Original article

Role of T-cell receptor V beta 8.3 peptide vaccine in the prevention of experimental autoimmune uveoretinitis

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Background T-cell receptor (TCR) plays an important role in the development of autoimmune diseases. Recently, it was reported that immunization of animals with TCR peptide derived from the pathogenic cells could prevent autoimmune diseases. The aim of this study was to investigate whether vaccination with a synthetic peptide from the hypervariable region of TCR V_β 8.3, an experimental autoimmune uveoretinitis (EAU)-associated gene, was able to prevent the disease.

Methods EAU was induced in Lewis rats by immunization with IRBP R16 peptide emulsified in complete Freund's adjuvant (CFA). The clinical and histological appearances were scored. Delayed type hypersensitivity (DTH) and lymphocyte proliferation were detected. Cytokine levels of aqueous humour, supernatants of cells from spleen and draining lymph nodes were measured by enzyme linked immunosorbent assay (ELISA). Gene expression of TCR V_β 8.3 on CD_4^+ T cells was examined by real time quantitative polymerase chain reaction (PCR).

Results After vaccination, the intraocular inflammation was significantly mitigated, antigen specific DTH and lymphocyte proliferation responses were suppressed, interleukin (IL)-2 in aqueous humour, interferon (IFN)-γ and IL-2 produced by the spleen and draining lymph node cells were significantly decreased, whereas the production of IL-4 and IL-10 were increased. The response of draining lymph node cells to TCR V_β 8.3 peptide was enhanced after vaccination. Inoculation with CFA alone did not affect the severity of EAU and the above parameters. The suppression of EAU was much stronger in the group of four fold inoculations than the group of two fold inoculations. The expression of TCR V_β 8.3 gene was significantly reduced in the group of fourfold inoculations.

Conclusion Vaccination with the synthetic TCR V_β 8.3 peptide could remarkably inhibit the development of EAU.

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Experimental autoimmune uveoretinitis (EAU) is an organ specific, intraocular inflammatory disease induced in susceptible species by immunization with retinal specific antigens such as interphotoreceptor retinoid binding protein (IRBP)[1,2] or its immunodominant epitopes,[3] and has been considered as a model for human autoimmune uveitis. Studies on human uveitis and its animal counterpart have revealed that autoreactive CD_4^+ T lymphocytes are primarily responsible for the development of this disease.[4] Therefore, a desirable therapy may involve selective inactivation or elimination of these pathogenic T cells.

Uveitis is one of the leading causes of blindness in the world. The prevention of uveitis and other autoimmune

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diseases has been intensively studied during the past decades. Of special interest are the promising results in the study of regulating antigen specific responses in experimental allergic encephalomyelitis (EAE), an organ specific autoimmune animal model for multiple sclerosis. It was reported that immunization of Lewis rats with synthetic peptides derived from T-cell receptor (TCR) of encep halitogenic T cell clones could prevent the develop ment of EAE.[5,6] The underlying mechanisms may include the induction of regulatory T cells and modulation of Th_1/Th_2 balance.[7] Yet, it is not clear whether EAU, like EAE,[8] can be prevented by disease associated TCR peptide vaccinations. Egwuagu and coworkers[9] examined the expression of genes coding for the 20 variable regions of TCR V_β families in Lewis rats and found that TCR V_β 8.3 was most strongly associated with the pathogenic T cell subline. Therefore, we investigated the effect of vaccination with EAU specific TCR V_β 8.3 peptide on uveoretinitis in Lewis rats. We also examined the expression of TCR V_β 8.3 gene in the vaccinated rats to determine its contribution to the effect of vaccination. Our results supported the validity of TCR V_β 8.3 peptide as a vaccine in the prevention of EAU with underlying mechanisms similar to those suggested in the EAE model. The additional mechanism we propose is the suppression of the EAU-associated TCR V_β 8.3 gene expression after multiple vaccinations.

METHODS

Animals

Eight to ten weeks old, inbred female Lewis rats, weighing 150 g to 180 g, were used in this study. These rats were treated according to institutional and federal guidelines. All animal experimental procedures adhered to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the use of animals in ophthalmic and vision research.

