the residues are involved in a β-sheet structure. In SCIII there are four amide resonances found at very low field: G111 NH (10.4 ppm); Y112 NH (8.0 ppm); I113 NH (9.4 ppm); and D114 NH (8.8 ppm). These latter three residues, Y112 to D114, also have significantly shifted αCH resonances located at 5.2 (Y112) and 5.3 ppm (I113 and D114). All of these ^{1}H NMR assignments for SCIII are in excellent agreement with those of Ca-bound rabbit TnC (*15*). In x-ray studies of TnC, the αCH protons of D114 and R148 and F112 and D150 of the β sheet are proximate, and in solution an nOe is present between these two protons (*15*). For SCIII, the analogous contact would be between D114 and Y112′ (and Y112 and D114′). An nOe between these protons was observed (Fig. 3B), indicating that the β sheet is present between SCIII peptides in solution (*16*).

In this report we present conclusive ^{1}H NMR evidence that two 34-residue site III peptides from TnC have assembled only in the presence of Ca to form a symmetric dimer in solution (*17*). The nOe results also suggest that the structure of the dimer is similar, if not identical, to that for the carboxyl-terminal domain of TnC. These results substantiate the use of synthetic peptides to study the folding and assembly of proteins and allow fundamental studies for protein design to be explored with this synthetic protein. Of particular interest for this study is the energetics of the hydrophobic interactions between helices of the SCIII peptides. In effect, we have simplified the 70-residue carboxyl-terminal domain of TnC to 34 residues without sacrificing tertiary structure. This should allow the interaction of drugs and other components of the troponin complex (*18*) to be efficiently studied with this peptide alone as the carboxyl-terminal half of TnC.

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16. The x-ray structure of turkey TnC (*5*) reveals that the β sheet formed between sites III and IV forms only two hydrogen bonds between residues I113 and I149. Furthermore at either end of the β sheet a water molecule separates the two strands. As a result the intermolecular distance between the two strands is larger than for a classical antiparallel β sheet. The average distance between α-carbons of residues 112 and 150 and of 114 and 148 is 4.72, implying 2.76 Å for the α-protons. From Fig. 3B, the calculated distance measured from volume integration is 2.97 Å, in good agreement with the x-ray findings.
17. Further support for the assembly of two peptides is derived from Ca titration experiments. During these experiments, the upfield-shifted aromatic resonances and several methyl resonances (Fig. 1) are monitored by 1-D ^{1}H NMR as a function of added Ca. We observe only two species in slow exchange; the unfolded apo-peptide and the structured dimer. Titration curves have shown that only 1 mol of Ca is required to fold 2 mol of SCIII peptide. Upon addition of a second mole of Ca we observe no further spectral changes in these resonances. These observations are consistent with a conformational change in SCIII upon binding of Ca, dimerization of a Ca-bound SCIII with an apo SCIII and subsequent conformational change in the apo SCIII, and finally binding of a second Ca molecule to the SCIII dimer. The resonances used to monitor these interactions reflect changes in the conformation of the peptides and especially the formation of the hydrophobic core of the dimer, more so than simply the binding of Ca. It is possible that other regions of the ^{1}H spectrum are more sensitive to the presence of Ca (that is, the β-methylene protons of coordinating amino acids) and would show slight differences in the dimer with one molecule of Ca. This report reflects nOe spectra for two-peptide, two-Ca dimer.
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19. The nOe build-up curves from NOESY spectra taken at 75, 150, and 300 ms showed that the nOe intensity was approximately linear with the mixing time for the cross peaks shown.
20. Residue 100 in chicken TnC, originally thought to be Asp, has been subsequently shown to be Asn (L. B. Smillie, personal communication).

21. We thank O. Herzberg and M. N. G. James for providing the x-ray coordinates for turkey TnC, R. Reid for use of the program MUTATE, and P. Semchuk for assistance in the peptide synthesis. Supported by a grant from the Medical Research Council of Canada and an Alberta Heritage Foundation for Medical Research Fellowship (G.S.S.).

5 March 1990; accepted 21 May 1990

Characterization of Naturally Occurring Minor Histocompatibility Peptides Including H-4 and H-Y

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Minor histocompatibility (H) antigens can be peptides derived from cellular proteins that are presented on the cell surface by major histocompatibility complex (MHC) class I molecules. This is similar to viral antigens, because in both cases cytotoxic T lymphocytes (CTLs) recognize artificially produced peptides loaded on target cells. Naturally processed minor H peptides were found to be similar to those artificial CTL-epitopes, as far as size and hydrophobicity is concerned. The peptides studied were isolated from a transfectant that expressed a model CTL-defined antigen, β-galactosidase, from male cells that express H-Y, which has been known operationally since 1955, and from cells that express H-4, known since 1961.

