Mimetics of a T Cell Epitope Based on Poly-N-acylated Amine Backbone Structures Induce T Cells in Vitro and in Vivo*

Received for publication, August 7, 2001, and in revised form, October 1, 2001
Published, JBC Papers in Press, October 12, 2001, DOI 10.1074/jbc.M107552200

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Peptidomimetics of the major histocompatibility complex (MHC) class I-restricted ovalbumin-derived T cell epitope SIINFEKL were generated by replacing parts of the peptide backbone by a poly-N-acylated amine (PAA) backbone with aromatic, heteroaromatic, and pseudo-aromatic side chains that branch off of the main chain at the amine nitrogen. The structure of the PAAs was designed to position this side chain in the central epitope anchor pocket of the MHC molecule. A number of biologically active PAAs were found that induced cytolysis by the mouse cytotoxic T cell clone 4G3. Competition experiments with independent peptides that are known to bind to the restricting MHC molecule H-2Kb suggest that the PAAs are bound by the MHC molecules at the same site as conventional peptide epitopes. The PAAs were active also in vitro and induced primary cytotoxic T cell responses in mice.

The endogenous peptides enkephaline and β-endorphin with agonist properties similar to opiates were the first reported example of ligands for peptide receptors mimicked by non-peptide compounds (1). Around 1975 and during the following years it was shown that these endogenous peptides and the opiates address the same receptor family (1, 2). Since this early work, a large number of non-peptide antagonists of peptide receptors have been found; some are used pharmaceutically (3), most as enzyme inhibitors, and some were developed as negative immune modulators (4). In contrast, very few non-peptide mimetics have been identified that exhibit agonist activity (1). Among these are ligands for G protein-coupled receptors such as angiotensin II (5), bradykinin (6), and growth hormone receptor agonists (7, 8) as well as for tyrosine kinase receptors. In most cases, these agonists are based on a peptide backbone structure and contain unusual or modified amino acid side chains. Several of these agonists were developed on the basis of previously established antagonists for the same receptor.

In earlier studies we had investigated the requirements for peptide binding by major histocompatibility complex class I (MHC-I) molecules (9, 10). MHC molecules are peptide receptors that control antigen-specific immune responses by T cells. They bind peptides derived from proteins through limited proteolysis and present them at the surfaces of cells (11). There are two classes of MHC molecules; the class I molecules (MHC-I) present peptides derived predominantly from internally expressed proteins, and the class II molecules present peptides derived mostly from endocytosed proteins. MHC-I molecules are heterodimers of a 45-kDa α-chain encoded by the polymorphic genes of the MHC and the non-covalently associated invariant 12-kDa β2-microglobulin (12). The peptides are usually 8–10 amino acids long (13) and are bound in a groove framed by two α-helices on top of a β-sheet pleated sheet (14). They are bound in extended conformation with the C- and N-terminal charges compensated by complementary MHC residues. Extensive hydrogen bonding between the peptide main chain and MHC side chains contribute to sequence-independent binding, whereas peptide sequence-specific binding is controlled largely by polymorphic MHC side chains that form MHC allele-specific pockets inside the peptide binding groove that accommodate two dominant and several subdominant anchor amino acid side chains of the T cell epitopes (15). The conformational stability of the MHC molecule largely depends on the presence of peptide, which, therefore, can be seen as an integral part of the protein (16). The energy required for melting the protein structure is tripled by the incorporation of a suitable peptide (17). The structural requirements for peptide selection by MHC molecules follows rules that are reminiscent of the packing of the core of a typical globular protein rather than for typical receptor ligand interaction. On average, more than 80% of the molecular surface of the peptide is buried inside the MHC molecular structure, and less than 20% is exposed to the outside and accessible for binding by the antigen receptor (T-cell receptor (TCR)) of MHC I-restricted cytotoxic T lymphocytes (CTL). Thus, MHC-dependent antigen recognition by T cells is based on a highly constrained one-ligand-two-receptors system that is focused on the constitutional elements of peptide structures.

