The critical role played by the CD28/CD152-CD80/CD86 costimulatory molecules in mediating T cell activation and suppression provides attractive targets for therapeutic strategies. CD28 and CD152 share a conserved polyproline motif in the ligand-binding region. Similar proline-rich regions in globular domains preferentially adopt a polyproline type II (PPII) helical conformation and are involved in transient protein-protein interactions. Interestingly, in the human CD80-CD152 complex, PPII helix was integrated in the design of novel peptide agents referred to as CD80 competitive antagonist peptides. Structural and functional studies suggest potential therapeutic value for select CD80 competitive antagonist peptides.

The interaction between cell surface costimulatory molecules on antigen-presenting cells and the T cells is critical in modulating cell-cell communication (1). Two structurally and functionally well characterized T cell surface costimulatory molecules are CD28 and the CTLA4 (cytotoxic T lymphocyte-associated antigen)/CD152, both of which bind the same ligands, CD80 (B7-1) and CD86 (B7-2), on the antigen-presenting cells. Whereas signaling via CD28 mediates T cell activation, ligation of CD152 down-regulates T cell proliferation and function (2). Thus, CD80/CD86-CD28/CD152 costimulatory molecules are potential therapeutic targets for modulating T cell responses.

Analyses of multiple receptor-ligand interactions suggest that in exposed domains involved in protein-protein interactions that mediate cell signaling, the intermolecular interfaces often present extended shallow clefts on ligand surfaces (3). Hence, molecules whose shapes complement the protein interaction clefts of the ligand surfaces are likely to block receptor binding (4). Previously, several linear peptides with extended polyproline type II (PPII)1 conformation have been shown to block ligand-acceptor complexes involved in molecular recognition (5–7). Acceptors are typically large proteins with measurable affinity for specific ligands; the latter can be presented as a small peptide sequence within an exposed loop on the surface of a large protein. Structurally, ligands may exist in extended PPII helical conformation, allowing the backbone atoms of the peptide to form hydrogen bonds with protein acceptor at the interface of the protein-peptide complex (8, 9). Examples of complexes where the ligand is in PPII conformation include the EFPPPPT peptide, which interacts with the VASP (vasodilator-stimulated phosphoprotein) EVH1 domain (Protein Data Bank code 1Q68), and the SOS (Son of Sevenless) peptide (KHYRPLPPLP) that interacts with Grb2 (growth factor receptor-bound protein 2) Src homology 3 domain (Protein Data Bank code 1CKB) (10, 11).

In the CD80-CD152 complex, the CD80 presents a shallow hydrophobic pocket, receptive to the highly conserved solvent-accessible polyproline sequence99MYPPPY104 localized in the complementarity-determining region (CDR)-3 like loop region of the CD152 (12). Interestingly, it is observed that Pro102 restricts the preceding proline in a PPII helical conformation. The PPII helix is located very close to type II β turns and β strand in Ramachandran plot with backbone dihedrals of (φ, ψ) = (–75, +145). The geometry of the PPII helix allows the polypeptide chain to progress immediately from this conformation to a β-sheet as observed in CD152 (13). A significant fraction of the unordered residues in many globular proteins have been shown to exist in left-handed PPII conformation (14). Although proline is most commonly observed, nonproline residues also form PPII helix driven by steric interactions between backbone and the solvent (15). Whereas the backbone solvation stabilizes the conformation, the side-chain interactions of the PPII helix in the local environment determine the specificity of the protein-protein interactions (8).

Based on PPII helical mimicry of the ligand binding conformation of the receptor protein at the CD80-CD152 interface, novel peptide agents referred to as CD80 competitive antagonist peptides (CD80-CAPs) were designed. Contact preferences of the amino acids to be at the interface of protein-protein interactions and the propensity of each residue to form PPII helix were integrated in the design of the CD80-CAPs (3, 16). Select CD80-CAP competitively inhibited CD80-CD28/CD152 binding and suppressed T cell activation, suggesting a potential therapeutic value.

