Update on D-Ala-Peptide T-Amide (DAPTA): A Viral Entry Inhibitor that Blocks CCR5 Chemokine Receptors

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Abstract: Peptide T, named for its high threonine content (ASTTTNYT), was derived by a database search which assumed that a relevant receptor binding epitope within *env* (gp120) would have sequence homology to a known signaling peptide. Binding of radiolabeled gp120 to brain membranes was displaced by peptide T and three octapeptide analogs (including "DAPTA", Dala1-peptide T-amide, the protease- resistant analog now in Phase II clinical trials) with the same potency that these four octapeptides blocked infectivity of an early passage patient isolate. This 1986 report was controversial due to a number of laboratories' failure to find peptide T antiviral effects; we now know that peptide T is a potent HIV entry inhibitor selectively targeting CCR5 receptors with minimal effects on the X4 tropic lab adapted virus exclusively in use at that time. Early clinical trials, which demonstrated lack of toxicity and focused on neurological and neurocognitive benefits, are reviewed and data from a small ongoing Phase II trial---the first to assess peptide T's antiviral effects---are presented. Studies using infectivity, receptor binding, chemotaxis, and blockade of gp120-induced neurotoxicity *in vitro* and *in vivo* are reviewed, discussed and presented here. Peptide T and analogs of its core pentapeptide, present near the V2 stem of numerous gp120 isolates, are potent ligands for CCR5. Clinical data showing peptide T's immunomodulation of plasma cytokine levels and increases in the percentage of IFNγ secreting $CD8^+$ T cells in patients with HIV disease are presented and suggests additional therapeutic mechanisms via regulation of specific immunity.

Keywords: Peptide T, entry inhibitor, immune reconstitution, antiviral, IFNγ, chemokine, cytokine, reservoir

DISCOVERY

By 1985, our National Institutes of Health laboratory had realized that the central nervous and immune systems shared many highly conserved receptors for peptide-mediated intercellular communication [78]. When we heard that CD4 was the receptor which HIV used for viral entry, we correctly assumed that this receptor would also be found in brain and hoped that our neuropharmacology laboratory might use its receptor methodological expertise to identify an effective receptor antagonist therapy against AIDS. In 1986, we identified an octapeptide inhibitor of gp120 binding and infectivity [76].We arrived at this octapeptide via a database search which assumed that a relevant receptor binding epitope within *env* (gp120) would have sequence homology to a known signaling (neuro) peptide. Peptide T, named for its high threonine content (ASTTTNYT), was derived from amino acids 185-192 of the gp120 V2 region of the SF-2 isolate [91].

We assumed that peptide T was targeting the CD4 receptor, the only identified HIV receptor at that time [20, 47]. The first chemokine would not be identified for two more years, and the role of chemokine receptors in HIV entry awaited a further ten years of research. The binding of radiolabeled gp120 to brain membranes was displaced by peptide T and three analogs (including "DAPTA", Dala1-peptide T-amide, the protease resistant analog [44] now in Phase II clinical trials) in precisely the same order of potency that these four octapeptides blocked infectivity of an early passage patient isolate [76]. We now know that brain is a rich source of chemokine receptors [28, 37, 42] and that in our early studies peptide T was probably binding to those receptors and not to CD4 as we initially posited.

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Our 1986 report generated some interest and more controversy due to a number of laboratories' failure to find peptide T antiviral effects. The one publication that appeared [96] detailed the failure of peptide T to suppress the replication of large amounts of viral inocula (undiluted H9IIIB supernatants) that had been propagated in T cell lines, or to inhibit synctyia formation of this virus in T cell lines. These common assays of the time were being effectively used to screen for nucleoside inhibitors, but their ability to detect receptor blockade was unproven and even a potent receptor antagonist would fail to be detected in the presence of excessive competing ligand, in this case virions.

Even more salient is the fact that we now know that peptide T acts as a potent HIV entry inhibitor that selectively targets CCR5 receptors with absent or greatly reduced effects on the X4 tropic lab adapted virus exclusively in use at that time [90]. The fact that peptide T's interactions with CCR5 receptors were not understood and its mechanism of action erroneously attributed to CD4 [76] for a decade after its discovery unfortunately greatly slowed the pace of clinical trials.

EARLY CLINICAL TRIALS

Although peptide T was invented in 1986, this is the first time that there has been a clinical trial measuring viral burden. Peptide T was invented at the National Institutes of Mental Health (NIMH) and previous studies focused on neurological and cognitive clinical assessments, its controversial anti-viral effect precluding its acceptance for ACTG studies in 1987-1988. At present, a clinical trial of peptide T is continuing at Saint Francis Memorial Hospital in San Francisco. This trial involves HIV positive patients who are receiving DAPTA in concert with standard HAART therapies. Those individuals are being studied intensively for a number of immunological parameters as well as viral load changes. Phase I trials conducted by the NIMH between 1987 and 1989 revealed that DAPTA was completely nontoxic and also demonstrated a number of symptomatic improvements. Most striking of these were improvements in cognitive and neuromotor function in patients with moderate neuropsychologic impairment compared with controls. Improvements in constitutional symptoms, included weight gain averaging 2 kg, were also noted. [8, 9] However, a double blind placebo trial of intranasal DAPTA for the treatment of painful distal neuropathy associated with AIDS found no significant difference in pain- the primary endpoint [93]. At the time of this trial which had three sites in New York City and one in San Francisco, an underground distribution network of peptide T was flourishing in these cities. Reports of patients mixing their drugs seem probable since blinded HPLC analysis and subsequent radioimmunoassay of blood samples taken one-half hour after drug administration revealed the presence of peptide T in 45% of the placebo group at one New York City site.

Peptide T has been shown to have clinical benefits in reversing, not merely attenuating, deficits in memory and cognition associated with HIV infection in several placebo-controlled trials [9, 38, 53] and has caused MRI brain scan improvements [100, 101]. A multi-site, two hundred patient, placebo-controlled study of peptide T for cognitive endpoints was conducted by the NIMH [38]. This trial failed to find cognitive improvements for the cohort as a whole, but did find that peptide T treatment was associated with overall cognitive improvement in patients who had more severe and clinically significant levels of cognitive impairment (baseline global deficit Z scores of at least 0.5), while overall deterioration was more common among the placebo group ($P<02$). One explanation is that 2/3 of the cohort had such minimal cognitive deficits that improvements could not be detected ("ceiling effect"). The power of the study was diminished by the use of a comprehensive but burdensome battery of neurocognitive tests given over a two day period, many of which had not been validated for sensitivity to treatment-induced improvements in HIV patients. The NIMH trial was conducted from 1989 and 1995, when the NIMH, upon breaking the blind for the neurocognitive data, broadly publicized that "no clinical benefit" had occurred. Although frozen plasma suitable for viral analysis was carefully collected and stored by the NIMH, no viral level analysis has yet been made available. These facts further contributed to a loss of interest in peptide T for HIV disease which was not remedied until the discovery of the role of chemokine receptors prompted a re-examination.

EFFECT ON CCR5 – NOT CD4 – MEDIATED ENTRY INHIBITION

The envelope protein of HIV interacts with two major chemokine receptors, CXCR4 [25] and CCR5 [14] to enter cells and select chemokines inhibit infectivity [4, 16]. HIV-1 establishes infection primarily via interactions with the β-chemokine receptor CCR5 in macrophages [14, 22, 69] and viral strains, which use this receptor, predominate during initial spread and the early phases of infection. These new findings of chemokine co-receptor usage by HIV-1 prompted us to determine if peptide T might exert its anti-HIV/gp120 effects through targeting of chemokine receptors rather than CD4. The first studies performed to explore this exciting possibility utilized chemotaxis and showed that peptide T preferentially inhibits β-chemokine chemotaxis and is a partial inhibitor of ^{125}I-MIP-1β binding [84, 86].

The infection of monocyte-derived macrophages (MDMs) with BaL, a CCR5-tropic isolate, or early passage dual-tropic isolates in activated $CD4^+$ lymphocytes was inhibited by peptide T, while little to no inhibition was observed with lab adapted X4 viruses (such as IIIB, MN, or NL4-3) propagated in $CD4^+$ T cells. (Fig. **1**). Peptide T was a more effective antagonist of infection in MAGI-CCR5 cells compared to CXCR4 expressing MAGI cells (Fig. **2A**). Detection of antiviral effects becomes more difficult at higher viral concentrations, as would be expected in a receptor-mediated process, and CXCR4 antiviral effect (Figs. **2B,C**) was only detected at the lowest viral dose.

Virus inhibition ranged from 60-99%, depending on the assay, receptor target, viral isolate and amount of added virus. Peak inhibitory effects were detected at concentrations from 10^{-12} to 10^{-9} M and peptide T acted to block viral entry as it inhibited in the MAGI cell assay and blocked

A.

