Dominance of an alternative CLIP sequence in the celiac disease associated HLA-DQ2 molecule

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Abstract During assembly, HLA class II molecules associate with the invariant chain. As the result, the peptide-binding groove is occupied by an invariant chain peptide termed CLIP (class-II-associated invariant chain peptide; sequence MRMA(TM)PLLM). By mass spectrometry, we have now characterized peptides that are naturally present in HLA-DQ2. This analysis revealed that 22 variants of Ii-derived peptides are associated with HLA-DQ2. Strikingly, the large majority of those do not contain the conventional CLIP sequence MRMA(TM)PLLM, but instead a peptide that partially overlaps with CLIP, sequence TPLLMQALPM. Peptide binding studies indicate that this alternative CLIP peptide has superior HLA-DQ2 binding properties compared to the conventional CLIP and that the minimal nine-amino-acid binding core consists of the sequence PLLMQALPM, findings that could be corroborated by molecular simulation. The alternative CLIP peptide was also found to be present in HLA-DQ2 molecules isolated from human thymus. Moreover, the alternative CLIP peptide was also found in association with HLA-DQ8. Together, these results indicate that HLA-DQ2 and HLA-DQ8 associate with an alternative CLIP sequence, a property that may relate to the strong association between HLA-DQ molecules and human autoimmune diseases.

Keywords HLA class II · HLA-DQ2 · CLIP · Alternative binding

Abbreviations Ii invariant chain CLIP class-II-associated invariant chain peptide

Introduction

The MHC class II α and β subunits associate with an invariant chain (Ii) trimer shortly after biosynthesis in the endoplasmic reticulum, generating a stable complex (Roche et al. 1991). Part of the Ii-chain, commonly referred to as CLIP (class-II-associated invariant chain peptide), occupies the peptide-binding groove of MHC class II molecules, thus preventing peptide loading in the endoplasmic reticulum (Bakke and Dobberstein 1990). Moreover, Ii facilitates the transport of the MHC class II molecules to the endosomal compartment where Ii is degraded by proteases. Subsequently, CLIP is released from the groove by the catalytic action of HLA-DM and exchanged for immunogenic peptides derived from proteins that have entered the endosomal/phagolysosomal system (Rocha and Neefjes 2008). Several autoimmune diseases, including type I diabetes and celiac disease, are HLA-associated.
In a recent study, we have reported the characterization of a large number of naturally HLA-DQ2-bound peptides through mass spectrometry (Stepniak et al. 2008). In agreement with two previous studies, the most abundant peptide identified was the HLA-class-I-derived peptide IEQEGPEYW (van de Wal et al. 1996; Vartdal et al. 1996). Similarly, the second most abundant peptide was found to be invariant chain (Ii)-derived (van de Wal et al. 1996). Similarly, the second most abundant peptide was IEQEGPEYW (van de Wal et al. 1996, Vartdal et al. 1996). The most abundant peptide identified was the HLA-class-I-derived peptide TPLLMQALPM (CLIP 95–103) as the nine-amino-acid binding core.

To test the predicted binding frame of the DQ2-CLIP sequence, we next analyzed the binding of N- and C-terminally truncated length variants of CLIP 92–107 (MATPLMQALPMGALP; Fig. 2b). Deletion of P95 and M103 was found to abrogate binding almost completely, indicating PLLMQALPM (CLIP 95–103) for a proline at the p8 position (Stepniak et al. 2008).

Additionally, modeling of HLA-DQ2 complexed with the peptide PLLMQALPM showed that it fits well in the groove of HLA-DQ2 (Fig. 3a and b). Moreover, it is predicted to bind better than the conventional CLIP peptide MRMATPLLM (unpublished data), a prediction that is confirmed by the observed superior binding of DQ2-CLIP (Fig. 2a).

Since the spacing of the anchor residues in DQ2-CLIP is identical to that of the dominant HLA-class-I-derived peptide IEQEGPEYW (van de Wal et al. 1996; italicized amino acids are residues at anchor positions) as well as gluten-derived T cell stimulatory peptides (Vader et al. 2003), we conclude that the DQ2-CLIP peptide binds to HLA-DQ2 molecules in a similar binding register. In conclusion, the minimal core sequence within the DQ2-CLIP peptide that binds strongly to HLA-DQ2 is PLLMQALPM (residues 95–103) in which the italicized amino acids are residues at anchor positions.