The complex composed of the ovalbumin-derived octapeptide SIINFEKL (18) and the mouse MHC-I molecule H-2Kb has been dissected by various analyses on the protein chemical as well as crystallographic level (18). Peptides, naturally presented by H-2Kb, are preferentially octapeptides with an amino acid with an aromatic side chain at position 5 and an aliphatic side chain at position 8 (13). The binding of these motive amino acids into the binding pocket of the MHC molecules involves

* This work was supported in part by grants from the Deutsche Forschungsgemeinschaft (STE 366/7-4 TP1 and SFB 510, Projects C6 and D4) and the Volkswagen Foundation (I/75 325). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

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1 The abbreviations used are: MHC, major histocompatibility complex; TCR, T-cell receptor; CTL, cytotoxic T lymphocyte; PAA, poly-N-acylated amine; Fmoc, N-(9-fluorenylethoxycarbonyl); tBu, tert-butyl; HPLC, high pressure liquid chromatography; FCS, fetal calf serum; OVA, ovalbumin; VSV, vesicular stomatitis virus; BB, building block(s).
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hydrophobic effects and π-stacking in the case of the aromatic side chain and hydrophobic interaction in the case of the aliphatic side chain (14, 15). Positional scans done either by replacing the peptide amino acid with alanine or by using peptide libraries that carried a defined amino acid at one sequence position and randomized mixtures of the proteinogenic amino acids at all others have confirmed the importance of the anchor positions for peptide binding by H-2Kb (19, 20). In addition, positions 4, 6, and 7 were shown to be important for T cell recognition. These positions were found to be relatively tolerant to amino acid variations, whereas anchor positions 5 and 8 were the most restrictive sites. At positions 1, 2, 3, 5, and 6, the side chains branch off the amines of the structurally most constrained C-terminal part of the peptide (14, 15). Positional scans done either by replacing the peptide amino acid with alanine or by using peptide libraries that carried a defined amino acid at one sequence position and randomized mixtures of the proteinogenic amino acids at all others have confirmed the importance of the anchor positions for peptide binding by H-2Kb (19, 20). In addition, positions 4, 6, and 7 were shown to be important for T cell recognition. These positions were found to be relatively tolerant to amino acid variations, whereas anchor positions 5 and 8 were the most restrictive sites. At positions 1, 2, 3, 5, and 6, the side chains branch off the amines of the structurally most constrained C-terminal part of the peptide (14, 15, 19).

Non-peptide mimetics of T cell epitopes with agonist properties and improved biostability and bioavailability might become useful for the development of vaccines and immune therapeutics. To explore the possibilities for such non-peptide TCR agonists, we had designed and synthesized a series of oligomers derived from the T cell epitope SIINFEKL by replacing the C-terminal part of the peptide (positions 4–8) with poly-N-acylated amine (PAA) spacer (21). Briefly, all reagents, amino acids, and solvents were purchased from Fluka (Buchs, Switzerland), Aldrich (Milwaukee, WI), Novabiochem (Läufelfingen, Switzerland), or Merck (Darmstadt, Germany). Trypsin chloride resin was obtained from PepChem (Tübingen, Germany). The oligomers were synthesized manually in small syringes fitted with a frit up to the introduction of the last three N-terminal amino acids; this was performed on a simultaneous multiple peptide synthesizer (SMS 350, Zinsser Analytic, Frankfurt; Software Syro, MultiSynTech, Bochum, Germany), using Fmoc/tBu strategy. After the first C-terminal amino acid, the flexible spacer was introduced using a bromocarboxylic acid derivative and a free diamine. The latter was orthogonally protected with 2-acyldimedone at the primary amino function (21). Each PAA was acylated at the secondary amino group with the corresponding carboxylic acid building blocks (BB) (Fig. 1), preactivated with 1-hydroxybenzotriazole/diisopropylcarbodiimide in dimethylformamide for 30 min, and subsequently added to the resin. The products were analyzed by on-line HPLC-electrospray mass spectrometry. After being cleaved off the resin, the analytical RP-HPLC showed a purity of oligomers ranging from 60 to 81% with the exception of PAA17 with only 35% purity (Table I). The synthesis of PAA30 yielded a mixture of unidentified by-products. All of the compounds were tested without further purification. Also, the crude compounds PAA17 and PAA30 were used in the binding and cytolsis assays.

