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# Rapid Identification of Antigenic T-Cell Epitopes by Extracellular Acidification Rate Signals

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**Abstract** We used a silicon-based biosensor, a microphysiometer, to measure real-time extracellular acidification rate signals associated with T lymphocyte responses to peptide ligands interacting with the T-cell receptor (TCR). We compared these effector responses with those of interferon-γ (IFN-γ) production, and T-cell proliferation. Within minutes, major histocompatibility complex (MHC)-bound peptides on antigen-presenting cells (APCs) engaged the TCR to increase acidification rates of the extracellular media was measured by microphysiometer. We exposed two myelin peptide-specific human T-cell clones, MSF132E11 (DRB1*1501 restricted) and TOM3A6 (DRB5*0101 restricted), to truncated analogues of the parent MBP 84-102 peptide, in the presence of MHC restricted human antigen-presenting cells, and measured the extracellular acidification rate signal changes, IFN-γ production and T-cell proliferation. The core epitopes recognized by these clones were identified by microphysiometer and found to be MBP 88–100 and MBP 91–100, respectively. These epitopes were identical to those identified by the IFN-γ and proliferation assays. We conclude that measurement of real-time extracellular acidification rate signals by the microphysiometer may facilitate rapid identification of human T-cell epitopes involved in immune disorders and the development of specific T-cell antagonists. J. Cell. Biochem. 77:409–417, 2000. 

**Key words:** T-cell clones; microphysiometer; acidification rate signal; MHC-peptide; TCR interaction

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the human central nervous system (CNS) characterized by demyelination and local infiltration of the CNS by macrophages, plasma cells, and T cells [Allen, 1991]. In MS and other demyelinating diseases, T-cell clones specific for myelin basic protein (MBP), a component of the myelin sheath, lead to demyelination of nerve sheaths in the CNS. In addition to MBP-restricted T lymphocyte clones, demyelinating inflammatory lesions can contain multiple nonrestricted immune cells capable of mediating tissue injury [Zamvil et al., 1990; Wucherpfenning et al., 1991; Martin et al., 1992a,b]. Major histocompatibility complex (MHC) class II (DR) molecules are heterodimers on the surface of antigen-processing/presenting cells (APCs), which bind and present autoantigenic peptides to autoreactive $CD4^+$ T cells. In demyelinating disease, such as MS, MBP, proteolipid protein, and myelin oligodendrocyte antigen peptides complexed with disease-associated MHC class II (DR2) heterodimers on APCs stimulate autoreactive T cells [Buss et al., 1987; Allen et al., 1987]. These activated cells induce cellular and humoral effector mechanisms, leading to the destruction of the myelin sheath in MS patients [Todd et al., 1988; Spielman et al., 1982; Olerup et al., 1989].

Studies involving the identification of major autoantigenic peptides by proliferation or ELISPOT assays using MS patient PBMCs [Oat et al., 1990; McCutcheon et al., 1997] demonstrated that an increased number of autoreactive T cells respond to myelin basic peptides

Abbreviations used: MHC, major histocompatibility complex; APC, antigen-presenting cell; TCR, T-cell receptor; MS, multiple sclerosis; PBMC, peripheral blood monocytes; MBP, myelin basic protein; Ab, antibody; MAb, monoclonal antibody; IFN-γ, interferon-γ; TMB, 3,3′,5,5′-tetramethyl benzidine; HVS, *Herpesvirus saimiri*; MBHA, 4-(2,4-dimethoxyphenyl-F-moc-aminomethyl)phenoxyacetamidonorleucyl; HBTU, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate; HOBt, 1-hydroxybenzotriazole; PyBOP, benzotriazole-1-zyloxytris(pyrrolidino)phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; NMM, N-methylmorpholine.

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contained within the sequence MBP 80–105. Therefore, the isolation of human T-cell clones specific to peptides contained within this sequence has enabled us to better understand the autoantigen presentation and to begin to develop drugs with the potential to interfere with the autoantigen presentation and/or the effector mechanisms that arise out of this event in multiple sclerosis.

While the markers of antigen presentation are generally considered the release of one or more cytokines and/or the proliferation of T cells, many unconventional signaling events, including calcium flux and tyrosine kinase phosphorylation patterns, have been used as markers [Rabinowitz et al., 1996]. Ligand receptor interactions on the cell surface have been shown to induce rapid increases in extracellular acidification rates for both T cells and B cells [Nag et al., 1992; Renschler et al., 1995]. Rabinowitz et al. [1996] clearly showed that acidification rate changes in mouse T-cell clones are induced much earlier than the calcium flux. We asked whether these rate changes could be used as effective markers of antigen presentation in antigen specific MHC restricted human T-cell clones. To answer this question, we had to compare the acidification rate changes in human T-cell clones with other standard activation markers, such as IFN-γ and T-cell proliferation. In addition, we needed to show that any perturbation in the parent antigenic peptide, such as truncation or alanine substitution, affecting the antigen presentation, would be reflected in the acidification rate changes, in parallel with the cytokine profile and T-cell proliferation. In our earlier studies, we suggested a correlation of acidification rate changes with cytokine profiles and T-cell proliferation in HVS-transformed human T-cell clones [Arimilli et al. 1998]. In the present study, we confirmed that this correlation also exists in antigen-specific human T-cell clones.