Peptides

The TCR V_β $8.3_{39\text{-}59}$ (DMGHGLRLIHYSY-GSGSFENG), and a uveitogenic peptide IRBP R16 (ADGSSWEGVGVVPDV) were synthesized using Fmoc amino acid standard solid phase techniques (CS Inc, USA) according to the manufacturer's instruction.

Vaccine and vaccination

EAU was induced by injection of 30 μg IRBP R16 peptide emulsified in complete Freund's adjuvant (CFA) containing 2.5 mg/ml of *Mycobacterium tuberculosis H37Ra*, into the left hind footpads of the Lewis rats. The peptide vaccine was prepared by emulsification of 100 μg V_β 8.3 peptide in CFA containing 1000 μg Mycobacterium tuberculosis H37Ra in a volume of 0.1 ml. The vaccine was injected into the right footpads of the Lewis rats. The experimental rats were divided into 5 groups: the normal group (6 rats) receiving no treatments; noninoculated group (12 rats) only receiving IRBP R16 peptide emulsified in CFA; CFA inoculated group (12 rats) receiving injections of an emulsion containing phosphate buffered saline (PBS), bacteria and CFA for 4 times (on 30, 20, 10 and 0 days before R16 peptide immunization); two-fold inoculated group (12 rats) receiving injections of peptide vaccine on 20 and 0 days before immunization with R16 peptide in CFA; and fourfold inoculated group (12 rats) receiving the peptide vaccine on 30, 20, 10 and 0 days before immunization with R16 peptide in CFA. Six rats in the last 4 groups were followed up for 30 days, whereas the other six rats in these groups were sacrificed on day 14 for further studies as described below.

Clinical and histological assessment of EAU

All the rats were monitored daily. Clinical signs were scored by slit lamp biomicroscope as follows: 0, normal; 1, minimal signs of inflammation with occasional cells in the anterior chamber; 2, presence of mild exudate in the anterior chamber; 3, moderate exudate in the anterior or posterior chamber (posterior chamber hypopyon); 4, large exudate within the anterior chamber, or massive posterior chamber hypopyon; 5, gross orbital oedema and exophthalmos in addition to the manifestation listed in 4.[10]

On day 14 after IRBP R 16 peptide immunization, 6 rats chosen randomly from each group were sacrificed and the eyes of each rat were enucleated and fixed in 10% formaldehyde. Sections of 5 μm in thickness were stained with haematoxylin eosin and examined under a light microscope. Histological grading was as follows: 0, no inflammatory cell infiltration and no destruction of the retina; 1, minimal cell infiltration in the retina and choroid but no

destruction; 2, partial and mild destruction of the outer retina; 3, moderate destruction of the outer retina; 4, extensive and severe destruction of the outer retina and partial destruction of the inner retina; 5, complete destruction of the entire retina.[11]

Assay for delayed type hypersensitivity

Delayed type hypersensitivity (DTH) response to IRBP R16 peptide was determined by measuring the thickness of swelling ears. On day 15 after IRBP R16 immunization, 20 μg of R16 peptide in 20 μl PBS was injected into the right pinna of each rat. PBS was injected into the left pinna as a control. Ear thickness was measured with a micrometer 24 hours after R16 peptide challenge. DTH dependent ear swelling was calculated according to the following formula:

$$\text{Specific cell swelling } (10^{-3}\text{mm}) = \frac{\text{24 h measurement of right ear} - \text{0h measurement of left ear}}{\text{24 h measurement of left ear} - \text{0h measurement of left ear}}$$

Lymphocyte proliferation assay

The draining lymph nodes at the inguinal areas of the rats were collected, minced and passed through a wire mesh to obtain a single cell suspension. The lymphocyte proliferation assay (LPA) was performed in a 96-well plate in triplicate with RPMI 1640 medium (Gibco, NY, USA) containing streptomycin (100 μg/ml), penicillin (100 U/ml), 2 mmol/L L-glutamine, 5×10^{-5} mol/L 2-mercaptoethanol, 0.1 mmol/L nonessential amino acids, 1 mmol/L sodium pyruvate, and 1% syngeneic rat serum. The cells were seeded at a density of 2×10^5 cells per well and incubated with the medium alone, stimulated with IRBP R 16 or V_β8.3 peptide (10 μg/ml) at 37˚C in an atmosphere of 5% CO_2 for 72 hours. The culture wells were pulsed with 37 kBq of 3H-thymidine for the last 16 hours and harvested on glass fibre filters. The thymidine uptake was assessed by liquid scintillation on a beta counter (Packard, USA).

