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Characterization of murine T-cell epitopes on mycobacterial DNA-binding protein 1 (MDP1) using DNA vaccination

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Abstract

Mycobacterial DNA binding protein 1 (MDP1) is a major protein antigen in mycobacteria and induces protective immunity against *Mycobacterium tuberculosis* infection in mice. In this study we determined murine T-cell epitopes on MDP1 with MDP1 DNA immunization in mice. We analyzed interferon- γ production from the MDP1 DNA-immune splenocytes in response to 20-mer overlapping peptides covering MDP1 protein. We identified several CD4⁺ T-cell epitopes in three inbred mouse strains and one CD8⁺ T-cell epitope in C57BL/6 mice. These T-cell epitopes would be feasible for analysis of the role of MDP1-specific T cells in protective immunity and for future vaccine design against *M. tuberculosis* infection.

Key words: DNA immunization; T-cell epitope; *Mycobacterium tuberculosis*

1. Introduction

Tuberculosis (TB) has been one of the most serious infectious diseases in the world. There were estimated 9.2 million new causes and 1.7 million deaths from TB in 2006 [1]. One third of people in the world have been infected with *Mycobacterium tuberculosis* (Mtb), the causative agent of TB. Multidrug-resistant TB and co-infection of Mtb with human immunodeficiency virus are recent problems [1].

The only TB vaccine currently available is the attenuated *Mycobacterium bovis* strain Bacillus Calmette-Guérin (BCG). Although the BCG vaccine is the oldest and the most widely used vaccine [2], the effect of which has been questioned for preventing pulmonary TB in adults [3] and also to wane with time since vaccination [4]. Therefore, the improved vaccine is an urgent need against TB [5].

Cell-mediated immunity plays a pivotal role in the control of Mtb infection [6, 7]. There is mounting evidence that CD4⁺ type 1 helper T (Th1) cells are involved in the development of resistance to the disease, primarily through the production of macrophage-activating cytokines such as interferon (IFN)- γ and tumor necrosis factor (TNF)- α . In addition, CD8⁺ cytotoxic T lymphocytes (CTL) contribute to disease resistance since susceptibility to Mtb is increased in mice with deficient in CD8⁺ T cells [8].

Identification of protective antigens is a crucial step to develop effective vaccines against TB. Many protective antigen candidates have been reported. They include, secreted and membrane-bound proteins, virulence factors such as PE/PPE or EsX, or proteins expressed in host macrophages [9, 10]. Since Mtb causes both acute disease and asymptomatic latent infection, antigens expressed in the dormant state have been also focused as target antigens for therapeutic vaccination of latent tuberculosis. Persisting bacilli resides within the hypoxic environment of the lung granulomas. Therefore, it is generally accepted that low oxygen tension induces

dormancy program of Mtb. Proteins expressed at the dormant stage include, two-component response regulator, dormancy survival regulator (*DosR*; Rv3133c) [11] and at least 20 other proteins encoded by the *DosR* regulon [12].

MDP1 is a major cellular protein of slow growers of mycobacteria [13]. The cellular content of MDP1 in mycobacterial cells increases at stationary and low-oxygen tension-induced non-replicating dormant phases [14]. MDP1 is a histone-like DNA-binding protein binding to GC-rich DNA and considered to control gene expression in mycobacteria [13, 15]. MDP1 has an activity to suppress the growth rate of bacteria presumably by inhibiting macromolecular biosyntheses [16]. Recently, Lewin and colleagues [17] showed that reduction of MDP1 expression by antisense plasmid increased the growth of BCG in both broth culture and macrophages. They showed that the antisense DNA inhibited the aggregation of BCG and reduced expression of several proteins in hypoxic condition as well, suggesting a role of MDP1 in regulation of gene expression in dormant bacilli. In addition to the role in the cytoplasm, MDP1 is exported by unknown mechanism to the cell wall and control the mycolic acid transfer [18] and mycobacterial adherence to lung epithelial cells [19]. Thus, MDP1 is a pleiotropic protein which has strong impacts on the mycobacterial virulence.

MDP1 has been also reported to be one of immunocompetent antigens. Prabhakar and colleagues [20] identified this protein as an immunodominant protein in human healthy contacts with TB patients through T-cell blot assay. They designated this protein as histone-like protein of Mtb (HLP_{Mtb}), which is the same molecule as MDP1. Matsumoto and colleagues [21] found that CpG DNA enhances immunogenicity of MDP1, such as productivity of TNF- α and IL-6 from mouse macrophages. They showed that co-immunization of BALB/c and C3H/He mice with MDP1 and Mtb DNA elicited IFN- γ production specific for this protein and caused reduction of the bacterial burden following Mtb challenge [21].

