**Introduction**

Plasma membranes constitute a substantial fraction of the total cell mass that function in subdividing the cell into various organelles and in maintaining a constant cellular and sub-cellular milieu. The general view of the lipid-globular protein mosaic model of plasma membrane structure proposed several decades ago depicting a lipid bilayer consisting of an exoplasmic and cytoplasmic leaflet made up of phospholipids, glycolipids and cholesterol that act together as a solvent for retention of membrane proteins is changing. There is an increasing appreciation and understanding of the importance of membrane-protein function and the role the lipid bilayer plays in regulating important physiological processes.

Transmembrane (TM) receptors are integral membrane proteins, which reside and operate typically within a cell’s plasma membrane. Binding to a signaling molecule or sometimes to a pair of such molecules on one side of the membrane initiates a response on the other side. In this way they play a unique and important role in cellular communications and signal transduction. The hydrophobic TM domain of proteins transversing the cell membrane is constrained in space usually giving rise to an α helical staircase structure. The geometry of the α helix (when viewed from the top) and the amino acids that spin off from the central core structure, that dictates protein-protein interactions and helix packing, can be determined more easily when compared to globular proteins, thereby allowing for subsequent...
motifs to be defined that are crucial for protein contact and inter-face relations. The biophysical properties and molecular resolution of membrane proteins are slowly being resolved giving rise to important spatial and functional information that engenders optimism for future therapeutic targets.4,5

The TM region of receptors may interact to form dimers.6 Walters and DeGrado7 have demonstrated that the majority of helical TM protein interactions can be simplified into five tightly grouped pairs of interacting helices whose structural features can be understood in terms of helix-helix packing. The identification of these structural and sequential motifs could be very useful for the design of new therapeutic agents. One structural amino acid repeat extensively discussed in the literature is the GX3G motif noted in the TM domain of glycoporphin A8,9 in which glycines spaced four residues apart in proteins pack like "knobs-into-holes" resulting in the formation of stable helical pairs. Factors such as inter-helical angles, distances and composition of side chains are critically important and influence helix-helix association and subsequent packing. There are many other identified examples of sequence motifs that enable homo- and hetero-dimerisation of TM helices10 and various approaches have been devised to design peptides to target TM helices11 and prevent dimerisation from occurring hence interfering with function.

The selective ability of TM peptides to dissociate dimer pairs in vitro was demonstrated over a decade ago by Ng et al.12 This group showed the ability of peptides derived from the same amino acid sequence as the dopamine D2 receptor TM regions could affect the dimerisation of proteins and subsequent function. The peptides corresponding to the D2-TM region VI and VII of the dopamine receptor effectively dissociated the dimer in vitro. Peptide action on the dimer was receptor specific and region specific. This raised the possibility of dissociating dimers within the TM with significant clinical implications. This review focuses on the transmembrane region and not at small molecule drugs that can disrupt protein-protein interactions outside of the confines of the TM (discussed elsewhere).13

**Therapeutic Application of TM Peptides**

The ability of TM peptides to modulate or interfere with membrane protein function, and consequently cellular function, or to act as vehicles to deliver drugs or biomarkers, has drawn much current interest because of their potential pharmacological application. Interference with membrane protein function can be the result of helical-helical interactions between receptor and peptide mimetics that has been widely reported for G-coupled receptors, tyrosine kinases and integrins. Currently, GPCRs comprise more than 30% of all therapeutic drug targets and it is likely that this will only increase as more orphan GPCRs are identified (reviewed by Rosenbaum et al. 2009).14 GPCR privileged structural motifs have been successfully used by many pharmaceutical companies to design and synthesize combinatorial libraries, which are subsequently tested against novel GPCR targets for lead finding. The opportunity that dimerisation provides for enabling novel drug discovery include new drug targets for diseases such as neurological diseases, schizophrenia and pre-eclampsia, and increases the breadth and depth of receptors available for therapeutic intervention.18,19,20,21 Similarly, the ability of tyrosine kinase receptors (RTKs) to dimerise on ligand binding makes the TM domain a site of focus to target potentially new anticancer agents.22-25 Dimerisation takes place between two identical receptors (homodimerisation) or between different members of the same receptor family (heterodimerisation) and is essential for RTK activation.26 The best studied examples of oncogenic RTK are the epidermal growth factor receptor family (EGFR) associated with many carcinomas and the ErbB receptor family, in particular HER2/ErbB2, which is overexpressed on the surface of several breast cancers.27 For further reading on TM peptides and RTKs and integrins the reader is directed to the following articles.28-41