MHC CLASS I–RESTRICTED CTLs are essential for immunity to intracellular pathogens (*1*). In addition, CTLs are also involved in rejection of transplanted foreign tissues. If tissue donor and recipient are matched for MHC genes, but not for other genes, recipient CTLs may recognize donor minor H antigens (*2*). CTLs are thought to recognize complexes of MHC class I molecules and peptides derived from cellular proteins, since MHC-restricted CTLs can recognize artificially produced peptides on suitable target cells and since

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crystallography suggested a peptide binding site on MHC molecules (*3, 4*). How MHC-binding antigenic determinants are produced naturally inside the cell is not known. Neither the proteases involved nor their cellular compartment is known. To study the biochemical pathways of MHC class I–restricted peptide presentation, we have set out to isolate and characterize naturally occurring CTL-recognized peptides representing minor H antigens H-Y (*5*) and H-4 (*6*) and a model CTL-defined antigen, β-galactosidase, expressed in transfectants (*7*).

CTL line 0805B specifically recognizes L^d-expressing tumor cells transfected with the gene for *Escherichia coli* β-galactosidase [such as P13.1 cells, which are β-galactosidase–transfected P815 cells (*7*)], or non-transfected tumor cells incubated with artificially produced β-galactosidase peptides (*7*) (Fig. 1, A and C). An acid elution technique similar to a method that has been shown to dissociate peptides from MHC class II molecules (*8*) was used to isolate CTL-recogniz-

