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Absence of peripheral blood T cell responses to 'shared epitope' containing peptides in recent onset rheumatoid arthritis

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Abstract

***Objectives*—To determine if peptides containing the 'shared epitope' sequence, QKRAA, from either endogenous, HLA-DRβ1(0401), or exogenous, *Escherichia coli* dnaJ, sources activate T cells in recent onset rheumatoid arthritis (RA).**

***Methods*—Peripheral blood mononuclear cell (PBMC) proliferative and whole blood cytokine responses to shared epitope containing peptides from DRβ1(0401) and *E coli* dnaJ, to control peptides from DRβ1(0402) and hsp40 and to the recall antigen, tetanus toxoid, were tested in 20 untreated, recent onset RA subjects, 20 HLA, age, and sex matched healthy controls and 18 other subjects with inflammatory arthritis. PBMC proliferative responses to a second *E coli* dnaJ peptide (with the shared epitope at the N-terminus) and two peptides from type II collagen with high affinity for DR4(0401) were tested in a further 16 recent onset RA and 17 control subjects.**

***Results*—PBMC proliferation and whole blood interferon γ or interleukin 10 production in response to the shared epitope containing and control peptides were not different between the disease and control groups. On the other hand, compared with controls, RA subjects had significantly higher proliferation to a collagen II (aa 1307-1319) peptide, but significantly lower proliferation and interferon γ production to tetanus toxoid.**

***Conclusion*—Recent onset RA subjects had no demonstrable increase in peripheral blood T cell reactivity to shared epitope containing peptides. However, a proportion had increased T cell reactivity to a peptide of similar length from a candidate RA autoantigen, collagen type II. Their impaired responses to tetanus are in keeping with evidence for general T cell hyporesponsiveness in RA.**

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Rheumatoid arthritis (RA) is a chronic inflammatory joint disease with a prevalence of around one per cent.[1] The concordance in monozygotic twins of up to 30% indicates that genetic factors predispose to RA.[2] The major known genetic contribution is from the highly polymorphic HLA DRβ1 alleles in the major histocompatibility complex (MHC).[3] Approximately 90% of RA patients express HLA DRβ1 alleles (*0401, *0404 or *0101) characterised by the amino acid sequence QK/RRAA from position 70 to 74 in the third hypervariable region of the encoded β chain.[4] It is not known how this sequence, termed the 'shared epitope', contributes to the development of RA. One hypothesis is that the endogenous shared epitope is presented on self MHC and is itself a target of T cells in RA. This argument has some indirect support. Firstly, a large proportion of peptides eluted from class II MHC proteins are from self MHC protein[5] and therefore could, potentially, be recognised by T cells. If these self MHC peptides played a part in positively selecting T cells in the thymus,[6] then presumably autoreactive T cells would either have to be deleted by negative selection or regulated by peripheral mechanisms of tolerance. Secondly, a commensal gut bacterium, *Escherichia coli*, expresses a heat shock protein, dnaJ, which also contains the shared epitope sequence.[7] It has been reported recently that gut mucosal lymphocytes preferentially bind to synovial high endothelial venules, possibly using unique adhesion molecule.[8] Thus, if T cells were activated by the shared epitope region of *E coli* dnaJ in the gut they might then migrate to synovial tissue and cross react with endogenous shared epitope whose presentation might be increased within the inflammatory lesion.

Two groups have recently considered this hypothesis. Salvat *et al*[9] reported that peripheral blood mononuclear cells (PBMCs) from neither established RA nor control subjects who expressed DRβ1(0401) proliferated in response to a shared epitope containing peptide from DRβ1(0401). Albani *et al*[10] confirmed this negative result in recent onset RA subjects compared with control subjects partly matched for age, sex, and HLA status, but reported increased proliferation in RA subjects to an *E coli* dnaJ peptide with the shared epitope sequence at the N-terminus. At the same time, we have investigated the 'shared epitope' hypothesis by measuring PBMC proliferative and whole blood cytokine responses to shared epitope containing peptides from HLA DRβ1(0401) and *E coli* dnaJ, to control peptides from HLA DRβ1(0402) and hsp40 and to tetanus toxoid protein, in recent onset, untreated RA, and age, sex, and HLA matched control subjects. In addition, we sought to show that PBMC proliferation could detect peptide epitopes by