DAPTA (M)	p24 (pg/ml) ± s.e.m.	Inhibition (log 10)
0	8792 ± 766	
10^{-12}	146 ± 98	-1.8
10^{-9}	255 ± 66	-1.5
10^{-7}	287 ± 54	-1.5

B.

Virion (Rc Usage)	Treatment	p24 (pg/ml)/sem	%Inhibition
MN (X4)	None	2270 ± 385	-
"	DAPTA (10^{-10}M)	2243 ± 216	0
"	DAPTA (10^{-8} M)	2074 ± 426	9
92HT596 (R5/X4)	None	2412 ± 174	-
"	DAPTA (10^{-10}M)	140 ± 66	94
"	DAPTA (10^{-8} M)	574 ± 122	76

Fig. (1). Selective R5 antiviral activity of peptide T. Panel A. Inhibition of HIV-1_{BaL} production from monocyte derived macrophages (MDMs) by peptide T. Monocytes were cultured for five days to generate monocyte-derived macrophages (MDMs) which were then infected with HIV-1_{BaL} virus (1ng/culture), with or without (vehicle only control) peptide T at the indicated concentrations. Supernatants were sampled and tested on d7 for p24 antigen production by ELISA kit. Results are adapted from [90]. Panel B. Inhibition of early passage R5/X4 (dual-tropic), but not lab-adapted HIV-1 isolates in primary $CD4^+$ T cells by peptide T. Purified $CD4^+$ T cells were activated with PHA and cultured in IL-2 containing media for three days prior to treatment with peptide T ("DAPTA", Dala1-peptide T-NH2) and infection with HIV-1 isolates with varied receptor tropism, as indicated. P24 levels were determined at day 7 post-infection and are the means of replicate cultures. Peptide T was partially suppressive for 92HT593, highly suppressive for the 92HT596, but did not inhibit the R4-tropic laboratory isolates MN and NL4-3. Results are adapted from [90].

infection in the luciferase reporter assay using HIV virions pseudotyped with ADA envelope [90].

Peptide T therefore has greatly reduced efficacy for X4 compared to R5 tropic HIV isolates. The failure of peptide T to act on X4 HIV isolates adapted to grow in cultured cell lines compared to the uncharacterized patient isolate (probably of R5 or dual-tropic receptor phenotype) used in our initial report [76] explains the early failure to replicate peptide T inhibition [88] using infected H9IIIB cells [96]. Infection in this assay occurs via X4 receptors (with CD4), an important detail which was not understood at the time.

PEPTIDE $T_{(4-8)}$ AND RELATED GP 120 DERIVED PENTAPEPTIDES: A ROLE IN RECEPTOR BINDING?

The identification of receptor binding domain(s) of gp120 is an important goal which has relevance to the development of receptor targeted therapeutics, as well as vaccines. The eight amino acid peptide T sequence was derived from a variable region (V2), an observation at odds with the assumption that a receptor active binding epitope would be conserved in identical form, in all HIV isolates [96]. We noted [88] that many endogenous peptide ligands exist in variant forms which preserve receptor potency and action. As additional HIV isolates were sequenced, we observed that five terminal amino acids of the peptide T sequence of gp120, located in the V2 loop, near the stem, are in fact, highly conserved [77]. Related pentapeptide sequences are comprised of sterically and chemically related amino acids present in similar location in other gp120 isolates [88]. (See also Figure 3) We synthesized many of these pentapeptides and showed they are potent chemoattractants [89] and gp120 antagonists [5, 86, 88] that suppressed viral replication and inhibited gp120 binding [88]. These pentapeptides share the activity of peptide T (DAPTA) to function as selective CCR5 entry inhibitors, as they block infection in the MAGI reporter assay, which is sensitive to entry inhibition [46, 82] (Fig. **3**).

The conservation of the peptide T pentapeptide motif in gp120 which, not only blocks infectivity, but also blocks gp120-induced neurotoxicity [5], gp120 signaling [58, 104] and β-chemokine induced chemotaxis [84, 86] suggests that this site in *env* mediates receptor binding to chemokine receptors. The active, synthetic, peptide T analogs have been shown to have a stable β-turn and substantial tertiary structure [19, 81] which is required for receptor potency. Large proteins often have small, discreet binding domains and small peptide antagonists derived from these larger polypeptides have been created [1, 34].

One hypothesis is that the peptide T epitope represents a receptor binding domain of gp120 [76], although most discussions of the determinants of chemokine receptor binding implicate the V3 region [43], or other sites near the V2 stem [87], and exclude a binding role for V2 based upon continued infectivity, albeit of lower efficiency, of virus with deletions in the V1/V2 region [11, 98]. No demonstration of direct receptor binding of V2 deleted gp120 has been reported so that it is difficult to compare receptor potency of mutant compared to native envelope

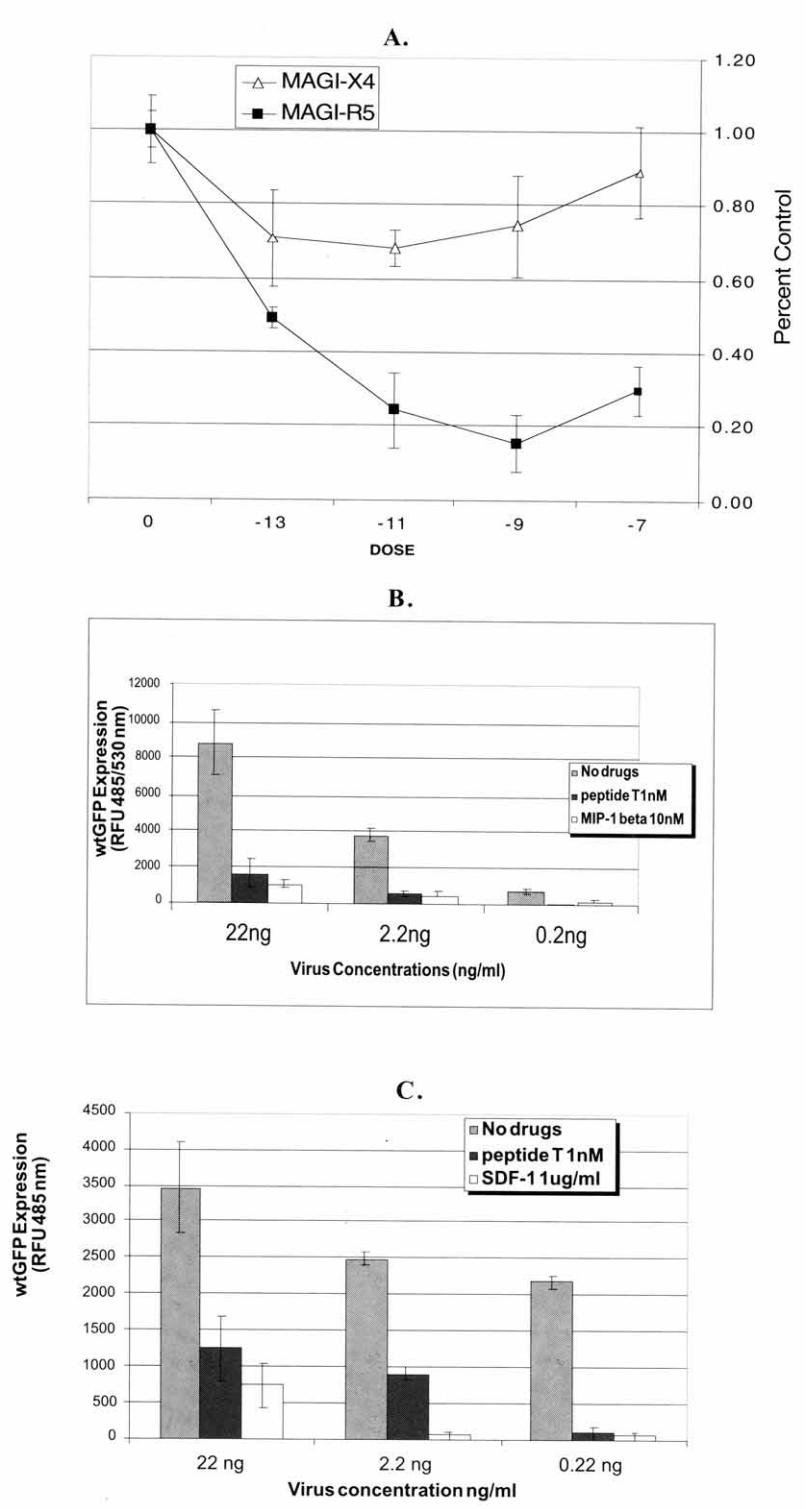

Fig. (2). Peptide T preferentially inhibits CCR5 mediated HIV entry. Panel A. Cells (MAGI) which expressed the two main entry chemokine receptors (CCR5 and CXCR4) were infected with HIV-1 (R5/X4 isolate 92HT596) with or without peptide T, as described [90], and blue focus forming units, indicating infected cells, were determined. Data presented are the mean and standard deviation of triplicate determinations. Statistical analysis was by t-test (*p,.05), for peptide T vs. vehicle treated cultures. The apparent chemokine receptor selectivity of peptide T depends on the amount of input viruses. GHOST cells expressing CCR5 (Panel B) or CXCX4 (Panel C) co-receptors were infected with HIV-1 (92HT596; 22.0, 2.2 and 0.22 ng/ml) in presence of peptide T 1nM or the relevant antagonist (SDF-1α or MIP-1β) overnight at 37^0C. After washing out of unabsorbed virus, cells were incubated in complete medium for 96h. Infection is represented as OD_{485} values of the wt GFP expression. Uninfected cultures were included in each experiment and subtracted as a background of infected cultures. Data are the mean from three experiments, + s.e.m.

