Nasal application of a naturally processed and presented T cell epitope derived from TCR AV11 protects against adjuvant arthritis

Esther A. E. van Tienhoven, Chris P. M. Broeren, Alida Noordzij, Joseé P. A. Wagenaar, Willem van Eden and Marca H. M. Wauben

Institute of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, PO Box 80165, Yalelaan 1, 3508 TD Utrecht, The Netherlands

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Abstract

Reactivity towards TCR peptides plays an important role in the regulation of several experimental autoimmune diseases. In a previous paper, we showed the TCRAV11 usage by an arthritogenic T cell clone isolated from a rat with adjuvant arthritis (AA). Moreover, we identified three immunogenic peptides in AV11: AV11 24–40, 41–55 and 66–80. In the present study, we show that T cells directed towards all three epitopes are part of the immune repertoire. The strongest delayed-type hypersensitivity (DTH) reaction was observed against the peptide derived from the third framework region, peptide AV11 66–80. DTH reactions to this peptide were detectable in naive rats and increased significantly after AA induction. Interestingly, modulation of the AV11 66–80 T cell response by nasal AV11 66–80 administration resulted in reduced DTH responses and in a strong inhibition of AA. These findings suggest that during the natural course of AA, T cells directed towards the third framework region of AV11 do not have a disease regulatory function, but instead play a role in the deterioration of AA.

Introduction

In autoimmune diseases, on the one hand, pathogenic self-reactivity of T cells plays an important role, while, on the other hand, self-reactivity is needed to regulate auto-aggressive responses (1). It has been shown that vaccination with modified auto-aggressive T cells protected against adjuvant arthritis (AA) and experimental autoimmune encephalomyelitis (EAE) (2,3). This suggested the activation of a T cell-specific regulatory T cell population. Indeed, anti-TCR-specific T cell responses were detected during the natural course of several autoimmune diseases (4–7). Moreover, it has been shown that T cells reactive to epitopes derived from the TCR of pathogenic T cells play a role in the regulation of autoimmunity (7–10). These findings indicate that although TCR-derived peptides are self-antigens, clonal deletion of TCR peptide-specific T cells in the thymus is not complete and that such cells are activated during autoimmune diseases. Furthermore, in the experimental autoimmune models EAE and collagen-induced arthritis (CIA), it appeared that autoreactive T cells used predominantly BV8S2 (TCR Vβ8.2) (11,12). Interestingly, immunization with a peptide derived from the third framework region of BV8S2 prevented the induction of both EAE and CIA in mice (10).

The usage of TCR AV regions in experimental autoimmune diseases is less well documented. Previously, we showed the usage of AV11 by an arthritogenic T cell clone (A2b) specific for heat shock protein 60 (13). This AV11 sequence contains a number of immunogenic regions as determined by immunization with overlapping peptides. Furthermore, T cell lines reactive to these peptides recognized the recombinant AV11 protein (13). Interestingly, in SWR and DBA/1 mice, two polymorphisms in the AV11 gene have been observed to be associated with resistance to CIA induction (12,14). Moreover, vaccination with recombinant TCR AV11 decreased the arthritis incidence in CBA/1 mice (15).

In the present paper, the AA model was used to study naturally occurring T cell reactivity towards the AV11 immunogenic regions. The strongest T cell reactivity was observed with a peptide derived from the third framework region of AV11, AV11 66–80. Interestingly, nasal administration of AV11 66–80 led to a significant reduction of AV11 66–80-specific
DTH reactions during AA and to a significant inhibition of disease. These data suggest that AV11 66–80–specific T cells play a role in the enhancement rather than in the down-regulation of the arthritic process.

Methods

Animals

Male inbred Lewis rats (RT11) were obtained from the University of Limburg (Maastricht, The Netherlands). Rats were 6–9 weeks old at the start of each experiment.

Peptides

AV11 26–40 (SIITTTVQWFRONPR), 41–55 (GLSLNLFYLVPGTKE), 66–80 (KERJTYLISNAQVE) and myelin basic protein (MBP) 87–99 (VHFFKNIVPTRP) were synthesized via automated multiple peptide synthesis (16). Ovalbumin (OVA) 323–339 (ISQAVHAAHAEINEAGR) and MBP 72–85 (QKSQRSQDENPV) were synthesized by standard solid-phase Fmoc chemistry (17). The peptides were purified by reversed-phase HPLC and analyzed via FAB/MS. The MBP peptides, used as marker peptides in the MHC binding assays, were biotinylated during peptide synthesis.

