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3-Layer-based analysis of peptide–MHC interaction: *In silico* prediction, peptide binding affinity and T cell activation in a relevant allergen-specific model

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ABSTRACT

CD4⁺ T cells recognize peptides bound to major histocompatibility complex (MHC) class II molecules on the surface of antigen presenting cells by their T cell receptor (TCR). Using a well-characterized allergen-specific model we studied peptide/MHC (pMHC) interactions by combining computational methods with experimental analyses. A 12-mer and an 18-mer peptide, both containing the human leukocyte antigen (HLA)-DR1-restricted, immunodominant T cell epitope of Art v 1, the major mugwort pollen allergen, were compared. A Molecular Dynamics simulation for a real time of 20 ns using GROMACS was performed. To this aim, the peptides were modelled into the binding groove of HLA-DRB1*0101 using different amino acid substitution tools. Binding of synthetic peptides to purified HLA-DRB1*0101 molecules was analysed in competition assays. The potency of the peptides to activate Art v 1-specific T cells was assessed using oligo- and monoclonal Art v 1-specific T cell cultures expanded from mugwort allergic individuals.

All approaches revealed that the 18-mer peptide possessed higher HLA DR affinity as compared to the 12-mer. Computer modelling indicated that a loop-like structure within the additional N-terminal peptide flanking region of the 18-mer contributed to the pMHC interaction. Our approach, to combine computational methods validated by experimental results, demonstrates that Molecular Dynamics simulation may be a useful tool for the prediction of pMHC interactions in the future with possible applications in T cell-based immunotherapy e.g. in Type I allergy.

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1. Introduction

The peptide binding groove of MHC class II molecules accommodates naturally processed peptides containing 12–26 amino acids (AA) (Rammensee, 1995; Rudensky et al., 1991; Suri et al., 2006). A core of nine consecutive AA interacts with the MHC molecules at certain anchor residues (Stern and Wiley, 1994). This peptide core

can be flanked by varying numbers of AA residues at the amino- and/or carboxyl terminus reflecting proteolytic steps and trimming during antigen-processing (Suri et al., 2006). The function of these peptide flanking regions (PFR) is not entirely clear. PFR can enhance the processing of epitopes and modulate the activation of T cells (Godkin et al., 2001; SantAngelo et al., 2002), or can serve as direct TCR contact residues, thereby either enhancing immunogenicity (Carson et al., 1997) or hindering TCR interactions (Moudgil et al., 1998) or indirectly influence TCR/pMHC interaction (SantAngelo et al., 2002).

CD4⁺ T helper (Th) cells play a major role in induction and maintenance of Type I allergy (Romagnani, 1994). Allergic individuals react to allergens with an excessive Th2 response, characterized by the production of e.g. interleukin-4 and interleukin-13, which cause immunoglobulin (Ig) class switching to IgE. Cross-linking of allergen-specific IgE bound to mast cells triggers the release of inflammatory mediators causing typical allergic symptoms. Mugwort (*Artemisia vulgaris*) pollen causes the most prevalent pollen allergy in late summer in Europe and parts of Asia and contain only

Abbreviations: APC, antigen presenting cell; MHC, major histocompatibility complex; pMHC, peptide/major histocompatibility complex; TCR, T cell receptor; AA, amino acid; PFR, peptide flanking region; MD, Molecular Dynamics; HB, hydrogen bond; HLA, human leukocyte antigen; PDB, Protein Data Bank; RMSD, root mean square deviation; RMSF, root mean square fluctuation; Ig, immunoglobulin; IC₅₀, concentration causing half-maximum inhibition.

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one major allergen, Art v 1 (Himly et al., 2003), which harbors one single immunodominant T cell epitope, Art v 1_{25–36} (Jahn-Schmid et al., 2002). The T cell response to Art v 1_{25–36} is strongly associated with the expression of HLA-DRB1*01 (Jahn-Schmid et al., 2005). TCRs that are specific for Art v 1_{25–36} and their minimal epitopes have been intensively investigated (Jahn-Schmid et al., 2005; Leb et al., 2008).