Cytokine assays

Approximately 20 μl of aqueous humour was obtained from both eyes of each rat by paracentesis before it was sacrificed. Cells from the spleens and draining lymph nodes were incubated in 24-well tissue culture plates (2×10^6 cells/well) with 10 μg/ml of R16 in 2 ml of serum free medium as described above. The supernatants collected 48 hours after the incubation were assessed for IFN-γ, IL-2, IL-4 and IL-10 and the aqueous humour was assessed for IL-2 by a specific sandwich ELISA kit (R&D Systems Inc, USA), according to the manufacturer's procedure.

Detection of TCR V_β 8.3 expression by real time quantitative PCR

CD_4^+ T cells of the spleens were isolated by means of magnetic beads using two kinds of antibodies, mouse antirat CD_4 monoclonal antibody and antimouse IgG antibody, conjugated to the magnetic beads (Dynal, Norway). The quantitative assessment of mRNA levels was performed using PE Prism 7000 sequence detection system (Perkin-Elmer Applied Biosystems, USA). The standard sample was serially diluted to perform real time quantitative PCR. The correlation between Ct values and copy numbers is linear, with a correlation coefficient of 0.999. The final reaction was performed in 10 μl 5×quantitative PCR buffer, 2 μl (25 μmol/L) primers, 1 μl dNTPs (10 mmol/L), 1 μl fluorescent probe (20 μmol/L), 2 μl Taq polymerase (2 U/μl), 5 μl cDNA, in a total volume of 50 μl. An initial step was 93˚C for 2 minutes. Cycling conditions were: 40 cycles of melting at 93˚C for 1 minute and annealing extension at 55˚C for 1 minute. Upstream primer (5'-GGCATGGTCTGAGGCTGA-TC-3'), downstream primer (5'-ACCCCTCAGGG-ATATCTCCATT-3'), and probe sequence (5'-ATTAC-TCATATGGTTCTGGC-3') were used for the detection of TCR V_β 8.3 expression.

Statistical analysis

Means and standard deviations were calculated for the clinical and laboratory measurements. Statistical analyses were performed by one way ANOVA or Student's *t* test. EAU scores were analyzed by the Snedecor and Cochran's test for linear trends in proportions. Probability valuesless than 0.05 were considered statistically significant.

RESULTS

Inhibition of IRBP R16 -induced EAU by the TCR V_β 8.3 peptide vaccine

After IRBP R16 peptide challenge, the clinical scores of EAU were evaluated by slit lamp biomicroscope examination (Figs. 1 and 2). A severe uveitis, as evidenced by ciliary congestion, meiosis, inflammatory