DNA vaccination with gene gun bombardment is a reliable method to induce reproducible

T-cell responses [22] and has been used for identification of T-cell epitopes of Mtb antigens, antigen (Ag) 85A [23-25], Ag85B [25], Ag85C [25], MPT51 [26, 27], and *DosR* regulon-encoded proteins [28]. Here, we identified murine T-cell epitopes on MDP1 with a strategy using inbred mouse strains, gene gun immunization with expression plasmid DNA encoding MDP1, overlapping synthetic peptides spanning the entire mature MDP1 amino acid (aa) sequence, and major histocompatibility complex (MHC) binding peptide prediction algorithms.

2. Materials and methods

2.1. Animals

Inbred mouse strains, BALB/c, C57BL/6, and C3H/He, were purchased from Japan SLC (Hamamatsu, Japan). The mice were kept under specific pathogen-free conditions and fed autoclaved food and water *ad libitum* at the Institute for Experimental Animals of the Hamamatsu University School of Medicine. Two to three-month-old female mice were used in all experiments. Animal experiments were performed according to the Guidelines for Animal Experimentation, Hamamatsu University School of Medicine.

2.2. Plasmid

The DNA encoding MDP1 molecule was inserted between EcoRI and Xho I sites located downstream of cytomegalovirus immediate-early enhancer/promoter region of eukaryotic expression plasmid, pCI (Promega, Madison, WI, USA). The integrity of the nucleotide sequence was validated by automated DNA sequencing with ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA) using a dye primer cycle sequencing kit (Applied Biosystems).

2.3. Peptides

Peptides spanning the entire MDP1 aa sequence of BCG (205 aa residues) were synthesized as 20-mer peptides overlapping by 10 residues, with the exception of P6 (p46-65) and the carboxyl-terminal P21 (p186-205) ([15], Fig. 1). MDP1 p23-31 and p46-60 peptides were synthesized by Hayashi kasei (Osaka, Japan). All peptides were dissolved in phosphate-buffered saline (PBS) at a concentration of 10 mg ml⁻¹ and stored at -80°C until use.

2.4. Prediction of T-cell epitopes by MHC binding peptide prediction algorithms

For the prediction of murine T-cell epitopes, following MHC binding peptide prediction algorithms were used through their web sites. These are, National Institutes of Health BioInformatics and Molecular Analysis Section (BIMAS) ([29], http://bimas.dcert.nig.gov/cgi-bin/molbio/ken_parker_comboform), SYFPEITHI program ([30], <http://www.syfpeithi.de/>), and RANKPEP program ([31], <http://bio.dfci.harvard.edu/Tools/rankpep.html>).

2.5. Immunization of mice

For DNA immunization with Helios gene gun system (Bio-Rad Laboratories, Hercules, CA, USA), preparation of the cartridge of DNA-coated gold particle cartridge was followed to the manufacturer's instruction manual. Finally, 0.5 mg of gold particles was coated with 1 µg of plasmid DNA and the injection was carried out with 0.5 mg gold per shot once. Mice were injected with 1 µg of plasmid DNA four times at 1-week intervals. Mice were also immunized subcutaneously with 10⁶ CFU of BCG (Tokyo strain) twice at a 2-week interval.

2.6. Preparation of splenocyte culture supernatants

Spleen cells were harvested from mice. Recovered cells were cultured with RPMI medium

supplemented with 10% fetal calf serum in 96-well plates at 2×10^6 cells per well in the presence or absence of $5 \mu\text{g ml}^{-1}$ of each MDP1 peptide at 37°C with 5% CO_2 atmosphere. Supernatants were harvested 48 h later and stored at -20°C until they were assayed. Concentration of IFN- γ in the culture supernatants was determined by a sandwich enzyme-linked immunosorbent assay (ELISA)

2.7. Quantification of IFN- γ with ELISA

The 96-well ELISA plates (EIA/RIA Plate A/2; Costar, Cambridge, MA) were coated with $2 \mu\text{g ml}^{-1}$ of capture antibody (Ab) (anti-murine IFN- γ monoclonal Ab [mAb] R4-6A2; BD Biosciences, San Jose, CA, USA) at 4°C overnight, washed with PBS supplemented with 0.05% Tween 20 (PBS-Tween), and blocked with Block One Blocking solution (Nakalai Tesque, Kyoto, Japan) at room temperature for 45 min. After washed with PBS-Tween, the culture supernatants were added to the plates and the plates were incubated at 4°C overnight. After washed with PBS-Tween, $0.5 \mu\text{g ml}^{-1}$ of biotin-labeled anti-murine IFN- γ mAb XMG1.2 (BD Biosciences) was added to the plates, and the plates were incubated for 1 h at room temperature. After washed with PBS-Tween, horseradish peroxidase-conjugated avidin (Bio-Rad Laboratories) was added and incubated for 30 min at room temperature. After washed, the plates were added with TMB one component HRP microwell substrate (BioFX laboratories, Owings Mills, MD, USA) to detect bound horseradish peroxidase-conjugated streptavidin. After 5 min, the absorbance of each well was measured at 630 nm using an EZS-ABS Microplate Reader (Asahi Techno Glass Tokyo, Japan).