## MATERIALS AND METHODS

### Cell Lines, Antibodies, and Chemicals

Human transformed B cells, B2A and B2B, expressing DRB5*0101 and DRB1*1501, respectively, were a generous gift from Dr. Roland Martin (Neuroimmunology, NIH, Bethesda, MD). Human myelin basic protein (MBP) was obtained from Chemicon. Mouse anti-human IFN-γ MAb and rabbit anti-human IFN-γ polyclonal Ab were obtained from Endogen (Woburn, MA), Peroxidase-conjugated goat IgG and rabbit IgG were purchased from Jackson Immunoresearch Laboratories (West Grove, PA). Human IFN-γ was obtained from Boehringer-Mannheim (Indianapolis, IN). TMB (3,3′,5,5′-tetramethylbenzidine) was obtained from Moss (Pasadena, MD). Endotoxin-free bovine serum albumin (BSA) was obtained from Calbiochem-Norabiochem (La Jolla, CA).

### Synthesis of MBP Peptides

All peptides prepared for these studies were acetylated at the N-terminus and amidated at the C-terminus. The MBP (83–102)$Y^{83}$ peptide with the sequence, Ac-YDENPVVHFFKNIVTPRTPP, was synthesized by the standard solid-phase method, using side-chain-protected Fmoc amino acids on an Applied Biosystems 431A automated peptide synthesizer. The deprotected crude peptides were purified by reverse-phase high-performance liquid chromatography (HPLC). The homogeneity and identity of the purified peptides were confirmed by mass spectrometry. All terminally truncated and alanine analogue MBP peptides were synthesized by solid-phase peptide synthesis, using Fmoc chemistry. All chemicals, including the Rink amide MBHA resin and side chain-protected Fmoc amino acids, were obtained from Nova Biochem (San Diego, CA). HBTU/HOBt or PyBOP/NMM activation chemistry was employed for protected amino acid coupling on a ABI 431 A automated peptide synthesizer or a ABIMED/GILSON AMS 422 multiple peptide synthesizer, as described earlier [Luu et al., 1996]. After the peptides were synthesized on solid phase, they were cleaved by TFA containing 5% 4-methoxybenzenethiol and 5% 4-methylmercaptophenol as scavengers. The crude peptides were precipitated by pentane:acetone (4:1, v:v) mixture and isolated by centrifugation. The peptides were washed with pentane:acetone mixture three times, followed by pentane, and then dried under vacuum. They were purified by reverse-phase HPLC, using a $C_{18}$ column; the pure fractions were pooled and lyophilized. Finally the peptides were characterized by electron spray mass spectrometry.

### T-Cell Cultures

Ag-specific T-cell clones, designated E11, were generated from MSF132 patient blood

that was previously HLA-typed and shown to express DRB1*1501. MSF132 T lymphocytes were reactive to MBP (83–102)$Y^{83}$ peptide and whole MBP protein. E11 clones were generated by plating $2 \times 10^5$/well/100 μl PBMCs from the patient blood in Yssel's Media, along with 30 μg/ml purified MBP protein. Cells were restimulated twice with irradiated (3,300-rad) patient PBMCs before screening for MBP reactivity by proliferation. A second T-cell clone TOM3A6, restricted to DRB5*0101, was a generous gift from Dr. Roland Martin (Bethesda, MD). Both E11 and TOM3A6 T-cell clones were maintained in culture by restimulating one cycle with Ag-specific stimulation, followed by a nonspecific restimulation by PHA. In the specific round of stimulation, $4 \times 10^6$ APCs (DRB1*1501 & DRB5*0101), irradiated with 3,300 rad, were pulsed with 50 μg/ml MBP (83–102)$Y^{83}$ for 2 h, washed, and added to $1 \times 10^6$ T cells in 1 ml Yssel's media in a 24-well plate. For nonspecific stimulation, $2 \times 10^5$ T cells and $1 \times 10^5$ B2A & B2B cells were irradiated with 5,000 rad each, $1 \times 10^6$ APCs (DRB1*1501 & DRB5*0101) were irradiated with 3,300 rad, and 0.5 μg/ml PHA in 1 ml Yssel's Media were mixed in a 24-well plate. Recombinant human IL-2 was added at days 4 and 7, after Ag-specific and nonspecific stimulation. T-cell clones were maintained at a density of $1 \times 10^6$/ml and restimulated every 10 days. At each restimulation, the clones were tested for specificity by a standard proliferation assay.

### Proliferation and Cytokine Assay

In this study, $2 \times 10^5$ APCs (DRB1*1501 & DRB5*0101) were pulsed with different truncated and alanine substituted peptides for 2 h, washed and irradiated at 3,300 rad and mixed with $2 \times 10^4$ T-cell clones per well in 200 μl of Yssel's media. After 32 h, 75 μl of the cell supernatants was collected. and IFN-γ was measured by the method described earlier [Arimilli et al., 1995]. The cells were then pulsed with 0.1 μCi of $^3$H-labeled-thymidine, harvested at 48 h and counted in a β-counter.