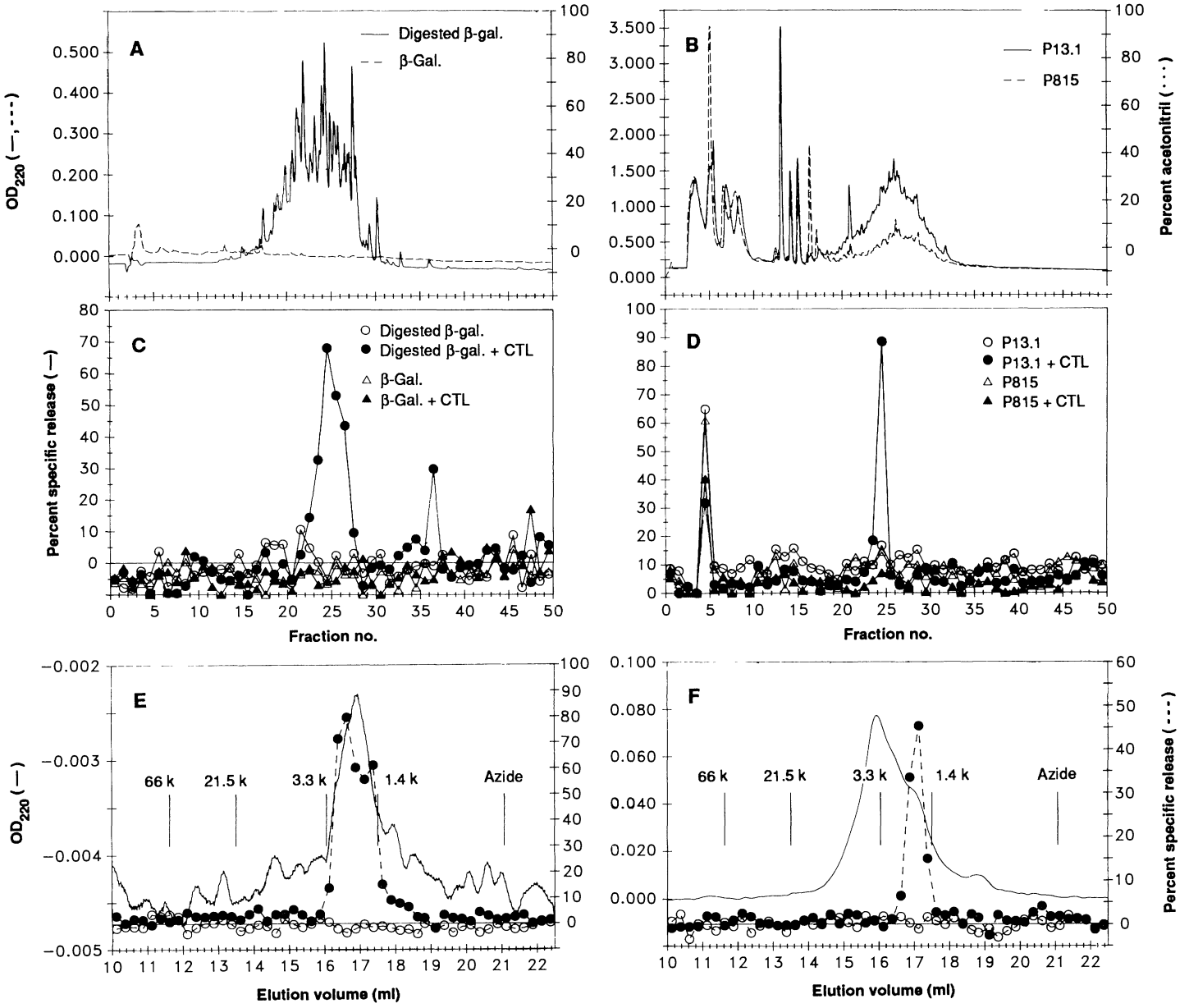

Fig. 1. Detection of naturally occurring CTL-epitopes in β-galactosidase–transfected cells (*22*). (A, C, and E) are profiles of acid extracts of digested or native β-galactosidase from *Escherichia coli*, whereas (B, D, and F) are profiles of acid extracts from the β-galactosidase–transfected mouse cell line P13.1 and from the P815 cell line. (**A**) Acid extracts of pepsin-digested (———) and undigested (– – – –) *E. coli* β-galactosidase (β-Gal.) (*23*) and (**B**) cell extracts of β-galactosidase–transfected P13.1 (———) and untransfected P815 (– – – –) were separated by reversed-phase HPLC. Fractions of the (**C**) undigested (△, ▲) and digested (○, ●) β-galactosidase preparations and (**D**) cell extracts of P13.1 (○, ●) and P815 (△, ▲) were incubated with P815 target cells and screened for CTL recognition with the β-galactosidase–specific CTL line 0805B (*7*) (closed symbols) and without CTL (open symbols). Fraction 25 from the (**E**) pepsin-digested *E. coli* β-galactosidase and (**F**) from the P13.1 cell extract were subjected to gel filtration and tested for recognition by 0805B CTL (●) and without CTL (○). P815 and P13.1 (*7*) tumor cells were homogenized in 0.1% (v/v) trifluoroacetic acid (TFA) in a dounce homogenizer followed by ultrasonication (Branson), kept on ice for 30 min, and pelleted (10 min, 10,000*g*). Supernatant was separated on a G25-column (Pharmacia). Eluant, 0.1% TFA; flow rate, 0.5 ml/min. Material of M_r <5000 was collected and separated on an analytical HPLC reversed-phase column (Superpep S, Pharmacia LKB) (*24*). Fractions were dried, suspended in 0.5 ml of phosphate-buffered saline (PBS), and resolubilized, then incubated with P815 tumor cells and tested for recognition by 0805B CTL (*25*). Fractions 25 (100 μl each) of the P13.1 cell extract and of digested β-galactosidase were separated on a Superose 12 HR 10/30 fast protein liquid chromatography column (Pharmacia LKB). Eluant, PBS; flow rate, 0.5 ml/min; fraction size, 250 μl. Fractions were tested for CTL recognition as before. Elution volume of several marker proteins and peptides is indicated in (E) and (F) (*26*).

able β-galactosidase–derived peptides that occur naturally in P13.1 cells. Low relative molecular mass material ($M_r \leq 5000$) extracted by acid elution from P13.1 or P815 cell lysates was separated by reversed-phase high-performance liquid chromatography (HPLC) (Fig. 1B) and tested for recognition by 0805B CTLs (Fig. 1D). Material from P13.1, but not P815, cells was recognized. The active fractions of P13.1-eluted material and of pepsin-digested β-galactosidase were subjected to gel filtration. Peptides that were recognized by CTL 0805B eluted at similar positions, which indicated that the M_r of the natural peptide was about 2000 (Fig. 1, E and F). CTL 0805B recognized both the artificial and the natural (*9*) peptides in a concentration-dependent fashion (Fig. 2, A and B); the natural peptide was not recognized by a K^d-restricted CTL line specific for an influenza nucleoprotein peptide (Fig. 2C).

