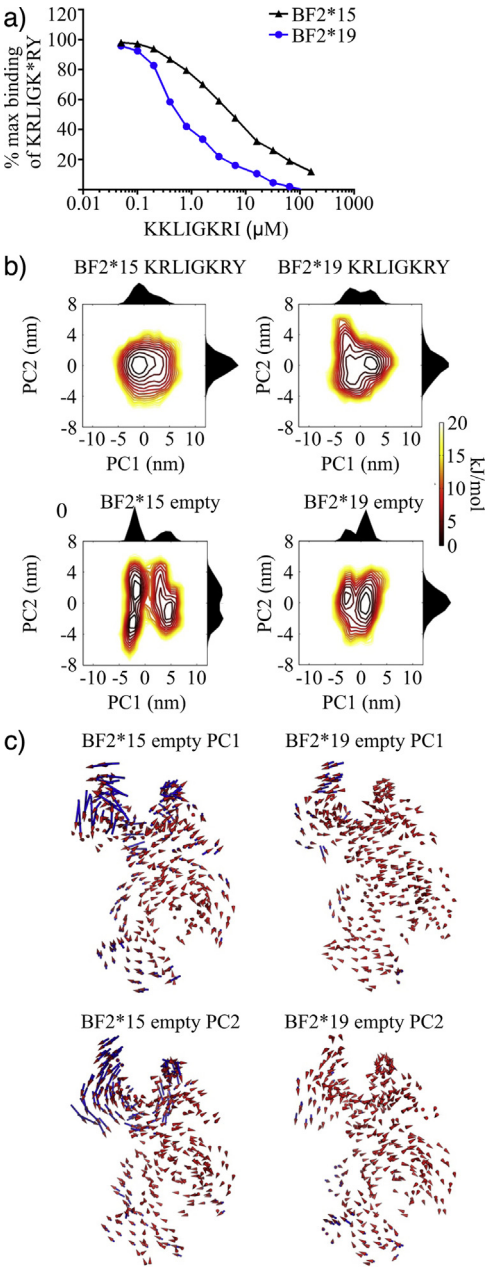

Figure 1a. (a) BF2*15 and BF2*19 differ in their intrinsic ability to select high affinity peptides. A final concentration of 0.5 μM BF2*15 or BF2*19 molecules refolded with UV conditional ligand (produced as in van Hateren et al., 2013) were exposed to ~360 nm light for 20 min at 4 °C in the presence of 10 μM chicken β_2-microglobulin. The UV-exposed proteins were then added to 0.1 μM high affinity peptide KRLIGK*RY (K* represents 5′ Tamra labelled Lysine) mixed with various concentrations of the low affinity competing peptide KKLIGKRI (0–160 μM) in a total volume of 60 μl. Fluorescence polarisations measurements were taken after being left at room temperature overnight as described in van Hateren et al. (2013) and plotted as a percentage of the maximum polarisation value (observed in the absence of KKLIGKRI). (b) BF2*15 and BF2*19 differ in their abilities to explore the conformational landscapes in the peptide-empty state. Gibbs free energy landscapes were generated from the principal coordinates of Principal Component PC1 and Principal Component PC2 and transformed by treatment as a Boltzmann ensemble as in Bailey et al. Individual probability densities for PC1 and PC2 are plotted on the outside adjacent axes. (c) The global dynamics of MHC I identified by Principal Component Analysis. 420 nanosecond MD simulations of BF2*15 and BF2*19 were performed using a common peptide free backbone structure as in Bailey et al. Porcupine plots indicate the magnitude and direction of motion for each backbone atom along Principal Component PC1 (top panels) and Principal Component PC2 (bottom panels) in the peptide free state. The arrows indicate the direction of the motion of each atom along this mode. The length of the arrow tail indicates the magnitude of the motion.

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