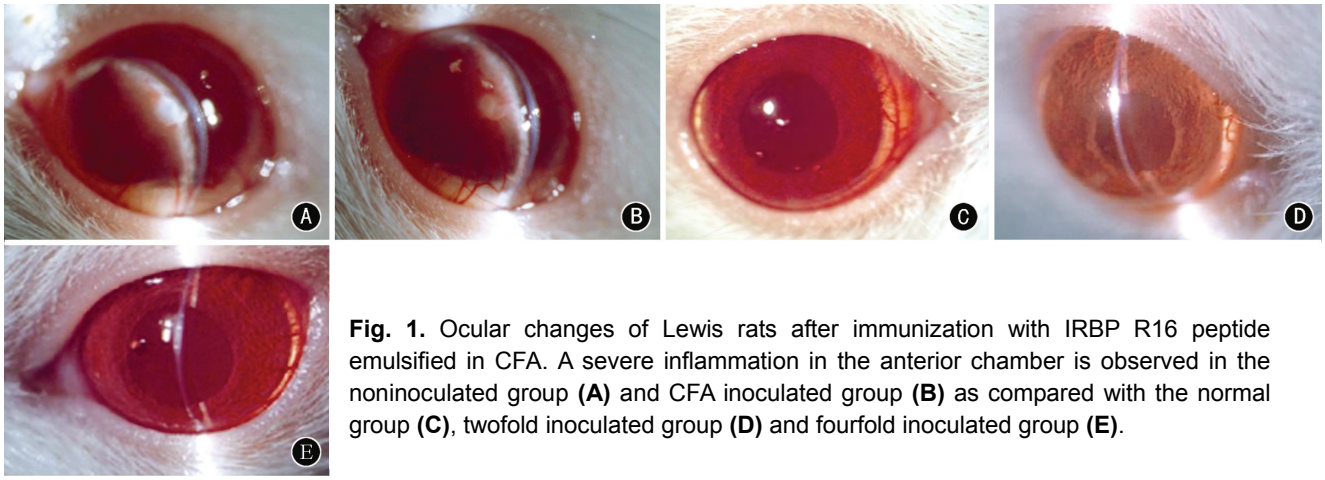

Fig. 1. Ocular changes of Lewis rats after immunization with IRBP R16 peptide emulsified in CFA. A severe inflammation in the anterior chamber is observed in the noninoculated group **(A)** and CFA inoculated group **(B)** as compared with the normal group **(C)**, twofold inoculated group **(D)** and fourfold inoculated group **(E)**.

cells and exudate in the anterior chamber, was observed in the noninoculated rats and CFA inoculated rats. In a marked contrast, no clinical intraocular inflammation was found in 3 rats of the fourfold inoculated group, and a mild inflammation was observed in the other 3 rats of this group. A moderate inflammation was noted in all of the rats in the twofold inoculated group. However, a shorter duration and delayed onset of EAU was observed in the two vaccination groups as compared with the CFA inoculated group as well as the noninoculated group (Fig. 2). The fourfold inoculation appeared to be more effective than the twofold inoculation. As controls, the severity and course of EAU in the CFA inoculated rats were not significantly different from those in the noninoculated rats.

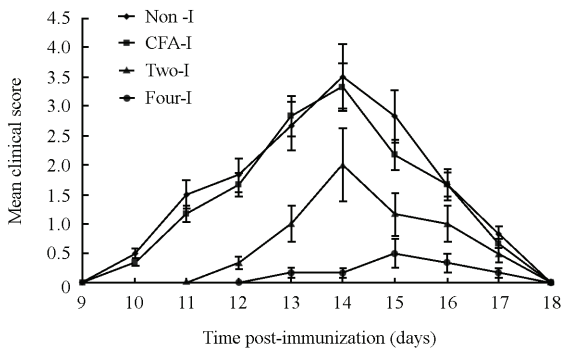

Fig. 2. Kinetics of EAU in different groups. Mean clinical scores are shown for each group. Non-I: noninoculated group; CFA-I: CFA inoculated group; Two-I: twofold inoculated group; Four-I: fourfold inoculated group.

A histological analysis was performed on day 14 after R16 immunization. Consistent with the clinical evaluation, severe retinal destruction associated with infiltration of numerous inflammatory cells was observed in the sections from both the noninoculated group and the CFA inoculated group (Figs. 3A, 3B and 4). Twelve eyes from the twofold inoculated group exhibited mild to moderate inflammation (Figs. 3c and 4). In the fourfold inoculated group, six eyes from the rats with no clinical signs of uveitis did not show obvious evidence of intraocular inflammation, whereas the other six eyes from the rats with clinical scores of 1 to 2 only showed scattered inflammatory cells (Figs. 3D and 4).

Suppression of systemic responses after TCR V_β 8.3 peptide vaccination

In addition to ocular inflammation, immunization with IRBP has been shown to induce concurrently systemic Th_1 mediated responses. To evaluate the impact of TCR V_β 8.3 peptide vaccination on the immune system, we measured DTH *in vivo* and lymphocyte proliferation *in vitro*. DTH response is typically manifested by the swelling of ear pinnae, which was readily seen in the noninoculated and the CFA inoculated groups. In contrast, DTH responses to the IRBP R16 peptide were significantly suppressed in the vaccinated groups, especially in the fourfold inoculated group (Fig. 5).