For isolation of naturally occurring peptides that represented classical minor H antigens, we chose H-Y, an antigen recognized by D^b-restricted CTLs on male, but not female, mouse cells (*5, 10*), and H-4^b, encoded on chromosome 7 and recognized by K^b-restricted CTLs (*6*). Spleen cells of C57BL/6 males (B6; *H-2*^{b}, *H-4*^{a}), BALB.B males [*H-2*^{b}, *H-4*^{x} (whose product is recognized by H-4^b–specific CTLs) (*4*)], or BALB.B females were subjected to the acid elution procedure described above. The extracts were separated by reversed-phase HPLC (Fig. 3, A to C) and tested for recognition by an H-Y–specific CTL line, 11P9, and by an H-4^b–specific CTL line, B21W9 (Fig. 3, D to F). H-Y–specific CTLs recognized fraction 28 from B6 male and BALB.B male, but not BALB.B female,

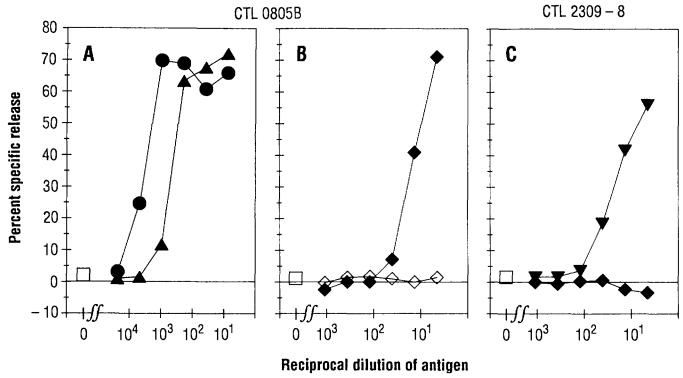

Fig. 2. Characterization of CTL-epitopes extracted from β-galactosidase–transfected cells. Titrated concentrations of reversed-phase HPLC-derived fractions 25 (●) and 26 (▲) of (**A**) pepsin-digested *E. coli* β-galactosidase and fraction 25 from cell extracts of (**B**) β-galactosidase–transfected P13.1 (♦) and untransfected P815 (◇) were incubated with P815 target cells and tested for recognition by CTL 0805B. (**C**) Titrated amounts of fraction 25 of the P13.1 cell extract (♦) and of synthetic peptide NP147-158(R$^-$) (100 ng/ml) (▼) were tested for recognition by the influenza-specific CTL line 2309-8. ^{51}Cr-labeled P815 target cells were incubated with titrated amounts of fractions or peptide and tested for recognition by CTL as in Fig. 1. Spontaneous release of target cells ranged between 13 and 19%; the effector-to-target ratio was between 7:1 and 10:1. EL4 target cells (H-2^b) incubated with the active fractions were not recognized by CTL 0805B (*12*), indicating MHC-restricted recognition. The CTL line 2309-8 was derived by repeated stimulation of spleen cells from a BALB/c mouse who had been immunized with influenza virus. Cultures contained syngeneic feeder cells and synthetic peptide TYQRTRALVTG at 0.1 μg/ml, according to a sequence defining a CTL-epitope in influenza nucleoprotein (*27*).