Table 1 Details of inflammatory arthritis subjects in studies 1 and 2

Subject	Age	Sex	ACR criteria	RhF	Symptoms duration (months)	HLA DRβ1	Diagnosis	Study number
2	63	F	4	N	1	1,2	RA	1
3	27	F	4	Y	3	0405,11	RA	1
4	57	F	5	Y	1	1,0401	RA	1
5	47	F	4	Y	10	0404,0407	RA	1
7	51	M	5	Y	5	7,12	RA	1
12	29	F	4	Y	2	2,3	RA	1
14	30	F	4	Y	1	1,0401	RA	1
17	25	F	4	N	1	0404,11	RA	1
18	51	F	4	Y	3	2,11	RA	1
21	75	M	4	N	3	0401	RA	1
24	44	F	4	Y	3	1,0401	RA	1
26	52	F	4	Y	4	2,9	RA	1
27	50	F	4	Y	2	1,0401	RA	1
28	31	F	4	Y	12	0404,6	RA	1
29	55	F	5	Y	9	0401,6	RA	1
31	43	M	4	N	6	1,3	RA	1
33	60	M	4	Y	3	3,0404	RA	1
36	74	M	4	N	2	1,0401	RA	1
37	44	F	4	N	4	2,10	RA	1
38	74	M	4	Y	6	7,0401	RA	1
1	42	F	3	Y	6	ND	Undifferentiated	1
9	33	F	3	Y	2	ND	Undifferentiated	1
10	62	M	3	Y	2	ND	Undifferentiated	1
15	54	F	3	N	6	1,7	Undifferentiated	1
19	24	F	3	Y	3	7,11	Undifferentiated	1
20	56	F	3	Y	4	1,7	Undifferentiated	1
22	48	M	3	N	4	0401	Undifferentiated	1
23	21	M	3	N	2	11	Undifferentiated	1
30	25	F	3	Y	4	ND	Undifferentiated	1
34	16	F	3	N	6	3,13	Undifferentiated	1
6	57	F	4	N	1	ND	Self limited	1
13	35	M	4	N	1	11,13	Self limited	1
25	87	F	4	N	2	2,3	Self limited	1
35	44	F	3	N	1	3,0401	Self limited	1
8	68	F	4	N	2	ND	PMR	1
11	34	F	4	Y	2	ND	Sjögrens	1
16	54	F	4	N	1	ND	Enteropathic	1
32	57	F	3	N	3	2,0401	Psoriatic	1
1a	26	F	4	Y	3	01,0401	RA	2
2a	79	F	4	Y	4	01,07	RA	2
3a	29	F	4	Y	9	01,02	RA	2
4a	46	F	4	Y	3	01,0401	RA	2
5a	73	F	4	Y	3	03,0401	RA	2
6a	63	F	4	Y	3	02,13	RA	2
7a	88	F	4	N	2	01,03	RA	2
9a	58	F	4	Y	9	03,13	RA	2
11a	72	F	4	Y	4	02,03	RA	2
12a	77	M	4	Y	3	02,03	RA	2
13a	61	F	4	Y	3	02,11	RA	2
14a	72	M	5	Y	3	0401	RA	2
16a	70	M	4	Y	4	0103,07	RA	2
17a	30	F	4	Y	2	02,03	RA	2
18a	72	M	4	N	3	0404,07	RA	2
21a	78	M	4	Y	3	02,0407	RA	2

RhF = rheumatoid factor, ND = not done, PMR = polymyalgia rheumatica.

including DR4(0401) binding peptides from collagen type II, an autoantigen in RA.

Methods

SUBJECTS

Subjects were recruited from the Rheumatology Outpatient Clinics at the Royal Melbourne Hospital and from local rheumatologists. Blood samples were obtained with the approval of local ethics committees and the informed consent of the subjects. Subjects with an inflammatory arthritis for less than 12 months considered by the referring rheumatologist to be RA, who were not taking slow acting anti-rheumatic agents or corticosteroids, were sequentially enrolled into two studies. Study 1 used PBMC proliferation and whole blood cytokine assays to test four peptides, HLA-DRβ1(0401 and 0402), *E coli* dnaJa and hsp40 and tetanus toxoid. Study 2 used the PBMC proliferation assay alone to test three peptides, *E coli* dnaJb, and two collagen II peptides. Subjects with inflammatory arthritis were divided into those who fulfilled the 1987 American College of Rheumatology criteria[11] for RA (RA subjects) and those who did not (non-RA subjects). All subjects were venepunctured between 9 and 11am and the blood processed immediately.

PEPTIDES

The following peptides were purchased from Macromolecular Resources (Colorado State University, Colorado) and shown to be greater than 90% pure by high performance liquid chromatography (HPLC) analysis: HLA-DRβ1(0401) aa 65-79 (KDLLEQKRAAVDTYC), HLA-DRβ1(0402) aa 65-79 (KDILEDERAAVDTYC), *E coli* dnaJa aa 56-70 (VLTDSQKRAAYDQYG), hsp40 aa 54-68 (VLSDPRKREIFDRYG). A second *E coli* peptide, dnaJb aa 61-75 (QKRAAYDQYGHAAFE) and two peptides from collagen type II aa 1307-1319 (TFLRLLSTEGSQN) and aa

1350-1362 (VEIRAEGNSRFTY), which bind HLA-DR4(0401) with high affinity[12] were synthesised in an Advanced ChemTech 396 multiple peptide synthesiser using Fmoc chemistry and solid phase synthesis, and purified to >90% homogeneity by HPLC. Peptides were dissolved at 1 mg/ml in human tonicity phosphate buffered saline (HT-PBS) and stored at −70°C before use. Aliquots of reconstituted peptide were shown to be endotoxin free by the quantitative Limulus lysate assay.[13]

PBMC PROLIFERATION ASSAY

In study 1, PBMCs were isolated on a Ficoll-Hypaque density gradient, washed, and resuspended at 10^6 cells/ml in HT-RPMI 1640 and 10% autologous serum. Aliquots of 200 µl (2×10^5 cells) were transferred into 96 well round bottomed plates with and without preloaded peptides to achieve final concentrations of 0.5, 5, and 50 µg/ml and tetanus toxoid (CSL) at 2 Lfu/ml, in sextuplicate. Plates were incubated at 37°C in 5% CO_2 for six days and 3H-thymidine (0.25 µCi) added for the last six hours. Cells were then harvested on glass fibre filters and incorporated radioactivity measured by liquid scintillation spectrometry. In study 2, PBMC proliferation assays were performed as above except peptides were preloaded at final concentrations of 10 µg/ml.