In the present study, we wanted to concentrate on pMHC interactions and the impact of PFR. Since the N-terminal peptide region appears to be important for MHC interaction (Rudensky et al., 1991; Pu et al., 2004), we compared the originally characterized immunodominant 12-mer Art v 1_{25–36} (KCIWEKAQHGA) (Jahn-Schmid et al., 2002; Jahn-Schmid et al., 2008) with the 18-mer Art v 1_{19–36} (NKKCDKKCIWEKAQHGA) which included an additional, 6AA long, N-terminal extension. Here, an *in silico* approach for the prediction of pMHC interactions was complemented with experimental MHC binding assays and T cell proliferation assays.

Interestingly, the immunodominant epitope of Art v 1 had not been predicted by standard algorithms (Rammensee et al., 1999; Sturniolo et al., 1999) as strong HLA-DR1 binding peptide. Therefore, we used Molecular Dynamics (MD) simulations (Karplus and Petsko, 1990; Hansson et al., 2002), an *in silico* technique which provides detailed insight into the interaction of molecules on an atomistic level and elucidate spatial rearrangements over time.

2. Methods

2.1. Peptides

Art v 1_{25–36} (KCIWEKAQHGA) and Art v 1_{19–36} (NKKCDKKCIWEKAQHGA) were obtained from Thermo Electronics (Ulm, Germany). Purity was >90% as confirmed by HPLC.

2.2. Computer simulation

We used MD software package GROMACS (Lindahl et al., 2001) to simulate the interaction of Art v 1_{25–36} and Art v 1_{19–36} with the HLA-DR1*0101 molecule. To our knowledge so far no X-ray structure of an 18-mer peptide binding to HLA-DR1 exists. Therefore, the first step was to model the structure of both Art v 1-derived peptides into the binding groove of the MHC molecule using Protein Data Bank (PDB) (Bergman et al., 2000) identification 1sjh. As we have shown previously for MHC class I, it is sufficient to substitute the side chains of the peptide and leave the backbone untouched in the core region (Knapp et al., 2008). In this case we started to model from the X-ray structure of 1sjh which containing a 13-mer peptide (PEVIPMFSALESG). For Art v 1_{25–36} a deletion of the C-terminal G in combination with side chain substitution via SCWRL (Canutescu et al., 2003) was sufficient to model the complex. For Art v 1_{19–36} the backbone of 1sjh was too short. Therefore, we additionally modelled the necessary backbone elongation by using the graphical interface and the prediction score of SPDBV (Guex and Peitsch, 1997).

The predicted structures were immersed into an artificial water cube of 20 Å side length (Omasits et al., 2008) with periodic boundary conditions. A steepest descend energy minimisation was performed to avoid spatial clashes. Thereafter, the system was warmed up to 310 K and the MD simulation was started. In total, it took 3 months to compute the spatial dynamics of the systems for a real time of about 20 ns. The calculations were performed on the Aurora computational cluster of the Technical University of Vienna.

The resulting trajectories were evaluated using XScore (Wang et al., 2002) and the GROMACS functions *g_rms* and *g_rmsf*. The root mean square deviation (RMSD) for three-dimensional structures is

defined as

$$\text{RMSD} = \sqrt{\frac{1}{n} \sum_{i=1}^{i=n} (A_{ix} - B_{ix})^2 + (A_{iy} - B_{iy})^2 + (A_{iz} - B_{iz})^2}$$

n represents the number of atoms, *i* the current atom, *A* the first complex and *B* the second one. The root mean square fluctuation (RMSF) is a similar method in which the average is taken over a certain time range giving a value for each particle, while the RMSD gives specific values for two time points.

The hydrogen bonds between peptide and MHC are calculated using the *g_hbond* function of GROMACS.

2.3. Peptide/MHC binding

HLA-DRB1*0101 molecules were purified from homozygous EBV cells by affinity chromatography using mAb L243 as described before (Texier et al., 2000, 2001). Peptide binding to HLA-DR molecules was assessed by competitive ELISA. Dilutions were prepared in 10 mM phosphate, 150 mM NaCl, 1 mM *n*-dodecyl β-D-maltoside, 10 mM citrate (pH 6.0). The biotinylated reporter peptide (HA_{306–318}) was incubated with HLA-DRB1*0101 molecules in the presence of serial dilutions of Art v 1_{25–36} or Art v 1_{19–36}. After 24 h of incubation at 37 °C, peptide/MHC class II complexes were added to 96-well plates coated with L243 antibody and incubated at RT for 2 h. Bound biotinylated peptides were detected by Streptavidin-alkaline phosphatase conjugate (GE healthcare, Saclay, France) and 4-methyl-umbelliferyl phosphate (Sigma-Aldrich, St. Quentin Fallavier, France) as substrate. Emitted fluorescence was measured at 450 nm upon excitation at 365 nm by a Gemini fluorometer (Molecular probes, St Grégoire, France). Data are expressed as peptide concentrations that prevented binding of 50% of the labelled reference peptide (IC₅₀) HA_{306–318} (PKYVKQNTLKLAT). Three independent experiments were performed to evaluate competitive binding. Unlabelled HA_{306–318} peptide was used to assess the validity of each experiment.