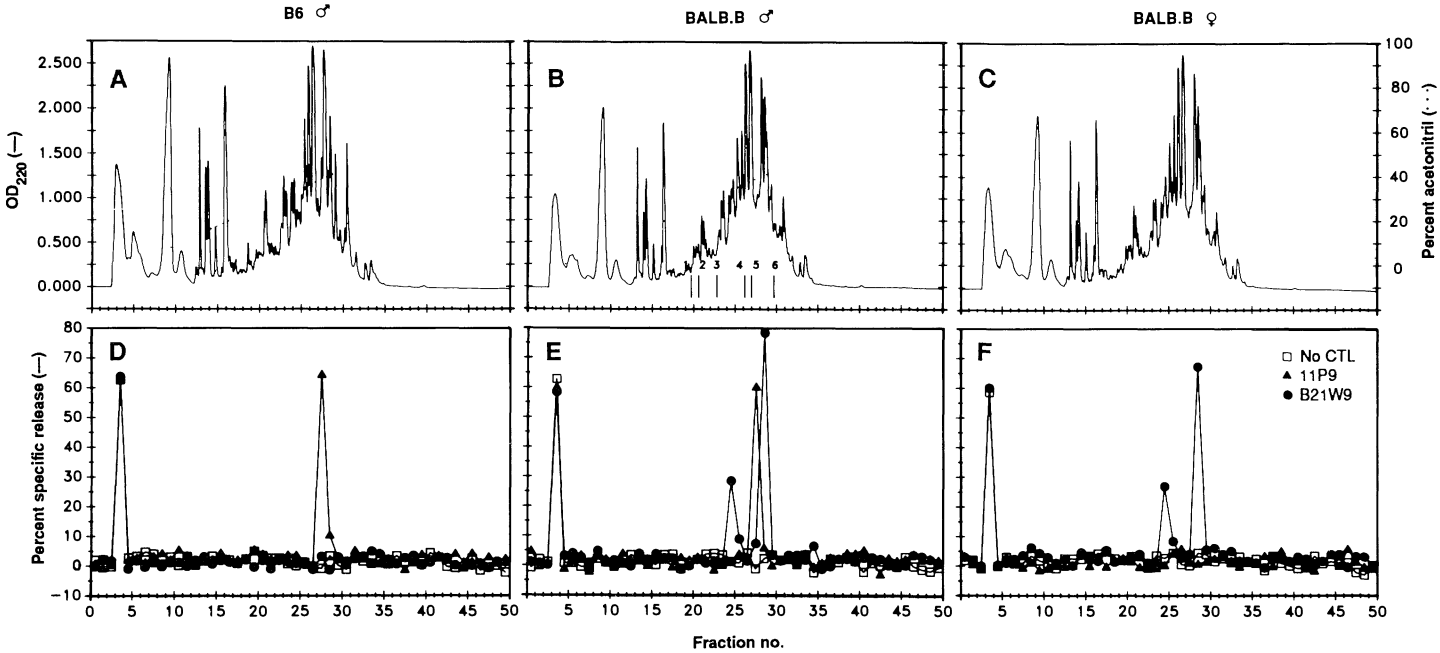

Fig. 3. Detection of naturally occurring minor H peptides representing H-4 and H-Y. TFA extracts of spleen cells from (**A** and **D**) male B6, (**B** and **E**) BALB.B, and (**C** and **F**) female BALB.B mice were separated by reversed-phase HPLC (A, B, and C). Fractions were incubated with EL-4 target cells and tested for CTL recognition (*25*) with H-Y–specific CTL line 11P9 (▲), H-4–specific CTL line B21W9 (*4*) (●), and without CTL (□) (D, E, and F, respectively). For comparison with these naturally occurring minor H peptides, several synthetic peptides representing MHC class I–restricted CTL-epitopes [indicated with numbers 1 to 6 in (B)] were subjected to the same column under identical conditions. Four male B6, two male BALB.B, and two female BALB.B spleens were homogenized and separated on G25 and the reversed-phase HPLC column as for the tumor cells in Fig. 1. Each synthetic peptide (50 μg at 1 mg/ml) was subjected to reversed-phase HPLC. Elution was performed under the same conditions as described for cell extracts. The retention time was determined by measuring optical density at 220 nm. The following synthetic peptides were used (*28*): 1, TYQRTRALVRTG [influenza nucleoprotein residues 147 to 158, K^d-restricted (*27*)]; 2, TYACRVKHDSMAE [β_2-microglobulin, *a* allele, residues 77 to 89, H-2^b–restricted (*29*)]; 3, IASNENMETMESSTLE [influenza nucleoprotein residues 365 to 380, D^b-restricted (*1*)]; 4, SDYEGRLIQNSLTI (influenza nucleoprotein, 50 to 63, K^k-restricted (*1*)]; 5, RYWAIRTRSG [influenza nucleoprotein, 384 to 393, B27-restricted (*30*)]; 6, MVVKLGEFYNQMM [influenza nucleoprotein, 82 to 94, A2-restricted (*1*)]. Spontaneous release of EL-4 targets was 17%, the effector-to-target ratio was 17:1 for B21W9 and 22:1 for 11P9 (*31*).

profiles, whereas H-4^b–specific CTLs recognized fraction 29 and, to a smaller extent, fraction 25 from BALB.B female or male, but not B6, profiles. H-4^b–specific CTLs also recognized fraction 29 from B10.129–*H-4^b*, the strain defining H-4, and from 129/Sv, the strain of origin of the *H-4^b* allele in B10.129–*H-4^b*–congenic mice (Fig. 4). CTL recognition of all these antigens is concentration-dependent (Fig. 4). Both H-4 and H-Y material is of peptidic nature, since it can be destroyed by proteases (Fig. 4, A and C). Recognition is MHC class I–restricted (Fig. 4, B and D).