### Measurement of T-Cell Metabolic Acidification Rate Signals

All the reagents required for the microphysiometer experiments were obtained from Molecular Devices (Sunnyvale, CA). Freshly cultured E11 clones with B2B cells and TOM3A6 clones with B2A cells at a density of $5 \times 10^5$ T cells mixed with $5 \times 10^4$ B cells were immobilized into microphysiometer cell capsules as described earlier [Nag et al., 1992]. Briefly, the mixture of T-cell clones and B cells was suspended in a serum-free loading medium (low-buffering RPMI 1640 containing 10% fatty acid-free endotoxin-free BSA). The T and B cells were collected by centrifugation and resuspended in 7.5 μl of loading medium. Low-melt agarose, melted and stored at 37°C, was added to the suspended cells in a ratio of 1:4; 10 μl of the agarose/cell mixture was immediately spotted into the center of the cell capsule cups held in a 12-well culture plate. After 5 min, 2 ml of loading medium was placed in the capsule cup over the solidified agarose, and a membrane insert was placed over the cells. The assembled cell capsule was loaded in the cytosensor chamber at 37°C and perfused at 50 μl per min with low-buffering RPMI 1640 medium containing 10% of BSA per ml without Hepes or bicarbonate buffers. Extracellular acidification rate signal measurements were made in the cytosensor microphysiometer, as described earlier by collecting potentiometric measurements for 45 s every 2 min [McConnell et al., 1992]. Acidification rate data (μV/s) were normalized to 100% before cell stimulation, permitting comparison of the data from cells in separate chambers.

## RESULTS AND DISCUSSION

The aim of this work was to compare and correlate the extracellular acidification rate signal changes with proliferation and IFN-γ release during antigen presentation in human T-cell clones. In addition, we sought to determine whether this method could be used to identify the minimum peptide epitope recognized by the T-cell clones. In vitro antigen presentation to T cells is a two-step event involving MHC class II peptide binding (MHC-P) and MHC-P-TCR complex formation, along with costimulatory events leading to production of cytokines and T-cell proliferation. Extracellular acidification rate signals in T cells can be detected within minutes of their activation or TCR ligation and is a general marker of cellular activity [Weiss et al., 1984a,b; Nag et al., 1992; McConnell et al., 1995]. Activation of most known signal transduction pathways can trigger increases in

**A.**

| | |
|---|---|
| MBP(83-102) | Ac-Y D E N P V V H F F K N I V T P R T P P NH2 |
| MBP(88-100) | Ac-V V H F F K N I V T P R T NH2 |
| MBP(89-100) | Ac-V H F F K N I V T P R T NH2 |
| MBP(90-100) | Ac-H F F K N I V T P R T NH2 |
| MBP(91-100) | Ac-F F K N I V T P R T NH2 |
| MBP(92-100) | Ac-F K N I V T P R T NH2 |
| MBP(91-101) | Ac-F F K N I V T P R T P NH2 |
| MBP(91-102) | Ac-F F K N I V T P R T P P NH2 |

**B.**

| | | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parent Sequence | | | | | | | | | | | | | | | | | | | | | | | |
| | Bt-Y | D | E | N | P | V | V | H | F | F | K | N | I | V | T | P | R | T | P | P-$NH_2$ | | |
| Substitution | | | | | | | | | | | | | | | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | | |
| A84 | Ac-Y | A | E | N | P | V | V | H | F | F | K | N | I | V | T | P | R | T | P | P $NH_2$ | | | 84 |
| A85 | Ac-Y | D | A | N | P | V | V | H | F | F | K | N | I | V | T | P | R | T | P | P $NH_2$ | | | 85 |
| A86 | Ac-Y | D | E | A | P | V | V | H | F | F | K | N | I | V | T | P | R | T | P | P $NH_2$ | | | 86 |
| A87 | Ac-Y | D | E | N | A | V | V | H | F | F | K | N | I | V | T | P | R | T | P | P $NH_2$ | | | 87 |
| A88 | Ac-Y | D | E | N | P | A | V | H | F | F | K | N | I | V | T | P | R | T | P | P $NH_2$ | | | 88 |
| A89 | Ac-Y | D | E | N | P | V | A | H | F | F | K | N | I | V | T | P | R | T | P | P $NH_2$ | | | 89 |
| A90 | Ac-Y | D | E | N | P | V | V | A | F | F | K | N | I | V | T | P | R | T | P | P $NH_2$ | | | 90 |
| A91 | Ac-Y | D | E | N | P | V | V | H | A | F | K | N | I | V | T | P | R | T | P | P $NH_2$ | | | 91 |
| A92 | Ac-Y | D | E | N | P | V | V | H | F | A | K | N | I | V | T | P | R | T | P | P $NH_2$ | | | 92 |
| A93 | Ac-Y | D | E | N | P | V | V | H | F | F | A | N | I | V | T | P | R | T | P | P $NH_2$ | | | 93 |
| A94 | Ac-Y | D | E | N | P | V | V | H | F | F | K | A | I | V | T | P | R | T | P | P $NH_2$ | | | 94 |
| A95 | Ac-Y | D | E | N | P | V | V | H | F | F | K | N | A | V | T | P | R | T | P | P $NH_2$ | | | 95 |
| A96 | Ac-Y | D | E | N | P | V | V | H | F | F | K | N | I | A | T | P | R | T | P | P $NH_2$ | | | 96 |
| A97 | Ac-Y | D | E | N | P | V | V | H | F | F | K | N | I | V | A | P | R | T | P | P $NH_2$ | | | 97 |
| A98 | Ac-Y | D | E | N | P | V | V | H | F | F | K | N | I | V | T | A | R | T | P | P $NH_2$ | | | 98 |
| A99 | Ac-Y | D | E | N | P | V | V | H | F | F | K | N | I | V | T | P | A | T | P | P $NH_2$ | | | 99 |
| A100 | Ac-Y | D | E | N | P | V | V | H | F | F | K | N | I | V | T | P | R | A | P | P $NH_2$ | | | 100 |
| A101 | Ac-Y | D | E | N | P | V | V | H | F | F | K | N | I | V | T | P | R | T | A | P $NH_2$ | | | 101 |
| A102 | Ac-Y | D | E | N | P | V | V | H | F | F | K | N | I | V | T | P | R | T | P | A $NH_2$ | | | 102 |
| | | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | A | |