V2-derived peptide (1nM)	Isolate	BFU (% Control)	
		MAGI-CXC4	MAGI-CCR5
d-ASTTTNYT-NH2 (DAPTA)	SF-2	58+/- 4	26+/-3
TTNYT	SF-2	45+/-7	23+/-4
TTSYT	LAV	35+/-7	23+/-5
NTSYG	RF	60+/-8	45+/-5
ETWYS	HIV-2	65+/-5	21+/-7

Fig. (3). V2 region derived pentapeptides inhibit HIV entry. The gp120 V2 region of diverse HIV-1 and 2 isolates encodes small peptides, homologous to the C-terminal five amino acids of peptide T (ASTTTNYT), which share the ability to block HIV entry in a CCR5 selective manner. Pentapeptide sequences are indicated by their single-letter codes. These synthesized peptides were tested for their ability to block HIV (92HT596; R5/X4 isolate) entry in MAGI cells via either the CCR5 or CXCR4 co-receptor pathways, with details as described in [90]. Infection was determined by counting blue focus-forming units (BFU) 48 hrs later. Data are the means and s.e.m of triplicate cultures and results are expressed as BFU's, percent of control (treated/vehicle x 100).

proteins. An explanation for replicative activity of the V2 deletion mutants may be that redundancy exists within gp120, such that additional chemokine receptor-mediated entry sites exist outside of V2. Other evidence also supports a role for V2 in co-receptor binding [36, 49, 55]. Substitutions in the peptide T region of V2 allow envelope proteins to assume a conformation competent for CD4-independent CCR5 binding [51, 92] suggesting an *env* interaction with CCR5 mapping, in part, to the V2/peptide T site. Hoffman [40] has searched for positions in gp120 that are linked to entry phenotype–associated changes in V3 in a large set of gp120 sequences. They identified 15 positions in gp120 outside of V3 that have significantly different variability or representation of specific amino acids among X4 and CCR5 sequences. Six of these positions fall between amino acids 190 and 204 at the C-terminal end of V2 which includes the peptide T site, therefore also identifying an important receptor function in V2. The biology of these V2-pentapeptides remains largely unexplored as only a few examples [88, 89] (Fig. **3**) have been studied.

PEPTIDE T BLOCKS GP120 BINDING TO CCR5 RECEPTORS

Another observation that suggests that specific peptide T related sequences near the V2 stem are important in gp120 biology is that the peptides block CD4 dependent iodo-gp120 binding [76, 88]. Further studies showed that binding of ^{3}H-peptide T is competed by V2 peptides, as well as gp120 (SF-2 isolate, NIAID AIDS Repository). Various specificity controls were performed in order to validate the binding methods, chiefly a structure-activity analysis which correlated rank order potency of V2 pentapeptides in binding inhibition with their rank order potency in the monocyte chemotaxis bioassay [94], a criterion for receptor-relevant binding. Peptides were active in binding inhibition in the nM and lower concentration range, while chemotactic EC_{50} was 10 pM [62, 86, 89].

We have recently begun to re-examine V2 peptide effects on gp120 binding with emphasis on chemokine receptor interactions, with initial focus on peptide T. Thus, using a rapid filtration gp120 binding method modeled after that used for numerous neuropharmacological receptor studies and similar to our initial report, with modifications and enhancements as perfected by Doranz [23], we show that

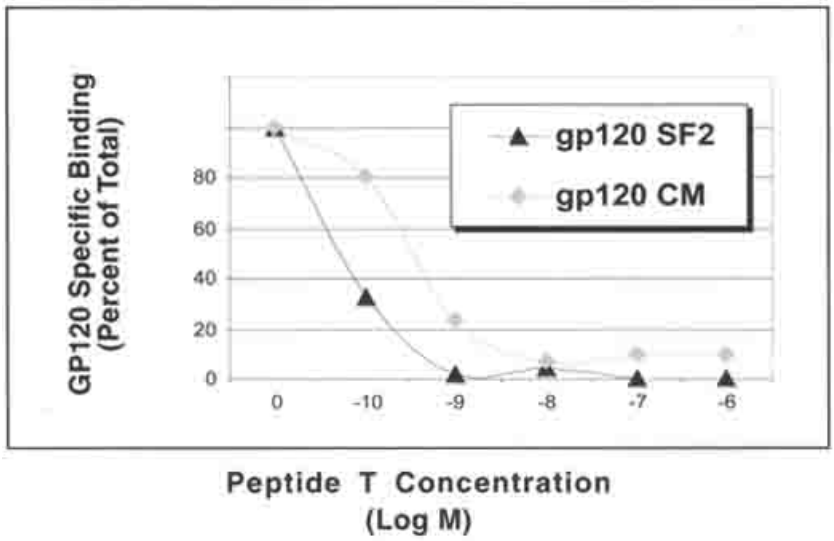

Fig. (4). Peptide T dose dependent inhibition of gp120 *Env* protein binding to CCR5 receptors. Fluorescent-labeled (FITC-gp120) *Env* proteins (CM or SF-2) were bound to Cf2Th/synCCR5 cells ($CD4^{-}/CCR5^{+}$) in the presence of 100nM sCD4 and the indicated amounts of peptide T (DAPTA). Peptide T inhibits binding of gp120 CM or SF-2; ($p=0.01$), with an IC_{50} of less than 0.1 nM. MIP-1β blocked gp120/sCD4 complex binding on Cf2Th/synCCR5 cells at10nM ($p=0.01$). Data are the means and s.e.m from triplicate determinations. Other conditions were taken from [23].

Cell Type	Receptor Expression	Added sCD4	Binding Competitor	Specific GP120 Binding (%) ± s.e.m
Cf2Th	$CD4^-/ R5^-$	100nM	-	0
Cf2Th/CCR5	$CD4^-/R5^+$	100nM	-	100±12
Cf2Th/CCR5	$CD4^-/R5^+$	10 nM	-	74 ±8
Cf2Th/CCR5	$CD4^-/R5^+$	0	-	2±6
Cf2Th/CCR5	$CD4^-/R5^+$	100nM	$gp120_{Bal}$ 1nM	19±5
Cf2Th/CCR5	$CD4^-/R5^+$	100nM	MIP-1β 10 nM	20±6
Cf2Th/CCR5	$CD4^-/R5^+$	100nM	Peptide T 1nM	22±7

Fig. (5). CD4-dependent binding of $GP120_{BaL}$ to CCR5 is inhibited by peptide T. Binding of R5-tropic HIV-1_{BaL} (FITC-$gp120_{Bal}$) envelope protein to CD4 negative canine thymocyte cell lines, with or without expressed CCR5, was performed according to Doranz et al [23]. Specificity of binding is shown in that FITC-$gp120_{Bal}$ binds to only $CCR5^+$ cells, and requires the presence of sCD4. Unlabeled $gp120_{BaL}$ (1nM) monomeric protein inhibited binding of FITC-$gp120_{BaL}$, as did MIP-1β (10nM) and peptide T (1 nM). Results are adapted from Ruff et al., 2001, Second HIV NCI, DRP Symposium.

peptide T inhibits FITC-labeled gp120 (CM or SF-2) to CCR5 receptor transfected Cf2Th/synCCR5 cells ($CD4^-/CCR5^+$) expressing high levels of CCR5 receptors in the presence of 100nM sCD4 Fig. (**4**)**.** Peptide T was a potent inhibitor of gp120 binding to CCR5 with a mean IC_{50} ranging from .08 to .5nM. Peptide T was a more potent antagonist of gp120 from the SF-2 compared to CM isolate.

Gp120 binding in this assay was saturable and dependent upon CD4. Fig. **5**. Binding of the R5-tropic $gp120_{BaL}$ did not occur to CCR5 negative Cf2Th cells, but both unlabelled $gp120_{BaL}$, MIP-1β as well as peptide T (1nM), inhibited binding of FITC- $gp120_{BaL}$ to Cf2Th/synR5 cells. These results showed peptide T to be a highly effective antagonist that potently and substantially inhibited CD4 dependent binding of gp120 isolates CM, SF-2, and BaL to CCR5.

