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CD4 receptor binding peptides that block HIV infectivity cause human monocyte chemotaxis

Relationship to vasoactive intestinal polypeptide

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The octapeptide Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr (peptide T) and two structural analogs are potent agonists of human monocyte chemotaxis, evincing identical rank potency orders as was previously shown for their inhibition of human immunodeficiency virus (HIV) envelope binding and T cell infectivity. Chemotactic activity could be inhibited by anti-CD4 monoclonal antibodies (Mabs), but not other mononuclear cell Mabs. The core peptide required for chemotactic activity is a pentapeptide related to the sequence Thr-Thr-Asn-Tyr-Thr. Homologous pentapeptides, identified by computer search, were detected in several other non-HIV-related viruses as well as the neuropeptide vasoactive intestinal polypeptide (VIP). The CD4 molecule, therefore, appears to be a recognition molecule for a small signal peptide ligand whose active sequence is a homolog of peptide T [4–8] and which may be the neuropeptide VIP.

Receptor-binding peptide; Receptor; Chemotaxis; Human immunodeficiency virus

1. INTRODUCTION

Recent reports indicate that viruses may utilize unique and specific cell surface recognition molecules as their initial attachment sites. For example, vaccinia binds to the epidermal growth factor (EGF) [1], rabies the acetylcholine receptor [2], Epstein Barr (EB) the complement receptor [3], and rheo the β-adrenergic receptor [4]. The human immunodeficiency virus (HIV), the etiologic agent of AIDS, has been shown to bind to a surface molecule known as CD4 (or T4), present on T lymphocytes, macrophages and other cells [5,6]. Binding to specific surface receptors forms a basis for viral tropism to particular tissues and cells, and plays an important role in viral pathogenesis. We have recently identified the subregion of the HIV external glycoprotein molecule, gp120, responsible for binding to brain membrane and human T cells. We have also demonstrated that de novo synthesized low-M_r peptides in the nanomolar range are capable of inhibiting gp120 binding and preventing human T cell infectivity [7].

Utilizing a sensitive in vitro human monocyte chemotaxis bioassay, we now describe potent picomolar chemotactic activity for peptides previously shown to inhibit HIV binding and infectivity. We have found that several other viruses, not obviously related to HIV, have amino acid sequences analogous to these peptides. Vasoactive intestinal polypeptide (VIP) also contains a similar peptide sequence within its structure. Peptides with these sequences have been synthesized and are po-

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tent chemoattractants. Our results suggest that the CD4 molecule could function as a recognition molecule for a small signal peptide.

2. MATERIALS AND METHODS

2.1. *Monocyte chemotaxis*

Neuropeptide receptor-mediated chemotaxis was performed as described [8,9]. Briefly, mononuclear cells were isolated from heparinized blood of normal human volunteers by sedimentation over Ficoll-Paque (Pharmacia). Migration was studied utilizing blind-well microchemotaxis chambers in which the upper and lower compartments were separated by a 5 μM pore sized polycarbonate membrane. Cells (5.5×10^4) in RPMI media with 1% bovine albumin were placed in upper wells and peptide attractants in the lower wells. At the end of a 90 min incubation at 37°C, monocyte migration was assessed by fixing, staining and counting the cells adherent to the distal membrane. Three triplicate fields were counted utilizing an optical image analyzer. Data are presented as a stimulation index, the ratio of migrating cells in the test compared to buffer-alone wells. Migration in the presence of buffer alone was 20–50 cells/field in these experiments. Peak response migration for fMet-Leu-Phe (FMLP) occurred at 10^{-8} M and was 220–280 cells/field in these experiments (stimulation index 9.2–11.6) representing a percentage of migrating cells of 5–8%, typical for this type of assay. Migration in this assay is chemotactic in nature since a 'checkerboard' analysis [8], performed on peptide T, revealed migration to occur primarily in response to a positive concentration gradient. All experiments have been repeated 3–8 times. When indicated, monoclonal antibodies (Mabs) were included in both upper and lower wells concurrently with test attractants.

2.2. *Peptides*

Peptides were synthesized by the Merrifield solid-phase technique and purified by HPLC at Peninsula Laboratories (CA). Thin-layer chromatography, high-voltage electrophoresis, amino acid analysis and analytical HPLC indicated the peptides employed in these studies are >95% homogeneous. In the case of VIP [1–12], the peptide was generated by tryptic digestion of VIP, separated by HPLC and characterized by amino acid analysis and sequencing using an ABI 470A gas-phase sequencer with a 120A on-line HPLC.