2.4. Allergen-specific T cell lines (TCL) and T cell clones (TCC)

Peripheral blood mononuclear cells were obtained from heparinized blood of mugwort pollen-allergic individuals with typical clinical history, i.e. recurrent rhinitis/conjunctivitis during late summer, positive skin prick test (≥3 mm) to mugwort pollen extract (ALK-Abello, Hørsholm, Denmark) and allergen-specific IgE (CAP-FEIA ≥3) to mugwort pollen (w6; Phadia, Uppsala, Sweden). All patients were sensitised to Art v 1 (data not shown) as determined by IgE-immunoblots as described (Jahn-Schmid et al., 2002). The study was approved by the ethics committee of the Medical University of Vienna (EK No. 497/2005).

Art v 1_{25–36}-specific TCL were established from peripheral blood mononuclear cells by allergen-specific stimulation with either mugwort pollen extract or recombinant Art v 1 (Biomay, Vienna, Austria) for 5 days and expansion with interleukin-2 as previously described (Jahn-Schmid et al., 2002). TCC were established from these TCL by limiting dilution and specificity of TCC was assessed by proliferation assays as described (Jahn-Schmid et al., 2002). To compare the reactivity of TCL or TCC to Art v 1_{25–36} or Art v 1_{19–36}, duplicates of 5 × 10⁴ T cells were stimulated with irradiated (60 Gy) APC expressing HLA-DRB1*0101 and peptides at a concentration of 3 μM, if not indicated otherwise. After 48 h of incubation at 37 °C and 5% CO₂, proliferation was assessed by [³H]-thymidine uptake for 16 h. Cells were harvested onto glass fibre filters and incorporated radioactivity was determined by β-scintillation counting. Results are shown in delta cpm (dpm = mean cpm of T cell proliferation with peptide – mean of T cell proliferation without peptide).