To test the effect of serum on PBMC proliferation, aliquots of 2×10^5 PBMCs from a normal subject in 180 µl of medium alone were transferred into 96 well plates made up with and without tetanus toxoid (2 Lfu/ml) in sextuplicate. Serum (20 µl, final concentration 10%) from the PBMC donor, or from nine RA, or nine normal subjects, was added to the replicates and the plates incubated, pulsed, and harvested as above.

Table 2 Details of control subjects in studies 1 and 2

Subject	*Age*	*Sex*	*HLA DRβ1*	*Study number*
1c	34	M	3,12	1
2c	65	M	2,0405	1
3c	28	F	3,10	1
4c	72	F	7,13	1
5c	34	F	6,15	1
6c	36	M	1,15	1
7c	68	F	1,3	1
8c	30	F	3,4	1
9c	38	M	4,15	1
10c	44	M	4	1
11c	35	M	2,4	1
12c	34	M	4,13	1
13c	27	F	4,15	1
14c	25	F	4	1
15c	38	M	1,4	1
16c	50	M	4,11	1
17c	40	M	4,11	1
18c	32	F	4,11	1
19c	36	F	3,4	1
20c	52	F	6,11	1
21c	26	F	3,4	2
22c	45	F	3,4	2
23c	40	M	3,4	2
24c	33	M	3,4	2
25c	47	F	3,4	2
26c	35	F	3,4	2
27c	34	M	3,4	2
28c	20	F	4	2
29c	38	F	1	2
30c	45	F	4	2
31c	29	M	4	2
32c	44	F	3,4	2
33c	42	F	3,4	2
34c	25	M	3	2
36c	41	F	3,4	2
37c	47	M	3,4	2
38c	46	F	3,4	2

WHOLE BLOOD ASSAY

Fresh heparinised blood (280 µl) was aliquoted into 96 well flat bottomed plates preloaded with peptides to achieve final concentrations of 0.33, 3.3 or 33 mg/ml, tetanus toxoid at 1.3 Lfu/ml, or PBS alone, in quadruplicate.[14] The plates were incubated at 37°C in 5% CO_2 for 48 hours and plasma supernatants carefully harvested and frozen at −70°C before cytokine assays.

CYTOKINE ELISAS

Interferon γ in harvested plasma supernatants was measured with an ELISA kit (Commonwealth Serum Laboratories, Melbourne). Interleukin 10 (IL 10) was measured by ELISA using monoclonal antibodies JES3-9D7 and biotinylated JES3-12G8 (PharMingen).

HLA TYPING

HLA alleles were typed by the standard microlymphocytotoxic method for all recognised class I and class II alleles. Class II results were confirmed using sequence specific oligonucleotide typing, according to the XI International Histocompatibility Workshop protocol, using oligoprobes for each DR allele and subtypes of DR4.

STATISTICS

Proliferation assay results were expressed as stimulation indices (that is, median cpm with antigen/cpm without antigen). Intergroup analyses were performed by the Mann-Whitney U test.

Results

SUBJECTS

Fifty four subjects with inflammatory arthritis were recruited over 12 months (table 1). Thirty six subjects fulfilled criteria for RA, 10 had undifferentiated arthritis, four self limited disease, and four other diagnoses (table 1). Thirty seven control subjects were studied during the same period (table 2). In study 1, 20 RA, 18 non-RA, and 20 age, sex, and HLA matched control subjects were tested with four peptides, HLA-DRβ1(0401 and 0402), *E coli* dnaJa, and hsp40, and tetanus toxoid, in PBMC proliferation and whole blood cytokineassays. In study 2, 16 RA and 17 control subjects were tested with three peptides, *E coli* dnaJb, and two collagen II peptides, in the PBMC proliferation assay alone.

PROLIFERATIVE AND CYTOKINE RESPONSES TO SHARED EPITOPE AND CONTROL PEPTIDES

In study 1, PBMC proliferation and whole blood cytokine responses to shared epitope containing peptides from DRβ1(0401) and *E coli* dnaJ were compared with responses to control peptides from the third hypervariable region of DRβ1(0402) and from the equivalent region in the human homologue of dnaJ, hsp40, which do not contain the shared