2.8. Depletion of CD4 $^+$ or CD8 $^+$ T-cell subsets

CD4 $^+$ or CD8 $^+$ T-cell subsets of peptide-reactive T cells were determined by depletion of CD4 $^+$ or CD8 $^+$ T cells, respectively. We used BD IMag system (BD Biosciences). Briefly,

spleen cells was mixed thoroughly with anti-mouse CD4 particles-DM or anti-mouse CD8a particles-DM (BD Biosciences, 50 μ l particles for 10^7 cells) and placed at 4°C for 30 min. The labeled cells were placed on the BD IMagnet and incubated for 8 min. Supernatant was carefully removed. This supernatant contains the cell fraction which was depleted CD4+ or CD8+ T cells.

2.9. MHC stabilization assay

MHC stabilization assay is originally described in Ljunggren and colleagues [32]. RMA-S cells ([33], 10^6 cells/well) were cultured at 26°C overnight and were then incubated for 1 h in the presence or absence of peptide (10, 50, or 100 μ M). The cells were then transferred to 37°C for 2 h and washed with FACS buffer, and cell surface expression of H2-D^b molecules was detected by flow cytometry by using phycoerythrin (PE)-conjugated mouse MAbs specific for H2-K^dD^b (28-14-8; eBioscience, San Diego, CA, USA). The results were expressed as the mean fluorescence intensity (MFI) ratio, which was determined as follows: MFI ratio = (MFI observed in the presence of peptide at 37°C — MFI observed in the absence of peptide at 37°C) / (MFI observed in the absence of peptide at 26°C) \times 100 (%).

2.10. Statistics

Data from multiple experiments were expressed as the means \pm S.E.M. Data were analyzed with Student's unpaired t test. *p* value of 0.05 or less was considered significant.

3. Results

3.1. IFN- γ production in response to overlapping synthetic peptides from MDP1 by splenocytes of pCI-MDP1 DNA-immune mice

Splenocytes from mice immunized with DNA vaccine encoding MDP1 (pCI-MDP1) were

stimulated with the overlapping MDP1 peptides for 48 h and IFN- γ concentration of culture supernatants was measured by ELISA. As shown in Fig. 2A, robust IFN- γ production was observed in splenocytes from MDP1 DNA-immune C57BL/6 mice (H2^b haplotype) in the presence of peptides P3 (aa 21 to 40), P9 (aa 71 to 90), and P11 (aa 91 to 110). Similarly, significantly higher IFN- γ production from splenocytes of MDP1 DNA-immune BALB/c (H2^d haplotype) and C3H/He (H2^k haplotype) mice was observed in response to two peptides, P5 (aa 41 to 60), P6 (aa 46 to 65) and three peptides, P5 (aa 41 to 60), P13 (aa 111 to 130), and P16 (aa 141 to 160), respectively (Fig. 2B and C). In C3H/He mice, P11 (aa 91 to 110) and P12 (aa 101 to 120) could induce relatively high IFN- γ production, but the value were not statistically significant.

In order to examine whether the same peptide induce IFN- γ following natural mycobacterial infection, splenocytes from mice immunized with BCG were examined for IFN- γ production in response to MDP1 peptides. Two peptides, P5 and P6, also induced significant IFN- γ production from splenocytes of BCG-immune BALB/c mice (Fig. 2B), but the level of IFN- γ produced in BCG-immune mice was lower than that in MDP1 DNA-immune mice. We were not able to detect significant IFN- γ production from splenocytes of BCG-immune C57BL/6 and C3H/He mice (Fig. 2A and C). In this experimental condition, DNA immunization with MDP1 DNA was superior to BCG vaccination in terms of IFN- γ production level from splenocytes.

3.2. T-cell subset analysis of T cells by the depletion of CD4⁺ or CD8⁺ T cells

Next, we examined which T-cell subsets responding to MDP1 peptides. CD4⁺ or CD8⁺ T cells were removed with magnetic beads and residual cells were stimulated with MDP1 peptides and resultant IFN- γ production was compared. As shown in Fig. 3A, IFN- γ production from splenocytes of C57BL/6 mice in response to P3 (aa 21 to 40) was significantly decreased by depleting CD8⁺ T-cell subset. In contrast, IFN- γ production in response to P9 (aa 71 to 90) and P11 (aa 91 to 110) was decreased by depleting CD4⁺ T cells. These results indicate that P3

contains CD8⁺ T-cell epitope, and P9 and P11 contain CD4⁺ T-cell epitopes. Intracellular IFN- γ staining results showed that IFN- γ -producing CD8⁺, but not CD4⁺ T cells were observed in response to P3 in C57BL/6 mice. And IFN- γ -producing CD4⁺, but not CD8⁺ T cells were observed in response to P9 and P11 (data not shown). Since C57BL/6 mice have a deletion of H2-E α gene and do not express H2-E molecules on the cell surface [34], two CD4⁺ T-cell epitopes in these peptides are exclusively considered to be presented on H2-A^b.

