Altered influenza virus haemagglutinin peptides inhibit T cell responses to type II collagen in rheumatoid arthritis

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Rheumatoid arthritis (RA) is a T cell mediated autoimmune disease and associated with HLA-DR4 or HLA-DR1 subtypes.[1 2] Type II collagen (CII) has been implicated as an autoantigen of RA, and CD4+ T cell responses to CII or CII derived peptides are mainly presented by HLA-DR4/1 molecules.[3 4] Inhibition of antigen presentation by HLA-DR4/1 molecules can interfere with T cell mediated autoimmune responses in RA.

Our previous studies have suggested that altered CII263–272 peptides inhibited CII263–272-induced T cell activation by blocking antigen presentation.[5 6] In this study we examine the role of the altered influenza virus haemagglutinin (HA) 308–317 peptides (altered peptide ligands (APLs)) with single or multiple substitutions of T cell receptor (TCR) contact residues in T cell responses of peripheral blood mononuclear cells (PBMC) and inhibitory effects of APLs on CII263–272-induced T cell activation in RA.

Twenty seven HLA-DR4/1 positive patients with RA (21 female, 6 male; mean (SD) age 53.6 (13.3) years; mean (SD) disease duration 10.4 (8.4) years) were included in the study. All patients fulfilled the American College of Rheumatology revised criteria for the classification of RA. Of 27 patients with RA, 24 (89%) were positive for DR4 and 3 (11%) for DR1.

Sequences of three APLs and CII263–272 were YVAQNTLKLA (APL1), YAKQATLKLA (APL2), YAKQATLALA (APL3), and FKGEQGPKGE, respectively. T cell proliferation experiments were performed by [^{3}H]thymidine incorporation assay. PBMC (2.0×10^5/well) were incubated with CII263–272 or APLs at 10 μg/ml for 5 days. In competitive studies, PBMC were preincubated with various concentrations of APLs as indicated for 2 hours before addition of CII263–272. Cultures were pulsed with [^{3}H]thymidine (0.25 μCi/well) before the last 12 hours. The data are presented as the stimulation index (SI). Enzyme linked immunosorbent assay (ELISA) kits were used to detect the levels of interferon γ (IFNγ) or interleukin (IL) 4 in the supernatants.

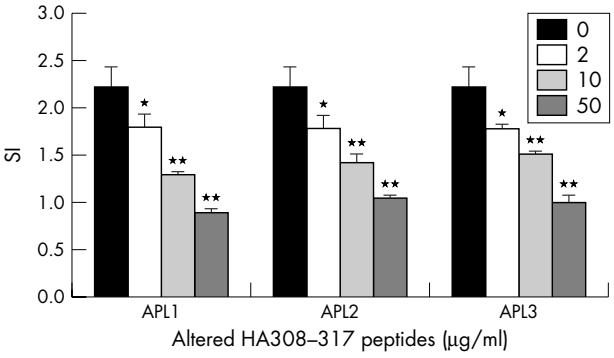

Figure 1 Inhibitory effects of altered HA308–317 peptides on T cell responses to CII263–272. PBMC from patients with RA were cultured for 5 days in the presence of 10 μg/ml CII263–272 and different concentrations of altered HA308–317 peptides (0, 2, 10, and 50 μg/ml, respectively). Results showed that altered HA308–317 peptides suppressed T cell responses to CII263–272 in a range from 2 μg/ml to 50 μg/ml (*$p<0.05$,**$p<0.01$).

Table 1 IFNγ and IL4 production by PBMC of RA stimulated by altered HA308–317 peptides

Stimulators (10 μg/ml)	IFNγ (pg/ml) (n = 10)	IL4 (pg/ml) (n = 10)
Medium	32.1 (15.8)*	25.9 (3.5)
CII263–272	77.3 (60.8)	28.1 (4.9)
APL1	37.8 (14.1)*	26.5 (5.1)
APL2	37.1 (23.5)*	29.6 (4.0)
APL3	34.8 (19.0)*	28.9 (6.9)

Results are shown as mean (SD).
To find whether the altered HA308–317 peptides affected cytokine profile, supernatants of PBMC were evaluated for IFNγ or IL4 production from 10 patients with RA with the altered HA308–317 peptides or CII263–272 stimulation. The levels of IFNγ in the supernatants from PBMC stimulated with CII263–272 were significantly higher than those incubated with medium alone(*$p<0.05$). In contrast, aPL down regulated IFNγ production in the supernatants from PBMC, compared with CII263–272 (*$p<0.05$). No differences of IL4 productions in the supernatants were found when T cells in PBMC from patients with RA were stimulated with CII263–272 or APLs.