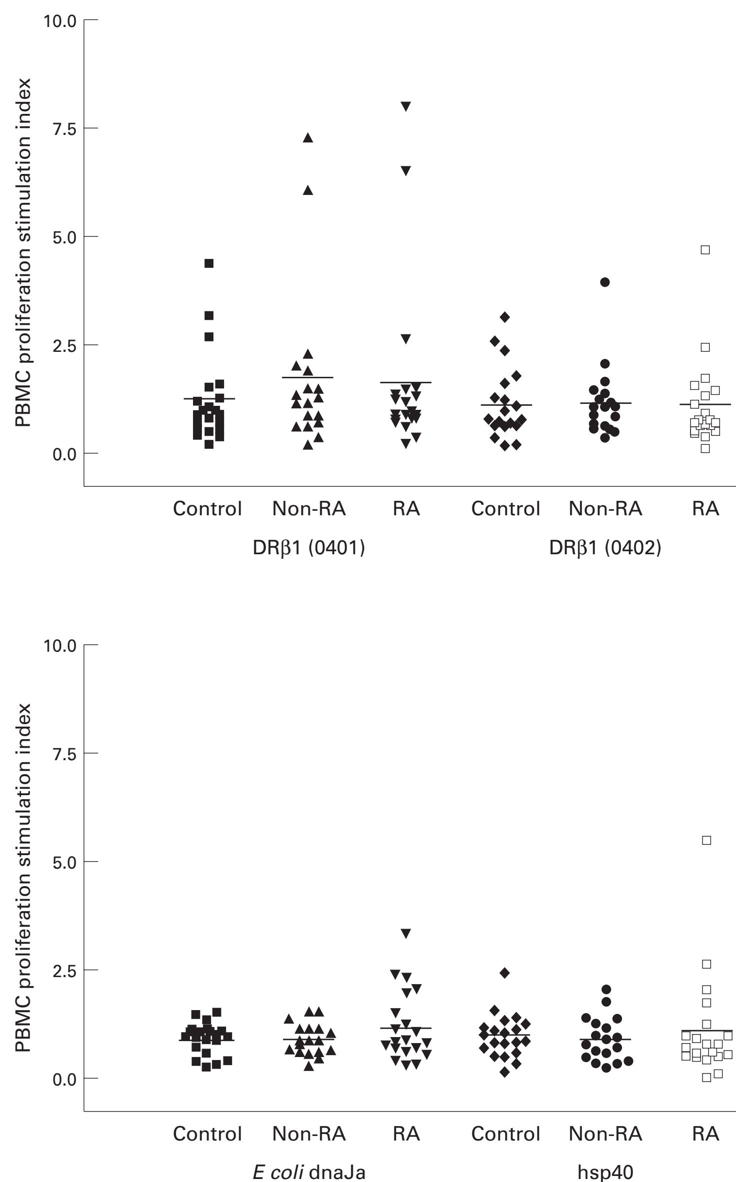

Figure 1 Stimulation indices for proliferation of PBMCs from RA, non-RA disease control, and healthy control (study 1) subjects after incubation with 50 μg/ml of DRβ1(0401), DRβ1(0402), E coli *dnaJa (aa 56-70) or hsp40 peptides.*

epitope. The stimulation indices of PBMCs from RA, non-RA, and normal control subjects in response to DRβ1(0401), *E coli* dnaJa, DRβ1(0402) or hsp40 peptides were not significantly different (fig 1). In addition, there was no difference between the three groups in the whole blood production of interferon γ or IL 10 (table 3) in response to the same peptides. These results were unchanged when RA and control subjects who expressed shared epitope containing DRB1 alleles were analysed separately (data not shown). To correct for any general variation in immune responsiveness, stimulation indices to peptides were expressed as ratios of the tetanus toxoid induced stimulation index for each subject. The corrected indices of the RA subjects were greater than the controls but there were no significant differences between the responses to shared epitope containing and control peptides (data not shown).

During study 1, it was reported that PBMCs from RA subjects proliferated significantly to *E coli* dnaJb.[10] In study 2, therefore, we measured the response of PBMCs from a further 16 RA and 17 control subjects to the dnaJb peptide. In addition, two collagen type II peptides, aa1307-1319, which bind both the RA associated HLA-DR4(0401) and the RA unassociated HLA-DR4(0402) molecule with high affinity, and aa1350-1362, which binds HLA-DR4(0401) alone, were tested as potential positive controls. The dnaJb and collagen (aa1350-1362) peptides failed to elicit significantly higher responses in RA subjects (fig 2A and B). The collagen (aa1307-1319) peptide, however, elicited signficantly higher responses in RA subjects compared with controls (fig 2C). PBMCs from six of 16 (38%) RA subjects in study 2 had responses to the collagen (aa1307-1319) peptide that exceeded the mean plus two standard deviations of the 17 control subjects.

PROLIFERATIVE AND CYTOKINE RESPONSES TO TETANUS TOXOID

PBMC proliferation in response to tetanus toxoid was significantly lower in RA compared with control subjects (19.4 *v* 50.3; p=0.0012) (fig 3A). Autologous (basal) proliferation was identical between the three groups (data not shown). The difference was still present between RA and control subjects who were HLA-DR4 (17.7 *v* 61.0; p=0.002) (fig 3B). Interferon γ production in whole blood in response to tetanus toxoid (1.3 Lfu/ml) was also significantly lower in RA subjects (7.5 *v* 290 U/ml; p=0.01) (fig 3C). IL 10 production in response to tetanus toxoid was also lower in RA subjects, but did not reach statistical significance (data not shown). In the absence of peptides or tetanus toxoid, interferon γ and IL 10 concentrations in the whole blood plasma supernatants were almost always undetectable and not different between the groups.