In BALB/c mice, IFN- γ production in the presence of P5 (aa 41 to 60) and P6 (aa 46 to 65) was significantly reduced by depleting CD4⁺ T cells. Similarly, IFN- γ production in the presence of P5 (aa 41 to 60), P13 (aa 111 to 130), or P16 (aa 141 to 160) was significantly reduced by depleting CD4⁺ T cells in C3H/He mice (Fig. 3B and 3C). These results indicate that these peptide regions contain CD4⁺ T-cell epitopes.

3.3. Identification of minimal T-cell epitopes in the responsive peptide regions of MDP1

Generally, CD8⁺ T cells recognize peptides of 8 to 11 aa residues on MHC class I molecules and CD4⁺ T cells recognize peptides of 12 to 18 aa residues on MHC class II molecules. Several MHC binding peptide prediction algorithms are available on internet. We employed BIMAS and SYFPEITHI programs for prediction of CD8⁺ T-cell epitope(s) in P3 in C57BL/6 mice and RANKPEP program for CD4⁺ T-cell epitope(s) in P5 and P6 regions in BALB/c mice (Table 1). MDP1 p23-31 9mer peptide (AAVENVVDVT) in P3 region showed the highest score (108) for H2-D^b binding in BIMAS program (Table 1). In BALB/c mice, RANKPEP algorithm predicted MDP1 p52-60 as the core motif of H2-A^d-restricted CD4⁺ T-cell epitope (Table 1). Since both P5 (p41-60) and P6 (p46-65) peptides let the immune splenocytes produce IFN- γ , we prepared overlapping 15mer peptide (p46-60) and examined the capacity to induce IFN- γ production from immune splenocytes.

As shown in Fig. 4A, CD8⁺ T cells of MDP1 DNA-immune C57BL/6 mice produced

significant amounts of IFN- γ in response to MDP1 p23-31 peptide. Splenocytes of the immune mice in the absence of the peptide and splenocytes of naïve C57BL/6 mice with or without the peptide did not produce significant level of IFN- γ (data not shown). Similarly, CD4⁺ T cells of MDP1 DNA-immune BALB/c mice produced significant amounts of IFN- γ in response to MDP1 p46-60 peptide (Fig. 4B). Splenocytes of the immune mice in the absence of the peptide and splenocytes of naïve BALB/c mice with or without the peptide did not produce significant level of IFN- γ (data not shown). These results indicate that MDP1 p23-31 is a minimal *bona fide* CD8⁺ T-cell epitope in C57BL/6 mice and MDP1 p46-60 is CD4⁺ T-cell epitope in BALB/c mice, respectively.

As for C3H/He mice, RANKPEP program predicted that MDP1 p41-60 contains MDP1 p46-54 peptide which showed the highest score (10.8) in MDP1 peptides presented on H2-A^k. However, the score was less than the peptide binding threshold value (14.2), suggesting no binding. The SYFPEITHI program, which has the prediction program for H2-A^k and E^k, predicted MDP1 p44-58 15mer peptide as H2-E^k binder with the highest score (24) in the program (Table 1). RANKPEP program also predicted three core 9mer peptides restricted by H2-E^k, MDP1 p113-121, p116-124, and p121-129 in P13 (p111-130) region and two core peptides restricted by H2-E^k, MDP1 p142-150 and p145-153 in P16 (p141-160) region, respectively (Table 1).

3.4. Identification of an MHC class Ia restriction molecule for MDP1 p23-31 in C57BL/6 mice

Since MDP1 p23-31 was found to be a CD8⁺ T-cell epitope for C57BL/6 mice, we examined MHC binding assay to determine H2 restriction molecule for the peptide. As shown in Fig. 5, The MFI ratio of PE-conjugated anti-H2-D^b mAb increased in the presence of 10 μ M of MDP1 p23-31 peptide. This value further increased up to 42.5% in the presence of 100 μ M of the peptide. This result confirmed that MDP1 p23-31 peptide does bind to H2-D^b.