T cell proliferative responses to APLs in PBMC from RA were 7.4% for APL1, 3.7% for APL2 or APL3, which were lower than for CII263–272 (62.2%). The mean (SD) SI values for T cell responses to APLs were 1.2 (0.4) for APL1, 1.3 (0.4) for APL2, and 1.1 (0.4) for APL3, which were significantly lower than for CII263–272 (2.0 (0.8)). In addition, it was shown that T cell proliferative responses to CII263–272 were suppressed by APLs in a dose dependent manner in a range from 2.0 μg/ml to 50 μg/ml (fig 1).

The levels of IFNγ were significantly increased when stimulated with CII263–272 (77.3 (60.8) pg/ml), compared with the levels of 37.8 (14.1) pg/ml for APL1, 37.1 (23.5) pg/ml for APL2, and 34.8 (19.0) pg/ml for APL3, which were similar to the level for unstimulated control (32.1 (15.8) pg/ml). No significant differences were found in IL4 production when PBMC were stimulated with APLs or CII263–272 (table 1).

In this study we assessed T cell responses to APLs in patients with RA and demonstrated that T cell responses to CII263–272 could be inhibited by the APLs with substitutions of the TCR contact residues. HA306–318 peptide can bind to HLA-DR4/1 molecules with a much higher affinity than CII263–272.[7] Altered HA306–318 peptides with the substitutions of TCR contact residues are not recognised by HA-specific T cell clones, although these peptide analogues still bind to DR4/1.[8] Therefore, altered HA peptides might be more efficient antagonist peptides in the inhibition of immune responses induced by HLA-DR4/1-specific peptides.

The mechanism by which APLs antagonise T cell responses cannot be based on HLA-DR blockade only. Alternatively, APLs may alter the cytokine production profile of T cells.[9 10] In this study we showed that APLs down regulated the production of IFNγ compared with CII263–272, which

promoted IFNγ secretion. These results suggest that APLs are not Th1 stimulators. It is not clear whether they regulate Th2 cells because no effects on IL4 production were found in the present study. Further studies are necessary to investigate whether APLs effectively inhibit T cell activation in vivo, such as in the HLA-DR4 transgenic animal model.

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Relationship between 5,10-methylenetetrahydrofolate reductase C677T gene polymorphism and methotrexate related toxicity in patients with autoimmune diseases receiving folic acid supplementation

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The common polymorphism C677T of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene reduces enzyme activity and it has recently been associated with increased incidence of methotrexate (MTX) related toxicity in patients with cancer and rheumatoid arthritis.[1–5] Considering that folate supplementation may reduce toxicity without affecting MTX efficiency, we conducted a retrospective study to analyse the effect of this polymorphism in patients with autoimmune diseases receiving folate supplementation.

Sixty three patients (F/M: 44/19, mean age 53.6 years, range 20–81) with autoimmune diseases who had been treated with MTX (7.5–15 mg/week, mean duration 35.8 months, range 2–121), were selected from the outpatient clinic between January and June 2004. Five of them had discontinued MTX treatment at the time of selection, because of adverse events or inefficiency. Thirty nine of the patients were receiving a combination of MTX with corticosteroids and/or other disease modifying antirheumatic drugs. All the patients were prescribed supplementary 2.5 mg folic acid the day before and the day after MTX treatment. All participants were informed and consented to take part in the study. Table 1 shows the characteristics of the patients.

Genomic DNA was extracted from peripheral blood, and analysis of the MTHFR C677T polymorphism was performed

Table 1 Patients' characteristics

Characteristics	Adverse effects	
	Present (n = 15)	Absent (n = 48)
Age (years)		
Mean (SD)	56.8 (10.8)	52.6 (16.6)
Range	33–72	20–81
Sex (F/M)	11/4 (2.75)	33/15 (2.2)
Disease		
Rheumatoid arthritis	13	33
Psoriatic arthritis	2	10
Ankylosing spondylitis	–	3
Polymyositis	–	2
Disease duration (months)		
Mean (SD)	36.6 (30.1)	35.6 (29.6)
Range	2–121	5–120
Additional drugs (No (%) of patients)		
Corticosteroids (only)	2 (13)	7 (15)
Other DMARDs (± corticosteroids)	8 (53)	22 (46)
MTHFR genotype, No (%)		
CC (wild type)	10 (67)	13 (27)
CT (heterozygous)	3 (20)	28 (58)
TT (homozygous)	2 (13)	7 (15)

DMARDs, disease modifying antirheumatic drugs (included ciclosporin: 9 patients, hydroxychloroquine: 7 patients, and infliximab: 14 patients).