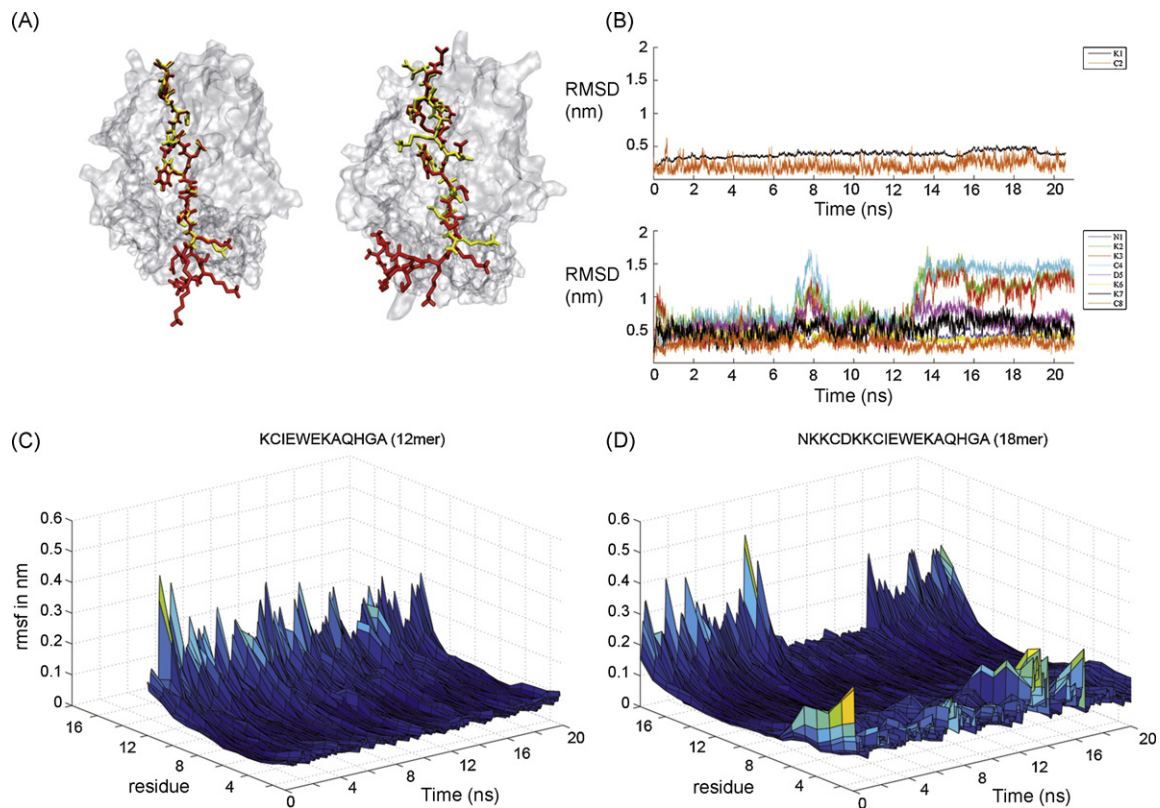


Fig. 1. Comparison of pMHC interactions of Art v 125–36 and Art v 119–36 with HLA-DRB1*0101 molecules using MD simulation. (A) Structure of the 12-mer and 18-mer peptide within the binding groove of an HLA-DR1 molecule at simulation time 0 ns (left) and 20 ns (right). Blue: MHC, red: Art v 119–36, yellow: Art v 125–36. (B) RMSD for all residues from the N-terminus to the P1 anchor residue (I3 and I9, respectively). Upper panel: Art v 125–36 shows almost no activity; lower panel: Art v 119–36 shows peaks at 8 and 14 ns. (C) RMSF for Art v 125–36. (D) RMSF for Art v 119–36. Numbering of the AA residues in (C) and (D) start at the N-terminus with 1 and end at the C-terminus with 12 or 18, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

3. Results

3.1. Computer simulation of peptide binding to HLA-DRB1*0101

In order to study pMHC/TCR interactions, the single immunodominant T cell epitope of Art v 1 was used as a model system in which TCR recognition had been well characterized in previous studies (Jahn-Schmid et al., 2002). The interaction between HLA-DRB1*0101 molecules and the 12-mer Art v 125–36 (KCIEWEKAQHGA), which contains the immunodominant T cell epitope of Art v 1, and the 18-mer Art v 119–36 (NKKCDKKCIEWEKAQHGA), comprising the 12-mer and 6 additional N-terminal AA was investigated.

For computer simulation of the pMHC interactions, the peptide anchors were first defined by (i) alignment to known structures of HLA-DR1-molecules obtained from the PDB (Bergman et al., 2000) (PDB-IDs: 1aqq, 1jwu, 1klg, 1kluU, 1pyw, 1seb, 1sjh, 1t5w), (ii) visualization of the peptide fitting into the respective peptide binding pockets and (iii) NetMHCII core prediction engine (Nielsen et al., 2007). These approaches revealed I27 as anchor residue in P1 for both peptides. Using the obtained peptide anchors for adjusting the core peptide within the HLA-class II binding groove, MD was then performed for both peptides. The last frames of the MD simulations were extracted from the trajectory and energetically minimised with a threshold of 5000 computational steps to obtain a favourably spatial arrangement. The superimposed structures of the peptides at 0 and 20 ns both positioned within a model of HLA-DRB1*0101, are depicted in Fig. 1A.

Next, binding affinity for the last frames was predicted using XScore resulting in an affinity score of 8.26 for Art v 125–36, and of 9.84 for Art v 119–36. Furthermore, evaluating frames within

the time range of 15 and 20 ns, Art v 125–36 had a lower average binding affinity (7.7) as compared to Art v 119–36 (9.6). In addition, the median number of hydrogen bonds (HB) between peptide and MHC-molecule was calculated over the simulation trajectory, which yielded 10 HB for Art v 125–36 and 16 HB for Art v 119–36. Normalising the number of HB for the total peptide length, the relative number of HB obtained for Art v 125–36 was 0.83 HB/AA, and 0.89 HB/AA for Art v 119–36. In Fig. 2 the distribution of HB within the 6 N-terminal AA is illustrated showing a significantly increasing number of HB during the last 8 ns of the simulation.