The lymph node cells (LNCs) from noninoculated and CFA inoculated groups showed a strong proliferation after further IRBP R16 peptide stimulation *in vitro* (Table 1). In comparison, the proliferative responses of LNCs to R16 peptide were significantly reduced after immunization with V_β 8.3 peptide. The inhibition of proliferation was more pronounced in the fourfold inoculated group [(17.9±5.1)% of proliferation] than the twofold group[(30.8±

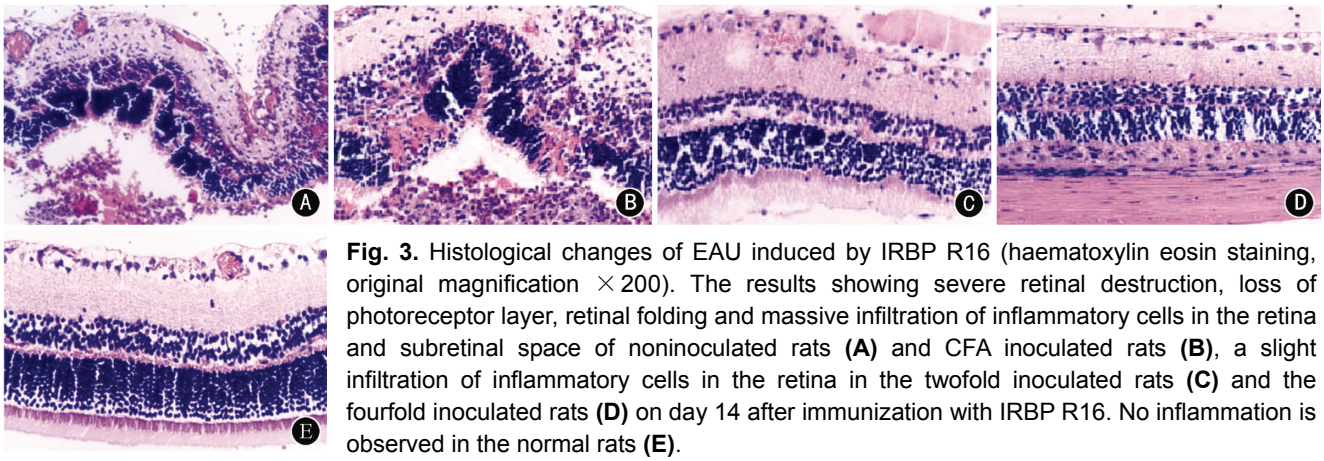

Fig. 3. Histological changes of EAU induced by IRBP R16 (haematoxylin eosin staining, original magnification ×200). The results showing severe retinal destruction, loss of photoreceptor layer, retinal folding and massive infiltration of inflammatory cells in the retina and subretinal space of noninoculated rats **(A)** and CFA inoculated rats **(B)**, a slight infiltration of inflammatory cells in the retina in the twofold inoculated rats **(C)** and the fourfold inoculated rats **(D)** on day 14 after immunization with IRBP R16. No inflammation is observed in the normal rats **(E)**.

Table 1. Effect of vaccination with V_β 8.3 peptide on the proliferative responses of lymphocyte proliferation assay (mean±SD, cpm of triplicate wells)

Stimulants	Noninoculated group	CFA inoculated group	Twofold inoculated group	Fourfold inoculated group	*F* value	*P* value
Medium	3.51±1.73	4.3±1.8	3.6±1.3	3.9±1.7	2.03	0.142
V_β 8.3 peptide	3.29±2.56$^\Delta$	4.9±2.7$^\Delta$	13.8±4.9*	22.5±5.3*	258.91	<0.001
IRBP R16 peptide	33.27±7.24*	31.6±7.5*	10.5±5.2$^\#$	6.3±2.1$^\#$	3.15	0.048

* *P*<0.05 compared with the values without stimulant. $^\#$ *P*<0.01 compared with noninoculated group and CFA inoculated group when stimulated with R 16 peptide. $^\Delta$ *P*<0.01 compared with noninoculated group and CFA inoculated group when stimulated with V_β 8.3 peptide.

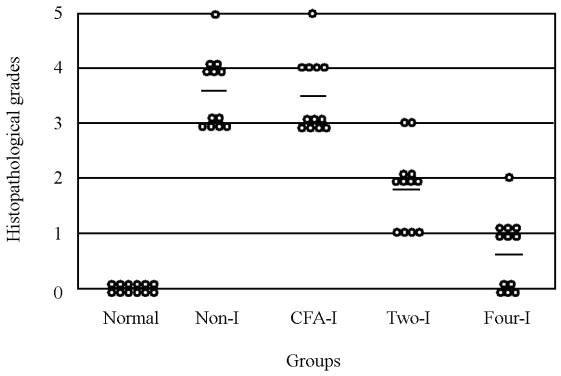

Fig. 4. Histological grades of the rats 14 days after immunization with IRBP R16. Each point is the score of an individual animal. The average scores of the group are denoted by the horizontal bars. Non-I: noninoculated group; CFA-I: CFA inoculated group; Two-I: twofold inoculated group; Four-I: fourfold inoculated group.