**Fig. 1.** Schematic representation of terminal truncated and alanine analogue MBP (88–102) peptides. **A:** Sequence of terminal truncation of MBP (83–102)$Y^{83}$ peptide. **B:** Sequence of various alanine substituted MBP (88–102)$Y^{83}$ peptides used in this study.

acid release [McConnell et al., 1992]. Unlike T-cell proliferation and interleukin-2 (IL-2) production, however, the extracellular acidification rate changes are early signaling events during antigen presentation. In fact, in the hierarchy of signaling events in T cells, acidification rate changes fall between partial calcium flux and full calcium flux [Rabinowitz et al., 1996]. Such a hierarchy can potentially help us understand T-cell agonist and antagonist activities of antigenic peptides, the minimum epitopes of T cells and

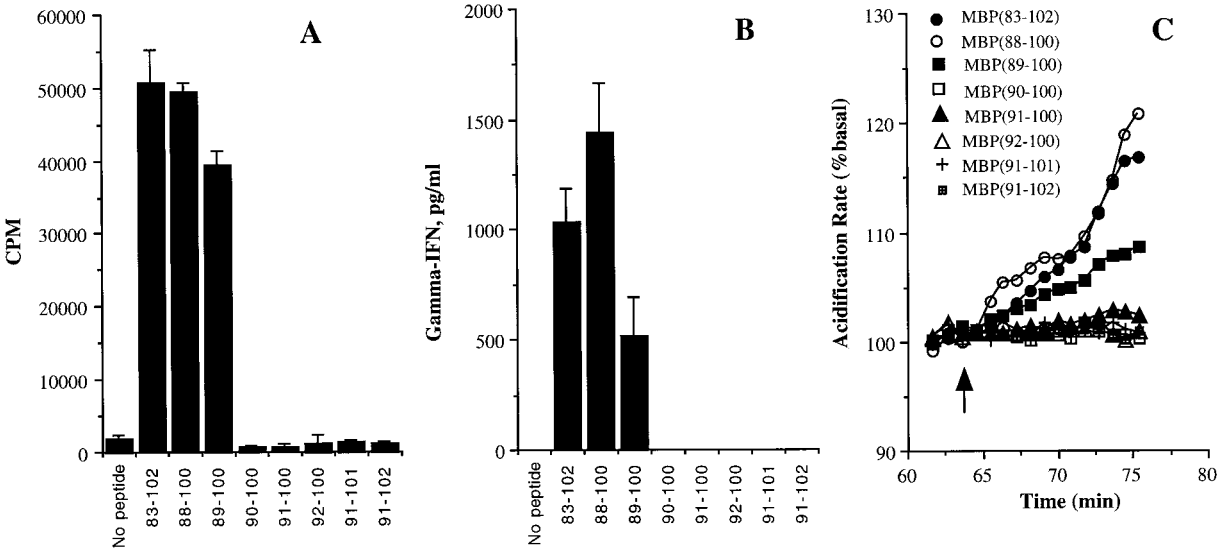


**Fig. 2.** Comparison of proliferation, interferon-γ (IFN-γ) response, and acidification rates of E11 cells with terminal truncated MBP peptides. Activation of E11 T-cell clones by terminal truncated peptides was shown by increase in cell proliferation, IFN-γ secretion and acidification rates. Proliferation was measured by $^{3}$H-thymidine incorporation, levels of IFN-γ by enzyme-linked immunosorbent assay (ELISA), and acidification rates by microphysiometer, as described under Materials and Methods. **A– C:** Proliferation, IFN-γ levels, and acidification rates of various truncated peptides.