In conclusion, we have in three cases isolated naturally occurring peptides recognized by MHC class I–restricted CTLs. These molecules are similar to those artificial or synthetic peptides used to define CTL epitopes of virus- or minor H–specific CTLs (*1, 3, 4*), as judged by size estimates (Fig. 1) and, more significantly, by similar behavior on reversed-phase HPLC columns (Fig. 3). These natural peptides are likely to bind to nascent MHC class I molecules in the endoplasmic reticulum (*11*). We have preliminary evidence that β-galactosidase peptides can be eluted from membrane fractions but not from the cytosol of P13.1 cells, which express β-galactosidase protein in the cytosol (*12*). With our model CTL-defined antigen, it should be possible now to follow the occurrence of protein, peptides, and MHC molecules through the cellular compartments. In addition, having minor H molecules available will make the production of minor H–specific antibodies feasible.

The male-specific antigen, H-Y, was first described by Eichwald and Silmser in 1955 (*5*), who observed that female mice would reject skin grafts from syngeneic males. The H-Y antigen has been used to investigate several basic biological issues, the highlights of which are the demonstration of MHC class I–restricted recognition by minor H–specific CTLs (*10*), the first and so far only correlate of a mouse minor H antigen in humans (*13*), studies on immunological tolerance (*14*) and, most recently, the work of von Boehmer and colleagues demonstrating both positive and negative thymic selection during T cell ontogeny using mice transgenic for an H-Y–specific T cell receptor (*15*). This has all been done in the absence of any information on H-Y molecules or genes. Our demonstration of H-Y molecules may lead to further refinement of those issues.

The H-4 gene was the third minor H gene to be operationally identified with the use of congenic strains. The first minor H molecule to be identified was the H-3–encoded β_2-microglobulin (*16*). Almost 50 other minor H genes have been mapped, most of them without any molecular information (*2, 17*); the most recent numbered ones being H-45 to H-47 (*18*). H-4 congenic strains have also been used to study immunological problems, such as polymorphism and complexity of minor H genes and immunodominance (*18, 19*).

With our new technique for isolating CTL-recognized peptides, combined with novel sensitive analysis methods, such as tandem mass spectroscopy, the rapid identification not only of minor H antigens, especially human minor H antigens, but also of other CTL-defined antigens, such as tumor, parasite, bacterial, or viral antigens (*1, 20*), should be feasible in the near future, thereby spurring basic biological as well as clinical understanding and synthetic vaccine design (*21*).