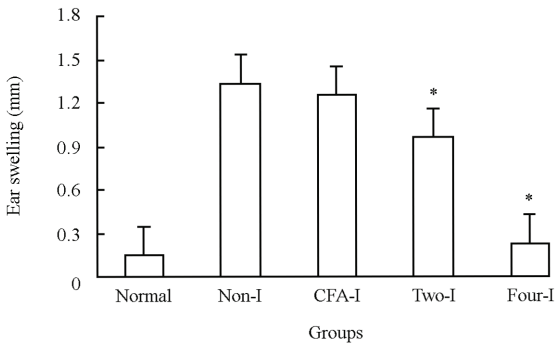

Fig. 5. DTH responses to IRBP R16 at each group. Significantly, suppressed DTH responses to IRBP R16 are noted in the fourfold inoculated group and the twofold inoculated group as compared with those in the noninoculated group and CFA inoculated group. *P<0.05. Non-I: noninoculated group; CFA-I: CFA inoculated group; Two-I: twofold inoculated group; Four-I: fourfold inoculated group.

6.9)%]. To examine immunogenicity of the peptide used for vaccination, LNC were exposed again to V_β 8.3 peptide *in vitro*. The responses of LNC from the vaccinated rats to the V_β 8.3 peptide were significantly higher than the noninoculated and CFA inoculated rats.

Skewing of Th_1/Th_2 cytokine balance after vaccination

To examine the possibility that TCR V_β 8.3 peptide vaccination induces an alteration of Th_1 dominant response that is known to mediate EAU, the cytokine concentrations in the aqueous humour and supernatants were measured by ELISA. As shown in Fig 6A, the concentration of IL-2 in aqueous humour of the V_β 8.3 peptide inoculated groups [twofold (212.5±31.9) pg/ml and fourfold (83.5±12.5) pg/ml] was significantly lower than that in the noninoculated group [(452.3±67.8) pg/ml] and CFA inoculated group

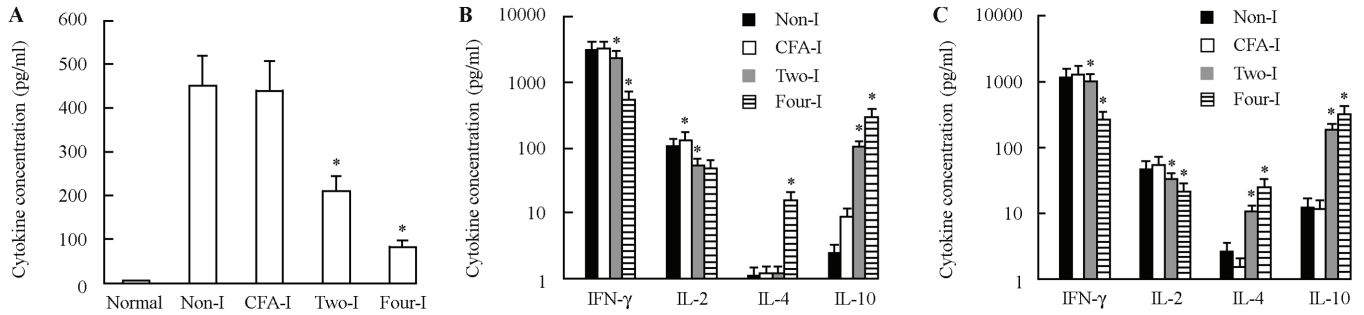

Fig. 6. Cytokine concentrations in aqueous humour and supernatants of cultured cells from drained lymph node and spleen. Cytokine concentrations were measured in duplicate via ELISA. The results represent the average of each group. Ordinate is a log scale. $^*P< 0.05$ compared with noninoculated group and CFA inoculated group. **A:** IL-2 concentrations in aqueous humour; **B:** cytokine concentrations in the supernatants of cultured cells from draining lymph node; **C:** cytokine concentrations in the supernatant of cultured splenocytes. Non-I: noninoculated group; CFA-I: CFA inoculated group; Two-I: twofold inoculated group; Four-I: fourfold inoculated group.