DIRECT PEPTIDE T BINDING TO CCR5

The most parsimonious explanation for the R5 specific antiviral and gp120 binding inhibitory effects is a direct interaction of peptide T with CCR5, although indirect effects involving functionally, or even physically interacting G-protein coupled receptors (GPCRs) may occur [31]. To assess these possibilities we prepared a novel FITC-peptide T derivative and studied binding Fig. (**6**) to CCR5 positive (Cf2Th/synR5, panels A,B) and CCR5 negative (Cf2Th, panels C,D) cells via FACS analysis. Since these cells do not express CD4, any binding by FITC-Peptide T must occur via another moiety. Cells were stained with anti-human CCR5 mAb (2D7), (all four panels), and FITC-peptide T, was included in panels B and D. Our results show negligible binding of FITC-peptide T to CCR5 negative cells (<1%), but up to 30% of the CCR5 positive (Cf2Th/synCCR5) cells were stained with FITC-peptide T [90]. Although, additional competition and structure-activity studies with peptide T analogs, envelope proteins, and chemokines will be required to extend these results, this data is consistent with the simple notion that peptide T blocks viral entry and gp120 effects by binding to CCR5 receptors.

PEPTIDE T PENTAPEPTIDES ARE POTENT CHEMOATTRACTANTS AND CCR5 ANTAGONISTS

In addition to the chemotactic activities of peptide T and related V2 peptides, a number of reports by us and others have documented the activities of these ligands as monocyte attractants [63, 64, 71, 89]. Migration caused by peptide T and homologous V2 pentapeptides is receptor-mediated as subtle modifications of structure or amino-acid substitution can greatly reduce activity [61, 89]. The peptides are typically potent, with EC_{50} in the pM range. Pharmacologically, the peptides are partial agonists as stimulation indices are usually about three to five fold over background migration; full agonists such as formyl-met-leu-phe will yield migration of 10-fold over background. Further evidence that these peptides are partial or "mixed agonist-antagonists" is the fact that they not only are active alone on CCR5 receptors, but also can block CCR5 mediated MIP-1β and gp120 chemotaxis. [86] Chemotactic activity is enhanced by culture of monocytes for 48-72 hrs in the presence of GM-CSF, a regimen which up-regulates expression of monocyte CCR5.

A further test of the ability of peptide T to direct CCR5 mediated chemotaxis was performed using the CCR5 positive (Cf2Th/synR5) and CCR5 negative (Cf2Th) cells in a chemotaxis assay. Using our previously reported method employing pre-labeling of cells with a fluorescent dye and following migration in response to a concentration gradient of ligand, [86] we showed that both MIP-1β and peptide T caused chemotaxis to the CCR5 positive cells, but not the CCR5 negative cells Fig. (**7**). Peptide T therefore causes CCR5 dependent chemotaxis, and antagonizes chemotaxis caused by CCR5 ligands, which is also consistent with peptide T binding to CCR5.

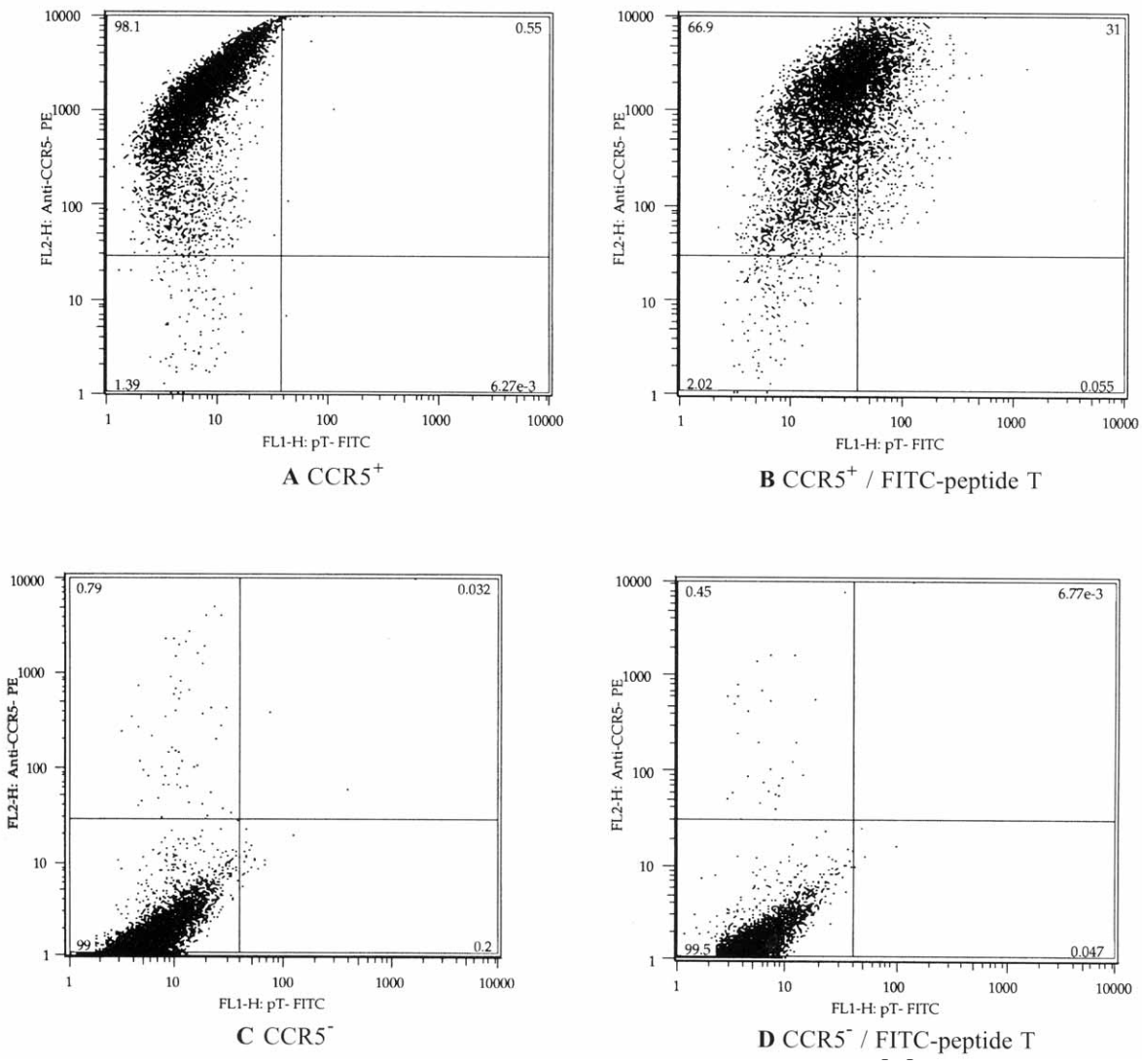

Fig. (6). Direct Binding of peptide T-FITC to Chemokine CCR5 Expressing Cells. The binding of a novel FITC-derivative of peptide T (DAPTA) was studied by flow cytometry (FACs) on transfected CCR5 positive (Cf2Th/synCCR5) and negative (Cf2Th) canine thymocytes. These cells do not express significant amounts of CD4. CCR5 was verified by staining with a PE-conjugated mouse anti-human CCR5 IgG (2D7) using a IgG1-PE isotope control (Pharmingen, San Diego, CA), (panels A and B compared to C and D, with PE-staining on the vertical axis). Results showed FITC-peptide T binding (horizontal axis) occurred only with CCR5 positive cells (B), and did not occur with CCR5 negative cells (D). Data are a representative fax plot showing the frequency of pT-FITC binding to CCR5 expressing cells after two hours of incubation.

Some of the peptide structures of Fig. 3 contain the glycosylation motif T/S-X-N for formation of N-linked glycosides and these sites may indeed be fully glycosylated in the mature gp120 molecule [50, 75]. Surprisingly, glycosylation does not destroy receptor functionality of V2-derived pentapeptides; in fact, glycosylated analogs maintain their chemotactic activity and even show enhanced stability to degradation [64].

PEPTIDE T, GP120 BLOCKADE, AND NEURO-AIDS

Neuro-AIDS can manifest itself in many forms including neuronal cell loss, neuropathies, inflammations, astrocytosis, memory loss, dementia, depression, psychosis and opportunistic infections such as Progressive Multifocal Leukoencephalopathy (PML). [3,83] CNS infection is typically latent and little virus is produced until late stages of disease. We first showed that gp120 induces neuronal apoptosis [7], which later was shown to be mediated through its high affinity interactions with chemokine surface receptors on brain and immune cells [29, 42]. Consistent with gp120's role in neuro-AIDS pathogenesis is the fact that it generally develops more frequently in later stages of HIV infection when the envelop protein has evolved broader receptor specificity [17] as well as increased CCR5 affinity [33]. GP120 therefore is considered to be a significant initiator of HIV pathologies in the brain via its direct actions on brain chemokine receptors, and therapies to treat brain disease may be developed which antagonize gp120 effects.