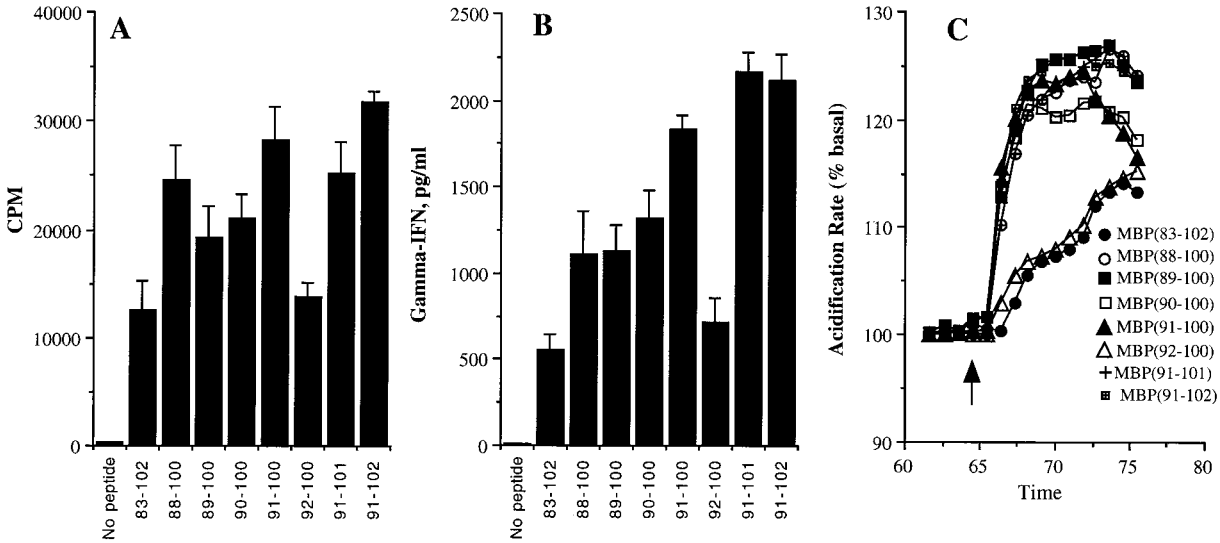


**Fig. 3.** Comparison of proliferation, interferon-γ (IFN-γ) response, and acidification rates of TOM 3A6 cells with terminal truncated MBP peptides. Activation of TOM 3A6 T-cell clones by terminal truncated peptides was shown by increase in cell proliferation, IFN-γ levels, and acidification rates. Proliferation was measured by $^{3}$H -thymidine incorporation, levels of IFN-γ by enzyme-linked immunosorbent assay (ELISA), and acidification rates by microphysiometer, as described under Materials and Methods. **A– C:** Proliferation, IFN-γ levels, and acidification rates, respectively, of various truncated peptides.

the contribution of individual amino acids in antigenic epitopes to T-cell activation.

Initially, the order of this hierarchy was investigated by measuring the effect of changes in antigen presentation and subsequent signaling events due to truncation and alanine substitution of the amino acid residues of parent antigenic peptide. In our earlier studies we showed that any changes in the antigenic peptide that led to a change in cytokine release

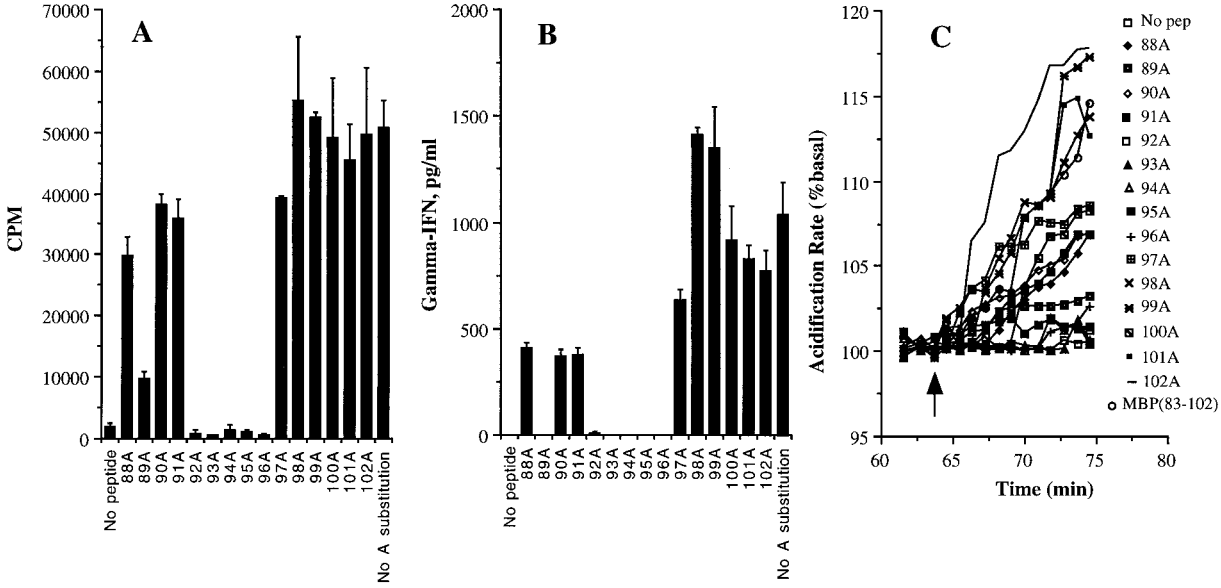


**Fig. 4.** Comparison of proliferation, interferon-γ (IFN-γ) response, and acidification rates of E11 cells with alanine substituted MBP peptides. Activation of E11 T-cell clones by alanine substituted peptides was shown by increase in cell proliferation, IFN-γ levels, and acidification rates. Proliferation was measured by $^{3}$H -thymidine incorporation, levels of IFN-γ by enzyme-linked immunosorbent assay (ELISA), and acidification rates by microphysiometer, as described under Materials and Methods. **A–C:** Proliferation, IFN-γ levels, and acidification rates, respectively, of various truncated peptides.

and T-cell proliferation is also reflected in acidification rate signal changes in immortalized HVS transformed human T-cell clones [Arimilli et al. 1998]. In this work, we confirmed our findings in T-cell clones and clearly show that the acidification rate signal changes can be used as a quick read-out in (1) perturbation in antigen presentation, (2) identification of minimum T-cell epitope, and (3) critical amino acid residues involved in MHC-Peptide-TCR interactions.

The peptides were acetylated at the N-terminus and amidated at the C-terminus to decrease the susceptibility of these peptides to protease degradation. The truncated and alanine substituted peptides used in in vitro experiments were >95% pure, as indicated by reverse-phase HPLC and mass spectrometry. The sequence of the peptides used in this study are listed in Figure 1A,B. E11 and TOM 3A6 human T-cell clones respond to the MBP (83–102)Y$^{83}$ parent peptide and are restricted to DRB1*1501 and DRB5*0101, respectively. Both DR alleles have been associated with MS [Todd et al., 1988; Spielman et al., 1982; Olerup et al., 1989]. MHC restriction of the two human T-cell clones was tested by using two different B cell transfectants having either DRB1*1501 or DRB5*0101 on their surface. These two MHC class II molecules show high affinity for binding to the parent peptide, MBP (83–102)Y$^{83}$. However, DRB1*0101 transfectants activate only TOM 3A6 clones, while DRB1* 1501 transfectants activate only E11 cells, in the presence of MBP (83–102)Y$^{83}$. This MHC restriction is seen in acidification rate signal changes in these T-cell clones (data not shown). In other words, although the acidification rate signal change is a nonspecific early T-cell activation marker, activation through the TCR ligation by a MHC peptide complex is certainly MHC restricted for a particular T-cell clone.

