Lymphocyte responses to DR1/4 restricted peptides in rheumatoid arthritis

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Abstract

Objective—To determine whether analog and unrelated DR1/4 binding peptides alter DR1/4 restricted responses of peripheral blood lymphocytes (PBL) from patients with rheumatoid arthritis (RA).

Methods—PBL from 25 patients with RA and 12 healthy controls were cultured with DR1/4 restricted peptides of the influenza haemagglutinin, amino acids 307-319 (HA) and matrix proteins, amino acids 17-29 (IM). Responses were determined by ³H-thymidine uptake proliferation assays and limiting dilution analysis. Competitor peptides were analogs HA-R₃₁₂ and HA-K₃₁₃ differing from HA by one amino acid at the 312 or 313 position respectively or unrelated peptides which bind to DR1/4.

Results—The responses of eight patients with RA to the two stimulatory influenza peptides did not differ significantly from controls and this was confirmed by the frequency estimate of T cells in PBL which responded to HA (mean frequency: 1 in 9.0×10^4 , $n = 5$, in DR1/4+ RA patients, 1 in 7.6×10^4 , $n = 5$, in DR1/4+ healthy controls). DR1/4 binding analogs of the HA peptide inhibited HA specific peptide responses of PBL from patients with RA and controls. Inhibition was also detected with unrelated peptides which bind to DR1/4 but to which the individual did not respond.

Conclusion—Similar responses to two DR1/4 restricted peptides were observed in patients with RA and controls. Both antigen analog- and unrelated peptide-major histocompatibility complexes (MHC) can result in the inhibition of antigen specific responses in multiclonal human lymphocyte populations. However, an analog peptide may be stimulatory in some individuals. These results provide some initial data for the development of a rational approach to MHC-specific immunomodulation in rheumatoid arthritis.

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The contribution of cell mediated immunity to joint inflammation and destruction in rheumatoid arthritis (RA) is poorly understood but there is evidence of a role for T cell-mediated immune responses in the pathogenesis of this disease.¹ Immunohistological

examination of the synovium has shown a prominent infiltrate of lymphocytes, the majority of which are activated T cells.² In addition, several experimental treatment strategies which act primarily through their effect on T cells are reported to be effective in the treatment of RA.¹⁻³

The association of RA with major histocompatibility genes is well established⁴ and the HLA DR4 (DRB1*04) and DR1 (DRB1*01) genes involved is well documented.⁵ Disease susceptibility has been localised to the third hypervariable region of the β chain of DR and in white groups is most commonly associated with Dw4 (DRB1*0401), containing the amino acid sequence LLEQKRAA at positions 67-74 and VG at residues 85-86.⁵ This disease-associated epitope is present in Dw4 (DRB1*0401), the most frequent DR subtype in white groups.⁵ Dw14.2 (DRB1*0408), Dw15 (DRB1*0405) and DR1.1 (DRB1*0101) have a single substitution at position 71, where a lysine replaces the arginine and Dw14.1 (DRB1*0404) has this sequence in the 67-74 region. These alleles also confer disease susceptibility particularly in ethnic groups where Dw4 is a less common allele.⁶⁻⁷

The role of the LLEQKRAA sequence in determining disease susceptibility to RA is not understood at present. This sequence lies within the peptide antigen binding groove of the MHC molecules. Little is known about the influence these residues have on T cells which recognise peptides and which may be involved in immune regulation in RA. Peptides which bind to such DR1/4 molecules may have a role in causing or perpetuating the disease. Thus peptides that are DR1/4 restricted can be used to analyse T cell responses dependent on the disease associated genes, and may be useful for comparison when studying putative disease associated antigens. Several investigators have used high affinity MHC-binding peptides to inhibit T cell activation by blocking the antigen-binding site of MHC molecules.⁸⁻⁹ It follows that unrelated DR1/4-binding peptides can potentially block the antigen presenting site of the disease associated MHC Class II molecules and thus the cascade of molecular events leading to the activation of arthritogenic T cells may be prevented.