[(440.8 ± 66.1) pg/ml]. After R16 peptide further stimulation *in vitro*, LNCs and splenocytes from both twofold and fourfold inoculated groups produced somewhat lower levels of IFN-γ and IL-2 and significantly higher level of IL-10 than those in the noninoculated and CFA inoculated groups (Fig. 6C). LNCs from the fourfold inoculated group, but not the twofold inoculated group, also secreted significantly more IL-4 than those from noninoculated and CFA inoculated groups. There was no significant difference between the noninoculated and CFA inoculated groups concerning cytokine levels in the culture super- natants and aqueous humour.

Expression profile of the TCR V_β 8.3 gene

Real time quantitative PCR was performed to analyze the impact of TCR V_β 8.3 vaccination on the expression of its gene in CD_4^+ T cells of the spleens. The expression of TCR V_β 8.3 gene on CD_4^+ T cells was indicated by copy numbers (Table 2). A significantly higher expression of TCR V_β 8.3 gene was found in the noninoculated, CFA inoculated and twofold inoculated groups than that in the normal group. The expression of TCR V_β 8.3 gene in the fourfold inoculated group was close to that in the control group and significantly lower than that in the noninoculated and CFA inoculated groups. A small reduction of V_β 8.3 gene expression was also observed in the twofold inoculated group as compared with the CFA inoculated and noninoculated groups. The expression of TCR V_β 8.3 gene in the noninoculated group and the CFA inoculated group was essentially identical.

Table 2. Expression of TCR V_β 8.3 gene on splenic CD_4^+ T cells of rats in each group

Groups	Copy numbers
Noninoculated group	$(3.39\pm2.17)\times10^7$
CFA inoculated group	$(3.06\pm0.83)\times10^7$
Twofold inoculated group	$(2.39\pm0.67)\times10^7$
Fourfold inoculated group	$(3.31\pm3.16)\times10^6$
Normal group	$(7.95\pm6.43)\times10^5$
F value	59.24
P value	<0.001

DISCUSSION

This study showed that EAU was prevented by immunization of rats with a synthetic TCR V region peptide. The TCR V_β 8.3 peptide emulsified with the strong adjuvant CFA was shown to be immunogenic and capable of suppressing the immune response to an uveitogenic IRBP peptide in Lewis rats. A high inoculation frequency, i.e. 4 injections with intervals of 10 days, was more effective than 2 injections with an interval of 20 days, in reducing the severity and duration of EAU. The adjuvant alone did not show any effect in the prevention of EAU.

Since autoreactive CD_4^+ T cells have been considered to be involved in the initiation of autoimmune diseases, utilization of a vaccine targeting these cells may represent a desirable strategy to prevent these diseases. Immunization with attenuated autoreactive T cells, known as T cell vaccine, has been shown to be effective to prevent EAE.[12] The study by Beraud et al[13] also showed a protective effect of T cell vaccination in the course of EAU. However, the use of T cell vaccines may be

limited because these cells are individually specific and should be inoculated with attenuated autologous autoreactive T cell clones to reach a protective effect. Therefore, recent studies have focused on vaccinations that allow a universal prevention of autoimmune diseases. TCR peptide vaccination seems to be one of the options.[14] Studies of T cell mediated diseases in animal models have indicated that the pathogenic T cells require a limited number of TCR gene elements for recognition of autoantigens.[15] This characteristic of autoreactive T cells suggests a possibility for selective immunotherapy. To test this assumption, a number of investigators have used synthetic peptide as vaccines comprising amino acid sequences from the TCR of autoreactive T cells in autoimmune disease models.[16-21] Immunization with some of these peptides showed promise in prevention of diseases, induced by autoantigens, such as autoimmune arthritis[18] and EAE.[19-21] However, in some reports, the efficacy was variable.[22-25] The discrepancy between these studies concerning the effects of the vaccine might be the result of the use of different types of adjuvants. TCR peptide vaccines provide a protection against autoimmune diseases only when injected with mycobacterium enriched CFA. The combination of TCR peptide with mycobacterium enriched CFA induces a sufficient number of peptide reactive regulatory T cells that mediate specific immune inhibition.[26]