1 The abbreviations used are: PPII, polyproline type II; CD80-CAP, CD80 competitive antagonist peptide; PBS, phosphate-buffered saline; bIIB, bovine collagen type II; r.m.s., root mean square; MOD, mean optical density; CDR, complementarity-determining region.
CD80 Competitive Antagonist

MATERIALS AND METHODS

Comparative Modeling—A three-dimensional modeling of the mouse CD152 incorporating more than 10 different CD80-CAP sequences and the mouse CD80 ECD was carried out with the Geno3D program on the Pole Bio-informatique Lyonnais server (available on the World Wide Web at http://geno3d.polebio.fr/) (17). This modeling system maps the known sequence onto selected templates and extracts homology derived spatial constraints based on interatomic distances and dihedral angles to generate protein three-dimensional structures by “topology mapping” (18). The free mouse CD152 (Protein Data Bank code 1DQT chain A) (19) and human CD152 (Protein Data Bank code 1AH1) (20) and ligand-bound human CD152 (Protein Data Bank code 1B4S chain C and Protein Data Bank code 1HTR chain D) (21, 22) were selected as the templates for modeling. The overlap for the secondary structure comparison of the modified CD152 queries and the three templates was between 51 and 67%. The solution structure of human CD80 (Protein Data Bank code 1DR9) (22), human CD86 (Protein Data Bank code 1NCC) (23), and the human CD80/CD86 monomer from the docked complexes (Protein Data Bank code 1ISL chain A and Protein Data Bank code 1ISB chain B) (12, 21) were specified as the templates for the mouse CD80 structure prediction. The structural overlap for the secondary structure comparison for the CD80 query and the four templates was between 48 and 53%.

Docking of mouse CD80-CD80-CAP—Docking of the mouse CD80 and the CD80-CAPs with the former as the target and the latter as the probe was performed using Bigger software that utilizes a soft docking algorithm. The docked geometries with maximal surface matching and favorable potential energy values were evaluated, and scored in terms of geometric complementarity of the surfaces, explicit electrostatic interactions, desolvation energy, and pairwise propensities of amino acid side chains to contact across the molecular interface, with a global score that ranks overall the docking results separating potentially nearly native solutions from other incorrect solutions (24). The top 100 docked structures generated were screened by superimposition with the CD80-CD152 complex. Potential docked structures with a root-mean-square deviation of <5 Å were identified, viewed with the SPDB viewer, and subjected to further energy minimization using GRAMMOS to optimize the binding conformation of the amino acids (available on the World Wide Web at us.expasy.org/spdbv/) (25).

Peptides—All peptides were synthesized on Rink amide resin by solid-phase peptide synthesis using Fmoc (N-9-fluorenly)methoxycarbonyl/dicyclohexylcarbodiimide/hydroxybenzotiazole methodology at the Biochemistry and Biophysics facility, Indiana University School of Medicine (Indianapolis, IN), as described (26). The free NH2 group of the terminal amino acid residue was acetylated. A control hexapeptide with little or no PPII helical content was likewise synthesized. The peptides were purified by semipreparative reverse-phase high pressure liquid chromatography, and the identity of the purified peptide was confirmed by mass-assisted laser desorption/ionization time-of-flight mass spectrometry.

Circular Dichroism—CD measurements were recorded on a JASCO model J-710 spectropolarimeter (Jasco Inc., Easton, MD) as described previously (8, 26, 27). The samples were prepared by dissolving lyophilized CD80-CAP peptides at 100 μM concentration in citrate buffer (1 mM sodium citrate, 1 mM sodium borate, 1 mM sodium phosphate, 15 mM NaCl) with pH adjusted at 7.0. CD spectra were collected using a 1-cm path length quartz cuvette at 5 °C in the 190–270-nm wavelength range with a 0.5-nm resolution and a scan rate of 200 nm/min. Reported spectra represent the unsmoothed averages of 20 scans. Each spectrum was measured three times with individually prepared solutions. Raw CD signals (in millidegrees) were converted to mean residue molar ellipticity [θ] at the wavelength of maximum ellipticity [θmax], represents the observed ellipticity in degrees, c is the molar concentration of peptide, and n is the number of residues in the peptide. To determine 0% PPII helical content, CD spectra were recorded with CD80-CAP peptides dissolved in 6 M CaCl2 (28).

