

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

Geoffrey J McColl, Juergen Hammer, Leonard C Harrison

## 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  $\gamma$  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  $\gamma$  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.<sup>1</sup> The concordance in monozygotic twins of up to 30% indicates that genetic factors predispose to RA.<sup>2</sup> The major known genetic contribution is from the highly polymorphic HLA DRβ1 alleles in the major histocompatibility complex (MHC).<sup>3</sup> 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  $\beta$  chain.<sup>4</sup> 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<sup>5</sup> 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,<sup>6</sup> 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.<sup>7</sup> It has been reported recently that gut mucosal lymphocytes preferentially bind to synovial high endothelial venules, possibly using unique adhesion molecule.<sup>8</sup> 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*<sup>9</sup> 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*<sup>10</sup> 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

The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia  
G J McColl  
L C Harrison

Roche Milano Ricerche, Milan, Italy  
J Hammer

Correspondence to:  
Dr G J McColl, Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital PO, Parkville, Victoria 3050, Australia.

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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 pep-

ptides. Subjects with inflammatory arthritis were divided into those who fulfilled the 1987 American College of Rheumatology criteria<sup>11</sup> 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 (KDILEDRAAVDTYC), *E coli* dnaJa aa 56-70 (VLTDSQKRAAYDQYG), hsp40 aa 54-68 (VLSDPKREIFDRYG). A second *E coli* peptide, dnaJb aa 61-75 (QKRAAYDQY-GHAAFE) and two peptides from collagen type II aa 1307-1319 (TFLRLLSTEGSQN) and aa

1350-1362 (VEIRAEGNSRFTY), which bind HLA-DR4(0401) with high affinity<sup>12</sup> 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.<sup>13</sup>

#### PBMC PROLIFERATION ASSAY

In study 1, PBMCs were isolated on a Ficoll-Hypaque density gradient, washed, and resuspended at 10<sup>6</sup> cells/ml in HT-RPMI 1640 and 10% autologous serum. Aliquots of 200 µl (2 × 10<sup>5</sup> 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<sub>2</sub> 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<sup>5</sup> 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.

#### 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.<sup>14</sup> The plates were incubated at 37°C in 5% CO<sub>2</sub> 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 micro-lymphocytotoxic 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.

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

## 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 cytokine-assays. 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

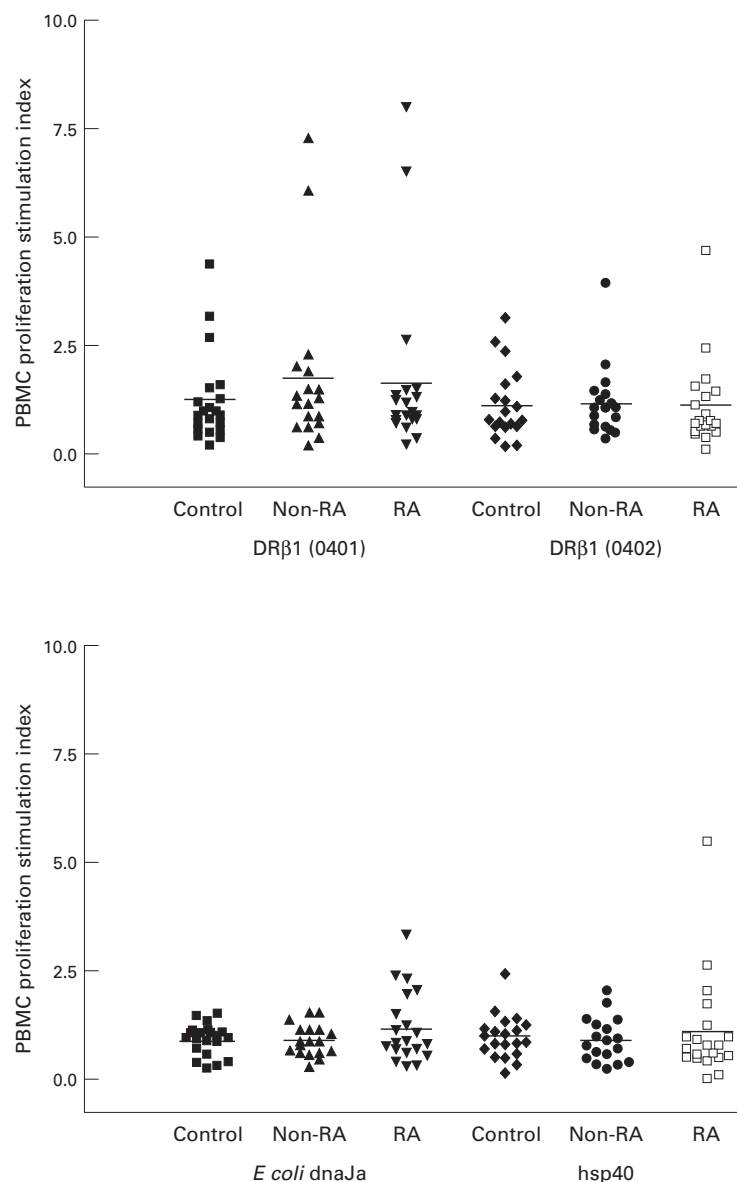


Figure 1 Stimulation indices for proliferation of PBMCs from RA, non-RA disease control, and healthy control (study 1) subjects after incubation with 50 µg/ml of DRβ1(0401), DRβ1(0402), *E coli dnaJa* (aa 56-70) or hsp40 peptides.