Although, the above exemplify interference of receptor function by non-charged peptide-protein helical-helical interactions, non-covalent interactions (electrostatic and charged) between charged amino acids within the TM are equally important and provide another indispensable means for the maintenance of structural stability and function of specific cell surface membrane receptors. Nowhere else is this point so well exemplified than in the multi-chain immune recognition receptors (MIRRs) present on immune cells such as T cells, B cells, mast cells and basophils (reviewed in ref. 42). The modeling and design of peptides that specifically bind natural proteins using charged amino acids in the membrane (arginine, lysine, glutamic acid) is an active area of research with important implications in health-related protein systems including inflammation, immunology, hematology, cardiology and infectious diseases.

The MIRR family group of receptors share common structural and functional features that include the presence of multi-subunits having a single TM spanning domain and containing one or more charged TM amino acids (Fig. 1). Feng et al.43 demonstrated that a common feature in the assembly of these diverse immune receptors (natural killer cells receptors and the Fc receptor for IgA, FcεR1) was based on polar TM-based embedded interaction sites. In the case of TCR the critical role of the TM environment and the molecular mechanisms underlying the assembly of the TCR-CBD3 complex has previously been defined by a series of elegant experiments by Wucherpfennig and co-workers and reviewed by Call and Wucherpfennig.44,45

The importance of TM charge in the assembly of the TCR has been known, however, for over two decades. Early experiments by Alcover et al.46 performing site-directed mutagenesis of Lys271 of the TCR-β chain, analyzed the capacity of the altered chain to assemble in a TCRβ-negative T-cell line. They convincingly demonstrated that substitution of this positively charged residue (Lys 271) with a different amino acid (alanine or glutamine) abolished formation of a TCR-α/β:CD3 complex and, consequently, receptor expression on the cell surface. These TM charged residues were not only important for assembly but later shown to dramatically influence the fate of membrane proteins by acting as degradation or retention signals targeting proteins to either the pre-lysosomal compartment or retention within the endoplasmic reticulum. The amino acids within the TM leading to retention were the same as those constituting the
binding motif involved with assembly. This led to the idea of co-localisation by pairwise assembly of opposing charges concealing the retention/degradation signals allowing progression into the next intracellular compartment while at the same time allowing progressive assembly of the subunits into a higher order complex until the receptor was assembled and transported to the cell surface.47,48 Based on multiple chain cotransfection of TCR chains in COS cells a hierarchy of formation of stable higher order partial complexes, ranging from a non-interactive pair to strong dimerisation, and the final assembly and expression of complete TCR on the surface of COS cells was reported by our group.49-51

Manolios et al. were able to demonstrate that the charged lysine and arginine on TCR-α TM formed critical interactions with opposing negatively charged aspartic acid on CD3-δ and was critical for assembly.52 This critical domain of interaction was confined to only 8 amino acids within the TCR-α TM region containing the charged groups which were necessary and sufficient for TCR assembly. Subsequent investigations defined the broad regions (TM, extra- and intra-cellular) of interaction between the individual TCR/CD3 chains involved with assembly.50 For one such pair (TCR-α/CD3-δ) where the site of interaction was defined and confined to the TM region experiments were extended and the smallest peptide necessary and sufficient to influence the function of the T cell was synthesized and tested for its ability to interfere with cell function in vitro and in vivo. This peptide was called core peptide (CP) and using CP as the lead peptide and the TCR as a model Manolios and coworkers investigated lipid-protein and protein-protein interactions within membranes and evaluated the potential clinical effects that CP conjugation with lipids and carbohydrate had on delivery, bioavailability and efficacy in vitro and in animal models of inflammation. These results are outlined below. As an evolving platform technology new approaches to therapy, delivery and clinical conditions are being explored, providing exciting new options to current therapies.