**Peptides**—The peptides were synthesized on solid phase using the Fmoc/tBu technology by EMCmicrocollections (Tübingen, Germany) as described elsewhere (20). The products were purified by reversed phase HPLC and their quality analyzed by analytical RP-HPLC and mass spectrometry.

**TABLE I**

| Compounds | R_t | ESI-MS | Purity | Compounds | R_t | ESI-MS | Purity |
|-----------|-----|--------|--------|-----------|-----|--------|--------|
| PAA6      | 19.2| 762.5  | 75     | PAA25     | 16.8| 799.0  | 69     |
| PAA13     | 17.5| 738.0  | 76     | PAA26     | 19.5| 801.0  | 75     |
| PAA14     | 14.7| 749.0  | 69     | PAA27     | 18.3| 805.0  | 74     |
| PAA15     | 16.1| 750.5  | 81     | PAA28     | 16.5| 805.0  | 74     |
| PAA16     | 18.2| 754.5  | 68     | PAA29     | 19.9| 806.0  | 75     |
| PAA17     | 14.9| 763.5  | 34     | PAA30     | ND  | ND     | ND     |
| PAA18     | 19.5| 778.0  | 78     | PAA31     | 21.7| 812.0  | 70     |
| PAA19     | 17.0| 778.5  | 75     | PAA32     | 22.4| 824.0  | 72     |
| PAA20     | 19.8| 780.0  | 71     | PAA33     | 20.3| 827.5  | 71     |
| PAA21     | 20.5| 788.0  | 73     | PAA34     | 22.3| 828.0  | 67     |
| PAA22     | 19.3| 792.0  | 80     | PAA35     | 20.2| 835.0  | 60     |
| PAA23     | 19.1| 792.5  | 79     | PAA36     | 22.9| 844.0  | 74     |
| PAA24     | 19.9| 794.0  | 74     | PAA37     | 22.6| 856.0  | 68     |

a RP-HPLC with a linear gradient of 0.1% trifluoroacetic acid in water (A) and 0.1% trifluoroacetic acid in acetonitrile (B), 10–100% B in 45 min at 0.3 ml/min flow rate; detection at 214 nm.

b Crude products after the cleavage from the resin.

c A trifluoroacetylated compound (R_t 19.6, molecular weight 859.5) was also present (34%).

d ND, not detected.
**MHC Stabilization Assay—H-2Kb** binding of the oligomers was tested by a stabilization assay as described in detail elsewhere (9) by making use of the dependence of the structural integrity of the MHC class molecules on the presence of ligand in their peptide binding groove. Briefly, the peptides and PAAs were incubated with the peptide transporter-deficient RMA-S cells, which then were tested for the expression of conformationally stable H-2Kb using the conformation-sensitive monoclonal H-2Kb-specific antibody B8.24.3. Ligand concentrations required for half-maximal H-2Kb stabilization (C50 stab) were calculated after linearizing the data by linear regression. Table II shows the C50 stab values for the oligomers tested (taken from Ref. 21).