EFFECT OF RA SERUM ON THE PROLIFERATIVE RESPONSE OF NORMAL PBMCS

To discover if a serum factor might be responsible for the impaired responses to tetanus in RA subjects, we examined the effect of different RA or normal control serum samples on PBMCs from a normal subject undergoing tetanus toxoid induced proliferation. Nine serum samples from RA subjects with the lowest responses to tetanus toxoid were compared with nine normal control samples. There was no reduction in the responses of PBMCs to tetanus toxoid in the presence of RA compared with control samples (data not shown).

Discussion

Recent onset, untreated RA subjects did not exhibit increased peripheral blood T cell responses to shared epitope containing peptides from endogenous, DRβ1(0401), or exogenous, *E coli* dnaJ (aa 56-70 or 61-75) sources. However, proliferative responses to a collagen II peptide (aa 1307-19) were increased and proliferative and interferon γ responses to whole tetanus toxoid decreased

Table 3 Interferon γ and IL 10 production (mean(SD)) in whole blood from study 1 subjects after incubation with 33 μg/ml of DRβ1(0401), DRβ1(0402), E coli *dnaJa (aa 56-70) or hsp40 peptides*

Cytokine	Peptide	Cytokine production in response to peptide			p Value
		Control	Non-RA	RA	
Interferon γ (U/ml)	*0401	18.5 (16.2)	8.7 (25.7)	4 (16.9)	NS
	*0402	38.8 (152.4)	6.0 (23.2)	1.8 (7.2)	NS
	dnaJa	10.0 (37.8)	4.0 (14.9)	3.5 (14.9)	NS
	hsp40	19.6 (77.2)	3.7 (14.7)	1.8 (5.9)	NS
IL 10 (ng/ml)	*0401	0.05 (0.13)	0.04 (0.09)	0.04 (0.10)	NS
	*0402	0.05 (0.18)	0.03 (0.06)	0.02 (0.04)	NS
	dnaJa	0.02 (0.06)	0.03 (0.06)	0.01 (0.04)	NS
	hsp40	0.02 (0.08)	0.03 (0.07)	0.02 (0.04)	NS

compared with HLA matched controls. After correction for the impaired responses to tetanus toxoid, responses were higher in the RA subjects in response to both the shared epitope containing and control peptides, indicating that a peptide specific difference was not masked by the apparent hyporesponsiveness in RA.

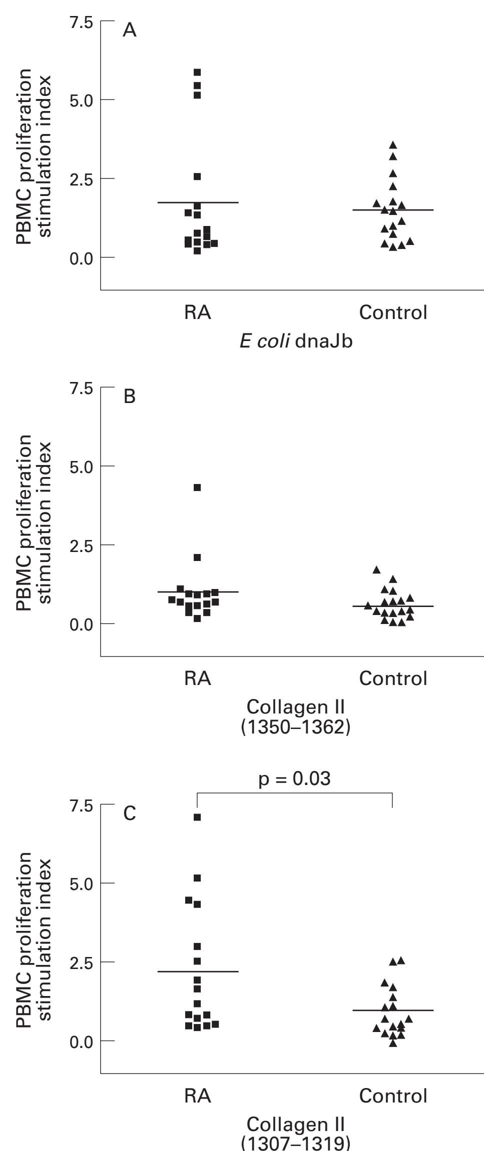

Figure 2 Stimulation indices for proliferation of PBMCs from a second group of RA and control (study 2) subjects after incubation with (A) E coli *dnaJb (aa 61-75) peptide;(B) collagen type II (aa 1350-1362) peptide; (C) collagen type II (aa 1307-1319) peptide.*