4. Discussion

T cells play pivotal role in induction of protective immunity against intracellular pathogens such as Mtb [6, 7]. The protective immunity induced by MDP1 immunization would be mainly attributable to T-cell responses evoked by the immunization. We here determined murine T-cell epitopes of MDP1, which would give the concrete basis of the protective immunity by MDP1 immunization. The peculiar immunogenic feature of MDP1 is DNA-dependent augmentation of antigenicity. MDP1 elicited protective immune responses when it was vaccinated to BALB/c and C3H/He mice with genomic DNA derived from *M. tuberculosis* [21]. Up-regulation of antigen-presenting cell functions induced by the interaction between MDP1 and CpG DNA was suggested in the protective immune responses [21]. CpG DNA is a key component of DNA vaccines for evoking significant immune responses against antigens. Therefore, we considered that DNA vaccination of MDP1 induce substantial immune responses, because produced MDP1 proteins may bind to CpG DNA in plasmid backbone and enhance the adjuvant effects.

In this study, we found at least seven T-cell epitope candidates peptides upon MDP1 DNA immunization. By contrast, only one peptide region (MDP1 p41-65) was found with *M. bovis* BCG vaccination (Fig. 2B). Several reasons for this difference would be speculated. First, MDP1 localizes in the cytoplasmic space, or is tightly attached to the cell wall of the live *Mycobacterium*. Non-secreted antigens like MDP1 would be difficult to be immunoreactive in the form of BCG vaccine. Second, BCG vaccine has been reported to be inefficient in terms of MHC class I antigen presentation. The fact may also cause the difference in the T-cell responses. Other possible explanation is that living mycobacteria have the mechanism to hide immunogenicity of MDP1, because strong immune response to MDP1 causes bactericidal host response.

In C57BL/6 mice, we found one CD8⁺ T-cell epitope, MDP1 p23-31 and two CD4⁺ T-cell

epitopes, MDP1 p71-90 and MDP1 p91-110. MDP1 p23-31 (AAVENVVDT) was speculated to be presented on H2-D^b with prediction algorithms and this was confirmed with MHC binding assay. Reported dominant peptide binding motif of H2-D^b consists of asparagine at position 5 (P5) and hydrophobic C-terminal residue such as isoleucine or leucine (P9 or P10) [35, 36]. P5 of MDP1 p23-31 is asparagine, but the C-terminal residue does not fit the motif. P5 (asparagine) and P10 (isoleucine) of MDP1 p23-32 fit the motif, suggesting that MDP1 p23-32 peptide also works as CD8+ T-cell epitope.

In BALB/c mice, MDP1 p46-60 was identified as H2-A^d-restricted CD4+ T-cell epitope. Since both P5 (p41-60) and P6 (p46-65) peptides, but not P7 (p51-70) peptide, let the immune splenocytes produce IFN- γ , we reasoned that the N-terminal aa residues (p46-50) are critical for the function. In C3H/He mice, at least three CD4+ T-cell epitopes were identified. RANKPEP or SYFPEITHI programs predicted P5 (p41-60), P13 (p111-130), and P16 (p141-160) bind to H2-E^k.

In conclusion, we identified murine T-cell epitopes of MDP1, an immunogenic major cellular mycobacterial protein causing protective immunity. We identified several CD4+ T-cell epitopes in three inbred mouse strains and one CD8+ T-cell epitope in C57BL/6 mice. Previously, we reported murine T-cell epitopes of MPT51, which is one of major secreted mycobacterial proteins at acute phase TB. MDP1 is a very abundant cellular protein and has been reported to be even up-regulated in dormant stage mycobacteria. Therefore, T-cell epitopes of MPT51 and MDP1 would be feasible for analysis of T-cell responses in different stage Mtb and for future TB vaccine design.

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Figure legends

Fig. 1. Schematic representation of the 21 overlapping synthetic peptides from MDP1 of BCG. All peptides covering entire MDP1 of BCG (205 aa residues) were synthesized as 20-mer molecules overlapping by 10 amino acids with the neighboring peptides.

Fig. 2. IFN- γ production from splenocytes stimulated with overlapping peptides of MDP1. Inbred mice [C57BL/6, (A), BALB/c (B) and C3H/He (C)] were immunized with plasmid DNA encoding MDP1 using gene gun four times at 1-week interval (filled bars) or with *M. bovis* BCG two times at a 2-week interval (hatched bars). The splenocytes (2×10^6) were stimulated with overlapping peptides ($5 \mu\text{g ml}^{-1}$) 2 weeks after the last immunization. Naïve mice (open bars) were used as controls. IFN- γ concentration of supernatant was analyzed by sandwich ELISA 48 h later. The means \pm S.E.M. from three (C57BL/6, BALB/c) or six (C3H/He) mice are shown. Asterisks (*) indicate $p < 0.05$ compared with the value without peptide (-) with Student's unpaired *t* test.