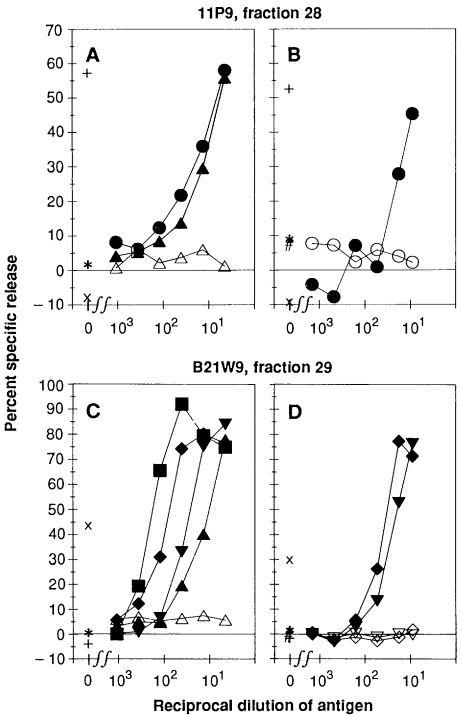

Fig. 4. Further characterization of H-4 and H-Y peptides. (**A**) Titrated concentrations of reversed-phase HPLC-derived fractions 28 each of male B6 (●) or BALB.B (▲) and protease-digested fraction 28 of male BALB.B (△) were incubated with EL-4 target cells and tested for CTL recognition with H-Y–specific CTL line 11P9. (**B**) The same was done with fraction 28 of male B6 with EL-4 (●) and P815 (○) as target cells. (**C**) Titrated concentrations of fraction 29 of B10.129–*H-4^b* (■), 129/Sv (▼), female BALB.B (◆), or male BALB.B (▲) extracts and of the protease-digested fraction 29 of male BALB.B (△) were incubated with EL-4 target cells and tested for recognition by H-4^b–specific CTL line B21W9. (**D**) Fractions 29 of female BALB.B (◇, ◆) and 129/Sv (▼, ▽) were incubated with EL-4 (filled symbols) or P815 (open symbols) target cells and tested with B21W9 CTL. For control purposes, concanavalin A–induced splenic blast cells of female BALB.B (x), male B10 or B6 (+), EL-4 (*), and P815 (#) tumor cells were used as targets in the absence of antigenic fractions (A to D). Fractions 28 and 29 (50 μl each) of BALB.B were incubated with 25 μl of proteinase K (2 mg/ml) (745723, Boehringer Mannheim) and pronase E (0.5 mg/ml) (33635, Serva) in PBS (37°C, 4 hours). For inactivation of proteases, the mixtures were boiled for 3 min. For control, 50 μl of fraction and 25 μl of PBS were boiled without proteases. This preparation is represented by (▲) in (A) and (C). Spontaneous release of tumor cells ranged between 11 and 19%, that of concanavalin A blasts between 26 and 36%, the effector-to-target ratio between 10:1 and 22:1 for B21W9 and between 20:1 and 22:1 for 11P9 (*31*).

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22. The results shown in Figs. 1 to 4 are typical of those from several independent preparations. P13.1 cells, for example, were extracted on three occasions; BALB.B male cells were extracted more than ten times. In all cases, the elution profiles as well as the position of CTL-recognized material were remarkably similar.
23. To make sure that the detection of β-galactosidase–derived CTL-epitopes in cell extracts is not due to fragmentation during the preparation, we suspended 1 mg of *E. coli* β-galactosidase (G-2513, Sigma) in 20 ml of 0.1% trifluoroacetic acid (TFA), dounced, and sonicated as described for the cell extracts. After two rounds of lyophilization, the material was resuspended in 0.1% TFA. The centrifugated supernatant was set aside, and the pellet was dried by speed-vac centrifugation, digested with pepsin (P-7012, Sigma) (1 ml, 0.1 mg/ml, 50 mM sodium acetate, pH 2.5, for 3 hours at 37°C), lyophilized, and suspended in 1 ml of 0.1% TFA. Both the soluble TFA extracts of undigested and digested β-galactosidase were subjected to reversed-phase HPLC exactly as described for the cell extracts. Fractions were dried and tested for recognition by CTL. As can be seen in Fig. 1, A and C, TFA-treatment of β-galactosidase did not lead to the production of fragments or CTL-epitopes.
24. Eluant A, 0.1% TFA; eluant B, acetonitril containing 0.1% TFA; gradient, 0 to 60% B; flow rate, 1 ml/min; fraction size, 1 ml.
25. CTL assays were performed according to standard methods (7). Briefly, ^{51}Cr-labeled P815 tumor cells were incubated in a total volume of 150 μl of a fraction diluted 1:3 in α–minimum essential medium (α-MEM) (Gibco), containing 10% fetal bovine serum, for 90 min at 37°C. Effector cells (50 μl) were added, and the assay was incubated for 6 hours (37°C, 5% CO_2). Radioactivity released into the supernatant was determined in a gamma-counter. Spontaneous release of target cells ranged between 12 and 16%; the effector-to-target ratio was between 1:10 and 1:30.
26. Marker proteins: 66 kD, bovine serum albumin; 21.5 kD, soybean trypsin inhibitor; 3.3 kD, secretin; 1.4 kD, synthetic peptide from influenza nucleoprotein, residues 147 to 158. These marker proteins behaved as theoretically expected. However, we observed that several other peptides did not elute in a volume that would have been expected according to their relative molecular mass. Retardation (for example, of somatostatin, M_r = 1638) could not be overcome by changes of eluant or column, so that size estimations of small peptides by gel filtration should generally be interpreted with caution.
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28. Single-letter abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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31. Generation of the H-Y–specific CTL line 11P9: A female B6 mouse was immunized intravenously with 10^7 irradiated (33 Gy), male B6 spleen cells. Three weeks later, recipient spleen cells (2×10^7) were stimulated in vitro against irradiated (33 Gy) male B6 spleen cells (2×10^7) in α-MEM-medium (Gibco) containing 10% fetal bovine serum, β-mercaptoethanol, glutamine, and antibiotics in a 5% CO_2 atmosphere at 37°C for 7 days. Thereafter, surviving cells were restimulated weekly with irradiated male B6 spleen cells in interleukin-2–supplemented medium.
32. We thank J. Klein for support; P. Bohley for helpful discussions; T. Schechinger for help in setting up the HPLC system; H. Kalbacher, K.-H. Wiesmüller, K. Deres, and G. Jung for synthetic peptides; F. Carbone and M. Moore for P13.1 cells; H. Schild for CTL and for reading the manuscript; C. M. Müller for artwork; and L. Yakes and P. Wiedmann for preparing the manuscript. Supported by Sonderforschungsbereich 120.