### Cytotoxicity Assay—(120) The SIINFEKL-specific CTL clone 4G3 was cultured in Dulbecco’s modified Eagle’s medium containing 10% FCS and growth factor (supernatant of concanavalin A-stimulated rat spleen cells at a concentration of interleukin-2 in culture of 50 units/ml) at 37 °C in a humidified atmosphere with 8% CO2. The CTL were re-stimulated biweekly with the OVA transgenic thymoma cell line EG7.OVA. Tumor cells, RMA-S (H-2b), EG7.OVA (H-2b, OVA) and LB (H-2bKb) were grown in Dulbecco’s modified Eagle’s medium containing 5% FCS. In the case of EG7.OVA, 100 μg/ml G418 was included in the medium to maintain the selection for the transgene. The indicated target cells were labeled with 51Cr and pulsed or not with the peptides or oligomers at the indicated concentrations in α-MEM, 0.1% bovine serum albumin. After 30 min the CTL were added in α-MEM, 20% FCS at the indicated effector to target ratios, and the cultures were continued at 37 °C for 5 h. Radioactivity released from the target cells was measured in scintillation plates using a 96-well plate β-counter (Packard Instrument Co.). The percent specific 51Cr release was calculated as follows: (experimental cpm – background cpm)/(total cpm – background cpm) × 100.

### RESULTS

**Induction of Cell-mediated Cytolysis by PAA Oligomers**—The PAAs used in the present study were derived from the H 2Kb-restricted OVA T cell epitope SIINFEKL by replacing the backbone of the C-terminal part of the peptide including sequence positions 4–8 by a poly-N-acylated amine structure. To establish the optimal length of this new backbone, a series of oligomers had been synthesized previously and tested for binding to the MHC-I molecule H-2Kb. These new ligands were characterized by aromatic, heteroaromatic, and pseudoaromatic side chains (Fig. 1) at a central position, a leucine residue at the C-terminal position, and polyanine spacer of different lengths and compositions between the two anchoring positions (21). PAA6 bound most efficiently to and stabilized H-2Kb. To then identify the optimal side chain constitution at the central and the C-terminal anchor position, a combinatorial library based on PAA6 was prepared by introducing a mixture of the building blocks BB6 and BB13–BB37 into the central and a mixture of the aliphatic protein amino acid side chains into the C-terminal position (21). This doubly randomized oligomer library was deconvoluted by, first, establishing the optimal C-terminal side chain and, second, with a leucine residue at this position, testing the N-acylated side chains shown in Fig. 1 at the central anchor position. All of the resulting oligomers bound to and stabilized H-2Kb, however, with different levels of efficiency, as reported earlier (21). In the present study these 26 oligomers, derived from the library de-convolution, were tested for their capacity to stimulate T cell responses. Prerequisite for such responses is the simultaneous binding of the oligomers by the MHC-I molecule H-2Kb and the antigen receptor of the T cell. 4G3, which was established by immunizing mice with the OVA-expressing EG7.OVA cells and which is specific for the T cell epitope SIINFEKL was employed for these initial analyses. To enhance the efficiency of MHC binding, RMA-S were used as target cells in these experiments (9, 20). These cells lack the transporter associated with antigen processing and are, therefore, devoid of internal MHC-bound peptides. 51Cr release from radiolabeled target cells upon cytolysis by 4G3 was used as the read-out for T cell activation. The results of these analyses are shown in Fig. 2. The responses to the control peptides are shown in the first panel. SIINFEKL is the cognate peptide for the CTL clone 4G3 and induces a potent response. SIINFEDL is an antagonist of SIINFEKL and, as the independent VSV-derived epitope RGYVYQGL (23), was not capable of inducing cytolyis by 4G3. Already the parental oligomer PAA6 stimulates 4G3 to lyse the target cells. It is, however, surpassed in its potency by the derivative PAA22. PAA13, -15, -17, -25, -27–30, -32, -33, and -36 also induce high levels of CTL responses, which are comparable with the activity found for PAA6. PAA19–23, -26, -31, and -34 induced only low levels of cytolyis at high concentrations. PAA14, -16, -18, -20, -24, -35, and -37 were completely inactive.