Fig. 3. Analysis of T-cell subsets responsive to MDP1 peptides. Mice were immunized with MDP1 DNA on the same schedule as Fig. 2. Splenocytes were treated with magnetic beads specific for CD4 or CD8. Cells (2×10^6) of the negative fraction were stimulated with peptides ($5 \mu\text{g ml}^{-1}$). Amounts of IFN- γ in the supernatant were analyzed by sandwich ELISA 48 h later. The mean \pm S.E.M. of three mice are shown. ** $p < 0.01$, * $p < 0.05$ (Student's unpaired *t* test).

Fig. 4. IFN- γ production from T cells in the presence of predicted peptides, MDP1 p23-31 and p46-60 peptides. C57BL/6 and BALB/c mice were immunized with MDP1 DNA and the immune splenocytes were treated with magnetic beads specific for CD4 or CD8 α and purified the

negative fraction. Cells (2×10^6) of the fraction were stimulated with $5 \mu\text{g ml}^{-1}$ of MDP1 p23-31 and MDP1 p46-65. IFN- γ in the culture supernatant was analyzed by sandwich ELISA 48 h later. The mean \pm S.E.M. of three mice are shown. Asterisks (*) indicate $p < 0.05$ compared with the value without peptide (-) with Student's unpaired t test.

Fig. 5. MHC binding assay of MDP1 p23-31 to H2-D^b. The ability of MDP1 p23-31 for binding to H2-D^b was measured by determining the stabilization of class I molecules on the surfaces of TAP2-deficient RMA-S. RMA-S cells (10^6 cells/well) were cultured at 26°C overnight and then were incubated for 1 h in the presence or absence of peptide (10, 50 or 100 μM). The cells were then transferred to 37°C for 2 h and washed with FACS buffer, and cell surface expression of H2-D^b was detected by flow cytometry by using a phycoerythrin-conjugated mAb specific for H2-K^dD^b. The results were expressed as MFI ratio \pm SD.

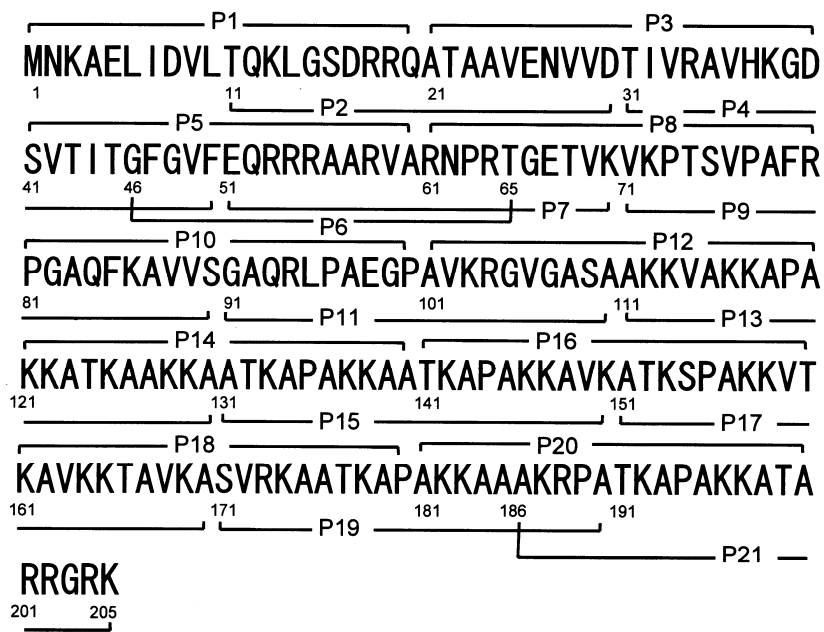


Fig. 1, Suzuki et al.