The results obtained in proliferation assays, IFN-γ production assays, and acidification rate signal changes in E11 cells by truncated peptides are presented in Figure 2. These results indicated that acidification rate changes match with the late signaling events such as proliferation and IFN-γ release induced by the parent peptide. In addition, the effect of truncated peptides MBP 88–100, 89–100 had little or no effect on T-cell stimulation, and the shorter peptides 90–100, 91–100, 92–100, 91–101, and 91–102 did not stimulate E11 T-cell clones, as indicated by the absence of proliferation and IFN-γ production. This is also shown by a lack

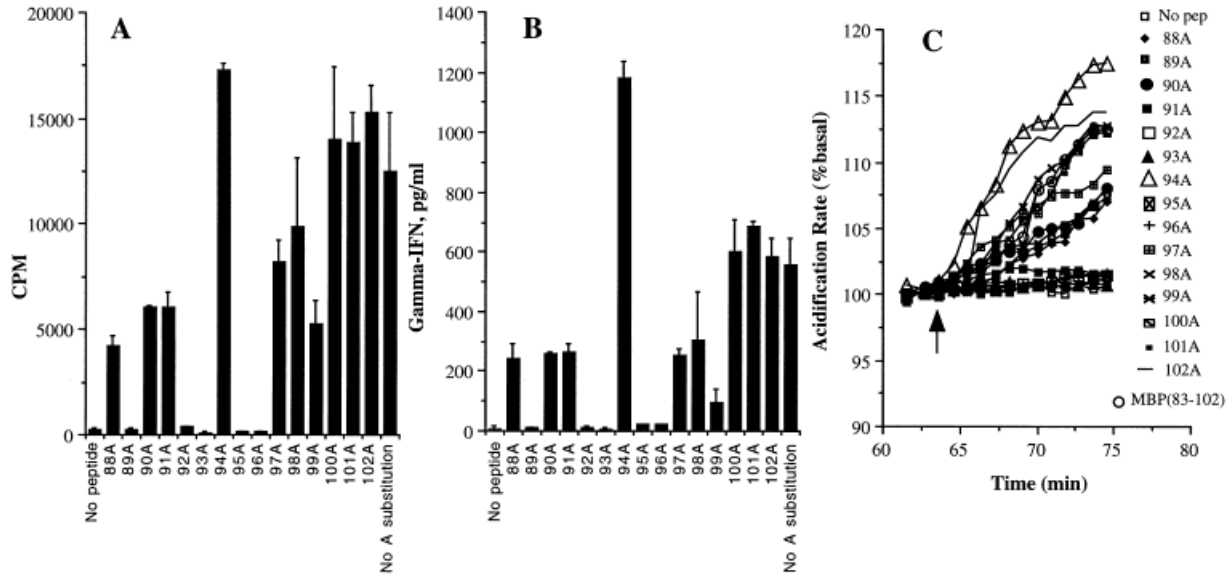


**Fig. 5.** Comparison of proliferation, interferon-γ (IFN-γ) response, and acidification rates of TOM 3A6 cells with alanine-substituted MBP peptides. Activation of TOM 3A6 T-cell clones by alanine substituted peptides was shown by increase in cell proliferation, IFN-γ secretion, and acidification rates. Proliferation was measured by $^{3}$H -thymidine incorporation, levels of IFN-γ by enzyme-linked immunosorbent assay (ELISA), and acidification rates by microphysiometer, as described under Materials and Methods. **A–C:** Proliferation, IFN-γ levels, and acidification rates, respectively, of various truncated peptides.

of acidification rate changes, after the trend seen in the late signaling events.

The DRB5*0101 restricted TOM 3A6 T-cell clones showed stimulation with all the truncated peptides. As shown in Figure 3, the proliferation and IFN-γ response signals are well correlated with acidification rate change signals. Truncation of parent peptide MBP (83–102)$Y^{83}$ had a positive effect on the T-cell stimulation, which is clearly reflected in all three assays. In fact, the parent peptide and MBP 92-100 showed less stimulation as compared to other peptides. An increase in the stimulation of T cells could be attributed to either a change in the MHC-peptide binding frame or MHC-peptide-TCR contact frame [Vogt et al., 1994]. In contrast to Vogt's findings, these T-cell clones can be stimulated by a nine amino acid peptide (MBP 92–100) as long as the peptide retains the MHC binding anchor residues [Vogt et al., 1994; Arimilli et al., 1998]. Peptide 91–100 showed an increase in the stimulation of T-cell clones as compared to 90–100 and 89–100. This finding suggests that the contribution of the added residues in 90–100 and 89–100 peptides have a negative effect if phenylalanine at position 92 is considered the anchor residue for binding to DRB5*0101. However, the added effect of the N -terminal residues before 91 in the parent peptide negatively affects T-cell stimulation.