Peptides which bind to DR1/4 have been described and include, residues 307-319 of the influenza haemagglutinin,¹⁰ residues 17-29 of the influenza matrix protein¹⁰ residues 1-15 of the 19-kd protein of *Mycobacterium tuberculosis* (MT)¹¹⁻¹² and residues 38-50 of the 18-kd

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Mycobacterium leprae protein (ML3)¹³ in addition to the naturally processed peptides derived from MHC-related molecules.¹⁴ Competition studies using analogs of such peptides, to inhibit human peptide-specific T cells, have been successful but have utilised peptide-specific T cell lines and clones. Before such techniques can be used to develop an immunotherapeutic approach to RA the lymphocyte responses of patients with RA to these peptides must be determined. In previous work, we analysed the responses of lymphocytes from patients with RA to the DR1/4 restricted epitope of MT.¹² In this report we describe the responses of peripheral blood lymphocytes (PBL) from healthy individuals and patients with RA to three other DR1/4 restricted peptides and show that proliferative responses can be altered by both non-stimulatory peptide analogs which bind to DR1/4 and by unrelated peptides to which an individual does not respond.

Patients and methods

PATIENTS

A total of 25 DR1/4+ patients with RA and 12 DR1/4+ healthy age-matched controls were entered into the study. All patients had seropositive erosive RA and fulfilled the American College of Rheumatology revised criteria for RA.¹⁵ There were 18 women and seven men aged 30–81 years. Their duration of disease ranged from one to 52 years. Most patients had at least four inflamed joints; eight patients had from 0 to 3 inflamed joints. Two patients were taking no medication, two patients took paracetamol, 17 patients took non-steroidal anti-inflammatory drugs (NSAIDs), eight patients had prednisone (2–10 mg/day), and 21 patients received anti-rheumatic agents (8 took methotrexate, 5 D-penicillamine, 3 intramuscular sodium thiomalate, 3 sulfasalazine and 2 azathioprine). PBL from all patients and controls were DR1/4 typed either with the assistance of M Roberts (Auckland Regional Blood Centre) or by a PCR technique.⁷ The HLA status was confirmed by DR4 subtyping using a single stranded conformational polymorphism technique¹⁶ recently developed in our laboratory for the HLA-DR4 group of alleles.

PEPTIDES

The amino acid sequences in brackets are given as single letter codes. The synthetic peptides from influenza haemagglutinin, amino acids 307–319 (PKYVKQNTLKLAT) and matrix, amino acids 17–29 (SGPLKAEIAQRLE), were synthesised by the Joint Protein Structure Laboratory, Ludwig Institute for Cancer Research, Melbourne, Australia. The analog peptides derived from HA⁸ were mono-substituted derivatives, HA-R₃₁₂, where the glutamine at position 312 was replaced by an arginine (PKYVKRNTLKLAT) or HA-K₃₁₃, where the asparagine at position 313 was replaced by a lysine (PKYVKQKTLKLAT) and were synthesised by Chiron Mimotopes

Pty, Clayton, Australia. The *M leprae* 18-kd peptide ML3, amino acids 31–50 (DAWREGEEFVVEFDLPGLK) and the *M tuberculosis* 19-kd peptide, D3, amino acids 31–50 (SGETTTAAGTTASPGAASGPK), were synthesised by the Protein Chemistry Unit, Massey University, New Zealand. All peptides were purified by reverse-phase high performance liquid chromatography and were greater than 92% pure.

LYMPHOCYTE PROLIFERATION ASSAY

Heparinised peripheral blood was subjected to Ficoll-Hypaque (Pharmacia, Piscataway, NJ) gradient separation. Lymphoid cells were harvested, washed three times in RPMI (Life Technologies, Grand Island, NY) medium and viable cells counted using trypan blue. The cells were cultured at 2×10^5 per well in 96 well round bottom plates (Nunc, Denmark) in a total volume of 200 μ l RPMI supplemented with 5% heat inactivated autologous serum (culture medium) and stimulated in triplicate with phytohemagglutinin (PHA) at 1 μ g/ml, peptide (HA, IM, ML3), or medium alone. Peptides were added to give a final concentration of 10 μ g/ml, unless otherwise stated, a dose in the optimal range for stimulating T cell proliferation.