CD28-CDO/CD86 Enzyme-linked Immunosorbent Assay—96-Well flat bottom plates were coated with anti-mouse IgG (Pharamingen, San Diego, CA). Biotinylated biotinylated peptide buffer (pH 9.4) overnight at 4 °C. The proteins were obtained from Ancell Corp. (Bayport, MN). After blocking with 1% bovine serum albumin in PBS for 1 h, human CD28-Fc at 300 ng/well was added and incubated at room temperature for 2 h and later washed three times in PBS. During this time, human CD80-Fc-biotin (51.3 kDa) or CD86-Fc-biotin (52.3 kDa) at a final concentration of 4 μM was preincubated with streptavidin-horseradish peroxidase at 1:1000 in PBS. Mixtures of a constant concentration of biotinylated CD80-Fc with increasing concentrations of specific CD80-CAP agents were added to the CD28-coated wells and incubated for 45 min at 37 °C. After washing five times in PBS, binding was detected by using TNB substrate (Kirkegaard & Perry, Gaithersburg, MD) in carbonate buffer (pH 9.4) overnight at 4 °C. The reaction was stopped by adding 25 μl of 2 M H2SO4. Absorbance at 405 nm was read in a microplate reader (model 680; Bio-Rad). For competitive experiments, absorbance was read at 605 nm over a period of time between 0 to 300 s with a mix time of 0.30 s and an interval of 5 s between readings prior to stopping.

CD152/CD80/CD86—Enzyme-linked immunosorbent assay experiments were performed similarly by adding mixtures of human CD80-Fc biotin/CD86-Fc biotin at a constant concentration of 0.4 μM and varying concentrations of CD80-CAP agents to the wells coated indirectly with 300 ng of human CD152-Fc.

Data Analysis—The kinetic velocity, which represents the slope of absorbance versus time curve calculated by linear regression, maximum velocity, or the highest velocity from overlapping segments of data points in the reaction and the change in absorbance with time was recorded and analyzed using microplate manager software 5.2 (Bio-Rad).

Animals and Induction of Collagen-induced Arthritis (CIA)—6–8-Week-old DBA/1 Lac J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and housed in a specific pathogen-free facility in the animal care facility at the Indiana University School of Dentistry bioresearch facility following approval of the animal care and use committee. For induction of CIA, the mice were inoculated subcutaneously at the base of the tail with 100 μg of bovine collagen type II (bCII) in 0.05 M acetic acid emulsified 1:1 with complete Freund’s adjuvant (4 mg/ml) (29). T Cell Proliferation Assays—The in vitro proliferation assays were done as described (26). Draining lymph nodes were removed from animals 1 week after bCII immunization, dispersed, and washed in RPMI 1640. Lymph node cells were cultured in RPMI 1640 containing 10% fetal calf serum, 25 mM HEPES, 2 mM L-glutamine, 50 units/ml penicillin, 50 mg/ml streptomycin, and 5 × 10−3 M 2-mercaptoethanol in round-bottom 96-well plates with bCII (20 μg/ml) for 72 h, including a final 16-h pulse with [3H]thymidine. Cultures contained CD80-CAP and control peptides in triplicate wells at concentrations ranging from 500 to 62.5 ng/ml. Cultures were harvested onto fiber mats using a Skatron harvester (Skatron, Sterling, VA), and the levels of [3H]thymidine incorporation were determined by liquid scintillation counting (Microbeta, Wallac, Turku, Finland). Results were confirmed by replicate experiments, and all data are expressed as cpm (counts/ min incorporated in antigen-stimulated culture − counts/min incorporated by control unstimulated culture) (20).

CD80/CAP Treatment in CIA—DBA/1 Lac J mice were induced CIA as described above. The CD80-CAP1 and the control peptides were dissolved in sterile PBS at a concentration of 5 μg/ml. For treatment in CIA, groups of bCII-immunized mice were administered intravenously in the tail vein 100 μl of sterile PBS or 500 μg of CD80/CAP1/control peptide. Mice were observed for clinical disease beginning 3 weeks after immunization and scored on alternate days as described (29). The severity of arthritis was recorded using an established macroscopic joint scoring system ranging from 0 to 4 as follows: 0, normal; 1, mild swelling with erythema; 2, significant joint swelling; 3, severe swelling and digit deformity; and 4, maximal swelling with ankylosis. Each joint was scored with a maximum possible score of 16 per mouse.

Statistical Analysis—For clinical score and in vitro proliferation analyses, a one-way analysis of variance with Tukey’s post hoc test was performed to determine the differences between groups.