**Dose-Response Relationships in PAA-induced Cytolysis**—To establish dose-response relationships for the oligomers, a more detailed titration of the concentrations required for the induction of cytolyis of RMA-S cells by 4G3 was done. The results are shown in Fig. 3 and Table II. PAA6 was chosen for these analyses as the original oligomer from which the others were derived. PAA22 was the most potent oligomer, PAA17 and PAA36 are representatives of the high efficiency ligands, PAA34 is a representative of the low efficiency group and PAA24 of the negative group. With concentrations between 0.289 and 12.3 nM required for half-maximal cytolyis of the target cells, the activity of the oligomers is in the same range found for many peptide agonists. Comparing PAA22 with the original PAA6, it was found to be ~42-fold more efficient in inducing T cell responses. Because the MHC binding of these two PAAs differs only slightly, this increased efficiency is largely caused by an improved binding of the MHC-PAA22 complex by the 4G3 T cell receptor. PAA34 is ~400-fold less efficient than PAA22 and is comparable with weak peptide agonists. PAA22 is about 46.000 times less efficient in
inducing cytolysis by 4G3 than the cognate peptide SIINFEKL. Taking into account that H-2Kb binds this oligomer
5,200-fold less efficiently than SIINFEKL, the H-2Kb-PAA22 complex in-
duces cytolysis by 4G3 9-fold less efficiently than the corre-
sponding complex with SIINFEKL.

**PAAs Compete with Cognate Peptides for Recognition by the T Cell 4G3**

Competition experiments were done to test whether the PAA oligomers bind to the MHC molecules at the
same site as known peptide ligands and whether the MHC-
oligomer complex and conventional MHC-peptide complexes
interact with the TCR in the same way (Figs. 4 and 5). The
VSV-derived T cell epitope RGYVYQGL, which binds to H-2Kb
(23) but is not recognized by the 4G3 T cell receptor, inhibits
4G3-mediated cytolysis of the RMA-S target cells induced by
SIINFEKL and by PAA22 and PAA36, the two most efficient
oligomers (Fig. 4). In a reverse setup, the oligomer PAA6 and
the library SII-X--L (21) also compete with SIINFEKL for bind-
ing to H-2Kb and induction of 4G3 activity as shown in Fig. 5.
The controls in this latter experiment were RGYVYQGL, as in
the previous experiment, a variant of this peptide with tyrosine
at the C terminus and SIINFEDL, which is a potent antagonist

**FIG. 2. Analysis of the capacity of PAAs to induce cytolytic T cell responses.** The PAAs were incubated with 51Cr-labeled RAM-S target cells and 4G3 cytotoxic T cells for 5 h at 37 °C. The effector to target ratio was 5:1. After 5 h, 100 μl of the supernatants were harvested and analyzed for the radioactivity. % Specific 51Cr release was calculated as described under “Experimental Procedures.”

**TABLE II**

| Oligomer | Oligomer concentration [M] for half-maximal: |
|----------|---------------------------------------------|
|          | MHC stabilization<sup>a</sup> | Cytolysis<sup>b</sup> |
| PAA6     | $231 \times 10^{-6}$ | $12.3 \times 10^{-9}$ |
| PAA17    | $321 \times 10^{-6}$ | $7.2 \times 10^{-9}$ |
| PAA22    | $175 \times 10^{-6}$ | $0.289 \times 10^{-9}$ |
| PAA24    | $335 \times 10^{-6}$ | No response |
| PAA34    | $278 \times 10^{-6}$ | $115 \times 10^{-9}$ |
| PAA36    | $93.8 \times 10^{-6}$ | $5.8 \times 10^{-9}$ |
| SIINFEKL | $0.337 \times 10^{-6}$ | $0.0000062 \times 10^{-9}$ |

<sup>a</sup> Taken from Ref. 21.
<sup>b</sup> Calculated from the results shown in Fig. 3.
of SIINFEKL. SII-X--L is less potent as an inhibitor than PAA6. Because both oligomers are agonists (weak agonists when compared with SIINFEKL), the cytolysis observed is a balance of competition with SIINFEKL for MHC binding and their own agonist activity. The difference between the results for PAA6 and SII-X--L is in accordance with the less efficient MHC-binding capacity of the latter ((21), Table II, and Fig. 5).