**Core Peptide (CP)**

The sequence of CP (GLRLILLKV) derived from the TCR-α chain has been shown to be an effective immunosuppressant.53 In animal studies, CP given subcutaneously significantly reduced the induction of T-cell mediated inflammation in animal models with adjuvant induced arthritis, allergic encephalomyelitis, delayed type contact hypersensitivity, and diabetes mellitus.53 Not only was it effective in preventing disease but also as a therapeutic given during active inflammation.

The application of topical CP peptide or CP cDNA in the treatment of T-cell mediated skin diseases (mouse and human) has been reported by Gollner et al.54 In murine models, direct topical application of the peptide inhibited the elicitation of contact sensitivity following application of a contact allergen in sensitized animals.54,55 When naked cDNA encoding the peptide sequence was injected into skin before application of contact allergen to sensitized animals, local immunosuppression was also observed.54,55 This implies that the expressed peptide is structurally intact and undergoes correct folding and post-translational modification. When the topical effects of CP were examined in humans with psoriasis, atopic eczema, lichen planus or contact dermatitis all patients except one reported a marked improvement of their skin disease. These data support peptide effectiveness and indicate that CP, delivered as peptide...
or cDNA might be a possible substitute for corticosteroids or immunosuppressive agents such as cyclosporine.

In a sophisticated set of experiments Mahnke et al. genetically engineered dendritic cells to secrete CP and noted antigen-specific immunosuppression in vivo. In a CD8-driven allergy model, the injection of these genetically engineered dendritic cells transduced with CP significantly reduced inflammation. In a CD4+ T cell-dependent model of multiple sclerosis (experimental allergic encephalomyelitis, EAE) injection of CP-secreting dendritic cells abrogated symptoms and prolonged survival. These effects were antigen-specific as transduced dendritic cells that did not express the respective antigen failed to convey protection. This implies that dendritic cells engineered to secrete CP are able to suppress T-cell activation in an antigen specific and localized manner without affecting other immune cells or other cell types. These elegant experiments indicate that CP may be a very useful new agent for in vivo immune suppression with widespread application beyond EAE.

Core Peptide Lipid/Sugar Conjugates

A number of modifications have been synthesized to test for improved bioavailability of CP. One such modification has been the synthesis of an all-D-amino acid analogue of CP (D-CP). This construct was shown to be recruited to the TCR complex and had retained the ability to inhibit T-cell activation. Moreover, D-CP manifested greater immunosuppressive activity than wild-type CP, attributed to the greater resistance to enzyme degradation, raising the possibility of its use as an oral agent. A number of modifications have been synthesized to test for antigen-specific as transduced dendritic cells that did not express the respective antigen failed to convey protection. This implies that dendritic cells engineered to secrete CP are able to suppress T-cell activation in an antigen specific and localized manner without affecting other immune cells or other cell types. These elegant experiments indicate that CP may be a very useful new agent for in vivo immune suppression with widespread application beyond EAE.

(1) N-myristoylation and tris lipidation. Lipid conjugation using 2-amino-2 (hydroxymethyl)-1,3 propanediol (Tris) as a linker to introduce mono-, di- and tripalmitate conjugates to improve the lipophilic character of therapeutic agents was reported by Wells et al. We have modified the C-terminal valine of CP by linking it to gly-tris-palmitate, -dipalmitate and -tripalmitate, and demonstrated using FITC as a fluoro chrome that both the CP and its lipopeptide conjugates translocated into fibroblasts and T cells. Tripalmitate conjugation resulted in improved inhibition of IL-2 production whilst the dipalmitate was not effective in these bioassays. Lipopeptides of the di and the tri-palmitate of a control peptide did not inhibit IL-2 production. It appears therefore that it is not only the palmitoyl moiety but the N-terminal valine of CP that is critical for exhibiting the biological effect. A more dramatic biological effect was noted clinically with lipopeptides in preventing the onset of arthritis in the adjuvant induced arthritis model. We believe that the number and type of lipid residues have a significant effect on membrane insertion of the peptide conjugates. Investigations on the insertion of lipid modified peptides in model membrane vesicles indicate that single lipid modification, N-myristoyl or S-farnesyl, does not contribute sufficient hydrophobicity for stable insertion of peptides into membranes and a second lipid moiety is required. Stable insertion could also be achieved by combining a single lipid residue with a cluster of positively charged amino acids which interact with the negatively charged phospholipid cell membrane. The nature of the fatty acid also has an effect on membrane binding; a palmitoyl group provides the peptides with fifteen times greater membrane affinity than myristoyl or geranyl-geranyl thioether residues. N-terminal myristoylation of CP (Myr) resulted in an increase in CP’s ability to inhibit IL2 production.