Gp120 neuronal cell death can be completely blocked *in vitro* with peptide T and short homologous pentapeptides, as well as peptides related to vasoactive intestinal peptide

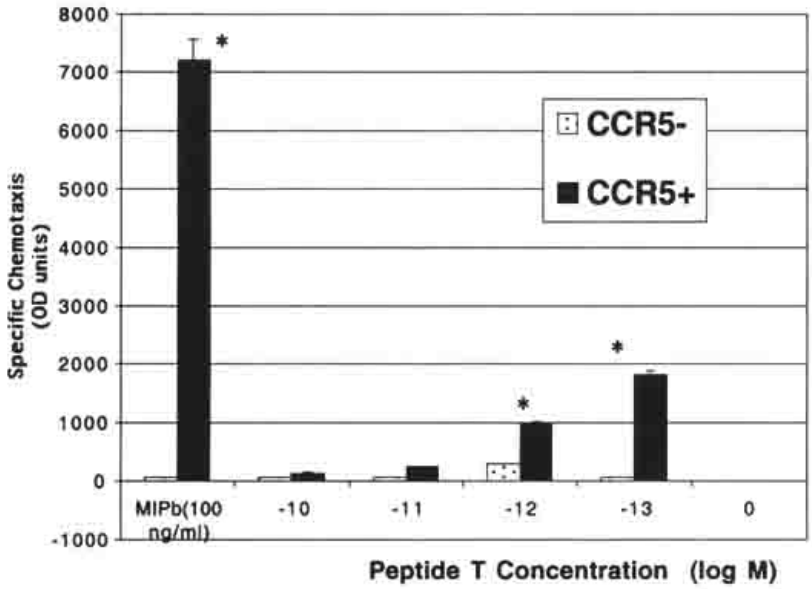

Fig. (7). Selective CCR5 Chemotaxis caused by Peptide T. Peptide T acts as a partial antagonist for chemotaxis on CCR5 expressing cells compared to MIP-1β. Peptide T induces cell migration to CCR5 expressing Cf2Th/synR5 cells, but not to these cells lacking CCR5. Cells were pre-labeled with calcein fluorescent dye and allowed to migrate in the presence of MIP-1β, an CCR5 ligand, and peptide T (Dala1-peptide T-NH2) for 4 hrs. Cells which migrated in response to a gradient (chemotaxis) were enumerated by fluorescence measurement in a plate reader (480/530nM), as described in [86]. Data are expressed as specific chemotaxis, OD units of drug treated minus vehicle only treated cells. Statistical comparisons of triplicate determinations for treated vs. vehicle only controls were by t-test, * $P<.05$

(VIP), which also shares homology with gp120 via the peptide T sequence [5]. A very extensive and often overlooked study, which demonstrated DAPTA's neuroprotective effects against gp120 *in vivo* was performed in neonatal rats. In these rats, administration of nanogram quantities of gp120 delayed the timing by 1-3 days of behavioral milestones and produced abnormal neuronal dearborization. With simultaneous administration of peptide T (DAPTA) and gp120, behavioral milestones were met at the normal time and normal neuronal arborization patterns were observed [39].

As already pointed out, the benefits of peptide T have not been confined to animal studies as human tests have shown clinical benefits in reversing, not merely attenuating, deficits in memory and cognition associated with HIV infection in several placebo-controlled trials [8, 38, 53]. Peptide T has also caused MRI brain scan improvements in AIDS patients [100, 101]. Recently, DAPTA was shown to acutely normalize the growth hormone secretion pattern of two children with AIDS [2] most likely by antagonizing gp120-induced suppression of growth hormone as previously shown in rats [72].

MECHANISMS OF PEPTIDE T ACTION IN THE BRAIN

The mechanisms of peptide T blockade of gp120 neurotoxicity or clinical improvements may include suppression of CCR5-tropic virus in the brain, as well as direct inhibition of free gp120 binding to brain CCR5 receptors [29]. Another possibility would be anti-gp120 effects through the release of specific chemokines. In studies to address these possibilities, we showed that neuronal killing in rat hippocampal cultures induced by gp120 obtained from five different strains of HIV can be partially or completely prevented by various chemokine ligand antagonists of gp120 binding, as well as peptide T [6]. Peptide T caused the release of β-chemokines, such as RANTES and MIP-1α, which are themselves neuroprotective, indicating that peptide T's neuroprotective effect in the brain may involve the indirect release of chemokines. Glia or neurons are the likely source of the chemokines as peptide T did not cause release of anti-viral chemokines from human MDMs, $CD4^+$ or $CD8^+$ T cells (unpublished observations). These studies also revealed a previously unknown action of chemokines to modulate neuronal viability in the non-pathological, normal, developmental state. The actions of chemokines, or peptide T itself, represents a novel action of these peptides, no doubt of some significance since modulation of neuronal survival plays a crucial role in brain development and learning.

PEPTIDE T EFFECTS IN OTHER BRAIN PATHOLOGIES

Alzheimer's disease (AD) associated with aging and of unknown etiology, is characterized by brain inflammation leading to neocortical atrophy. Neocortical atrophy and loss of large neocortical neurons are also common features of HIV infection of the brain [45] suggesting convergence of pathogenic pathways, most likely involving inflammatory

cells of the brain, such as the microglia [99]. Chronic (6 month) DAPTA treatment completely prevented reduction in cortical thickness and the loss of accompanying large cortical neurons in a well studied rat model of AD [95]. The ability of chronic DAPTA treatment to prevent reductions in cortical thickness and loss of accompanying large cortical neurons may involve a direct effect of DAPTA on neocortical neurons through stimulation of neuronal chemokine receptors, or via indirect pathways involving neurotrophin release by glia. These observations suggest new areas of research into the pathogenesis of dementing illness that involve neuronal loss, even when caused by disparate etiological agents (AIDS vs. AD).

PEPTIDE T EFFECTS IN PSORIASIS

We and others have examined the actions of peptide T in additional pathological models where HIV gp120 may be involved. In the case of psoriasis, studies were prompted by observations of pronounced ancillary symptomatic benefit in an HIV patient who received peptide T in an early study [101]. This benefit was not immediately explicable although it had been observed that some patients infected with HIV-1 experience several hyperproliferative skin disorders, including seborrheic dermatitis, ichthyosis, and psoriasis. Transgenic mice which expressed *env* developed skin lesions reminiscent of psoriasis [52]. Gp120 may thus be a mediator in AIDS related psoriasis. Resolution of psoriasis in AIDS has been observed with antiretroviral drugs such as AZT, further underscoring the role of HIV in this lesion. Additional clinical studies of peptide T in psoriasis have shown clinical and histological benefits in non-AIDS patients [21, 24, 65]. When lesions resolve with peptide T administration they remain normalized for up to 6 months despite withdrawal of the drug [65].

A defining feature of psoriatic lesions is a pronounced T cell infiltrate which promotes the epidermal hyperplasia. Up-regulation of RANTES has been observed in non-AIDS psoriasis and may recruit activated T cells [85], as may local release of gp120 in HIV. The ability of peptide T to antagonize RANTES chemotaxis [84, 86], or suppress inflammatory cytokines, may be associated with the observed clinical benefit. Mechanistically, the cutaneous lesions of psoriasis are associated with decreases in the levels of cAMP dependent protein kinases (PKA), which have been associated withcontrol of cellular differentiation and proliferation. Exposure of cultured psoriatic fibroblasts (but not normal fibroblasts) to peptide T resulted in time and dose dependent increases of PKA and maximal effects were detected at 1pM [58]. Conversely, gp120 (0.1pM) treatment of normal fibroblasts decreased PKA activity and peptide T blocked this effect. Thus peptide T normalizes (increased) PKA activity in psoriatic fibroblasts and blocks the action of gp120 to suppress PKA activity in normal human fibroblasts. Fibroblasts may express chemokine receptors [73], providing a model by which chemokine ligands, such at RANTES or gp120 may be antagonized by peptide T to suppress psoriatic lesions, and galacotosylceramide may act in lieu of CD4 to bind gp120 to these cells.

CYTOKINE CHANGES IN AIDS PATIENTS RECEIVING PEPTIDE T

The actions of cytokines during the course of HIV infection have been postulated to play a significant role in pathogenesis during AIDS progression [68]. Their alterations have been the focus of new theories to explain the indirect effects of HIV on T cell depletion and immune functional impairments [15, 60] the mental deficits of AIDS [67], as well as somatic changes including cachexia and the growth and neuroendocrine abnormalities in children [57]. Treatments that normalize cytokine production may have benefits in the management of HIV disease. Therefore the levels of 14 cytokines were determined after 6-8 weeks of treatment with peptide T (DAPTA).