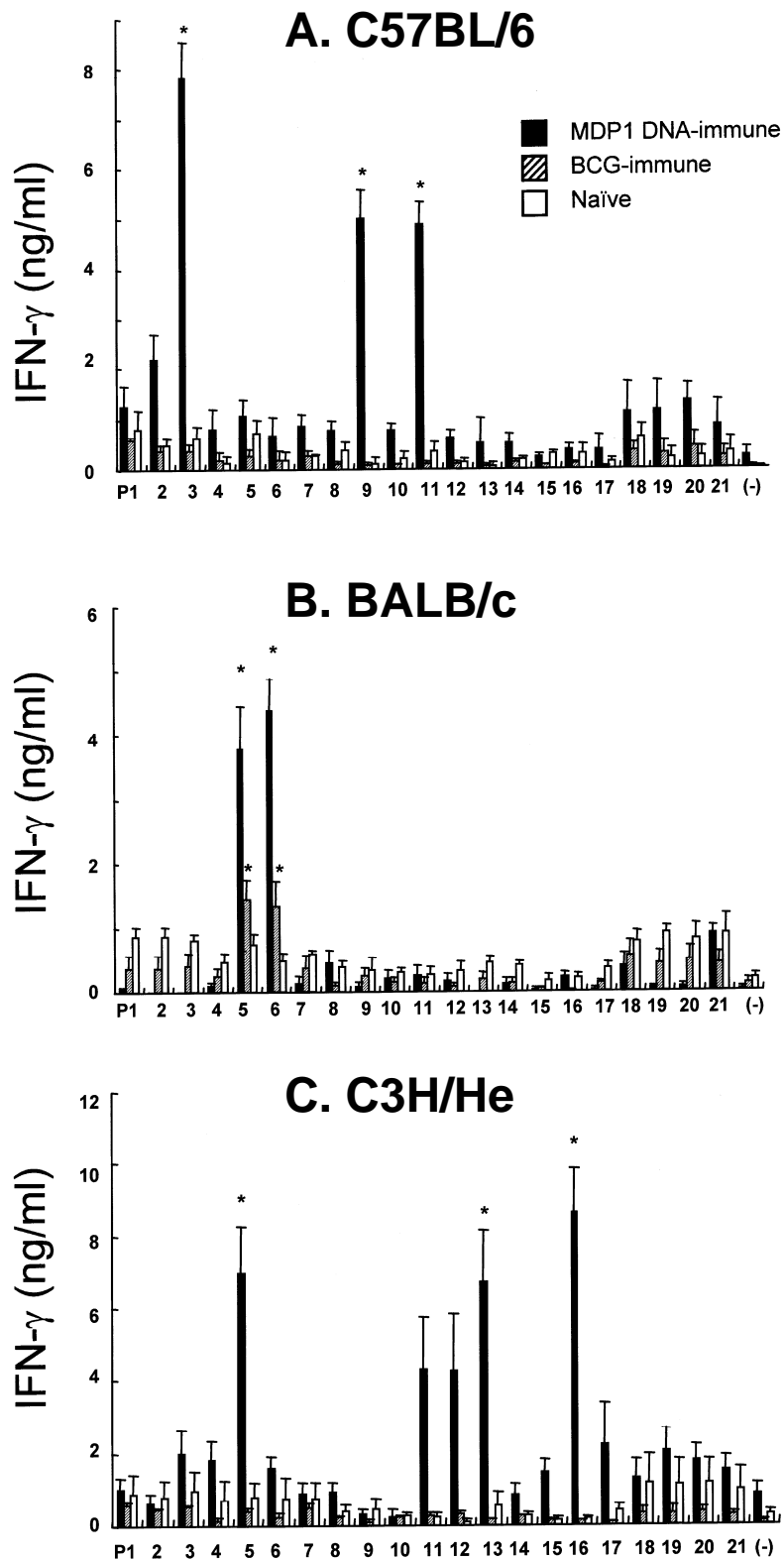


Fig. 2, Suzuki et al.

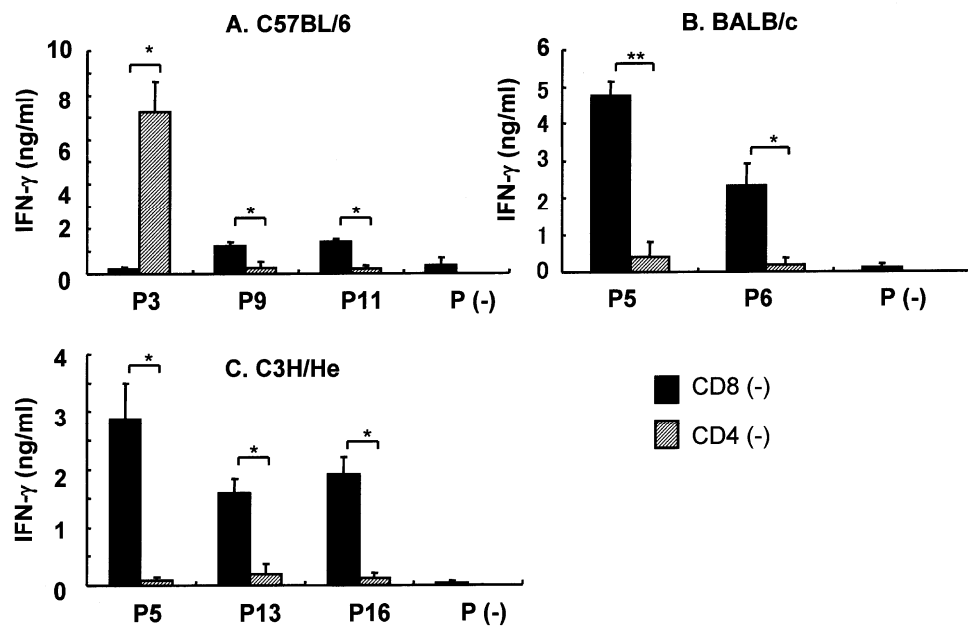


Fig. 3, Suzuki et al.