For competition studies, 2×10^5 PBL were preincubated with the blocking peptide at 10 μ g/ml, unless otherwise stated, in a total volume of 190 μ l before the addition of the stimulating peptide. Preliminary experiments indicated that blocking could be detected although to a lesser extent when both stimulatory and blocking peptides were added simultaneously but a four hour pre-incubation with the blocking peptide gave more consistent results and was therefore used throughout. A control peptide from the 19-kd protein of *M tuberculosis* (D3) to which DR1/4+ individuals do not respond¹² was included as a negative control in competition experiments and did not affect the response to stimulating peptides.

Cultures were incubated at 37°C in 5% CO₂ in air for 5–6 days and pulsed with 0.25 μ Ci ³H-thymidine (Dupont/NEN, Boston, MA) for the last 18 hours. Cells were thus harvested during the peak response to peptide (4–6 days) but not at the peak of the PHA response. They were harvested onto filters and uptake of radiolabel was determined using either an LKB Beta plate system (Wallac, Turku, Finland) or PHD cell harvester (Cambridge Instruments Inc, MA) and a liquid scintillation counter. The results are expressed as cpm or as the stimulation index (SI), which represents the counts per minute (cpm) obtained with peptide or PHA stimulation divided by the cpm in cultures of cells with medium alone. The standard deviations were usually less than 20% of the SI values. In all cases, 1.7 X SI exceeded 2 standard deviations of the background.

LIMITING DILUTION ANALYSIS

Graded numbers of PBL (from 6.4×10^1 to 2×10^5) were cultured with 5×10^4 autologous

γ -irradiated (40 Gy) PBL feeder cells in round bottom 96-well plates with peptide (10 μ g/ml). Eleven dilutions were used for each experiment with 24 microcultures per concentration. No exogenous growth factors were added. Cultures containing only feeder cells plus peptide or responder cells alone were included as controls. Plates were maintained at 37°C in an atmosphere of 5% CO₂ in air for six days and harvested as described above. Microcultures were scored as positive for proliferation to the peptide when the cpm was >3SD above the background. Minimal estimates of the frequencies of proliferating T cells were calculated by analysis of the Poisson distribution relationship between the number of responding cells cultured and the percentage of negative wells. At least four experimental points were used to fit data to the zero order term of the Poisson equation by the least squares method and results are expressed as the values determined by linear regression analysis as described previously¹⁷ where the slope is the frequency (f). Values of p were determined from the coefficient of correlation and thus indicate whether the data follows 'single-hit' kinetics.

Results

PROLIFERATIVE RESPONSES TO DR1/4 RESTRICTED PEPTIDES

Responses to the DR1/4 restricted peptide from the 19-kd protein of *M. tuberculosis* are similar in controls and patients with RA.¹² Experiments were carried out with other DR1/4 restricted peptides from influenza to determine whether this was a general phenomenon particularly as analogs of the haemagglutinin peptide are candidate blocking agents. PBL from 8 DR1/4 positive patients with RA and controls were cultured at 2×10^5 cells per well with 10 μ g/ml HA or IM for six days. The data are shown in table 1. Although proliferation in response to peptides differed in magnitude between individuals, there was no significant difference between the responses of the DR1/4+ patients with RA and controls. PBL from two patients with RA responded to only one of the peptides, while PBL from one

patient and two control subjects did not respond to both peptides. The one patient (RA1) whose PBL did not respond to both peptides was aged 30 years and was taking no medication. PBL from the other patient taking no medication (RA8, aged 68) responded to both peptides. All other patients whose PBL showed responses were taking NSAIDs and long-acting antirheumatic agents. The patients who were 'responders' included those with four to 26 inflamed joints and three patients with none, two or three inflamed joints.