2.3. *Antibodies*

Antibodies with specificity for T cell (OKT4, OKT8, OKT3) or monocyte (OKM1) determinants were purchased from Ortho Pharmaceuticals.

3. RESULTS

In our initial report [7] we described 'peptide T', ASTTTNYT, whose sequence is present in the ARV isolate of HIV [10], as competitively inhibiting HIV binding and infectivity. We have now examined the dose response and rank potency of peptide T and several analogs as agonists in an in vitro assay of human monocyte chemotaxis. Migration detected in this assay is primarily in response to a positive concentration gradient [8], a measure of chemotaxis. The results indicate that peptide T has potent chemotactic agonist activity with an EC_{50} of approx. 10^{-11} M (fig.1). The high bioactivity (maximal activity at 4×10^{-10}–8×10^{-11} M) and the biphasic dose-dependent response (inhibition at higher concentrations) of this peptide is also characteristic of other chemotactic neuropeptides [8]. The single amino acid modified analog [D-Ala^1]peptide T was slightly more active at 8×10^{-11} M than peptide T in this assay, and this may be related to this latter peptide's greater stability [7]. The carboxyl-terminal amino acid (e.g. amide form or DL isomer) appears to play a significant biological role since the analog [D-Ala^1,D-Thr^8]peptide T-NH_2 shows a reduced biopotency (EC_{50} of 2×10^{-10} M, with peak responses observed at 10^{-8} M). Higher concentrations of this peptide were even less effective (not shown). The peptide TTT is chemotactically inert and thus provides further evidence for specificity of the response. The rank potency order for chemotaxis, [D-Ala^1]peptide T > peptide T ≫ [D-Ala^1,D-Thr^8]peptide T-NH_2, is identical for both inhibition of ^{125}I-gp120 binding and human T cell infectivity by the HIV virus as reported by Pert et al. [7], consistent with the hypothesis that chemotaxis is mediated by the same receptor involved in HIV binding.

We were also interested in defining the essential

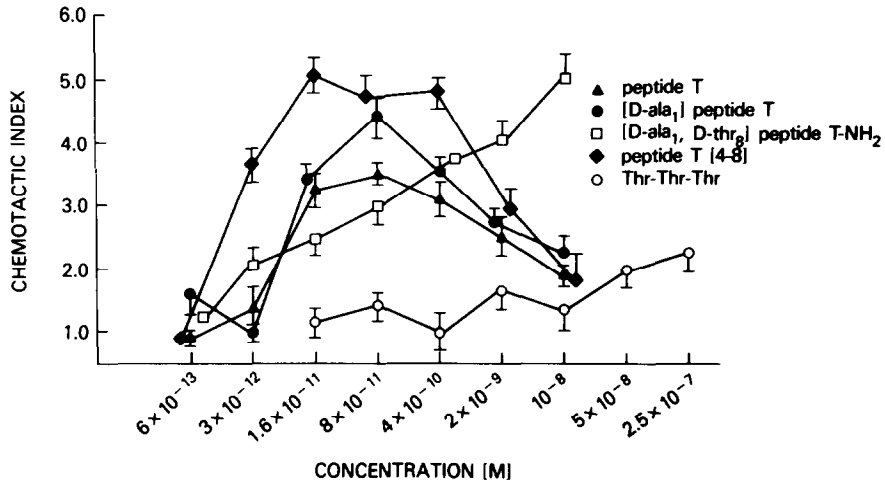


Fig.1. Peptides which block HIV infection are monocyte chemoattractants. The deduced peptide T (ASTTTNYT) and four analogs, indicated in the figure, were tested in an assay of human monocyte chemotaxis. Means ± SE of the chemotactic index vs peptide concentration are presented for a representative experiment, which was repeated 5 times. A chemotactic index of 9.2–11.6 was observed for peak response migration of FMLP which occurred at 10^{-8} M.

features of the core peptide responsible for bioactivity. An analysis of the peptide T-containing region of the gp120 from nine HIV isolates indicated that the only homologies were pentapeptides related to the sequence TTNYT [11]. We therefore tested this shorter analog of peptide T for activity in this assay. The results indicate that the peptide TTNYT has even greater chemotactic activity than peptide T itself, having an EC_{50} of 2×10^{-12} M with a peak activity in the range of 1.6×10^{-11} M (fig.1). Further truncation of this peptide (e.g. TNYT) destroyed the chemotactic activity (fig.3).