RESULTS

Design and Structure of CD80-CAP—The interface between both CD152/CD80 and CD152/CD86 is large, burying a total of 1255 and 1290 Å of the solvent-accessible surfaces of CD80 and CD86, respectively (12, 21). The proline-rich region of the CD152 CDR-3-like loop packs against the hydrophobic patch of residues that form a shallow cavity on the front face of CD80 and CD86 (30). Similar proline-rich regions commonly occur in globular domains involved in transient protein-protein interactions (31). Typically, proline-rich regions preferentially adopt a PPII helical conformation (32). Most PPII helices in globular domains vary in length from 4 to 6 residues (14, 33). Significantly, in the complex with CD80, the Pro101 of CD152 is in PPII helical conformation with the dihedral angles of \( \phi \) and \( \psi \) 

\[ \text{MRW} = \frac{\text{counts in the sample}}{\text{reference counts}} \]
measuring −75 and 164, respectively (Table I). A competitive antagonist for this receptor ligand interaction should not only be small as to occupy the shallow binding site of CD80 (surface area of 655 Å), but it should also mimic the PP_{II} helical conformation of the physiological receptor (12). Amino acids exhibit varied frequencies of occurrence at the protein interface and distinct pairing preferences at sites of protein-protein interactions (16, 34). In addition, residues vary in the propensity to form PP_{II} helix (15). A polypeptide backbone possesses both α helix and PP_{II} helix propensity. The extent to which the PP_{II} helix is adopted is determined by the degree of backbone solvation and modulated by side chain interactions (8, 13). Studies of the synthetic peptide made up of MYPPPY sequence do not exhibit PP_{II} helical conformation and had no inhibitory potential in cellular assays (35). This may be attributed to the lower potential of aromatic amino acids to propagate PP_{II} helix (36). Integrating the residue preferences and propensities, novel CD80-CAP hexapeptides were designed so as to possess significant PP_{II} helical content in the context of the CD80 binding interface.

Substituting the CD80-CAP residues for the hydrophobic motif in the mouse CD152, comparative modeling of the modified CD152 was performed using the mouse CD152 (Protein Data Bank code 1DQT) as template (19, 20). This gives a structural representation of the CD80-CAP with reference to the adjacent residues of CD152. Since PP_{II} helix formation has been shown to be a locally driven event with little/no involvement of long range interactions, it is logical to presume that synthetic CD80-CAP with blocked charges will adopt a similar conformation as in the predicted model (37). Each CD80-CAP was superimposed with the ligand binding motif of free murine CD152 (Protein Data Bank code 1DQT) (19), free human CD152 (Protein Data Bank code 1AH1) (20), and CD80 (Protein Data Bank code 118L) (12)/CD86 (Protein Data Bank code 1185) (21) bound CD152. Superimposition of the CD80-CAP1 with the polyproline motif of the free murine CD152 (Protein Data Bank code 1DQT) (A), free human CD152 (Protein Data Bank code 1AH1) (B), and human CD152 complexed with CD80 (Protein Data Bank code 118L, chain C) (C) and CD86 (Protein Data Bank code 1185, Chain C) (D) presented as a trace of the sequences. The CD80-CAP1 is in blue. r.m.s. deviation for each superimposition is indicated.

The difference in the r.m.s. deviation values may be attributed to the structural differences between the monomer (Protein Data Bank code 1DR9) and homodimer (Protein Data Bank code 118L) of CD80. The recently developed docking program “BIGGER” was used to generate and evaluate plausible binding modes between the predicted mouse CD80 ECD and the CD80-CAP (24). The coordinates of each CD80-CAP were systematically rotated (in discrete steps of 15) and translated against the surface of CD80. The top 100 docked structures of each complex generated were screened by superimposition with the human CD80-CD152 complex (Protein Data Bank code 118L). Potential CD80-CAP-CD80-docked structures with r.m.s. deviation of <5 Å were then subjected to energy minimization to optimize the binding conformation of the amino acids by Gramm. Fig. 2 shows the energy-minimized CD80-CAP1 occupying the binding cleft of CD80. The conserved CD80 residues critical for binding (Tyr^{28}, Val^{89}, and Leu^{103}) are within 5 Å of the CD80-CAP1, suggesting near native docking (12). Incorporation of glutamine increased both the PP_{II} helical propensity and the potential for interaction with the interface residues (Val^{79} and Gln^{81}) at the binding pocket of CD80. The docked interface also suggests covalent interactions between methionine and cysteine of the CD80-CAP1 with the conserved Tyr^{28} and Gln^{81}, respectively, in the binding pocket of CD80.