The frequency of precursor cells proliferating in response to HA was determined by limiting dilution analysis. Increasing numbers of PBL were cultured with HA for six days and wells containing proliferating cells were scored. The frequency of precursor cells which responded to HA ranged from 1 in 3.52×10^4 to 1 in 1.49×10^5 in DR1/4+ RA patients and 1 in 6.19×10^3 to 1 in 1.94×10^5 in DR1/4+ controls. For comparison, two DR1/4- RA patients and three DR1/4- controls were included in the analysis. In 5 of 6 DR1/4- individuals (RA patients + controls), the data did not follow 'single hit' kinetics nor fit the Poisson distribution ($p > 0.1$) as the precursor frequencies were lower than estimated in table 2. However, precursor frequencies to HA are included in table 2 to demonstrate the difference in response between DR1/4+ [mean (SD) $f = 1$ in 8.2×10^4 (6.40), $n = 10$] and DR1/4- individuals [mean (SD) $f = 1$ in 2.92×10^6 (2.41), $n = 5$]. In summary these data demonstrate that the responses of PBL from DR1/4+ RA patients to the two DR1/4 restricted peptides from influenza do not differ to those from healthy age-matched controls.

RESPONSES TO HA 307-319 AND MONO-SUBSTITUTED ANALOGS

Mono-substituted analogs of HA 307-319 have been shown to bind to DR1 and inhibit HA stimulated proliferation of a DR1-restricted HA specific T cell clone but do not stimulate the T cell receptor (TCR) of this clone.⁸ Two such analogs, HA-R₃₁₂ and HA-K₃₁₃ were synthesised and tested for their ability to stimulate PBL to proliferate. Of the 14 DR1/4+ individuals tested, 3 RA patients responded to HA and both analogs (SI > 1.7), 2 RA patients responded to HA and the HA-R₃₁₂ analog, and the remaining 7 patients and 2 controls responded only to the HA peptide. The results from individuals who responded to one or both analogs are shown in table 3. The three patients with RA, RA1, 20, and 21, who only responded to HA are included for comparison.

EFFECT OF ANALOGS ON THE PBL RESPONSE TO HA

The peptides HA-R₃₁₂ and HA-K₃₁₃ were tested for their ability to inhibit multiclonal T cell responses specific for HA. Dose-response data using a constant concentration of stimulatory peptide with increasing amounts of HA-R₃₁₂ competitor peptide is shown in fig 1.

Table 1 Comparison of maximum proliferative responses of peripheral blood lymphocytes from patients with RA and healthy controls to influenza matrix (IM) and haemagglutinin (HA) peptides.*

Subjects	Nil	IM	HA	PHA
RA1	191	169 (0.9)	126 (0.7)	64188 (336)
RA2	318	453 (1.4)	478 (1.5)	15843 (50)
RA3	664	887 (1.3)	1358 (2.0)	13372 (20)
RA4	633	999 (1.6)	1912 (3.0)	53908 (85)
RA5	1785	2988 (1.7)	5579 (3.1)	49674 (28)
RA6	421	1108 (2.6)	864 (2.1)	9332 (22)
RA7	148	468 (3.2)	691 (4.7)	7845 (53)
RA8	207	1659 (8.0)	1515 (7.3)	23087 (111)
C1	518	501 (1.0)	646 (1.2)	9341 (18)
C2	596	571 (1.0)	430 (0.7)	8806 (15)
C3	1234	2452 (2.0)	2150 (1.7)	4073 (3.3)
C4	389	870 (2.2)	885 (2.3)	5949 (15)
C5	99	233 (2.4)	338 (3.4)	10311 (104)
C6	280	662 (2.4)	3319 (12)	12970 (46)
C7	254	1681 (6.6)	1324 (5.2)	26651 (105)
C8	470	6146 (13)	2411 (5.1)	8390 (18)

*Values are the mean cpm of triplicate cultures in which the SD did not exceed 20% and (stimulation index). Data with phytohaemagglutinin (PHA) are included for comparison.