Delayed-type hypersensitivity (DTH)

Peptide was dissolved in PBS (1 mg/ml) and 100 µl was injected in one ear. PBS was injected in the contra-lateral ear. The DTH reaction was determined by measuring the ear thickness 48 h after injection with a pressure-sensitive micro-meter. Data are expressed as the mean difference in ear thickness between the right and left ear in mm/100 ± SEM. The paired Student's t-test was performed on the mean difference to evaluate the effect of each peptide. The unpaired Student's t-test was performed to compare the mean differences in DTH reactions between the different groups.

Nasal peptide administration

Rats were lightly anesthetized with ether and 10 µl of 10 µg/µl peptide in PBS was administered nasally using a micropipette. This was done on days −15, −11, −7 and −3 preceding the induction of arthritis or EAE or the isolation of spleen and mandibular lymph nodes.

Induction and clinical evaluation of AA and EAE

AA was induced by intradermal injection in the base of the tail of 100 µl 5 mg/ml Mycobacterium tuberculosis (Mt, strain H37Ra; Difco, Detroit, MI) emulsified in incomplete Freund’s adjuvant (IFA; Difco). To measure DTH reactions after induction of AA, rats were immunized with 10 mg/ml Mt. Rats were examined in a blind set-up for clinical signs of arthritis. Severity of arthritis was scored by grading each paw from 0 to 4 based on swelling, erythema and deformation of the joints as described earlier (18). EAE was induced by s.c. injection of 50 µl of a 1:1 emulsion of peptide MBP 72–85 (1 mg/ml) with 4 mg/ml Mt in IFA in each hind footpad. In a blind set-up, rats were examined for EAE and severity was scored on a scale from 0 to 4: 0, no signs; 1, limp tail; 2, hind leg weakness; 3, paraplegia; 4, front and hind leg paralysis, moribund condition.

T cell proliferation assay

Three days after the last nasal peptide administrations, rats were sacrificed, and the inguinal, popliteal and mandibular lymph nodes, and the spleen were isolated. Proliferation of 2×105 cells/well was measured in flat-bottom 96-well plates (Costar, Cambridge, MA) in triplicate cultures. The cells were cultured in culture medium [IMDM (Gibco/BRL, Gaithersburg, MD), supplemented with L-glutamine (2 mM), β-mercaptoethanol (50 µM), penicillin (50 U/ml), streptomycin (50 U/ml) and 2% heat-inactivated normal rat serum] in the presence of a dose range of specific antigen or concanavalin A (2.5 µg/ml). Cultures were incubated at 37°C, 5% CO2 for 3 days and subsequently pulsed for 16–20 h with [3H]thymidine (0.4 µCi/well; Amersham, Little Chalfont, UK). [3H]Thymidine incorporation was measured using a liquid scintillation counter. Results are expressed as mean c.p.m. of triplicate cultures ± SD.

Peptide–MHC binding assay

The MHC class II–peptide binding studies were performed on affinity-purified detergent-solubilized MHC molecules as described previously (19). Briefly, rat RT1.B1 and RT1.D1 molecules were affinity purified from cell lysates of the MHC class II+ Z1a T cell line using the mAb OX6 and OX17. For competition studies purified RT1.B1 (3 µM) or RT1.D1 (1 µM) was incubated with respectively 100 nM of biotinylated marker peptide MBP 72–85 or 87–99 and a dose range of unlabeled competitor peptide for 40 h at room temperature at pH 5 in the presence of a protease inhibitor mix. The MHC–peptide mixtures were analyzed by SDS–PAGE under non-reducing conditions and followed by Western blotting. Biotinylated peptides were visualized through enhanced chemiluminescence (Western blot ECL kit; Amersham, Arlington Heights, IL). The IC50 value is the concentration of competitor peptide in µM resulting in 50% inhibition of the binding of 100 nM biotinylated marker peptide to 3 µM RT1.B1 or 1 µM RT1.D1 as calculated with the molecular Analyst software (BioRad, Hercules, CA).