**CD Spectrum of CD80-CAP**—The best way to unambiguously reveal the PP_{II} structure in solution is to use spectroscopies based on optical activity such as CD (13). CD spectra were recorded at 5 °C with CD80-CAPs dissolved in citrate buffer with pH adjusted at 7.0. As seen in Fig. 3, the CD spectrum of CD80-CAP1 presented a strong negative band (θ = −48,000) at 207 nm and a weak positive band (θ = 17,000) at 223 nm, reproducing the characteristic features of a PP_{II} helix (8, 15). The CD spectrum of CD80-CAP3 presented a much reduced negative band (θ = −19,000) at 209 nm and a weak positive band (θ = 17,000) at 225 nm suggestive of lower PP_{II} helical conformation.
content as compared with CD80-CAP1. The molar ellipticity minimum of both CD80-CAP1 and CD80-CAP3 at 207 and 209 nm, respectively decreased drastically in the presence of 6 M CaCl₂ due to the disruption of the PPII helix (13) (Fig. 3, data not shown). Additional CD80-CAPs with predicted structural similarity to the binding motif of CD152 in the docking studies lacked definitive secondary structure and exhibited random coil conformation (data not shown).

The CD80-CAP Competes with the Physiological Receptors for Binding CD80—The ability of the synthetic CD80-CAP to compete with physiological receptors (CD28/CD152) for binding the ligands (CD80/CD86) was evaluated by enzyme-linked immunosorbent assay. CD80-CAP1 and CD80-CAP3 inhibited significantly the binding of CD80 to both CD28-Fc and CD152-Fc (Fig. 4, A and B). Maximum decrease in the percentage of binding of CD80Fc to CD28 (43%) and CD152 (51%) was observed in the presence of CD80-CAP1 (500 μM). A dose response exhibiting greater decrease in the percentage of binding of CD80Fc to CD152-Fc was observed with increasing concentrations of CD80-CAP1 (Fig. 4B). However, neither peptide inhibited CD86 binding to either receptor proteins even at high concentrations (data not shown). This can be attributed to the differences in the binding pockets of CD80 and CD86 to accommodate the synthetic PP₆ helical peptide (21). The CD80-CAPs with predicted structural similarity but lacking PP₆ helical content did not inhibit CD80/CD86 binding to CD28/CD152 and were excluded from further studies (data not shown).

Previously, it has been shown that relative to its affinity for binding CD152, CD80 binds CD28 with slower kinetics and lower affinity (37, 38). A plot of change in absorbance with time showed that the maximum absorbance was reached at a later time point in the binding of CD80 to CD28 (220 s) than to CD152 (79 s), supporting the previous observations of slower association rate for the former interaction than the latter (39) (Fig. 4, C and D). Significantly, CD80-CAP1 at 25 μM drastically reduced the rate of association of binding of CD80 to both CD28 and CD152. The maximum velocity (mean optical density (MOD)/min) of CD80 binding to CD28 derived from overlapping segments of data points in the reaction was significantly reduced from 501.3 MOD/min to 258.3 MOD/min in the presence of CD80-CAP1 (25 μM) (Fig. 4E). Similar reduction in the maximum velocity was observed for the interaction between CD152 and CD80, from 575.9 MOD/min to 350.1 MOD/min in the presence of CD80-CAP1 (25 μM) (Fig. 4F). CD80-CAP3 (50 μM) also competed effectively, decreasing with the maximum velocity of CD80 binding to CD28 and CD152 to 435.5 and 406 MOD/min, respectively (data not shown). The optical density experiments showed significant reduction in the association rate of select CD80-CAP and to CD28 or CD152 consistently in multiple experiments. Taken together, these data suggest that both CD80-CAP1 and CD80-CAP3 can selectively block CD80-CD28/CD152 interactions. Previously, small molecule inhibitors thought to bind at the “MYPPPY” binding site on CD80 exhibited relatively weak inhibition of CD152-CD80 interactions as compared with CD28-CD152 interaction.