As illustrated in Fig. 1B the RMSD indicated that Art v 125–36 remained almost stable in the binding groove, while Art v 119–36 behaved very flexible at the N-terminus. At about 8 ns the peptide was trying to slap out of the binding groove but fell back immediately. At 14 ns a similar movement occurred, but at this time point the N-terminal AA (NKKCDK) remained outside and built a loop-like structure interacting with the α -chain of the MHC molecule as displayed in Fig. 1A. This activity was also reflected in the three-dimensional display of the RMSF (Fig. 1C and D), showing relative stability for the Art v 125–36 (Fig. 1C) and higher activity peaks at 8 ns as well as at 14 ns for Art v 119–36.

Taking all in all, the results of the different methods used (XScore, *g_rms*, *g_rmf* and *g_hbonds*) predicted that Art v 119–36 has a stronger affinity to HLA-DRB1*01 than Art v 125–36.

3.2. Peptide-binding to HLA-DRB1*0101 molecules

The binding capacity of Art v 125–36 and Art v 119–36 to HLA-DRB1*0101 molecules was assessed in competition studies using biotinylated HA_{306–318} as indicator. HA_{306–318} inhibited itself at an IC₅₀ of 3 nM (Fig. 3B). Art v 125–36 inhibited binding of HA_{306–318} at

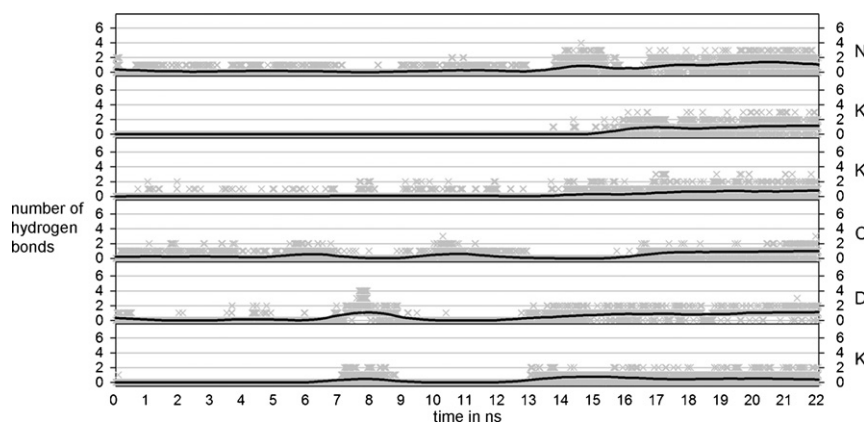


Fig. 2. Hydrogen bonds calculated for each residue of 6 N-terminal PFR of Art v 119–36 throughout the whole calculation period of 22 ns. The loop of the 18-mer peptide (see Fig. 1A) is formed by the 6 N-terminal AA residues (NKKCDK).

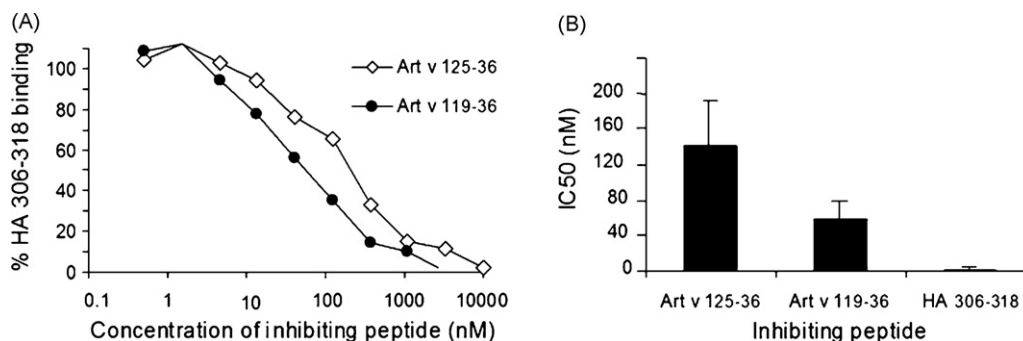


Fig. 3. Peptide binding to HLA-DRB1*0101 molecules. (A) Increasing concentrations of Art v 125–36 and Art v 119–36 and HA_{306–318} were tested for inhibition of binding of biotinylated HA_{306–318}. Inhibitions are shown in percentages. One representative experiment of 3 is shown. (B) Comparison of the binding capacity of Art v 1-peptides with the HA-reference peptide. IC₅₀ values plus standard deviations are shown as means of three independent experiments.

an IC₅₀ of 134 nM (Fig. 3A and B). Art v 119–36 inhibited HA_{306–318}-binding at an IC₅₀ of 58 nM (Fig. 3A and B) indicating superior binding of the 18-mer to HLA-DRB1*0101 molecules.