Additional evidence that these peptides exert their effects through the CD4 molecule is shown by the ability of a CD4 monoclonal antibody to block specifically peptide T-induced chemotaxis. The Mab OKT4a, capable of inhibiting HIV infectivity of monocytes via its binding to an epitope of the CD4 molecule [6], was used in these studies. Both [D-Ala^1]peptide T- and TTNYT-induced migration (fig.2A) was inhibited by a low dose (0.1 μg/ml) of OKT4a, but chemotaxis by the irrelevant attractant FMLP was not affected. At a concentration of 1.0 μg/ml non-specific inhibition of FMLP migration was also detected. Inhibition of chemotactic responses at high concentrations of antibody (fig.2) is not limited to anti-CD4 Mabs, but occurs with unrelated Mabs, e.g. anti-transferrin receptor (not shown) and, therefore, may be due to cell aggregation or other undefined post-receptor effects. Inhibition of peptide T-induced chemotaxis was only demonstrable with Mabs which were specifically directed to the CD4 molecule. Other Mabs such as OKT8, OKT3, or OKM1 directed to other T cell or macrophage surface antigens did not inhibit FMLP or peptide T chemotaxis (fig.2B) at 0.1 μg/ml.

A search in the Microgenie (Beckman) software revealed additional viral proteins containing sequences related to the protypic core peptide, TTNYT (see legend to fig.3). In addition to peptide T [4–8] (TTNYT) and the homologs represented in other HIV isolates [11], such as the sequence TTSYT, several single amino acid substituted pentapeptide sequences were identified in the viruses HTLV-I (STNYT), Epstein-Barr (TENYT), polio (TINYT) and in the neuropeptide VIP (TDNYT). To assess the relevance of these sequences with respect to CD4 activity, the bioactivity of these synthesized peptides was evaluated. A comparison of chemotactic activity of these compounds (fig.3) shows that the peptides TTSYT, STNYT and TENYT were comparably active to peptide T [4–8] with EC_{50} values in the 5×10^{-12}–10^{-11} M range and peak stimulation occurring at 10^{-10} M. The analog TDNYT, comprising VIP [7–11], was also quite active, but was slightly

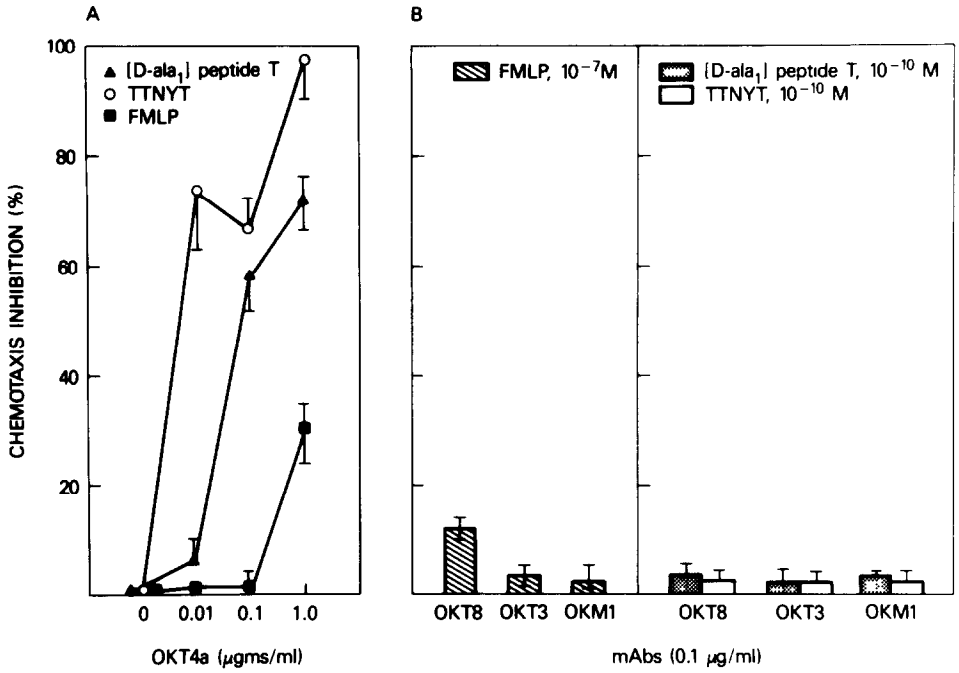
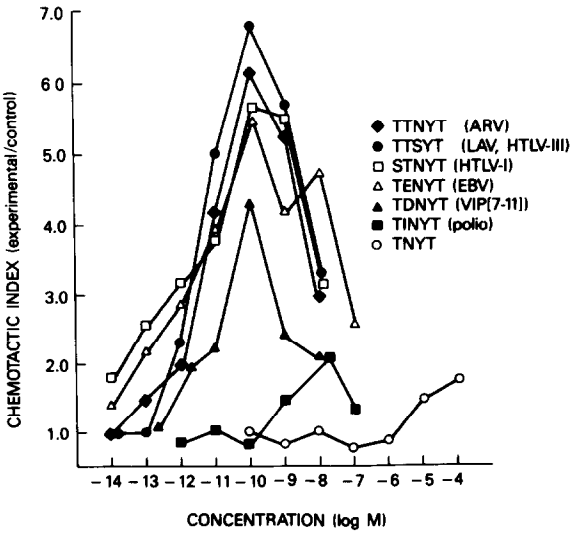