Table 2 The frequency (*f*) of peripheral blood lymphocytes proliferating to the DR1/4 restricted influenza haemagglutinin 307–319 peptide (HA)*

Patient	Reciprocal of <i>f</i>	<i>p</i>	Controls	Reciprocal of <i>f</i>	<i>p</i>
DR1/4+					
RA9	3.52×10^4	0.01	C2	6.19×10^3	0.01
RA10	5.82×10^4	0.001	C7	1.17×10^4	0.01
RA4	5.88×10^4	0.001	C6	7.24×10^4	0.001
RA11	1.49×10^5	0.001	C9	9.68×10^4	0.05
RA12	1.49×10^5	0.05	C8	1.94×10^5	0.01
DR1/4-					
RA13	3.44×10^5	>0.1	C10	2.04×10^6	>0.1
RA14	5.49×10^6	>0.1	C11	1.25×10^6	>0.01
			C12	5.46×10^6	>0.1

*The frequency, *f*, was derived from the zero order term of the Poisson distribution by linear regression analysis with *p* values derived from the correlation coefficient.

Table 3 Responses to the influenza haemagglutinin 307–319 peptide and two mono-substituted analogs

Subjects	Nil	HA	HA-R ₃₁₂	HA-K ₃₁₃
RA15	894	2734 (3.1)	2227 (2.5)	866 (1.0)
RA16	84	215 (2.6)	180 (2.1)	259 (3.1)
RA17	521	1638 (3.1)	3447 (6.6)	6021 (11.6)
RA18	1674	3656 (2.2)	4386 (2.6)	6156 (3.7)
RA19	378	3424 (9.1)	1692 (4.5)	318 (0.8)
RA1	642	2016 (3.1)	938 (1.4)	693 (1.1)
RA20	43	364 (8.5)	69 (1.6)	57 (1.4)
RA21	577	8099 (14.1)	640 (1.1)	543 (0.9)

Values are the mean cpm of triplicate cultures in which the SD did not exceed 20% and (stimulation index). All patients were DR4+. RA15 not able to subtype, all others were DRB1*0401. The data of RA 19, 1, 20 and 21 are also included in figure 2.

Figure 1 A single amino acid substitution of influenza haemagglutinin 307–319 peptide (HA) renders it non-stimulatory but effective as an inhibitor of an HA-specific response. The HA analog HA-R₃₁₂ was tested for its capacity to stimulate a proliferative response in peripheral blood lymphocytes (○) or to inhibit a response to 10 μg/ml HA (●) in a homozygous DR4+ (DRB1*0401 and DRB1*0404) RA patient. The mean cpm of triplicate cultures with SE are shown.

Effective inhibition was noted with 5–10 μg/ml of competitor peptide. PBL from DR1/4+ RA patients and controls were thus incubated with 10 μg/ml HA-K₃₁₃ or HA-R₃₁₂ before stimulation with 10 μg/ml HA. The results obtained from 8 patients with RA and two controls are shown in fig 2. Of the 10 individuals tested in this experiment, none responded to the HA K₃₁₃ (SI <1.7) and two of 10 responded to the HA-R₃₁₂ (SI >1.7). The HA-K₃₁₃ analog inhibited the response to HA in all 10 of 10 individuals whereas the HA-R₃₁₂ analog inhibited responses in eight of 10. Thus in this sample of patients and controls, the HA-K₃₁₃ analog appeared to be the more effective inhibitor. In one patient with RA (subject 19, fig 2) who responded to HA-R₃₁₂, the response

Figure 2 The inhibition of peripheral blood lymphocyte responses to influenza haemagglutinin peptide 307–319 (HA) by analog peptides. Two HA analogs HA-R₃₁₂ and HA-K₃₁₃ were tested for their capacity to inhibit the responses of peripheral blood lymphocytes from 10 individuals, 8 RA patients and 2 healthy controls to HA. Cultures contained the peptides as indicated. The results are shown as the mean cpm of triplicate cultures with SD. The mean cpm of cultures without peptide was 204, 291, 43, 31, 642, 378, 894, 136, 88, and 202 for subjects 21, 2, 20, 23, 1, 19, 15, 12, C8 and C9 respectively.

to both this analog and HA together was less than the response to either individual peptide alone. From the data it is clear that these HA analog peptides can inhibit HA specific responses of multiclonal T cells.