A lack of increased T cell reactivity to endogenous shared epitope containing peptides from DRβ1(0401) in RA and normal subjects who express HLA DR4 has recently been reported by two groups. Salvat *et al*[9] measured PBMC proliferation to the DRβ1(0401) aa 65-79 peptide in seven established RA and 14 control subjects who expressed DRβ1(0401) and found responses in neither group. In contrast, seven subjects who were DRβ1(1501), which does not contain the shared epitope, had increased proliferative responses to the DRβ1(0401) peptide irrespective of disease status. Albani *et al*[10] measured PBMC proliferation to peptides from DRβ1(0401) (aa 65-79 and aa 70-84) and *E coli* dnaJ (aa 56-70 and aa 61-75) in 21 subjects with recent onset, untreated RA and in 26 controls partially matched for age, sex, and HLA. Neither of the DRβ1(0401) peptides, which have a low affinity for native DR4(0401), stimulated proliferation in RA over control subjects. However, the dnaJ peptide with the shared epitope at the amino terminus (aa 61-75) (designated dnaJb in this study), which has a high affinity for native DR4(0401), significantly stimulated proliferation in RA compared with control and 18 juvenile RA subjects. The other dnaJ peptide, designated here as dnaJa, was reported to be 'less effective' in stimulating proliferation, which they attributed to its lower affinity for purified native DR4(0401). We confirmed the lack of an increased proliferative response to the DRβ1(0401) peptide and, in addition, showed that whole blood interferon γ and IL 10 production are not increased in response to this peptide. Similarly, responses to dnaJa were not increased. However, in contrast with Albani *et al*,[10] we were unable to demonstrate increased PBMC proliferative responses to the dnaJβ peptide in recent onset RA subjects. Nevertheless, 38% of the same subjects exhibited increased responses to a collagen type II peptide (aa1307-1319), which binds with high affinity to HLA DR4(0401).

In an autoimmune disease, binding of putative T cell epitopes to disease associated HLA molecules would be one criterion for establishing pathogenicity. Whether this criterion is weakened if such epitopes also bind disease unassociated or protective HLA molecules is unknown. In this study, the collagen type II peptide (aa1307-1319), which stimulated PBMCs from RA subjects, is known to bind both the RA associated HLA-DR4(0401) and

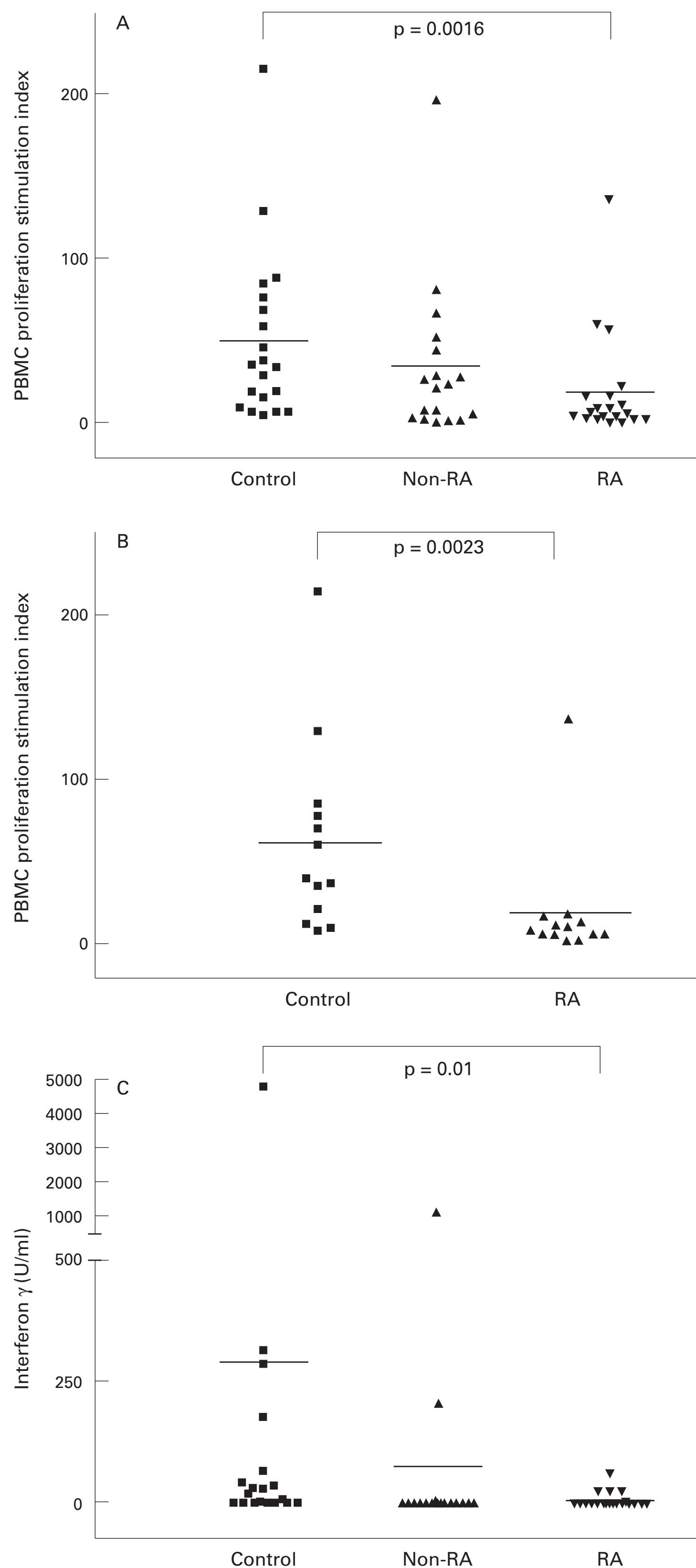

Figure 3 (A) Stimulation indices for proliferation of PBMCs from RA, non-RA disease control, and healthy control (study 1) subjects after incubation with 2 Lfu/ml of tetanus toxoid; (B) stimulation indices for proliferation of PBMCs from DR4 expressing study 1 subjects after incubation with 2 Lfu/ml of tetanus toxoid; (C) interferon γ production in whole blood from study 1 subjects after incubation with 1.3 Lfu/ml of tetanus toxoid.

the RA unassociated HLA-DR4(0402) molecule.[12] All we can conclude is that binding to HLA-DR4(0402) in addition to HLA-DR4(0401) does not militate T cell recognition in this context. That the collagen type II peptide (aa1350-1362), which failed to stimulate PBMCs from the same subjects, binds only HLA-DR4(0401) and not HLA-DR4(0402), simply means that while binding in vitro may be a prerequisite for T cell activation, it does not necessarily imply that peptide specific T cells are present or, if they are, can be detected by a proliferation assay.