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  $\gamma$  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*.<sup>10</sup> 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 significantly 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  $\gamma$  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  $\gamma$  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  $\gamma$  responses to whole tetanus toxoid decreased

Table 3 Interferon  $\gamma$  and IL 10 production (mean(SD)) in whole blood from study 1 subjects after incubation with 33  $\mu\text{g/ml}$  of DR $\beta$ 1(0401), DR $\beta$ 1(0402), *E coli dnaJ* (aa 56-70) or *hsp40* peptides

Cytokine	Peptide	Cytokine production in response to peptide			p Value
		Control	Non-RA	RA	
Interferon $\gamma$ (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
	<i>dnaJ</i>	10.0 (37.8)	4.0 (14.9)	3.5 (14.9)	NS
	<i>hsp40</i>	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
	<i>dnaJ</i>	0.02 (0.06)	0.03 (0.06)	0.01 (0.04)	NS
	<i>hsp40</i>	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.

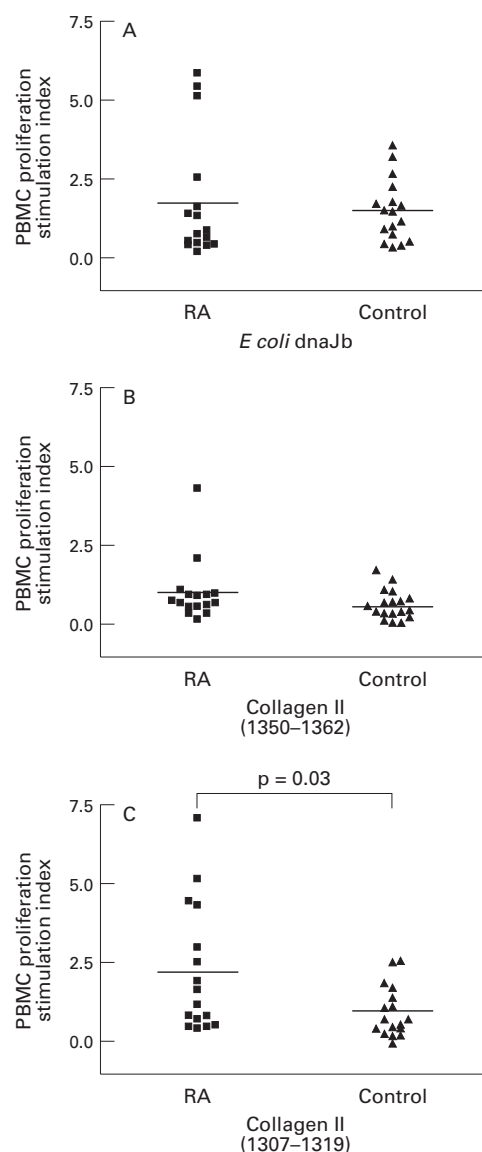
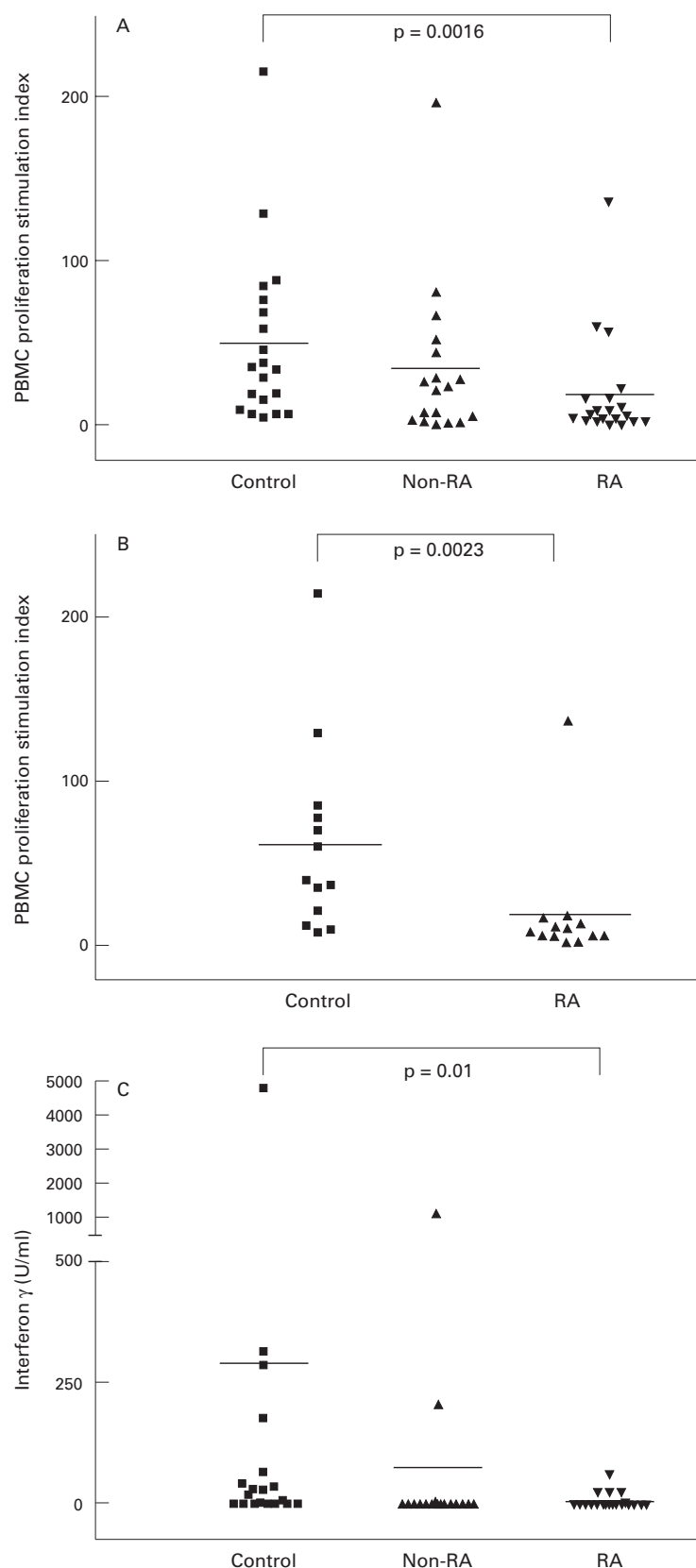