Results

Natural processing and presentation of TCR AV11 epitopes during AA

Previously, we have identified three immunogenic regions of the TCR α chain of A2b: AV11 26–40, 41–55 and 66–80 (13). T cell responses, induced after immunization with synthetic peptides comprised of sequences derived from these immunogenic regions, appeared to be MHC class II restricted (13). These findings raised the question whether during AA the AV11 peptides were naturally processed and presented in a MHC class II-restricted manner. To study this, we evaluated the presence of AV11-specific T cells after the onset of AA. 14 Days after AA induction, the three immunogenic AV11 peptides were tested for DTH reactivity. As shown in Table 1, all three AV11 peptides induced a significant DTH reaction, whereas no DTH reaction was observed with a highly immunogenic control peptide. This indicated that AV11 T cell epitopes are naturally processed and presented in vivo, and that AV11 specific T cells are part of the T cell repertoire.
Fig. 1. Binding affinity of AV11 peptides to MHC class II molecules. MHC RT1.B1 (3 μM) was incubated with biotinylated MBP 72–85 (100 nM) and a dose range of unlabeled AV11 26–40. RT1.D1 (1 μM) was incubated with biotinylated MBP 87–99 (100 nM) and a dose range of AV11 41–55 or 66–80. The peptide–MHC mixtures were analyzed by SDS-PAGE and Western blotting, and the biotinylated peptides were visualized through ECL.

Table 1. Natural occurrence of AV11 peptide specific T cells

<table>
<thead>
<tr>
<th>Peptides</th>
<th>TCR region</th>
<th>n</th>
<th>DTH reactiona</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV11 26–40</td>
<td>CDR1</td>
<td>12</td>
<td>12 ± 2c</td>
</tr>
<tr>
<td>AV11 41–55</td>
<td>CDR2</td>
<td>12</td>
<td>23 ± 2c</td>
</tr>
<tr>
<td>AV11 66–80</td>
<td>FR3d</td>
<td>12</td>
<td>29 ± 2c</td>
</tr>
<tr>
<td>OVA 323–339</td>
<td></td>
<td>3</td>
<td>2 ± 1</td>
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</tbody>
</table>

MHC binding affinity of AV11 epitopes

Previously, we demonstrated that blocking with OX6 (anti-RT1.B1) abrogated the proliferative response of AV11 26–40-specific T cells, while OX17 (anti-RT1.D1) inhibited AV11 41–55- and 66–80-specific T cell responses (13). Here, we determined in a competitive peptide–MHC binding assay the MHC binding affinity of AV11 26–40 for RT1.B1, and of AV11 41–55 and 66–80 for RT1.D1. Figure 1 shows the competitive inhibition of binding of the biotinylated marker peptides to purified MHC class II molecules by the addition of increasing concentrations of non-labeled AV11 peptides. AV11 26–40, which induced only a mild DTH reaction, appeared to be a strong binder for RT1.B1 (IC50 16–32 μM). Both AV11 41–55 and 66–80 induced a strong DTH reaction, and appeared to be a strong RT1.D1 binder (IC50 8–16 μM) and an intermediate RT1.D1 binder (32–64μM) respectively. These data indicate that there is no clear correlation between the MHC binding affinity of the peptide and the observed DTH reaction.

Table 2. AV11 66–80-specific DTH responses

<table>
<thead>
<tr>
<th>Autoimmune modela</th>
<th>AV11 66–80 nasal administrationb</th>
<th>n</th>
<th>DTH reactionc</th>
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<tbody>
<tr>
<td>–</td>
<td>–</td>
<td>8</td>
<td>7 ± 2d</td>
</tr>
<tr>
<td>AA</td>
<td>–</td>
<td>6</td>
<td>35 ± 2d,e</td>
</tr>
<tr>
<td>AA</td>
<td>+</td>
<td>10</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>EAE</td>
<td>–</td>
<td>10</td>
<td>27 ± 2d,e</td>
</tr>
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</table>

AA and EAE were induced as described in Methods.

Based on the strongest DTH reaction, AV11 66–80 was selected for further studies. First, we analyzed the DTH reaction towards AV11 66–80 in naive animals. As shown in Table 2, a mild DTH reaction was already observed in naive rats. Next, we analyzed whether AV11 66–80-specific T cell responses were increased before the onset of clinical disease. Indeed, a strong DTH reaction was observed 9 days after AA induction, when no clinical signs of arthritis could be observed yet (Table 2). This DTH reaction was comparable with the DTH reaction observed 14 days after AA induction (29 ± 2, Table 1), when all rats showed clinical signs. These data indicate that AV11 66–80-specific T cells already became activated before clinical onset of AA.