To evaluate the role of each amino acid residue in the parent peptide in T-cell stimulation and correlate the effect seen in T-cell proliferation and cytokine production with the acidification rate changes, we made alanine peptide analogues of the MBP 88-102 peptide. Proliferation, IFN-γ response and acidification rate signal changes of E11 T-cell clones by alanine substituted peptides are shown in Figure 4. Alanine substitution at positions 89, 92, 93, 94, 95, 96 showed very little or no proliferation and IFN-γ production. Similarly, there was no extracellular acidification rate signal change for these peptides, indicating a substantial correlation between the acidification rate changes and the proliferation and IFN-γ production for the alanine substituted peptides. Residues 94 and 96 were previously shown as critical TCR contact residues for another DRB5*0101 and MBP (84–102) peptide restricted T-cell clone [Wucherpfennig et al., 1994]. In TOM3A6 clones, peptide residues at position 89, 92, 93, 95, and 96 are critical and this is reflected in all three assays (Fig. 5A,B,C). The negative effect of these alanine substituted peptides on T-cell activation is due to either the lack of peptide binding to the MHC class II on the surface of

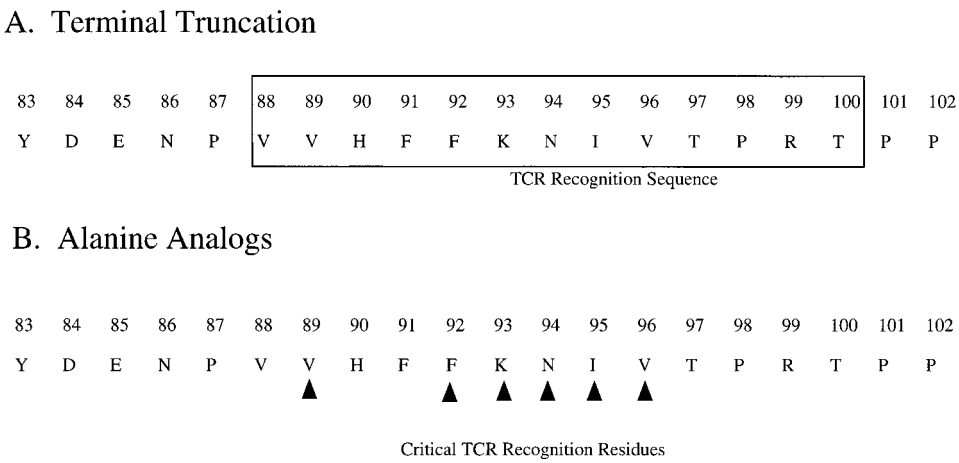


**Fig. 6.** Schematic representation of critical DR2 binding and TCR contact residues in E11 T-cell clones. **A:** Core TCR recognition sequence. **B:** Critical TCR recognition residues (arrows).

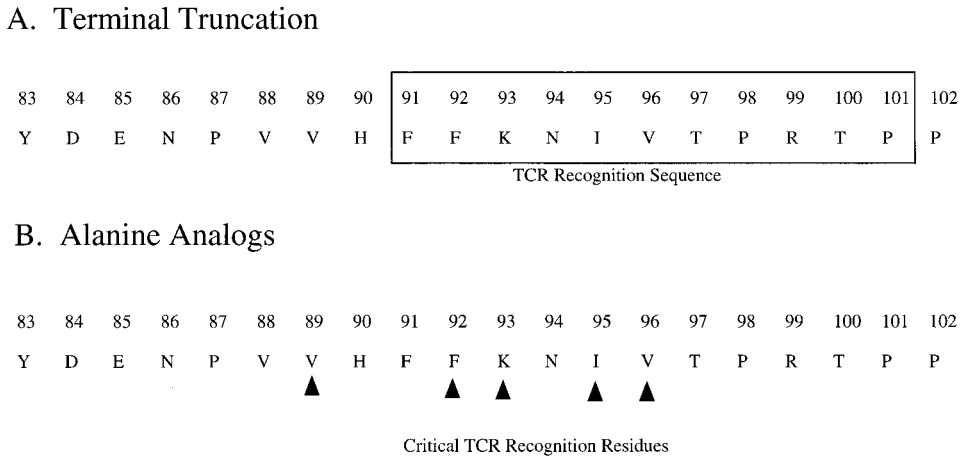


**Fig. 7.** Schematic representation of critical DR2 binding and TCR contact residues in TOM 3A6 T-cell clones. **A:** Core TCR recognition sequence. **B:** Critical TCR recognition residues (arrows).

the APCs or the alteration of TCR contact residues on the peptide.

Studies using truncated peptides in the three assays described above, indicated that the core or minimum sequence recognized by the E11 T cells is MBP 88–100 (Fig. 6A). By contrast, data from alanine-substituted peptides in E11 cells suggested that peptide amino acid positions 89, 92, 93, 94, 95, and 96 are important TCR contact residues in antigen presentation (Fig. 6B). Similarly, Figure 7A represents the core TCR recognition sequence as MBP (91–101) in TOM 3A6 cells and amino acid residues 89, 92, 93, 95, and 96 as critical contact residues (Fig. 7B).

In conclusion, with the help of MHC peptide-binding studies or the common peptide-binding motifs for MHC found in the literature, coupled with acidification rate signal change studies, one can predict the TCR contact residues in a given peptide ligand [Vogt et al., 1994]. In other words, the effect of alanine-substituted peptides on antigen presentation leading to TCR ligation can be measured rapidly and easily by using the extracellular acidification rate signal changes. Furthermore, core recognition sequences can be determined using truncated peptides. The information obtained by these experiments can help design and screen biologically active TCR agonists or antagonists that

could potentially alter the course of T-cell activation in immune disorders.