Figure 2 Stimulation indices for proliferation of PBMCs from a second group of RA and control (study 2) subjects after incubation with (A) *E coli dnaJb* (aa 61-75) peptide; (B) collagen type II (aa 1350-1362) peptide; (C) collagen type II (aa 1307-1319) peptide.

A lack of increased T cell reactivity to endogenous shared epitope containing peptides from DR $\beta$ 1(0401) in RA and normal subjects who express HLA DR4 has recently been reported by two groups. Salvat *et al*<sup>9</sup> measured PBMC proliferation to the DR $\beta$ 1(0401) aa 65-79 peptide in seven established RA and 14 control subjects who expressed DR $\beta$ 1(0401) and found responses in neither group. In contrast, seven subjects who were DR $\beta$ 1(1501), which does not contain the shared epitope, had increased proliferative responses to the DR $\beta$ 1(0401) peptide irrespective of disease status. Albani *et al*<sup>10</sup> measured PBMC proliferation to peptides from DR $\beta$ 1(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 DR $\beta$ 1(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 DR $\beta$ 1(0401) peptide and, in addition, showed that whole blood interferon  $\gamma$  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*,<sup>10</sup> we were unable to demonstrate increased PBMC proliferative responses to the *dnaJb* 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  $\gamma$  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.<sup>12</sup> 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  $\gamma$  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.<sup>15-22</sup> 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*),<sup>22</sup> in RA cohorts varies from 22%<sup>21</sup> to 40%.<sup>16</sup> This phenomenon has been associated with reduced CD4 and increased CD8 T cells in peripheral blood,<sup>21,23</sup> with more lymphocytic infiltration and synovial lining layer hypertrophy in synovial biopsy specimens,<sup>18</sup> and with a higher erythrocyte sedimentation rate.<sup>20</sup> In addition, hyporesponsive RA subjects have exhibited significant increases in proliferative responses to recall antigens after leucapheresis and anti-tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) monoclonal antibody treatment.<sup>15,22</sup> Tetanus toxoid induced interferon  $\gamma$  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  $\gamma$  production in whole blood and PBMCs from RA subjects.<sup>24-27</sup> 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,<sup>28,29</sup> were rendered hyporesponsive by local anti-inflammatory (IL 10 and transforming growth factor  $\beta$  (TGF $\beta$ )) and possibly pro-inflammatory (TNF $\alpha$ ) cytokines in this tissue, before re-entering the circulation. Several lines of evidence support this notion. Synovial lymphocytic infiltration is greater in hyporesponsive subjects.<sup>18</sup> Synovial tissue and fluid contains high concentrations of IL 10, TGF $\beta$ , and TNF $\alpha$ , all of which may anergise T cells<sup>15,30,31</sup> and hyporesponsiveness in RA PBMCs can be reversed by the addition of IL 2 or interferon  $\gamma$ .<sup>16,17,32</sup> 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 erythematosus, scleroderma, and Crohn's disease.<sup>26 33 34</sup>

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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