Reduced DTH responses and protection against AA after nasal administration of AV11 66–80

The early activation of AV11 66–80-specific T cells during the onset of AA raised the question whether such T cells play a role in deterioration or amelioration of the arthritogenic process. To study this, we applied AV11 66–80 nasally 4 times at 3-day intervals before disease induction and investigated the AV11 66–80-specific DTH reaction 9 days after AA induction. As shown in Table 2, nasal administration reduced the DTH response significantly to almost undetectable levels. Next, we evaluated the effect of nasal AV11 66–80 administration on the development of AA. As a control, we used the OVA 323–339 peptide. The peptides were applied nasally 4 times at 3-day intervals, starting 14 days before AA induction and investigated the AV11 66–80-specific DTH reaction 9 days after AA induction. Nasal administration of AV11 66–80 almost completely inhibited arthritis development, whereas administration of OVA 323–339 had no influence on AA development (Fig. 2A). As an additional clinical parameter, the percentage of weight loss was evaluated. As shown in Fig. 2(B), OVA 323–339-treated animals had a marked weight loss during active arthritis, while the AV11 66–80-treated animals only gained weight. To test whether the disease inhibitory effect of AV11 66–80 was
Fig. 2. Protection against AA after nasal administration of AV11 66–80. The peptides OVA 323–339 and AV11 66–80 were dissolved in 10 µg/µl PES and 10 µl was applied nasally 4 times at 3-day intervals, starting 14 days before AA was induced. AA was induced by s.c. injection of 100 µl 5 mg/ml Mt dissolved in IFA. (A) Arthritis score. (B) Percent weight loss. Each experimental group consisted of n = 5 rats. Data are derived from one representative experiment out of two. Data are expressed as mean ± SEM. ■, AV11 66–80; ▲, OVA 323–339.

Fig. 3. No protection against EAE after nasal administration of AV11 66–80. Peptides were nasally administered as described in the legend of Fig. 2. EAE was induced 3 days after the last nasal peptide administration. (A) EAE score. (B) Percent weight loss. Each experimental group consisted of n = 4 rats. Data are expressed as mean ± SEM. ■, AV11 66–80; ▲, OVA 323–339.

specific for AA, we analyzed the effect of nasal peptide administration on the induction of EAE in Lewis rats. At first, we measured the presence of AV11 66–80-specific T cells during the onset of EAE, as EAE is induced in the presence of the same adjuvant used for AA induction (Mt/IFA). As shown in Table 2, a strong DTH response was detected during the onset of EAE. Interestingly in contrast to the effect on AA, nasal peptide administration did not effect the development of EAE (Fig. 3A and B).

No deletion of AV11 66–80-specific T cells after nasal administration

Since we observed a clear inhibition of AV11 66–80 DTH responses after nasal peptide administration, we analyzed whether this was due to deletion of AV11 66–80-specific T cells. Therefore, 3 days after the last nasal administration of AV11 66–80 or control peptide OVA 323–339, spleen and mandibular lymph nodes were isolated and tested in a proliferation assay. In the mandibular lymph nodes, no proliferative responses were detected to the administered peptides (Fig. 4a). However, in the spleen, peptide-specific proliferative responses were detected (Fig. 4b). This indicated that nasal peptide administration led to a specific T cell activation and did not delete peptide specific T cells.

Discussion

In this paper, we report that T cells specific for three peptides derived from the AV11 region of an arthritogenic T cell clone are naturally present in the T cell repertoire of Lewis rats, as measured by DTH reactions during AA. The peptide AV11 26–40 induced only a mild DTH reaction, while peptides AV11 41–55 and 66–80 induced strong DTH reactions after AA induction. AV11 41–55 is localized in the second CDR and it has been described that in multiple sclerosis patients, CDR2 peptides derived from various variable regions of the TCR β chain appeared to be immunogenic (20). Furthermore, a CDR2 peptide derived from VB8S2 was found to be naturally processed during EAE and protected against EAE (4). Our AV11 41–55 data confirm the finding that CDR2 peptides are in general immunogenic (20).

AV11 66–80 is localized in the third framework region of the α chain. Interestingly, a polymorphism in this framework region
of AV11 was suggested to play an essential role in resistance and susceptibility to arthritis induction (14). Moreover, it has been described that TCR peptides derived from the third framework region of both mouse and rat BV8S2 protected against experimental autoimmune arthritis (6,10).

The observation that AV11-specific T cells were present in the immune repertoire indicated that these cells were not deleted in the thymus. T cell selection in the thymus is controlled by the number of peptide–MHC complexes, the affinity of the peptides for the MHC molecules and the affinity of the TCR for the MHC–peptide complex (21). The mild DTH reaction to AV11 26–40 could be due to the rather high affinity of the peptide for RT1.B1. However, AV11 66–80 induced the strongest DTH reaction and appeared to be a strong RT1.Db binder. Therefore, the lack of deletion of these AV11-specific T cells should be due to either the low frequency of specific epitope–MHC complexes within the thymus or the relatively low TCR affinity for the specific peptide–MHC complexes.