Five patients received peptide T (DAPTA) 3 mgs/day, by metered nasal spray for 6 to 8 weeks (mean 7+ 0.6 wks). Patients were not taking other concurrent anti-retroviral therapies, but were taking Chinese herbal medications. Their mean CD4 count was 205 + 39 at baseline, and 131+ 36 at the end of the study period (not significantly different, $p>.05$). At the beginning and end of the study, cytokine measurements on flash frozen, stored plasma samples were made after capillary electrophoresis isolation of individual protein peaks, followed by enzyme-linked immunoassay using specific antibodies [79]. Analysis was conducted on blinded samples. Those data are presented in Fig. **8**. The cytokines IL-1, IL-6, IL-8, TNFα were measured at greater than 100 pgms/ml at baseline in all five patients. The cytokines IL-2, IL-4, IL-10, IL-12, and IL-13 were low to barely detectable, while cytokines IL-3, IL-5, IL-7, IL-9, and IL-11 were largely not detectable in any of the cohort.

Following intranasal peptide T administration, highly significant reductions in the levels of IL-1, IL-6, IL-8, and TNFα were observed ($P<.005$) (Fig. 8A). One explanation for patient effects may be the direct action of peptide T on monocytes, most likely on CCR5, as it has been shown that peptide T suppressed TNF production in cultures of stimulated normal human macrophages [80]. A cascade of multiple and varied effects on release and receptor binding of cytokines could result from the simple modulation of one receptor, i.e. CCR5, with a mixed agonist-antagonist ligand like peptide T.

The cytokines IL-2, IL-4, IL-10, and IL-12 were increased significantly ($P<.005$) (Fig. 8B). Where significant changes occurred (increase or decrease), all five members of the cohort responded in the same direction. Cytokines that were undetectable prior to treatment generally remained undetectable. It is beyond the scope of this review to fully evaluate the clinical implications of these changes in cytokine levels, and the field is rich and complex. Elevated TNFα [30] and IL-6, [74] and reduced IL-2, IL-4 [35] are reported in AIDS patient lymph nodes and peripheral mononuclear cells. Those *in vitro* observations made on isolated cell populations were qualitatively consistent with the baseline *in vivo* levels observed in the current patients.

Cytokine action in the nervous system has been invoked as an explanation for the mental deficits and neurological impairments of AIDS since viral levels in the brain are low

A.

CYTOKINE	MEAN pgs/ml±s.e.m.		% CHANGE
	BASELINE	POST-DRUG	(**p<.005)
IL-1	320±85	175±26	-46**
IL-6	179±15	130±21	-28**
IL-8	207±68	144±76	-30**
TNFα	339±86	260±67	-23**

B.

CYTOKINE	MEAN pgs/ml±s.e.m.		% CHANGE
	BASELINE	POST-DRUG	(**p<.005)
IL-2	14±6	57±9	+395**
IL-4	6±2	28±8	+448**
IL-10	13±2	45±5	+350**
IL-12	9±3	33±7	+363**
IL-13	7±3	30±9	+416**

Fig. (8). Panel A. Cytokine decreases in 5 AIDS patients after 7±1 weeks intranasal peptide T treatment Cytokine measurements were made at baseline and at end of the study period from blinded plasma samples after capillary electrophoresis followed by ELISA. Patients were not taking anti-retroviral therapies, except for traditional Chinese herbal medicines. Patients were maintained on their treatments for 1 month prior to, and during, the period of added peptide T (3mgs/day). All members of the cohort (five of five patients) showed changes (decrease or increase, as indicated). The following cytokines were undetectable <5 pgms/ml in both pre and post peptide T plasma samples: IL-3, IL-5, IL-7, IL-9, IL-11. Data were presented in Ruff *et al.,* 1995, *AIDS Res. and Human Retro.,* 11, S163. Panel Panel B. Cytokine Increases In 5 Aids Patients After 7±1 Weeks Intranasal Peptide T Treatment.

and largely confined to monocyte derived cells [48]. The inflammatory cytokines IL-1, IL-6, and TNFα have been implicated in HIV brain pathologies [66] and levels of these inflammatory cytokines decreased in plasma following DAPTA treatment. Furthermore, the proinflammatory cytokines TNFα, IL-1, and IL-6 upregulate virus expression *in vitro* [27, 66] and the reduction of these cytokines with DAPTA treatment would favor less viral replication. The Th2 cytokines IL-4, IL-10,and IL-13, are increased by DAPTA (Fig. 8) and induce a potent virostatic state in infected macrophages *in vitro* [70], as well as inhibit production of the proinflammatory cytokines IL-1 and TNFα, which upregulate virus expression. Thus the elevation of Th2 cytokines by DAPTA treatment would favor less macrophage viral replication.

ENHANCED INTRACELLULAR IFNΓ SECRETION BY $CD8^+$ T CELLS IN ONGOING SAN FRANCISCO TRIAL

Cytotoxic T lymphocytes (CTLs) play a role in containing HIV spread. These effector lymphocytes are not fully functional in suppressing virus production, perhaps due to lack of CD4 help or HIV suppression of virus-specific $CD8^+$ cells. The ability of CTLs to secrete gamma interferon (IFNγ) is accepted to be an indicator of cytotoxic capability. In San Francisco, there is an ongoing study involving 11 men (with viral loads between 500 and 25,000) who are receiving intranasal DAPTA (6 mg per day) in concert with their HAART therapy. All patients were stable on their respective therapies. The percentage of IFNγ secreting $CD8^+$ T cells from six persons after 24 weeks treatment with the HIV entry inhibitor peptide T (DAPTA) was measured. Fig. (**9**). PBMCs were isolated and activated with PMA/ionomycin and IFNγ secreting/$CD8^+$ cells were enumerated by flow cytometric methods. Nine of eleven evaluable individuals showed a statistically significant increase (via a paired T-test) with peptide T treatment in IFNγ secreting cells from baseline (6 + 1.6 % positive cells) to week 8 (19 + 7 % positive cells, p=.04) and these increases were sustained at week 12 (26+ 7.7%, p=.003). At 24 weeks the increases had fallen back to baseline levels. The peak of peptide T response at 8-12 weeks, as well as its transient nature is typical of other immunomodulatory therapies, such as IL-2 [54]. The enhancement of HIV-specific, IFNγ-expressing, T cells would be most relevant for the augmentation of anti-viral immunity. A mechanism for peptide T effects on immunity in AIDS may be as an antagonist of gp120 at chemokine receptors, which earlier studies have shown suppresses T cell activation. [13, 32, 60]

IN VIVO ANTIVIRAL EFFECTS OF PEPTIDE T

Peptide T *in Vivo* Suppression Of Virus Replication in Peripheral Blood Monocytes

The chemokine receptor CCR5 (CCR5) is the major co-receptor for macrophage-tropic strains [22], which are predominate during the asymptomatic stages of infection and play a crucial role in transmission [10]. Monocyte-derived macrophages (MDMs) have the potential to act as long-lived reservoirs for HIV-1, even with effective HAART therapy [56, 97], and to disseminate virus to other tissues, such as brain [12]. Thus CCR5 is an attractive target for inhibition of CCR5 mediated HIV entry and CCR5 antagonists are anticipated to be a powerful new class of receptor-based therapeutic agents against HIV-1 infection.