Fig.2. Mab inhibition of peptide T chemotaxis. (A) The Mab, OKT4a, with specificity for an epitope of the CD4 molecule specifically inhibits chemotaxis by peptide T analogs [D-Ala^1]peptide T and TTNYT (both at 10^{-10} M), but not the unrelated attractant FMLP, at 10^{-7} M. (B) Other anti-mononuclear cell Mabs do not inhibit chemotaxis. Percent inhibition is defined as [{1 – (experimental – background)/(control – background)} × 100].

less potent than peptide T [4–8]. The sensitivity of receptor-mediated events to slight structural changes is demonstrated by the low chemotactic activity of peptide TINYT.



4. DISCUSSION

Structure-activity analysis indicates that human monocyte chemotactic activity for peptide T and its analogs (fig.1) follows the same rank potency orders previously described for inhibiting gp120 binding to rat brain membranes and inhibition of HIV infection of human T cells [7]. Furthermore, only Mabs for the CD4 molecule, such as OKT4a (fig.2) or OKT4 (not shown), specifically inhibit peptide T or TTNYT chemotaxis, but not that of the irrelevant peptide FMLP. These results suggest

Fig.3. Chemotaxis to core pentapeptides. Peptides were synthesized and tested as chemoattractants based on their relatedness to sequences present in the HIV isolates ARV (TTNYT); LAV and HTLV-III (TTSYT); and the other viruses HTLV-I (STNYT); Epstein-Barr virus (EBV) (TDNYT); and polio (TINYT); and the peptide VIP [7–11] (TDNYT). The sequences were retrieved with Microgenie software from Beckman Instruments and synthesized de novo, as described. Data are from three representative experiments which have been repeated numerous times.

that peptide T chemotaxis is mediated through the same molecular determinant that is responsible for both gp120 binding and HIV infectivity and which is recognized by anti-CD4 Mabs.

Other viruses such as HTLV-I and EBV, as well as one neuropeptide, VIP, share sequence homology with the prototypic core bioactive sequence TTNYT, but have a single amino acid substitution in the second position. These peptides are chemotactically active (fig.3) although their relevance to viral function is as yet unexplored. Interaction with the CD4 receptor may be necessary for viral replication. Alternatively, the commonality of these sequences may indicate a relatedness among these virions not associated with the CD4 receptor.

The identification of low-M_r peptides which inhibit HIV infectivity and gp120 binding suggested that the CD4 molecule might subserve an additional function as a recognition molecule for a soluble peptide ligand. Neuropeptide receptors are now recognized as shared components, commonly present on immune cells as well as nervous and endocrine cells [8,9,12]. Evidence exists that several of the immunological defects associated with HIV infection, such as the immunosuppression [13,14], and polyclonal B cell activation [15], are not due to direct virus infection, but are mediated by viral proteins [16], most notably the large envelope glycoprotein, gp120.

Our results suggest that a previously unrecognized activity, namely interaction of the CD4 molecule with the N-terminus of VIP may occur. Preliminary experiments (B.M., M.R., unpublished) indicate that the chemotactic activity of VIP [1–12] is indistinguishable from the peptide TDNYT (VIP [7–11]) (fig.3). These results suggest that the endogenous ligand for the CD4 receptor may be the peptide VIP or a yet to be described close analog of the chemotactically active deduced 'core' pentapeptides described in fig.3. By mimicking the action of these 'core' pentapeptides, viral proteins could exert hormone effects on CD4 receptor bearing cells throughout the body, including the CNS, and thus may have a role in the etiology of the progressive dementia in AIDS patients [17] as well as their immunological abnormalities. VIP receptors have been described on human T cells [18] and localized in rodent spleen [19] while functionally this peptide inhibits T cell mitogenic responses [20]. Also, a secretory type diarrhea is commonly associated with the AIDS syndrome [21] and a significant biological action of VIP is stimulation of water and Cl secretion in the bowel [22]. Further characterization of the biological properties of the peptides described in this report could facilitate efforts to control the AIDS epidemic and might provide further insight into the pathogenetic mechanisms of other viral illnesses.

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