Because celiac disease and type I diabetes are also associated with HLA-DQ8, we also analyzed naturally HLA-DQ8-bound peptides through mass spectrometry. The invariant chain was represented again by 22 peptides with a minimal core of ten amino acids present in all length variants, which is identical to that found in HLA-DQ2 [sequence TPLLMQALPM (CLIP 94–103); data not shown]. In addition, binding studies revealed that the peptide ATPLLMQALPMGALP (CLIP 93–107) binds slightly better than the peptide MRMATPLLMQALPMGALP (CLIP 90–101; data not shown). These results thus indicate that next to HLA-DQ2, HLA-DQ8 also appears to harbor an alternative CLIP peptide. Further studies will be required to determine the exact binding frame of this alternative CLIP peptide in HLA-DQ8.

It is now widely accepted that positive selection in the thymus is promiscuous (Ignatowicz et al. 1996), that is, a...
single self-peptide is able to mediate positive selection of large numbers of T cells with the ability to respond to various peptide antigens (Ignatowicz et al. 1997). It has also been demonstrated that in mice lacking DM molecules required for the dislocation of CLIP from MHC class II (Fukui et al. 1997; Martin et al. 1996), 15–50% of the normal number of CD4+ T cells are selected. Moreover, the T cell receptor repertoire in these mice was different from that in wild-type mice, suggesting that MHC class II–CLIP complexes can select a large and diverse repertoire of T

**Fig. 1** Identification of an alternative CLIP peptide in HLA-DQ2. A Alignment of the length variants of the invariant-chain-derived peptides present in HLA-DQ2 expressed on EBV-transformed B cells. The solid line marks the predicted alternative CLIP sequence specific for HLA-DQ2; the dashed line marks the CLIP sequence found in other HLA class II molecules. B Tandem mass spectrum of one of DQ2-CLIP peptides. The amino acid sequence of the peptide is indicated on top of the spectrum, including the expected b- and y-fragment ions. Observed fragment ions are underlined and indicated in the spectrum. The correct identity of the peptide was proven by tandem mass spectrometry of the synthetic compound.

| Identified invariant chain-derived sequences | Relative abundance [%] |
|---------------------------------------------|-------------------------|
| T P L L M Q A L P M G A L P               | 6.7                     |
| T P L L M Q A L P M G A L P Q            | 13.3                    |
| T P L L M Q A L P M G A L P Q G P        | 2                       |
| A T P L L M Q A L P M G A                | <0.01                   |
| A T P L L M Q A L P M G A L P            | 3.3                     |
| A T P L L M Q A L P M G A L P Q          | 4.9                     |
| A T P L L M Q A L P M G A L P Q G P      | <0.01                   |
| M A T P L L M Q A L P M G A              | 3.3                     |
| M A T P L L M Q A L P M G A L P          | 3.3                     |
| M A T P L L M Q A L P M G A L P Q        | 15.7                    |
| R M A T P L L M Q A L P M G A L P Q      | 2.3                     |
| R M A T P L L M Q A L P M G A L P Q      | 2.3                     |
| R M A T P L L M Q A L P M G A L P Q      | 5.6                     |
| R M A T P L L M Q A L P M G A L P Q      | 17.3                    |

| y_n | Intensity x 10^6 |
|-----|------------------|
| y_1 | 6                |
| y_2 | 4                |
| y_3 | 6                |
| b_1 | 1200             |
| b_2 | 1000             |
| b_3 | 800              |
| b_4 | 600              |
| b_5 | 400              |
| b_6 | 600              |
| b_7 | 1200             |
| b_8 | 1000             |
| b_9 | 800              |
| b_10| 600              |
| b_11| 400              |

(Fukui et al. 1997; Martin et al. 1996), 15–50% of the normal number of CD4+ T cells are selected. Moreover, the T cell receptor repertoire in these mice was different from that in wild-type mice, suggesting that MHC class II–CLIP complexes can select a large and diverse repertoire of T
cells. Given the dominant presence of DQ2-CLIP in HLA-DQ2, it is therefore possible that a substantial proportion of HLA-DQ2-restricted T cells in the periphery has been selected by HLA-DQ2–[DQ2-CLIP] complexes in the thymus. As HLA-DQ8 also appears to harbor an alternative CLIP peptide, this may similarly apply to T cell receptor selection in HLA-DQ8 individuals. It is tempting to speculate that this relates to the fact that HLA-DQ2 and HLA-DQ8 confer susceptibility for the common autoimmune diseases celiac disease and type I diabetes (Nepom and Kwok 1998; Todd et al. 1987).

Concluding remarks

In conclusion, we have observed that HLA-DQ2 molecules preferentially associate with an alternative form of the invariant-chain-derived CLIP peptide, DQ2-CLIP. The DQ2-CLIP has an at least four times higher binding affinity to HLA-DQ2 than the previously described classical CLIP sequence. Similarly, we observed that HLA-DQ8 harbors an alternative CLIP peptide. This property may relate to the strong association between these HLA-DQ molecules and human autoimmune diseases.
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