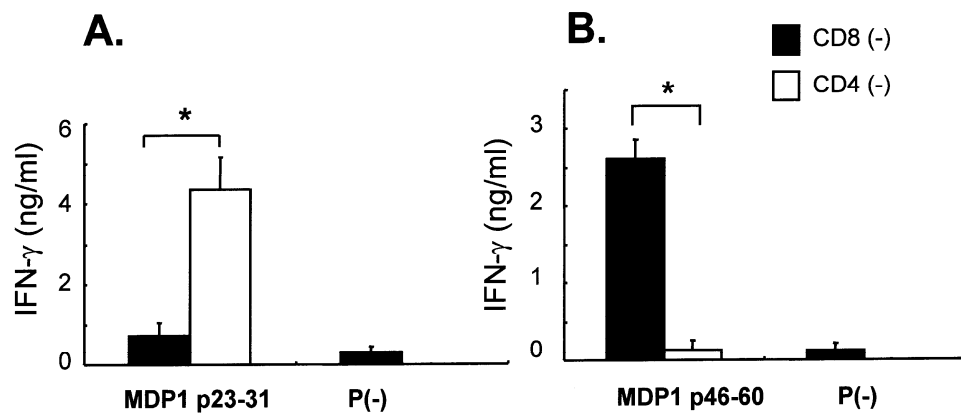


Fig. 4, Suzuki et al.

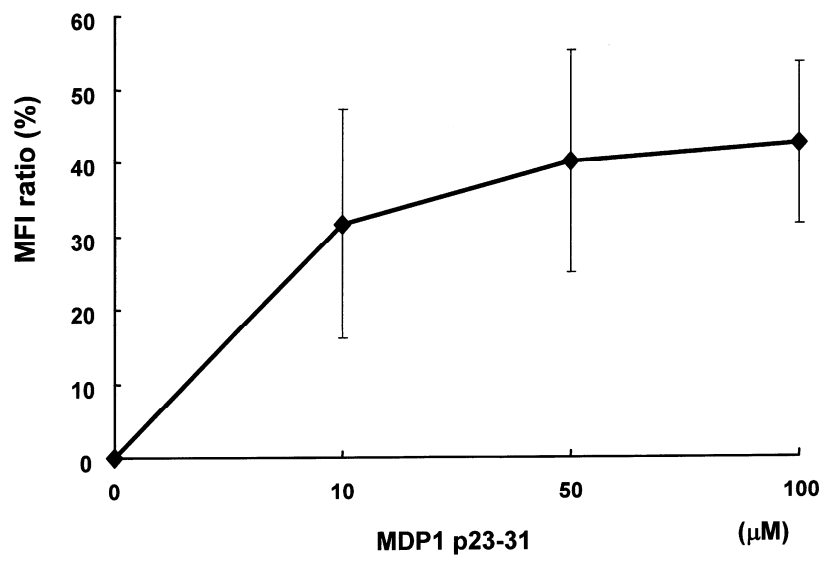


Fig. 5, Suzuki et al.

TABLE 1. T-cell epitope candidates in MDP1 molecule

Peptide	Amino acid sequence	Estimated scores for restriction molecules ^a	
		K ^b	D ^b
p21-40 (P3)	ATAAVENVVDTIVRAVHKGD		
p23-31 (9mer)	AAVENVVDT	0.13	108 <u>20</u>
p23-32 (10mer)	AAVENVVDTI	0.40	50.2 <u>33</u>
		A ^d	E ^d
p41-60 (P5)	SVTITGFGVFQRRRAARVA		
p46-65 (P6)	GFGVFQRRRAARVARNPRT		
p52-60	QRRRAARVA	11.9 (7.1)	— ^b
		A ^k	E ^k
p41-60 (P5)	SVTITGFGVFQRRRAARVA		
p44-58 (15mer)	ITGFGVFQRRRAAR		<u>24</u>
	GFGVFQRR	10.8 (14.2)	
p111-130 (P13)	KKVAKKAPAKKATKAAKKA	A ^k	E ^k
p113-121 (9mer)	VAKKAPAKK	—	17.9 (10)
p116-124 (9mer)	KAPAKKATK	—	25.4 (10)
p121-129 (9mer)	KKATKAAKK	—	18.7 (10)
p141-160 (P16)	TKAPAKKAVKATKSPAKKVT	A ^k	E ^k
p142-150 (9mer)	KAPAKKAVK	—	17.3 (10)
p145-153 (9mer)	AKKAVKATK	—	15.9 (10)

Epitopes predicted by computer algorithms are shown. ^aEstimated scores are derived from BIMAS (bold), SYFPEITHI (underlined), or RANKPEP (plain). Parentheses indicate threshold scores, scores above which suggest the peptide binding to the corresponding H2 molecule. ^b—; score not shown in the program.