Since peptide T has a CCR5 selective antiviral effect [90], its effect on active infection of circulating monocytes in patients was tested. Only a small fraction (5-10%) of the total circulating macrophage population is productively infected. [56, 97] We hypothesized that blocking infection of monocytes by peptide T would prevent and reduce the population of the infected differentiated macrophages, one of the viral reservoirs in HIV infected patients that persist even in the face of effective HAART therapy. Patients whose viral

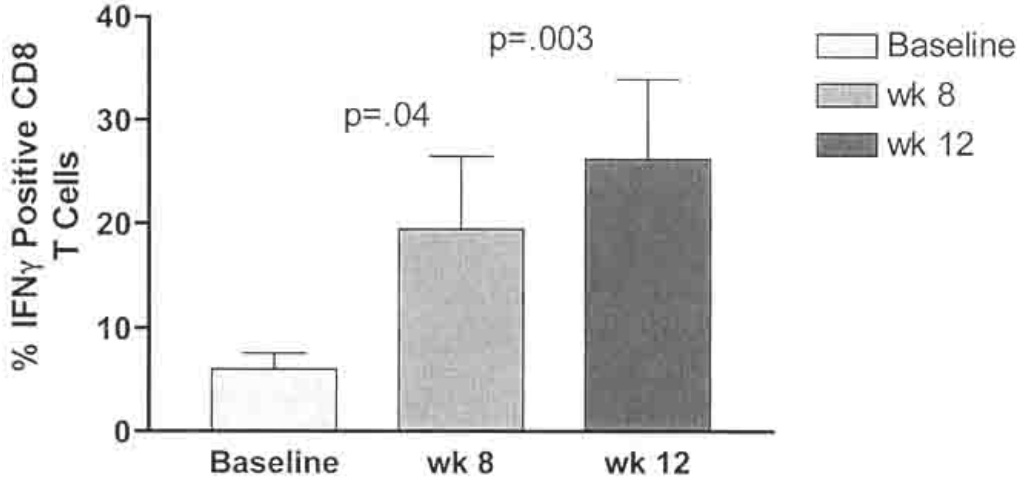

Fig. (9). Peptide T increases in IFNγ secreting CD8+ T- lymphocytes suggests restoration of immune function. PBMC's from HIV-1 infected patients under treatment with peptide T (6 mgs/day) were isolated and stained for intracellular IFNγ by activation with PHA overnight, then with phorbol ester and calcium ionophore in the presence of a protein transport inhibitor. The cells were stained with fluorescence-labeled control antibodies (immunoglobulins IgG1 and IgG2) or with fluorescence-labeled antibodies (anti-CD8 and mouse mAb antibody to human IFNγ, all from the Pharmingen) for 30 min on ice. Samples were read using a FacsCalibur Flow Cytometer (Becton-Dickinson) and data were analyzed by FloJo software (Tree Star, Inc., San Carlos, Calif.) The results are presented as percent of IFNγ expressing $CD8^+$ T-cells. Statistical comparisons for weeks 8 and 12 with baseline values were by paired t-test.

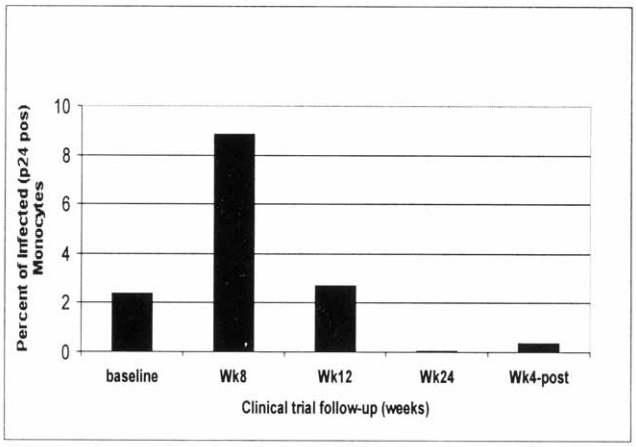

Fig. (10). Peptide T therapy suppresses infection of peripheral blood monocytes. PBMC's from HIV-1 infected patients under treatment with peptide T (patients had viral load at baseline between 500 and 25,000 copies/ml, and were taking concurrent HAART therapies. Peptide T was added, 6 mg/day, by metered nasal spray, for up to six months, followed by a 4 week off-drug period. PBMCs were isolated at indicated time points and freshly stained with monocyte specific anti-CD14 IgG1 (BD, Pharmingen) fluorescence-labeled antibody and isotope-matched control (IgG1-FITC (Pharmingen). HIV-1 core proteins were detected by Flow Cytometry measurement after intracellular staining with anti-core p24 antibody (KS57-RD1, Coulter Immunology) and Coulter clone isotope MsIgG2-RD1). Uninfected monocytes are used as a negative control, and an anti-HIV serum. used to block the binding of PE-labeled anti core (KC-57), is used as a further specificity control. Monocytes are gated using anti-CD14 and >20,000 events were collected. The results are presented as the mean percent of infected (p24 positive) cells from six persons completing 24 weeks therapy and a one month follow-up (off peptide T). Viral p24 was undetectable in any patients' peripheral blood monocytes at 24 weeks by this antibody based method.

RNA levels (Roche Amplicor test) were stable for 30 days on standard HAART treatments with between 500 and 25,000 RNAcopies/mL, received added peptide T (2 mg,3x/day) for up to 24 weeks. We therefore determined intracellular HIV core antigen in patient derived MDMs. Fig. (**10**)**.** We detected p24 expressing monocytes in our cohort and their levels ranged from undetectable to 8% of the total blood monocyte population. We noted an increase in numbers of monocytes expressing core antigens at week 8, an effect that abated at week 12 and thereafter decreased so that peptide T treatment for 24 weeks was associated with inability to detect viral antigens, a measure of active HIV

replication in the circulating monocyte (CD14) population. The increase of HIV core antigen positive monocytes at week 8, compared to baseline, occurred at the time of maximal IFNγ response, suggesting that immune activation may be the causefor the rise and subsequent decline in viral replication in the monocytes. Week 8 was also a time when cytokine changes favoring a virostatic state occurred (Fig. **8**). The apparent flushing of the monocyte reservoir is evidence for a cell-mediated antiviral response by peptide T.

Failure to Isolate HIV-1 from Patient PBMCs after 24 weeks of Peptide T

The synthetic peptide T (DAPTA) represents a new class of antiviral compound with *in vitro* potency against a step in viral entry. An initial clinical trial of peptide T for viral and immunological endpoints provides an opportunity to evaluate viral phenotype and emergence of resistant viruses *in vivo*. We therefore attempted the isolation and culture of virus from patients, pre and post peptide T treatment in order to determine any phenotypic or drug sensitivity changes. Generation of primary isolates from HIV-1 infected patients treated by peptide T was attempted by several methods such as recovery of virus from co-culture of patient PBMC's or plasma with activated PBMC's from healthy donors which had been CD8 cell depleted. Cultures were tested for p24 production for up to 21 days and were considered to be positive if this viral protein was detected at a level >10pg/ml. A quantitative co-culture method [18] was also used in which cryopreserved patient PBMC's are thawed and serially diluted with PHA-stimulated normal donor PBMC's ($CD8^+$ depleted) and cultured for up to 21 days.

To determine viral phenotype, supernatants from any positive cultures from the above methods were collected, stored (-70C°), and tested for their ability to infect indicator cell lines (GHOST cells) that express the specific HIV entry receptors CCR5 or CXCR4. Productive infection generates green fluorescent protein expression which can be determined in a plate reader and a signal of 2x background is deemed positive.

The results of these initial (unpublished) evaluations appear in Fig. (**11**)**.** We were able to isolate virus and determine their chemokine receptor usage from five of six patients at baseline. Five of these six persons completed 24 weeks of therapy with peptide T. No drug related toxicities were noted. All of the patients expressed an R5 or dual-tropic (R5/X4) phenotype. All of the patients that showed viremia by PBMC co-culture (5 of 6) at baseline became negative by 24 weeks of peptide T treatment: in fact, after twelve weeks of peptide T administration it became impossible to grow virus from most patients. In some cases (#16, 23) it became possible to isolate virus upon prolonged (>6 weeks) cessation of peptide T, although all patients remained on HAART therapies. Additional studies of PCR analysis of integrated proviral genome in patient PBMCs are currently under investigation and will further clarify how antiviral effects are associated with peptide T.

Failure to Develop Peptide T (DAPTA) Escape Mutants *in Vivo* or *in Vitro*

Culture of virus in the presence of peptide T *in vitro* for more than 30 days failed to elicit the generation of resistant isolates Fig. (**12**) as wild-type virus (HIV-1 SF-2 isolate) did not differ in its sensitivity to peptide T inhibition when compared to peptide T selected virus. We have also cultured one patient virus (#16), as well as the early passage R5/X4 isolate 92HT596 (NIH AIDS Research and Reagent Program) by similar methods and have not generated a resistant variant. These studies (Fig. **12**) suggest that development of resistant viruses, an important concern which can often limit therapies, was not readily induced *in vitro* by culture of virus with increasing amounts of peptide

Virus Detected by Co-Culture of Patient PBMCs					
Patient Number	**Viral Phenotype at Baseline**	**Baseline**	**Wk 8**	**Wk 12**	**Wk 24**
#16	R5	+	–	+[1]	–
#17	R5/X4	+	+	+	–
#18	R5/X4	+	+	–	–
#19	Not detected	–	+	–	–
#21	R5	+	–	withdrew	
#23	R5/X4	+	+	–	–

Fig. (11). Inability to isolate HIV-1 from patient blood mononuclear cells (PBMCs) after 24 weeks treatment with Peptide T. HIV was generally isolated at baseline, and thereafter with decreasing frequency until week 24 of peptide T administration. Repeated attempts proved negative for ability to co-culture patient virus with naïve, PHA-activated, CD8 depleted donor mononuclear cells. Cultures were positive if p24 (ELISA) detected at > 10 pg/ml at any culture time up to four weeks after initial infection. Fresh PHA-activated, CD8 depleted donor mononuclear cells were added to cultures weekly. If virus was detected it was propagated and then tested for chemokine receptor utilization by infecting CCR5 or CXCR4 expressing GHOST cell indicator lines. [1]Patient went off study medication (peptide T) previous 4 weeks.