COMPETITION BETWEEN UNRELATED DR1/4 RESTRICTED PEPTIDES

Peptides of different sequences have been shown to compete for presentation to T lymphocytes by the same MHC molecules both in vitro and in vivo in the mouse^{18 19} and in human T cell clones.¹⁸ To test whether this effect can be demonstrated in human PBL, these cells were cultured with 10 μg/ml HA, 10 μg/ml of a DR1/4 restricted peptide from the 18-kd protein of *M leprae* (ML3) or 10 μg/ml of both peptides. Most individuals responded to HA but not to ML3. In these individuals the simultaneous culture of PBL with ML3 resulted in inhibition of the HA response (fig 3). In only three of 37 individuals tested, such as RA patient 24, a response to ML3 was detected in the absence of a response to HA. In this situation, culture with both HA and ML3 resulted in the inhibition of the ML3 response (fig 3). Such data supports the notion that competition between unrelated DR1/4

Figure 3 An unrelated peptide can inhibit the response to another DR1/4 restricted peptide. The results are expressed as mean cpm of triplicate cultures with SD where peptide 1 is the stimulatory peptide and peptide 2 is the inhibitory peptide. For RA patients 4, 2 and controls C9 and C6, peptide 1 was HA and peptide 2 was ML3. For RA patient 2*, peptide 1 was IM. For RA patients 24, 1 and control C1, peptide 1 was ML3 and peptide 2 was HA. The mean cpm in cultures without peptide was 633, 318, 22, 318, 42, 247 and 16 for subjects 4, 2, C9, C6, 24, 1, and C1 respectively.

restricted peptides can occur in fresh human lymphocyte populations.

Discussion

New strategies for the immunotherapy of RA can be developed if the antigen presenting function of the disease-associated HLA molecule is examined. Our intention is to extend the hypothesis that antigen presentation by DR1/4 is critical to the pathogenesis of RA and can be blocked by DR1/4 binding peptides. Analog and unrelated peptides can inhibit specific T cell responses to stimulatory peptides both in vitro and in vivo.^{18,19} Nevertheless, little is known about the effect of such inhibitors on the responses of multiclonal populations of human T cells. In this report we have examined responses to DR1/4 restricted peptides in patients with RA and demonstrate that PBL T cell proliferative responses to these peptides can be inhibited by other peptides which bind to DR1/4.

To study the DR1/4 binding site, the ideal peptide is one which elicits a consistent response. T lymphocytes important in RA have presumably been activated and thus responses to an antigen that most individuals are exposed to each winter was selected. Responses of PBL to two DR1/4 restricted peptides from influenza virus were detected in five of eight patients with RA and six of eight healthy controls. One patient responded only to the haemagglutinin peptide. The absence of a

detectable response in some individuals may be due to the low frequency of antigen-specific precursor cells in the circulation which could change from time to time as suggested previously.¹² Analysis of the frequency of HA peptide specific precursors demonstrated that this could vary from 1 in 6.19×10^3 to 1 in 1.94×10^5 in DR4+ individuals. The range in precursor frequency also presumably accounts for the variation in the magnitude of the response to the two peptides and is dependant on factors such as previous exposure to influenza. Even so, the responses were consistent in that those of the healthy control group did not differ significantly from those of the patient group. Comparative frequency analysis of T lymphocytes has been useful in multiple sclerosis patients where the frequency of myelin basic protein reactive T cells in the blood is 10–100 times higher than in controls and in patients with RA or other neurological diseases.²⁰ Such analysis of DR1/4 restricted peptide responses may lead to a better understanding of the antigens involved in causing or prolonging the disease should a high precursor frequency be detected in the RA patient group.