3.3. Peptide-induced proliferation of Art v 125–36-specific TCL and TCC

In order to compare the potency of Art v 125–36 and Art v 119–36 to activate allergen-specific T cells, TCL containing enriched numbers of Art v 1-specific T cells were established from peripheral blood of mugwort pollen-allergic patients. Since these cultures are usually ‘oligoclonal’, i.e. contain allergen-specific T cells express-

ing different TCR clonotypes, also monoclonal Art v 125–36-specific TCC were produced by limiting dilution of Art v 1-specific TCL. In total 23 TCL and 7 TCC derived from 16 different patients were studied. First, five TCL and one TCC were tested for reactivity to different concentrations of both peptides. As shown in Fig. 4, the Art v 119–36 was the superior stimulant to Art v 125–36. Then, a larger number of TCL ($n=23$) was tested for proliferation in response to both peptides using the optimum concentration for Art v 125–36. In 17/23 TCL (73%), Art v 119–36 induced significantly higher T cell proliferations as compared to the 12mer peptide (median Art v 125–36: 11,503 dpm; Art v 119–36: 19,610 dpm; $p=0.005$, Wilcoxon Signed Ranks Test). In line, the proliferative response of the major-

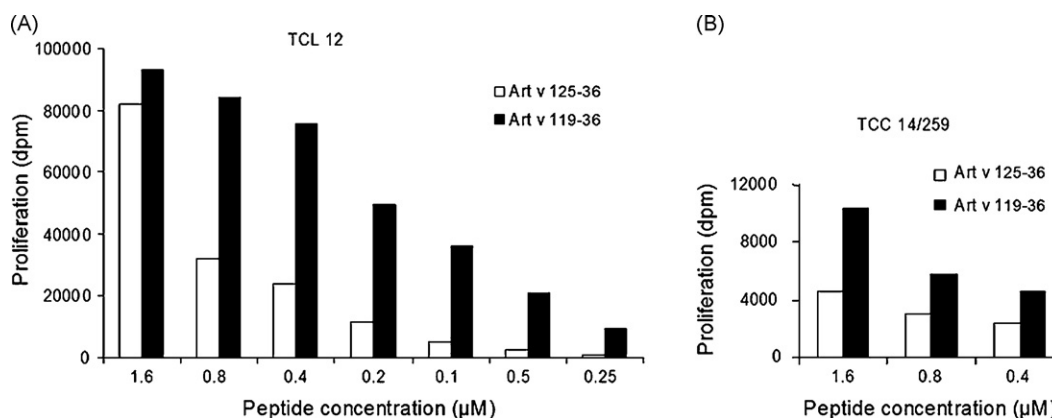


Fig. 4. Proliferative responses of Art v 125–36-specific T cells to different concentrations of Art v 125–36 and Art v 119–36 presented by HLA-DRB1*0101-expressing APC. (A) One representative TCL (A) and one TCC (B) is shown. Values for medium alone: 953 cpm (A) and 97 cpm (B).

ity (6/7; 86%) of TCC to the 18-mer peptide (median: 13,019 cpm) was superior stimulation induced by the 12-mer peptide (median: 8905 dpm).

4. Discussion

In the present study, we combined *in silico* prediction by means of MD, MHC/peptide binding assays and T cell activation assays to analyse pMHC interactions using Art v 1, a clinically important allergen which has a single immunodominant, HLA-DR1-restricted T cell epitope, as a model system. Two peptides with different PFR were compared: the 12-mer Art v 1_{25–36} (KCIWEKAQHGA) and the 18-mer Art v 1_{19–36} (NKKCDKKCIWEKAQHGA) both containing the immunodominant epitope (core: IEWEKAQHG), but differing at its N-terminal extension. MD simulations and experimental data reciprocally supported each other. Art v 1_{19–36} was superior in MHC class II-binding and T cell activation, while the simulation data also revealed a higher affinity of the 18-mer peptide which may be attributed to the 6 N-terminal AA, which predicted to form a loop-like structure conferring additional interaction with the MHC molecule.