To our knowledge, reduced proliferative responses and interferon γ production to a recall antigen like tetanus toxoid have not previously been reported in a cohort of untreated, recent onset RA subjects. Reduction of delayed type hypersensitivity and of proliferative responses in vitro to recall antigens and mitogens has been well described in RA subjects with established disease.[15–22] The frequency of hyporesponsiveness, defined as significantly reduced delayed type hypersensitivity or proliferative responses to soluble recall antigens (for example, PPD, streptodornase/streptokinase, tetanus toxoid or *Candida albicans*),[22] in RA cohorts varies from 22%[21] to 40%.[16] This phenomenon has been associated with reduced CD4 and increased CD8 T cells in peripheral blood,[21 23] with more lymphocytic infiltration and synovial lining layer hypertrophy in synovial biopsy specimens,[18] and with a higher erythrocyte sedimentation rate.[20] In addition, hyporesponsive RA subjects have exhibited significant increases in proliferative responses to recall antigens after leucapheresis and anti-tumour necrosis factor α (TNFα) monoclonal antibody treatment.[15 22] Tetanus toxoid induced interferon γ production in whole blood was also reduced in our RA subjects, consistent with their lower proliferative responses and previous reports of lower mitogen induced interferon γ production in whole blood and PBMCs from RA subjects.[24–27] Some of the undifferentiated non-RA subjects, who are clinically heterogeneous, also had low responses to tetanus and it will be of interest to determine if they develop classic RA.

The hyporesponsive phenotype of circulating cells from RA subjects could be explained if lymphocytes that homed to synovial tissue via addressin molecules expressed on high endothelial venules in response to the chronic inflammatory response,[28 29] were rendered hyporesponsive by local anti-inflammatory (IL 10 and transforming growth factor β (TGFβ)) and possibly pro-inflammatory (TNFα) cytokines in this tissue, before re-entering the circulation. Several lines of evidence support this notion. Synovial lymphocytic infiltration is greater in hyporesponsive subjects.[18] Synovial tissue and fluid contains high concentrations of IL 10, TGFβ, and TNFα, all of which may anergise T cells [15 30 31] and hyporesponsiveness in RA PBMCs can be reversed by the addition of IL 2 or interferon γ.[16 17 32] We found no evidence for a factor in RA serum that suppressed tetanus toxoid induced proliferation of normal PBMCs and no increase in

unstimulated IL 10 production in RA whole blood. Hyporesponsiveness is probably a non-specific response because it has also been reported in other chronic inflammatory diseases including systemic lupus erythematosis, scleroderma, and Crohn's disease.[26 33 34]

In conclusion, we find no evidence in recent onset RA for peripheral blood T cell recognition of either endogenous or exogenous shared epitope containing peptides that might support their role as antigens in the pathogenesis of RA.