Inhibition of DR1/4 restricted responses by competing peptides can be facilitated by analog peptides that differ from the stimulating peptide by as little as one amino acid or by peptides that are completely unrelated except for their MHC binding characteristics. Analogs of the HA peptide and the unrelated DR1/4 restricted peptide ML3 were both efficient at inhibiting the response of PBL to HA. Although it has been presumed that the mode of inhibition is MHC blockade,²¹ recent evidence suggests that antigen analog-MHC complexes may act as antagonists of the T cell receptor.²² An ideal peptide for immunotherapeutic development would presumably be one which has the ability not only to bind to DR1/4 but also to block responses at the level of the TCR. However, the design of such a peptide for RA may not be possible until the peptide which causes or perpetuates the disease is known. At present, it may be useful to consider naturally processed peptides such as the ones derived from MHC-related molecules that are bound to DR1.¹⁴

To assay for suitable candidates, the inhibitory peptide must itself not illicit a proliferative response, although for the purpose of blocking the presentation of a self peptide involved in the pathogenesis of RA it may not be important as to whether there is a response to the blocking peptide or not. The analogs of the HA peptide stimulated a response in only five of 14 DR1/4+ individuals and responses to ML3 were rare in the white New Zealand population (unpublished observation). In an individual who responded to the HA-R₃₁₂ analog, less proliferation occurred when both HA and HA-R₃₁₂ were included than in response to either peptide alone. It is difficult to envisage how MHC blockade could account for this result. A more likely mechanism might involve inhibition at the level of the TCR and several possibilities

can be considered. Firstly, induction of a hyporesponsive state^{23, 24} in the T cell could be induced by delivery of a negative signal. Secondly, only partial activation of the TCR could occur as shown by lymphokine production in the absence of proliferation.²⁵ Thirdly, the inhibitory capacity of the peptide-MHC complexes on the TCR could occur in the absence of any signal transduction comparable, in effect, to a pharmacological receptor antagonist. This third mechanism is supported by the work of De Magistris *et al*²² who achieved more effective inhibition of HA-specific T cell clones with similar analog peptides than with unrelated peptides.

Specific analog peptides have been used in an animal model of autoimmune disease, experimental allergic encephalitis (EAE), to prevent responses to the disease-causing peptide present in myelin basic protein.^{26, 27} As the peptide which causes or perpetuates RA is unknown it is more appropriate to consider blocking the DR1/4 binding site with an unrelated peptide. Competitive inhibition of the formation of autoantigenic peptide-MHC may interfere with the activation of T cells responsible for the disease particularly as DR4 Dw4 T cell recognition of self antigens has been reported in the rheumatoid synovial compartment.²⁸ Our results demonstrate that this type of blocking is feasible. It was possible to detect inhibition by two unrelated DR1/4 restricted peptides, ML3 and HA, by selecting individuals who would respond to only one of the peptides. Thus the ML3 peptide would inhibit responses to HA in HA responding subjects, whilst HA would inhibit ML3 specific responses in individuals responding to the ML3 peptide. Preliminary studies using synovial fluid T cells indicate that responses by these cells can be inhibited in a similar way (unpublished observation).

Biochemical studies of peptide binding to Class II MHC molecules in vitro showed that competition between peptides occurred at a single site.²⁹ This approach has since been used to assign MHC contact residues of several antigenic peptides.³⁰ Competition between peptides has been predicted to take place in vivo and is proposed as a mechanism for the observed immunodominance of particular T cell determinants within complex protein antigens. The hen egg lysozyme system has been used as a model for studying this competition in vivo in the mouse. From results obtained with this system Adorini *et al*³¹ proposed that 100 nmoles of an efficient competitor peptide is sufficient to completely saturate the available MHC Class II binding sites in each mouse. Thus in vivo competition in humans should not require exceedingly large amounts of peptide. In vitro studies allow the identification of peptides capable of blocking DR1/4 responses and provide the basis for designing peptides for future in vivo studies.

The result of competitor peptide administration may not simply be the inhibition of a single specific peptide response. Cohen and Young³² propose that a regulated autoimmune network exists in healthy individuals and that

autoimmunity results from the loss of suppressing elements. If competitor peptides change the immunodominance of an auto-antigen or alter the balance of dominant T cell clones they may allow the dysregulated autoimmune network to normalise. Future work aimed at the analysis of TCR V α and V β gene usage in multiclonal rheumatoid lymphocyte populations exposed to DR1/4 binding peptides, may help us to further understand the outcome of peptide competition.

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