**Induction of T Cell Responses to PAA in Mice**

To test the antigenic capacity of PAA22, mice were immunized with the oligomer as described above. The in vivo induced cytotoxic T cells that tested positive for CD8 (data not shown) were analyzed in vitro for their specificity using RMA-S cells as targets and PAA6 and -22 as antigens, as well as SIINFEKL and SIINFEDL as control peptides. As shown in Fig. 6, these cells lysed the target cells in the presence of PAA22. No cross-recognition of SIINFEDL was ever observed. Also, PAA6 and SIINFEKL were recognized by the PAA22-primed T cells, but the degree of cross-reactivity varied from experiment to experiment. Fig. 6 shows two representative experiments. The effector cell cultures were titrated in serial 2-fold dilutions as indicated.

**DISCUSSION**

MHC-restricted recognition of epitopes by TCRs is highly constrained and appears to depend largely on the typical molecular features of peptides (15). MHC-I molecules bind the peptides in extended conformation by forming a number of molecular interactions with the terminal main chain charges, specific side chains, and the peptide bonds of the main chain. The peptide is thereby forged into a conformation that exposes only a few side chain residues to the outside of the complexes for interaction with the T cell receptor. The specificity of the T cell response depends on these few residues (9, 24).
these constraints, it is possible, as demonstrated by the data presented herein, to design non-peptide mimics that bind to MHC-I molecules and induce T cell responses. The dose-response analyses for these oligomers reveals that they are about six orders of magnitudes less efficient in inducing responses by the T cell clone 4G3 than its cognate ligand SIINFEKL (18). However, half-maximal T cell-induced cytolysis is achieved with not concentrations of the oligomer, which is comparable with many T cell epitopes. A comparison of the peptide and PAA concentrations required for half-maximal saturation of the MHC molecule H-2Kb (21) shows that the reduced efficiency of the PAA for T cell stimulation is mainly due to their less efficient binding to the MHC-I molecule and to a lesser degree to the recognition of the resulting MHC-oligomer complex by the TCR. This observation is consistent with the high degree of rotational freedom of the PAA backbone compared with the more constrained peptide bonds. Once bound by the MHC molecule, PAs seem to compare well with peptides in their capacity to stimulate T cell responses.

The optimized PAs are not only recognized by the established T cell clone 4G3 but can also induce primary T cell responses in vivo in mice. In extensive experiments using combinatorial peptide libraries, 4G3 had been found before to display a relatively high degree of degeneracy (25). This finding might explain the relative ease and the relatively high yield of the identification of cross-recognized PAs in the experiments presented herein. Analyses of the specificity of T cell lines and clones induced in mice with PAA6 and -22 show that these two oligomers address different T cell repertoires, which also differ in vivo. In extensive experiments using combinatorial libraries, PAA6 and -22 show that these two oligomers address different T cell repertoires, which also differ greatly in their degree of degeneracy.