CD80-CAP Inhibits Antigen-specific T Cell Proliferation—The ability of the CD80-CAP to block the CD80-CD28/CD152 interactions on lymph node cells were assessed by T cell proliferation assays. LNC from bCII-sensitized mice were restimulated in vitro in the presence of varying concentrations of CD80-CAP. A significant decrease in the lymph node cell proliferative responses to collagen II was observed in cells treated with CD80-CAP1. A dose response was observed with maximum inhibition (55%) at 500 μM CD80-CAP1 (mean Δ cpm = 21,985) as compared with cells stimulated with bCII only (mean Δ cpm = 39,947) with the unstimulated cultures measuring 5648 cpm (Fig. 5). Interestingly, CD80-CAP3 was not inhibitory at all concentrations tested, as was the control peptide (Fig. 4). Although CD80-CAP3 adopted PPII helical conformation, none of the top 100 predicted docked structures exhibited significant proximity to the critical residues (Tyr²⁸ Val⁷⁹) at the binding pocket of CD80. This may explain the lack of inhibitory potential of CD80-CAP3 despite its ability to compete with CD80-Fc for binding CD28 and CD152.

Treatment with CD80-CAP Protects against CIA and Inhibits Primed T Cell Responses—The biological potential of CD80-CAP1 to block the development of inflammatory CIA during
antigen priming in vivo was tested. Groups of DBA/1 Lac J mice were induced CIA and administered a single intravenous injection of CD80-CAP1 or control peptide (500 μg) or PBS on the day of bCII immunization. The vehicle- and control peptide-treated arthritic mice exhibited a maximum mean disease severity index of 9.25. In contrast, a significant suppression of arthritis was observed in mice treated with CD80-CAP1 exhibiting a maximum mean disease severity index of 3.3 (Fig. 6). Collectively, these results suggest that CD80-CAP1 probably possesses the optimum PP1 helical content to achieve the bioactive conformation as it binds CD80 on the antigen-presenting cells.

DISCUSSION

The bias introduced in the combinatorial approach and the large number of peptides involved in random peptide screening by phage display, often compromise the process of identification and development of protein antagonists (4). Therefore, development of pseudoreceptors or minireceptors by rational modification of the interfaces in known receptor ligand complexes offers an attractive alternative approach (9). Here we present the design of a functional CD80 binding peptide antagonist (CD80-CAP) that may be regarded as a T cell inhibitory pseudoreceptor.

The importance of the polyproline motif in the FG loop of CD28 and CD152 in B7 binding has been substantiated by functional and structural studies (2, 12, 21, 30). The MYP-PPY sequence contributes 400 Å of protein surface to the binding interface of CD152-CD80 complex dominating the interaction. Similar nonrepetitive proline-rich sequences in the binding sites of actin receptor (XPPPP, X represents G/L/I/S/A) or phosphatidylinositol 3-kinase receptor (PPRPLPVAPGSSKT) have been shown to preferentially adopt PPII helical conformation (31). In a survey of 274 nonhomologous polypeptide chains from proteins of known structure, Stapley and Creamer (33) have shown that more than half of the polypeptide chains have at least one PPII region. It has been suggested that PP1 helix formation is a unidirectional local folding event driven by steric interactions between proline and the immediately preceding residue (37). Over 90% of PPII helices in globular domains are short, being 4–5 residues in length (14). Previously, synthetic peptides made up of residues from the binding sites of receptors for Src...
mice were immunized with 100 µg of bCII in CFA. Groups of mice were administered intravenously 500 µg of CD80-CAP1 or control peptide or vehicle on the day of immunization. The severity of arthritis was evaluated by assigning a score of 0–4 based on the degree of inflammation for each limb, with 4 indicating severe arthritis and 0 indicating no arthritis and a maximum score of 16 per mouse. The numbers of mice in each treatment group are indicated. Data are presented as mean severity of arthritic mice (total clinical score per group divided by the number of mice in each group.) (A) and average severity per arthritic mouse per group (B) (*, p < 0.05).

FIG. 6. Treatment with CD80-CAP1 prevents CIA. DBA/1 Lac J mice were immunized with 100 µg of bCII in CFA. Groups of mice were administered intravenously 500 µg of CD80-CAP1 or control peptide or vehicle on the day of immunization. The severity of arthritis was evaluated by assigning a score of 0–4 based on the degree of inflammation for each limb, with 4 indicating severe arthritis and 0 indicating no arthritis and a maximum score of 16 per mouse. The numbers of mice in each treatment group are indicated. Data are presented as mean severity of arthritic mice (total clinical score per group divided by the number of mice in each group.) (A) and average severity per arthritic mouse per group (B) (*, p < 0.05).

homology 3 domains or antigenic peptides binding the major histocompatibility complex have been shown to adopt PPII helical conformation and inhibit protein-protein interactions (6, 38). Based on residue interface preferences and PPII helical propensity, novel CD80-CAPs were designed so as to possess optimum PPII helical content in a bioactive conformation within the binding pocket of CD80. Molecular superposition is one of the most important means to interpret the relations between three-dimensional structures and activities of known active compounds (9, 39). The observed low r.m.s. deviation upon superimposition of CD80-CAP1 with the ligand binding motif of CD152, the active site that inhibits B7-CD28/CD152 interactions, suggests that the former represents a true structural mimic of the CD80 binding mode of CD152.