MD has become a standard technique to simulate biological molecules, as indicated by a rapidly increasing number of publications which have increased e.g. in PubMed citations from 163 in 1990 to 2175 in 2007. However, due to the computational expense current computer systems restrict the simulation time of MD to nanoseconds of real time. In addition, the size of the simulated systems is limited. In our study, we used a time period of 20 ns to simulate pMHC binding of the Art v 1-derived peptides. During the last 6 ns the RMSD was almost stable for both complexes, indicating that this simulation time was sufficient to reliably predict pMHC interactions (Fig. 1B–D). Furthermore, the binding affinity of both Art v 1-derived peptides predicted by MD was in line with the data found in peptide/MHC binding and T cell activation assays. The initial spatial settings for MD simulation were based on the prediction that I27 was the P1 position of Art v 1_{25–36} within the MHC binding groove. This P1 anchor position for HLA-DRB1*0101 had been previously confirmed by experimental data in peptide binding assays using Art v 1_{25–36} and mono-substituted peptides thereof (Jahn-Schmid et al., 2008).

All approaches employed in this study indicated that Art v 1_{19–36} has a higher affinity to HLA-DRB1*0101 than the 12-mer peptide: the computer MD simulations, as well as peptide binding to HLA-DR1 molecules and actual T cell stimulation experiments using a large number of TCL and TCC from 16 different allergic individuals. Applying a combination of computer based tools such as XScore, *g_rms*, *g_rmf* and *g_hbonds* for MD simulation resulted in a more detailed insight into the spatial binding geometry between the Art v 1-derived peptides and MHC which could not be provided by experimental methods alone. According to the MD simulation, the 6 N-terminal AA residues in Art v 1_{19–36} form a loop-like structure able to interact with the MHC class II molecule e.g. by HB (Fig. 2) and, thus, most likely provide an explanation for the experimental evidence that Art v 1_{19–36} binds stronger than Art v 1_{25–36}. Hence, in our study, experimental data verified the reliability of the computer simulations and, vice versa, MD simulations could explain the experimental findings. This mutual assistance demonstrates the benefit of structure-based MD methods in contrast to sequence-based techniques, which may be faster but do not give insight into the binding geometry and mechanisms of pMHC interactions. In general, a further advantage of MD methods in the context of pMHC interactions is that these simulations can be used for different MHC alleles just based on their (known) structure, while sequence-based approaches apparently do not perform equally well for all MHC alleles (Gowthaman and Agrewala, 2008). Mostly, sequence-based algorithms need to be recalibrated on a lot of experimental data.

Besides the basic need for pMHC interaction, the stability of the trimolecular complex pMHC/TCR is the subsequent determinant in T cell stimulation. In this respect, it has been claimed that PFR can also serve as TCR contact residues (Suri et al., 2006). At a clonal level it has been shown that the potency to stimulate T cells is not a simple function of peptide length, but depends also on TCR interaction (Cease et al., 1986). This view was supported in our study by the existence of a minority of allergen-specific T cells, which reacted preferentially to the 12-mer peptide. Thus, regarding certain TCR clonotypes, the PFR of the 18-mer peptide could also mediate a negative impact on TCR-recognition in spite of its positive effect on pMHC binding.

In summary, our 3-layer evaluation of pMHC-interaction based on computer simulation, HLA-binding assay and T cell activation led to convergent results: Art v 1_{19–36} binds clearly better to its restricting HLA-class II molecule, which results in a stronger T cell activation of the majority of peptide-specific T cells. Apart from insights into immunological mechanisms of pMHC interactions, we show that MD simulations may be applicable for the selection of suitable peptides for specific peptide immunotherapy in T cell-mediated diseases (Larche and Wraith, 2005). This promising form of treatment has been introduced in Type I allergy (Muller et al., 1998; Oldfield et al., 2002), because non-IgE-binding peptides would provide the advantage of safety, ease of production and standardization of the vaccine. Since Art v 1 contains only a single major T cell epitope, mugwort pollen allergy would be especially suited for a peptide immunotherapy approach. Our present study suggests that the 18-mer peptide Art v 1_{19–36} would be a good candidate for this application.

Conflict of interest

The authors declare no conflict of interests.

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