Previous studies on EAU have shown an increased expression of TCR V_β 8.3 gene on CD_4^+ T cells,[9] which was reproduced in the present study. These results suggest that TCR V_β 8.3-bearing cells may play an important role in the development of EAU and that a selective deletion or suppression of the V_β 8.3-expressing T cell subset could provide an effective therapeutic strategy without having to use a generalized immunosuppressive regime. Therefore, we investigated whether the vaccination of Lewis rats with a 21 amino acid sequence of TCR V_β 8.3 peptide comprising the core of the immunogenic activity,[27,28] together with mycobacterium enriched CFA as adjuvant, could block the development of EAU. Additionally, in an attempt to examine whether the adjuvant has influence on EAU, control groups included rats injected with CFA and PBS four times according to the protocol used in the fourfold inoculated group. Our data exclude the possibility that CFA alone prevents the induction of EAU. The protocols of 2 and 4 inoculations were used to examine the effect of TCR V_β 8.3 vaccination on inhibiting the development of EAU. Both protocols were effective in prevention against EAU, as judged by clinical and histopathological criteria. However, a stronger efficacy was consistently found in the fourfold inoculated group than in the twofold inoculated group, suggesting that the dosage of this type of vaccine is important in achieving an ideal effect.

DTH and LPA were used to determine the correlation between the severity of EAU and antigen specific cellular immune responses. The results showed that TCR peptide vaccination suppressed the DTH response as well as the lymphocyte proliferation to the IRBP R16 peptide. In addition, the draining LNCs showed a significant proliferation of the V_β 8.3 peptide in the inoculated groups. This demonstrated that the peptide vaccine was highly immunogenic and could induce an intensive cellular immune response against the R16-specific T cells, thereby inhibiting the development of EAU.[26,29]

Cytokines are essential components in the pathogenesis of EAU. It has been well documented that Th_1 cytokines exacerbate whereas Th_2 cytokines ameliorate the inflammatory process of EAU.[30] Therefore, we examined the possibility of cytokine shifting induced by vaccination. IFN-γ and IL-2, predominantly Th_1 derived, were decreased in production in the vaccinated groups. Th_2 derived and antiinflammatory cytokines IL-10 and IL-4 were in general upregulated after vaccination except for IL-4 produced by LNCs from the twofold inoculated group. The change in the Th_1/Th_2 cytokine profile paralleled the reduction in clinical severity of EAU. In view of the fact that inflammation occurs in the intraocular tissues, we measured the local concentration of IL-2, a Th_1 cytokine that has been shown to make an important contribution to the development of EAU.[31] The result revealed a significantly decreased IL-2 concentration in aqueous humour after TCR V_β 8.3 peptide immunization. They all suggest that TCR V_β 8.3 peptide might induce a switch from Th_1 to Th_2, which results in the inhibition of disease progression.[32,33]

To investigate the changes generated by the peptide

vaccine at molecular level, we also detected the expression of TCR V_β 8.3 gene using real time PCR. A marked decrease in the expression of V_β 8.3 gene was noted in the rats receiving fourfold inoculation as compared with the noninoculated and CFA inoculated rats. It is at present unclear whether the peptide vaccine directly or indirectly suppresses the expression of TCR V_β 8.3 gene. Further studies are needed to address this question.

We found in this study that some of the rats in the fourfold inoculated group could not achieve a complete prevention against EAU. Possibly TCR V_β 8.3 utilization is not exclusive and that other TCR V_β gene segments are involved in the initiation of EAU.[9,34] Vaccination with TCR V_β 8.3 peptide alone may not be sufficient to control ocular inflammation during the course of EAU. Therefore, studies are needed to investigate the potential of vaccination with other TCR V_β peptides in the prevention of EAU.

In conclusion, the present study favours the idea that sufficient vaccination with TCR V_β 8.3 peptide decreases the expression of TCR V_β 8.3 gene and inhibits the proliferation responses as well as DTH reactions specific to IRBP R16 peptide. TCR V_β 8.3 peptide also induces a cytokine switch from Th_1 to Th_2 dominance. These mechanisms together lead to an effective prevention against EAU induced by IRBP R16. The future investigation of the predominant usage of TCR V_β genes in patients suffered from autoimmune uveitis is likely to aid in designing a new strategy for the prevention and treatment of this kind of autoimmune disease.

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