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1 Harris EJ. Clinical features of rheumatoid arthritis. In: Kelley W, Harris E, Ruddy S, Sledge C, ed. *Textbook of rheumatology*. Philadelphia: WB Saunders, 1993:874-901.
2 Silman AJ, MacGregor AJ, Thomson W, *et al*. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. Br J Rheumatol 1993;32:903-7.
3 Nepom GT, Byers P, Seyfried C, *et al*. HLA genes associated with rheumatoid arthritis. Identification of susceptibility alleles using specific oligonucleotide probes. Arthritis Rheum 1989;32:15-21.
4 Gregersen P, Silver J, Winchester R. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum 1987;30:1205-13.
5 Harris PE. Self-peptides bound to HLA molecules. (Review). Crit Rev Immunol 1994; 14:61-87.
6 Rudensky A. Endogenous peptides associated with MHC class I and selection of CD4 T cells. Semin Immunol 1995;7:399-409.
7 Albani S, Roudier J. Molecular basis for the association between HLA DR4 and rheumatoid arthritis. From the shared epitope hypothesis to a peptidic model of rheumatoid arthritis. (Review). Clin Biochem 1992;25:209-12.
8 Salmi M, Andrew DP, Butcher EC, Jalkanen S. Dual binding capacity of mucosal immunoblasts to mucosal and synovial endothelium in humans: dissection of the molecular mechanisms. J Exp Med 1995;181:137-49.
9 Salvat S, Auger I, Rochelle L, *et al*. Tolerance to a self-peptide from the third hypervariable region of HLA DRB1*0401 in rheumatoid arthritis patients and normal subjects. J Immunol 1994;153:5321-29.
10 Albani S, Keystone E, Nelson J, *et al*. Positive selection of autoimmunity: Abnormal immune responses to a bacterial dnaJ antigenic determinant in patients with early rheumatoid arthritis. Nature Med 1995;1:448-53.
11 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.
12 Hammer J, Gallazi F, Bono E, *et al*. Peptide binding specificity of HLA-DR4 molecules: Correlation with rheumatoid arthritis association. J Exp Med 1995;181:1847-55.
13 Sharma S. Endotoxin detection and elimination biotechnology. Biotechnol Appl Biochem 1986;8:5-22.
14 Petrovsky N, Harrison L. Cytokine-based human whole blood assay for the detection of antigen-reactive T-cells. J Immunol Methods 1995;186:37-46.
15 Cope AP, Londei M, Chu NR, *et al*. Chronic exposure to tumor necrosis factor (TNF) in vitro impairs the activation of T cells through the T cell receptor/CD3 complex; reversal in vivo by anti-TNF antibodies in patients with rheumatoid arthritis. J Clin Invest 1994;94:749-60.
16 Emery P, Panayi G, Nouri A. Interleukin-2 reverses deficient cell mediated immune responses in rheumatoid arthritis. Clin Exp Immunol 1984;57:123-9.
17 Keystone E, Poplonski L, Snow KM, *et al*. Reconstitution of impaired autologous mixed lymphocyte reactivity in rheumatoid arthritis. Autoimmunity 1991;8:199-207.
18 Malone D, Wahl S, Tsokos M, Cattell H, Decker J, Wilder R. Immune function in severe, active rheumatoid arthritis. The relationship between peripheral blood mononuclear proliferation to soluble antigens and synovial tissue immunohistologic characteristics. J Clin Invest 1984;74:1173-85.
19 Paimela L, Johansson-Stephansson EA, Koskimies S, Leirisalo-Repo M. Depressed cutaneous cell-mediated immunity in early rheumatoid arthritis. Clin Exp Rheumatol 1990;8:433-7.
20 Pope R, Kniker W, Talal N, Dauphinee M. Delayed type hypersensitivity in patients with rheumatoid arthritis. J Rheumatol 1993;21:17-20.
21 Verwilghen J, Vertessen S, Stevens EA, Dequeker J, Ceuppens JL. Depressed T-cell reactivity to recall antigens in rheumatoid arthritis. J Clin Immunol 1990;10:90-8.
22 Wahl S, Wilder R, Katona I, *et al*. Leukapheresis in rheumatoid arthritis. Association of clinical improvement with reversal of anergy. Arthritis Rheum 1983;26:1076-84.
23 Haraoui B, Wilder R, Malone D, Allen J, Katona I, Wahl S. Immune function in severe, active rheumatoid arthritis: a relationship between peripheral blood monnuclear cell proliferation to soluble antigens and mononuclear cell subset profiles. J Immunol 1984;133:697-701.
24 Malaise MG, Franchimont P. Defective in vitro gamma-interferon production in rheumatoid arthritis. Arthritis Rheum 1987;30:230-1.
25 Reuter A, Bernier J, Vrindts GY, *et al*. Production of interferon gamma by peripheral blood mononuclear cells from normal subjects and from patients with rheumatoid arthritis. Clin Exp Rheumatol 1988;6:347-54.
26 Stolzenburg T, Binz H, Fontana A, Felder M, Wagenhaeuser FJ. Impaired mitogen-induced interferon-gamma production in rheumatoid arthritis and related diseases. Scand J Immunol 1988;27:73-81.
27 Zangerle PF, De Groote D, Lopez M, *et al*. Direct stimulation of cytokines (IL-1 beta, TNF-alpha, IL-6, IL-2, IFN-gamma and GM-CSF) in whole blood: II. Application to rheumatoid arthritis and osteoarthritis. Cytokine 1992; 4:568-75.
28 Freemont AJ. Molecules controlling lymphocyte-endothelial interactions in lymph nodes are produced in vessels of inflamed synovium. Ann Rheum Dis 1987; 46:924-8.
29 Freemont AJ. Functional and biosynthetic changes in endothelial cells of vessels in chronically inflamed tissues: evidence for endothelial control of lymphocyte entry into diseased tissues. J Pathol 1988;155:225-30.
30 Lipsky PE, Davis LS, Cush JJ, Oppenheimer-Marks N. The role of cytokines in the pathogenesis of rheumatoid arthritis. (Review). Springer Semin Immunopathol 1989; 11:123-62.
31 Cohen SB, Katsikis PD, Chu CQ, *et al*. High level of interleukin-10 production by the activated T cell population within the rheumatoid synovial membrane. Arthritis Rheum 1995;38: 946-52.
32 Seitz M, Napierski I, Kirchner H. Depressed PPD and tetanus toxoid presentation by monocytes to T lymphocytes in patients with rheumatoid arthritis: restoration by interferon gamma. Rheumatol Int 1988;8:189-96.
33 Tsokos GC, Boumpas DT, Smith PL, Djeu JY, Balow JE, Rook AH. Deficient gamma-interferon production in patients with systemic lupus erythematosus. Arthritis Rheum 1986;29: 1210-5.
34 Crow MK, DelGiudice-Asch G, Zehetbauer JB, *et al*. Autoantigen-specific T cell proliferation induced by the ribosomal P2 protein in patients with systemic lupus erythematosus. J Clin Invest 1994;94:345-52.