Since AV11 66–80 showed the strongest DTH reaction at day 14 after disease induction, this peptide was selected for further study. Both the DTH reactions at days 9 and 14 were significantly increased as compared with naive animals. This indicated that already during the induction phase of AA, before clinical signs of AA were visible, activation of TCR that deletion or neonatal tolerization of TCR-specific T cells had occurred. Taking into account the relatively high MHC binding affinity of the peptide, this could imply that a relative low number of MHC–AV11 66–80 complexes is present in naive rats due to, for example, crypticity of the epitope, while during the strong inflammatory process of AA, AV11 66–80 is unveiled. This would be in agreement with the observation by Di Rosa et al. showing that strong inflammatory processes can induce a change in the processing machinery of antigen-presenting cells (APC) (22). Alternatively, it is possible that in naive rats, due to the low frequency of AV11-bearing T cells, the number of specific MHC class II–AV11 66–80 complexes is low. During AA, an expansion and subsequent turnover of AV11+ T cells could result in uptake and presentation of AV11 peptides by professional APC (23) or in presentation of TCR peptides by MHC class II molecules on activated T cells (24).

To study the role of AV11 66–80-specific T cells during AA, we applied AV11 66–80 nasally before AA induction. This resulted in a strong inhibition of the AV11 66–80-specific DTH reaction and in protection against AA. The protection was specific, since nasal AV11 66–80 application could not protect against EAE. Moreover, we observed that 3 days after the last nasal peptide administration, a clear peptide specific proliferative response was present in the spleen. Although we cannot exclude that after AA induction, AV11 66–80-specific T cells were deleted, the proliferative response in the spleen directly after nasal administration argues against AV11 66–80-specific T cell deletion as the main protective mechanism. In several other autoimmune models, the induction of T cells producing regulatory cytokines (e.g. IL-4, IL-10 and transforming growth factor-β) after nasal administration has been observed (25–29). As other groups indicated that TCR peptide-specific T cells could play a role in the down-regulation of autoimmune diseases (30–32), it could be possible that nasal AV11 66–80 administration led to enhanced activation of the already pre-existing regulatory T cell response. However, the reduction of the naturally occurring AV11 66–80 DTH response during AA after nasal AV11 66–80 administration indicated that nasal administration modulated the natural AV11 66–80-specific T cell response. This finding, together with the finding that AV11 66–80-specific T cells were already activated before the clinical onset of arthritis, suggests that the naturally occurring AV11 66–80-specific T cell response during AA plays a role in the exacerbation or perpetuation of AA, rather than in the down-regulation of the disease process. This hypothesis is further substantiated by our recent findings indicating that 11 out of 36 rats immunized with AV11 66–80 in a Th1 skewing adjuvant (dimethyl-dioctade-cyl ammonium bromide) developed arthritis (33). Moreover, three out of five rats injected i.v. with AV11 66–80-specific T cells also developed arthritis, indicating the T cell-mediated character of the AV11 66–80-induced arthritis. Enhancement of experimental autoimmune diseases after TCR peptide immunizations has also been described by others (34–36). Yamamura and co-workers showed that an α chain TCR CDR3 peptide derived from an encephalitogenic T cell clone did not only enhance MBP- or proteolipid protein-induced EAE, but also AA (36). However, these findings and our findings are in contrast with data described by other groups, showing that deletion or neonatal tolerization of TCR-specific T cells or modulation of the TCR peptide-specific Th1 response towards a Th2 response all resulted in exacerbation of the experimental autoimmune diseases EAE and diabetes (37–41). There is no easy explanation why the outcome of TCR-based therapies is so variable. Differences could amongst others be based on the activation of pre-existing versus induced TCR-specific T cells, the timing of the intervention, the different model systems or the presence of MHC class II on rat T cells, which in contrast to mouse T cells express MHC class II (24).

In conclusion, we showed that AV11 peptide-specific T cells are part of the normal immune repertoire. Moreover, our data suggests that T cells specific for the third framework region AV11 66–80 play a role in the deterioration of AA, rather
Nasal application of TCR AV11 66-80 protects against AA than in the down-regulation of AA. Although the underlying mechanism has not been elucidated, these data urge to caution with respect to TCR-based immunotherapy of autoimmune diseases.

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Abbreviations

AA adjuvant arthritis
APC antigen-presenting cell
CDR complementarity-determining region
CIA collagen-induced arthritis
DTH delayed-type hypersensitivity
EAE experimental autoimmune encephalomyelitis
IFA incomplete Freund’s adjuvant
MBP myelin basic protein
Mt Mycobacterium tuberculosis
OVA ovalbumin

References

Nasal application of TCR AV11 66–80 protects against AA