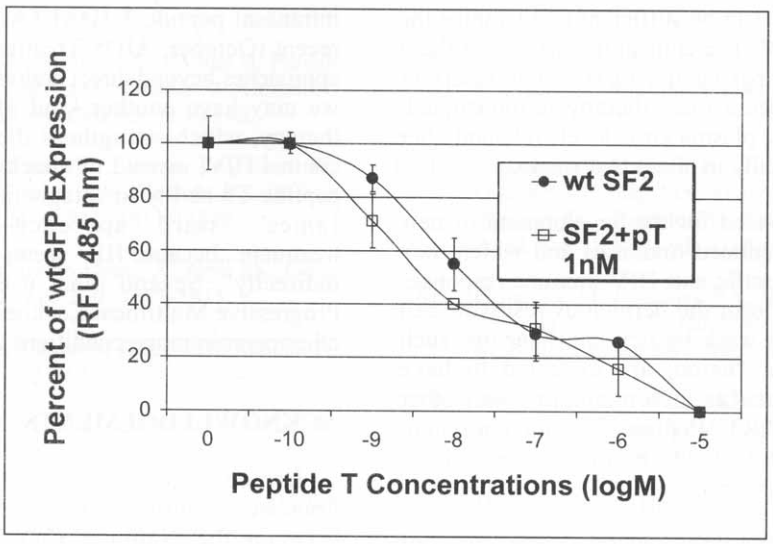

Fig. (12). Continued sensitivity of HIV-1_{SF-2} virus propagated in presence of peptide T. PM1 cells, a clonal derivative of HUT78 cells which are permissive for growth of macrophage and T-cell tropic viruses, were used to generate peptide T-resistant viruses *in vitro*. PM1 cells were infected with a $TCID_{50}$ amount of cell free SF-2 virus in the presence of peptide T (1 nM). Samples of cell supernatants were collected every four days and evaluated for virus production by p24Ag ELISA test (Coulter) with addition of fresh peptide T containing media. After passage 8 the peptide T concentration was increased to be 10nM. The sensitivity to inhibition by peptide T of SF-2 virus propagated more then one month in the presence of peptide T was evaluated on GHOSTCCR5 cells infected with wild type and viruses propagated long-term in the presence of different concentrations of peptide T, as indicated. Infection is represented as OD_{485} values of the wt GFP induced expression. Results presented are average data from two independent experiments.

T or *in vivo* in patients who received peptide T. *In vivo* there was no shift in viral receptor phenotype (e.g. from R5 to X4), a concern with receptor-based therapies, [69] which have seen this shift within 4-8 weeks.

CONCLUSIONS

To our knowledge, peptide T and its homologs are the only potent gp120-derived antiviral peptide entry inhibitors and gp120 binding antagonists yet reported. The simplest interpretation of all evidence is that the peptide T–like pentapeptide near the V2 stem comprises a binding epitope for a gp120 receptor, most likely CCR5. This hypothesis does not exclude other binding motifs elsewhere in gp120. Selective antiviral activity with peptide T occurs for physiologically predominating R5 compared to X4 tropic isolates. Laboratory X4 strains propagated in T cell lines are inefficiently, if at all, inhibited. Peptide T inhibits infectivity by binding to CCR5 and thus blocking gp120 binding. Gp120 binding to CCR5 in our assays is dependent upon CD4, and is competed by specific R5 tropic gp120 isolates, such as Bal or CM (Fig. **4**)**.** Peptide T seems to bind directly to CCR5 in that an analog (FITC-DAPTA) was specifically bound to CCR5 expressing cells, but not CCR5 negative cells. An interaction with CD4 was excluded as the target cells (Fig. 5) do not express this protein. Of interest are other G-protein coupled receptors such as the family of VIP/PACAP and growth hormone releasing hormone (GRHH) peptides, due to their shared homology with gp120 via the peptide T sequences (discussed in [72, 90]). No obvious gp120 sequences correlate with receptor tropism although Hoffman [40] implicates amino acids near and within the peptide T site in *env* in chemokine receptor tropism.

Advances in the treatment of HIV-1 infection have dramatically reduced the death rate from AIDS in the United States since 1996, when protease inhibitor-based combination therapy regimens, known as highly active antiretroviral therapy (HAART), came into widespread use [41]. The success of protease inhibitors exemplifies the power of rational drug design; however, toxicities and other problems limit their long-term use. An increasing number of patients interrupt continuous drug therapy, so-called structured treatment interruptions (STI), to avoid these toxicities. The clinical usefulness of the STI approach, however, remains to be determined and concerns have been raised [59].

The excellent suppression of viral replication achieved by many patients on HAART initially raised hopes for virus eradication due to the partial restoration of CD4 and CD8 T cell number and function during therapy. However, recently revised HHS Treatment Guidelines for AIDS have suggested delaying the start of HAART as a result of two findings [102]. First, it is now clear that HIV-1 persists in a small reservoir of latently infected resting memory CD4+ T cells and monocytes, which show minimal decay even in patients on HAART therapy [56, 97]. Second, there is evidence of ongoing virus production even in patients for whom viremia is suppressed to undetectable levels [26, 103], raising a concern of resistance development that would abrogate therapeutic efficacy.

The reservoir of infected cells that is resistant to the best of current therapies appears to be sufficient to guarantee the lifetime persistence of HIV. The clinical importance of these findings is that the stable persistence of HIV-1 in reservoir cells allows viremia to occur once therapy is interrupted. Thus, with rare exceptions, plasma virus levels rebound after cessation of therapy, typically in about two weeks.

Thus, there is a clear need for the development of new antiviral therapies with reduced toxicities and which may result in restoration of specific anti-HIV immune responses that may eliminate virus form the pernicious reservoir cell populations. Drugs which work by new mechanisms, such as blockage of entry, or fusion, are expected to have synergistic therapeutic benefits in combination with other treatments, such as HAART. Peptide T, with consistent findings of lack of toxicity, holds promise to be able to address these therapeutic concerns.

Peptide T seems to have three separate mechanisms of anti-viral action: 1) it is an entry inhibitor by blocking CCR5; 2) it is an immune modulator, increasing IFNγ-secreting CD8 cells; and 3) it is a gp120 receptor antagonist, thus blocking the neurotoxic and other harmful effects of gp120. The ability to culture virus from patient samples, and active replication (p24 Ag production) in the peripheral monocytes, was completely suppressed by peptide T after 6 months. Paradoxically, plasma viremia did not decrease significantly in our cohort of patients who continued to have a stable, low level (500-25,000 copies/mL) viremia even after many months or years on HAART therapy. In this respect, the cohort is a treatment resistant group as HAART typically makes virus levels undetectable in a few weeks. Two explanations for the paradox of continued plasma viremia (Roche PCR) with inability to culutre virus from PBMCs, may be a shift toward defective virus production or an effect on different viral “compartments” with peptide T treatment.

Patients also had significant increases in IFNγ secreting CD8 T cells, considered to be a marker of immune reconstitution and anti-viral defense, peaking at 8-12 weeks after initiation of therapy. These initial results are drawn from a small number of patients and require additional clinical studies and verification, but are congruent with the surprisingly large body of independent positive clinical experiences over the years which we have reviewed. For the future, these patient changes are of interest because of the treatment goals discussed above, namely new therapies that target “reservoirs” unreached by HAART.

Our initial attempts to create resistant mutants by *in vitro* culture in the presence of peptide T were unsuccessful, nor did resistant virus or phenotype switching (e.g. R5 to X4) arise in patients receiving peptide T for 6 months. *In vivo* such escape mutants may still be found. If it continues to prove difficult to elicit escape mutants –lack of resistance development- a further goal of enhanced treatment efficacy may be met with peptide T. Finally, further anti-viral studies should look at drug-naïve patients who have higher viral loads in contrast to this cohort that had been generally treated for many years with many combinations of drugs, and still had a persistent, unresponsive virus level typically below 10,000 copies/ml. Still, the results so far suggest that intranasal peptide T (DAPTA) already fulfills John James' recent (October, AIDS Treatment News) call for additional approaches beyond direct antiretroviral therapy: "In the future we may have another kind of treatment, immune- based therapy, which strengthens the immune system's ability to control HIV, instead of attacking the virus directly." Also, peptide T's ability to antagonize gp120-induced toxicities is James' "third approach---HIV harm reduction treatment...because HIV seems to cause most of its damage indirectly". Several other uses of peptide T, such as in Progressive Multifocal Leukoencephalopathy, psoriasis, and other neuroimmune conditions are in early trials or planned.

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