The binding of PAs to the MHC-I molecules H-2Kb of the active PAA seems to be similar to the way in which peptides bind. This conclusion is supported by the observation that independent H 2Kb-binding peptides are competitive inhibitors of the PAs and that the PAs, in turn, suppress the T cell response to the cognate epitope of 4G3 SIINFEKL. However, comparing the constitution of the aromatic, heteroaromatic, and pseudoaromatic building blocks that induce T cell responses with those that are negative reveals no clearly identifiable structure-function relationship. Neither the bulkiness of these side chains, the numbers of aromatic rings, nor the presence or absence of polar groups or heteroatoms correlate with the biological activity. The most potent PAA carries a p-methoxybenzyl side chain (PAA22). The small furan side chain (BB13) is as active as the very bulky biphenyl side chain (BB32), whereas the small thiophene side chain (BB16) is inactive. Similarly, the difference in activity between BB6 (benzyl side chain) and BB22 (p-methoxybenzyl side chain), both potent T cell inducers, and BB19 (p-hydroxybenzyl side chain) and BB20 (p-fluorobenzyl side chain), which are either very marginally active or completely negative, cannot be easily explained. If the PAs bind to the MHC molecules in the same way as peptides, then all of these BBs should be inserted into the pocket for the central aromatic anchor amino acid side chain of peptides and thus point into the MHC-I molecule and away from the T cell receptor. Yet, the different BBs have strikingly different effects on the responses of the T cells. In an earlier study we were able to show that hydroxylation of one of the nitrogens of the peptide backbone of the SIINFEKL epitope, which is also hidden inside the MHC molecule, can affect T cell responses and, for instance, render an agonist peptide into an antagonist (26). These two examples demonstrate that very subtle changes at epitope sites that are buried inside the MHC molecule and that are not accessible to the T cell receptor can result in differences in the binding of the complex by the receptor that translate into substantially different cellular responses.

Even more striking with respect to structure-function relationships is the fact that PAs induce specific reactions of the 4G3 T cell clone at all, although they lack side chains at the positions that correspond to the peptide sequence positions 4, 6, and 7. In SIINFEKL, these three sequence positions carry asparagine, glutamate, and lysine, respectively, which are the most prominently exposed side chains and crucially important for the induction of T cell responses. Various experiments have shown that the replacement of one of these amino acids by alanine can abolish the T cell response. It thus appears that the TCR of 4G3 interacts differently with the MHC-PAA complex than with the MHC-SIINFEKL complex. The interaction of a single TCR with two very different MHC peptide complexes has been shown for the T cell clone 2C (27, 28). In this case, an altered orientation of one of the complementarity-determining region loops of the TCR generates a different interaction surface with different binding properties.

PAs are readily synthesized on solid phase and thereby easily accessible (21). Their resistance to proteolysis should result in an improved biostability, which might be very interesting for in vivo applications. Also the capacity of the optimized PAs to exert biological activity at sub-nm concentrations supports the notion that they might constitute an interesting new class of T cells epitope mimetics suitable for in vivo applications. Moreover, these PAs testify to the fact that it is possible to generate agonistic non-peptide analogues of ligands for peptide receptors.

Acknowledgments—We are grateful to Arpenik Nashdeian and Karin Kälberer for technical support and to Patricia Zambon and Rodion Demine for assistance in preparing this manuscript.