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## REFERENCES

Allen IV. 1991. Pathology of multiple sclerosis. In: Matthews WB, Acheson ED, Batchelor JR, Weller RO editors. McAlpine's multiple sclerosis. Edinburgh: Churchill Livingstone. p 341.

Allen IV, Cosb SL, Kirk J, Martin SJ, Dinsmore S. 1987. Neuropathology and neurovirulence of canine distemper virus plaque isolates in the hamster. Neuropathol Appl Neurobiol 13:349–369.

Arimilli S, Cardosa C, Mukku P, Baichwal V, Nag B. 1995. Refolding and reconstitution of functionally active complexes of human leukocyte antigen DR2 and myelin basic protein peptide from recombinant alpha and beta polypeptide chains. J Biol Chem 270:971–977.

Arimilli S, Deshpande SV, Nag B. 1998. Identification of core structure and critical T cell receptor contact residues in an antigenic peptide by measuring acidification rates. J Immunol Methods 212:49–60.

Buus S, Sette A, Colon SM, Miles C, Grey HM. 1987. The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. Science 235:1353–1358.

Luu T, Pham S, Deshpande S. 1996. Automated multiple peptide synthesis: improvements in obtaining quality peptides. Int J Peptide Protein Res 47:91–97.

Martin R, McFarland HF, McFarlin DE. 1992a. Immunological aspects of demyelinating diseases. Annu Rev Immunol 10:153–187.

Martin R, Utz U, Coligan JE, Richert JR, Flerlage M, Robinson E, Stone R, Biddison WE, McFarlin DE, McFarland HF. 1992b. Diversity in fine specificity and T cell receptor usage of the human $CD4^+$ cytotoxic T cell response specific for the immunodominant myelin basic protein peptide 87–106. J Immunol 148:1359–1366.

McConnell HM, Owicki JC, Parce JW, Miller DL, Baxter GT, Wada G, Pitchford S. 1992. The cytosensor microphysiometer: biological applications of silicon technology. Science 257:1906–1912.

McConnell HM, Wada, HG, Arimilli S, Fok KS, Nag B. 1995. Stimulation of T cells by antigen-presenting cells is kinetically controlled by antigenic peptide binding to major histocompatibility complex class II molecules. Proc Natl Acad Sci USA 92:2750–2754.

McCutcheon M, Wehner N, Wensky A, Kushner M, Doan S, Hsiao L, Calabresi P, Ha T, Tran TV, Winkelhake J, Spack EG. 1997. A sensitive ELISPOT assay to detect low-frequency human T lymphocytes. J Immunol Methods 210:149–166.

Nag B, Wada HG, Fok KS, Green DJ, Sharma SD, Clark BR, Parce JW, McConnell HM. 1992. Antigen-specific stimulation of T cell extracellular acidification by MHC class II-peptide complexes. J Immunol 148:2040–2044.

Nag B, Wada HG, Passmore D, Clark BR, Sharma SD, McConnell HM. 1993. Purified beta chain of MHC class II binds to CD4 molecules on transfected HeLa cells. J Immunol 150:1358–1364.

Olerup O, Hillert J, Fredrikson S, Olsson T, Kam-Hansen S, Moeller E, Carlsson B, Wallin J. 1989. Primary chronic progressive and relapsing/remitting multiple sclerosis: two immunogenetically distinct disease entities. Proc Natl Acad Sci USA 86:7113–7117.

Ota K, Matsui M, Milford, EL, Mackin GA, Weiner HL, Hafler DA. 1990. T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 346:183–187.

Rabinowitz JD, Beeson C, Wulfing C, Tate K, Allen PM, Davis MM, McConnell HM. 1996. Altered T cell receptor ligands trigger a subset of early T cell signals. Immunity 5:125–135.

Renschler MF, Wada HG, Fok KS, Levy R. 1995. B-lymphoma cells are activated by peptide ligands of the antigen binding receptor or by anti-idiotypic antibody to induce extracellular acidification. Cancer Res 55:5642–5647.

Spielman RS, Nathenson N. 1982. The genetics of susceptibility to multiple sclerosis. Epidemiol Rev 4:45–65.

Todd JA, Acha-Orbea H, Bell JI, Chao N, Frone Z, Jacob CO, McDermott M, Sinha AA, Timmerman L, Steinman L, McDevitt HO. 1988. A molecular basis for MHC class II-associated autoimmunity. Science 240:1003–1009.

Vogt AB, Kropshofer H, Kalbacher H, Kalbus M, Rammensee HG, Coligan JE, Martin R. 1994. Ligand motifs of HLA-DRB5*0101 and DRB1*1501 molecules delineated from self-peptides. J Immunol 153:1665–1673.

Weiss A, Imboden J, Shoback D, Stobo J. 1984a. Role of T3 surface molecules in human T cell activation: T3-dependent activation results in an increases in cytoplasmic free calcium. Proc Natl Acad Sci USA 81:4169–4173.

Weiss MJ, Daley JF, Hodgdon JC, Reinherz EL. 1984b. Calcium dependency of antigen-specific (T3-Ti) and alternative (T11) pathways of human T-cell activation. Proc Natl Acad Sci USA 81:6836–6840.

Wucherfenning KW, Weiner HL, Hafler DA. 1991. T-cell recognition of myelin basic protein. Immunol Today 12: 277–282.

Zamvil SS, Steinman L. 1990. The T lymphocyte in experimental allergic encephalomyelitis. Annu Rev Immunol 8:579–621.