1 February 1990; accepted 10 May 1990

Prevention of HIV-1 Infection and Preservation of CD4 Function by the Binding of CPFs to gp120

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Infection by human immunodeficiency virus type–1 (HIV-1) is initiated when its envelope protein, gp120, binds to its receptor, the cell surface glycoprotein CD4. Small molecules, termed *N*-carbomethoxycarbonyl-prolyl-phenylalanyl benzyl esters (CPFs), blocked this binding. CPFs interacted with gp120 and did not interfere with the binding of CD4 to class II major histocompatibility complex molecules. One CPF isomer, CPF(DD), preserved CD4-dependent T cell function while inhibiting HIV-1 infection of H9 tumor cells and human T cells. Although the production of viral proteins in infected T cells is unaltered by CPF(DD), this compound prevents the spread of infection in an in vitro model system.

CD4, A SURFACE GLYCOPROTEIN found primarily on a subset of T lymphocytes, is a receptor for both the class II major histocompatability complex (MHC) antigens (*1, 2*) and the human immunodeficiency viruses (HIV) (*3, 4*). CD4 probably binds a monomorphic domain on class II MHC, thereby facilitating antigen recognition and enhancing T cell activation by increasing adhesion and signal transduction (*5*). HIV binds to CD4 through its envelope glycoprotein, gp120 (*4*). A COOH-terminal region of gp120 (amino acids 403–421), in particular Trp^{411}, has been implicated (*6, 7*). The most NH_2-terminal immunoglobulin-like domain of CD4 is sufficient to bind gp120 (*8*), although the second domain in the intact molecule also contributes to binding (*9*). The binding sites on CD4 for gp120 and class II MHC overlap (*10, 11*); however, the binding sites for class II MHC may be more extensive (*10, 12*). The binding of gp120 blocks CD4 binding to class II MHC and thus inhibits CD4-dependent lymphokine production (*2, 13, 14*).

Soluble forms of CD4 seem to be promising agents that can inhibit infection by binding to gp120 (*15–17*), without interfering with the binding of cellular CD4 to the class II MHC proteins (*16*). However, proteins may have delivery, stability, and expense problems that may not be associated with low molecular weight agents. A mutational analysis of CD4 (*8, 11*) implicated Phe^{43} in the interaction of CD4 with gp120; substitution of Leu for Phe^{43} completely abrogated gp120 binding (*11*). We therefore tested a series of small phenylalanine-containing molecules for inhibition of gp120 binding to CD4. Derivatives of the dipeptide prolyphenylalanine with an NH_2-terminal carbomethoxycarbonyl moiety and a COOH-terminal benzyl ester (Fig. 1), termed CPFs (*N*-carbomethoxycarbonyl-prolyl-phenylalanyl benzyl ester), blocked gp120 binding to CD4, reversed the inhibition by gp120 of CD4–class II MHC binding, inhibited infection by HIV, and preserved CD4-dependent lymphokine production in the presence of gp120. By examining a number of closely related compounds we have begun to identify those functional groups responsible for the inhibitory activity. Thus, CPFs are promising forerunners to an effective inhibitor of HIV-1 infection.

The pre–T cell leukemia line HSB-2 was transfected with CD4 and a high expressing clone, HSBCD4-M.23, was isolated (*18*). Binding of gp120 to CD4 on HSBCD4-M.23 can be detected by flow cytometry after staining with an antiserum to gp120 and a fluoresceinated antibody to immuno-

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