Based on analysis of structural complexes of protein-protein interactions wherein binding depends on the presence of one or more prolines, it has been suggested that the functionally critical proline residues in the interface of one protein often are in contact with one or more aromatic residues from the other component (40). In the complex between CD152 and CD80/CD86, the proline in the binding interface of CD152 is packed against the tyrosine in CD80 and phenylalanine in CD86. Molecular docking predicts close proximity between the edges of the Pro3 in the CD80-CAP1 with the face of Tyr28 in the binding pocket of CD80 (Fig. 2). This geometrical orientation has been more frequently observed for functionally important proline residues in the binding interfaces (40).

Structurally, the extended conformation of PPII helix has been shown to be an important secondary structure at the binding site of many protein/protein or peptide/protein interactions involved in transcription, signaling cascades, cytoskeletal rearrangements, and antigen recognition complexes (15, 32, 33). The weaker binding of proline-rich regions has been suggested to be advantageous in transient interactions as it permits large changes to be made in the Kd by small changes in the sequence of the proline-rich sequence or of its binding domain (31). Previously, it has been shown that the CD152 has higher affinity for binding CD80 than CD86 (1). Interestingly, it is observed that Pro101 of CD152 is restricted to the PPII space in the complex with CD80 (φ = −75) but not with CD86 (φ = −85). Backbone solvation, hydrogen bonding, and side-chain interactions are some of the factors involved in PPII helix formation (13, 15, 37). Thus, the local environment of the CD80 binding pocket and the orientation of functionally important Pro101 of CD152 may account for the differences in the strength of interactions between CD152 and CD80/CD86. In this context, it is interesting to observe that the synthetic CD80-CAP1 inhibits significantly the CD80 binding of receptors and has no effect on CD86 binding. This selective inhibition of CD80 binding by CD80-CAP1 could be attributed to its PPII helical content as well as the interresidue interaction and orientation within the smaller binding cavity of CD80 as compared with CD86. The protein-peptide interactions involving PPII structures are thought to be entropy-driven processes rather than enthalpy-induced associations as the key and lock model implies. Hence, PPII structure has been suggested to behave as an “adaptable glove” in order to get the correct recognition (31, 32).

The inhibitory potential of the CD80-CAP1 was tested in a T cell-dependent autoimmune disease model, CIA. Significantly, a single intravenous administration of CD80-CAP1 (500 µg) reduced the mean severity of collagen-induced arthritis, exhibiting a protective effect. The expression of CD80 is up-regulated in synovial T cells and antigen-presenting cells in rheumatoid arthritis (41, 42). However, administration of anti-CD80 antibody was not therapeutic in CIA (43, 44). The lower molecular weight and the optimal structure in relation to the binding cavity of CD80 probably facilitated greater tissue permeability and blocking efficacy of CD80-CAP1, accounting for its protective effect in CIA.

One of the approaches to identify lead agents is to map the receptor/ligand binding epitope onto a small peptide or peptidomimetic (39, 45). The validity of such an approach is supported by several functional immunomodulatory peptides designed to mimic the CDR-like regions of IgSF proteins (46, 47). In this report, the presence of functional proline at the binding site of CD152 and CD28 with the propensity to form PPII helix together with the interresidue contact preferences has been adopted in the design of novel T cell inhibitory agent that acts by blocking costimulation via CD80-CD28/CD152 interactions. Recently, it has been shown that conformational analyses of peptide and nonpeptide inhibitors of ovβ3 integrins by a hybrid method that integrates structure- and ligand-based drug design strategies can be adopted in the design of new potent ovβ3 integrin antagonists (48). Since the CD80-CAP assumes a bioactive conformation, as suggested by the binding and functional activity, it can be regarded as a starting point for the development of peptidomimetic, pseudopeptide, or small molecule inhibitors of T cell costimulation (39, 48, 49). The therapeutic potential of such an agent can extend to most T cell-mediated autoimmune diseases, transplant rejection, and chronic inflammatory conditions.