REFERENCES
1. Beeley, N. R. A. (2000) Drug Discov. Today 5, 354–363
2. Ashby, A., Birirrakis, N., Sakarellos-Daitistis, M., Sakarellos, C., and Marraud, M. (1989) Biochem. Biophys. Res. Commun. 25, 27–49
3. Evans, B. E., Rittle, K. E., Bock, M. G., DiPardo, R. M., Freidinger, R. M., Whitter, W. L., Lundell, G. F., Veber, D. F., Anderson, P. S., and Chang, R. S. (1988) J. Med. Chem. 31, 2235–2246
4. Falciioni, F., Ito, K., Vidovic, D., Belunis, C., Campbell, R., Berthel, S. J., Bolin, D. R., Gillespie, P. B., Huby, N., Olson, G. L., Sarabu, R., Guenot, J., Madison, V., Hamm, J., Sinapiglia, F., Steinmetz, M., and Nagy, Z. A. (1999) Nat. Biotechnol. 17, 562–567
5. Perlman, S., Schambey, H. T., Rivens, R. A., Greenlee, W. J., Hjorth, S. A., and Schwartz T. W. (1995) J. Biol. Chem. 270, 1493–1496
6. Aramori, I., Zenkoh, J., Morikawa, N., 5, Asano, M., Hatori, C., Sawai, H., Kayahiki, H., Satoh, S., Inoue, T., Abe, Y., Sawada, Y., Mizutani, T., Inamura, N., Iwami, M., Nakahara, K., Kojo, H., Oka, T., and Natu, Y. (1999) Immunopharmacology 45, 185–190
7. Smith, R. G., Cheng, K., Schoen, W. R., Pong, S. S., Hickey, G., Jacks, G., Butler, B., Chan, W. W., Chuang, L. Y., and Judof, F. (1993) Science 260, 1640–1645
8. Patchett, A. A., Nargund, R. P., Tata, J. R., Chen, M. H., Barakat, K. J., Johnston, B., Cheng, K., Chan, W. W., Butler, B., and Hickey, G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7001–7005
9. Udaka, K., Wiesmueller, K.-H., Kienle, S., Jung, G., and Walden, P. (1995) J. Biol. Chem. 270, 24130–24134
10. Priddun, L., Wiesmueller, K.-H., Kienle, S., Jung, G., and Walden, P. (1996) Eur. J. Immunopharmacol. 236, 249–253
11. Barber, L. D., and Parham, P. (1993) Annu. Rev. Cell Biol. 9, 163–206
12. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennety, W. S., Strominger, J. L., and Wilson, I. (1987) Nature 327, 562–567
13. Falk, K., Ro¨tzschke, O., Stevanovic, S., Jung, G., and Walden, P. (1996) Eur. J. Immunol. 27, 562–567
14. Fremont, D., Matsumura, M., Stura, E. A., Peterson, P., and Wilson, I. (1992) Science 257, 919–927
15. Matsumura, M., Fremont, D. H., Peterson, P. A., and Wilson, I. A. (1992) Science 257, 927–934
16. Bouvier, M., and Wiley, D. C. (1994) Science 265, 398–402
17. Fahnestock, M. L., Tamir, I., Narhi, L., and Bjorkman, P. J. (1992) Science 258, 1658–1662
18. Ro¨tzschke, O., Falk, K., Stevanovic, S., Jung, G., Walden, P., and Rammensee, H. G. (1991) Eur. J. Immunol. 21, 2891–2894
19. Fremont, D. H., Stura, E. A., Matsumura, M., Peterson, P. A., and Wilson, I. A.
Non-peptide T Cell Epitopes

(1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2479–2483
20. Udaka, K., Wiesmüller, K.-H., Kienle, S., Jung, G., and Walden, P. (1995) J. Exp. Med. 181, 2097–2108
21. Bianco, A., Brock, C., Zabel, C., Walk, T., Walden, P., and Jung, G. (1998) J. Biol. Chem. 273, 28759–28765
22. Borges, E., Wiesmüller, K. H., Jung, G., and Walden, P. (1994) J. Immunol. Methods 173, 253–263
23. van Bleek, G. M., and Nathenson, S. G. (1990) Nature 348, 213–216
24. Gundlach, B. R., Wiesmüller, K. H., Junt, T., Kienle, S., Jung, G., and Walden, P. (1996) J. Immunol. 156, 3645–3651
25. Walden, P., Wiesmüller, K.-H., and Jung, G. (1995) Biochem. Soc. Trans. 23, 678–681
26. Hin, S., Zabel, C., Bianco, A., Jung, G., and Walden, P. (1999) J. Immunol. 163, 2363–2367
27. Brock, R., Wiesmüller, K.-H., Jung, G., and Walden, P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13108–13113
28. Speir, J. A., Garcia, K. C., Brunmark, A., Degano, M., Peterson, P. A., Teyton, L., and Wilson, I. A. (1998) Immunity 8, 553–562
Mimetics of a T Cell Epitope Based on Poly-N-acylated Amine Backbone Structures Induce T Cells *in Vitro* and *in Vivo*

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*J. Biol. Chem.* 2001, 276:48790-48796.
doi: 10.1074/jbc.M107552200 originally published online October 12, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M107552200

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