(2) Lipoamino acids. Toth and his colleagues have undertaken a series of studies in designing novel lipidic conjugates which combined the structural features of amino acids and fatty acids. These lipoamino acids can be reacted either by their amino or by their carboxylic acid function, or both. They can be used in conventional solid phase peptide synthesis and can be linked in any position of the peptide sequence. This class of compounds combines the structural properties of peptides with the characteristics of lipids and membranes. Conjugation with a lipoamino acid on the N-terminal of its sequence enhanced CP’s (LA) ability to lower IL2 production considerably.

(3) Glycosylation. In an attempt to render CP more water soluble and therefore improve its bioavailability in aqueous environments, Toth has utilized the same technology used to create lipoamino acids to incorporate sugar groups to peptides. The potential of this technique is that it enables hydrophobic molecules to be rendered water-soluble, therefore opening the possibility of orally administered peptides. CP conjugation with a glucose succinate moiety (GS) had a profound anti-arthritic effect in animal models of arthritis.
Biophysical Characterisation of Core Peptide

A wide variety of biophysical techniques combined with the use of model membrane systems have been used to study CP peptide-membrane interactions. These techniques including CD, nuclear magnetic resonance (NMR), and surface plasmon resonance (SPR) have provided important information on the relationship between membrane-active CP peptide structure and CP biological function.

The technique of SPR was used by our group64 to define CP’s affinity for different membranes. The dibasic CP was found to bind to both zwitterionic and anionic model membranes as well as to a T-cell membrane preparation. By contrast, switching one or both of the basic residues to acidic residues on the peptide sequence led to a complete loss of binding to model membranes. In addition, the position of the charged amino acids in the sequence, the number of hydrophobic amino acids between the charged residues, and substitution of one or both basic to neutral amino acids were found to effect binding. These results when compared with in vitro T-cell stimulation assays and in vivo adjuvant-induced arthritis models showed very close correlation and confirmed the findings that both amino acid charge and location have a critical role in CP peptide activity. All lipid conjugates of CP exhibited irreversible binding to model membranes. All the Gly-Tris palmitate conjugates of CP bound stronger to model membranes than CP. Di- and tripalmitate conjugates of the control peptide exhibited no binding to model membranes in agreement with the result of the IL-2 inhibition assay.58 These results demonstrate that the charged amino acids in the peptide sequence and lipid conjugation are both important factors in CP’s function.59

Secondary structure analysis of CP using CD revealed that in aqueous solution the peptide contains only a small percentage of α-helical and about 75% random coil structures, while CP in a hydrophobic solution (Trifluoroethanol) has about 40% α-helical structure. CD spectrum in the presence of dimyristoyl phosphatidyl choline/dimyristoyl phosphatidyl glycerol (DMPC/DMPG) membrane (70:30 ratio) was found to be characteristic for peptides primarily in the β-conformation at lower concentration, whereas at higher CP concentration the spectrum was typical for an exclusively β-structure, attributed to aggregated peptides associated with the negatively charged DMPG components.65 CD studies also revealed a dose-dependent conformational change of CP from a dominantly random coil structure to that of β-structure as the concentration of DMPG increased relative to CP.66

In the study of CP both solution and solid-state NMR spectroscopy has been applied. The effect of CP on model membranes (liposomes) was assessed by 31P and 2H solid state NMR spectroscopy.65 31P and 2H NMR measurements were performed to investigate whether CP is capable of perturbing model membrane structure. These experiments found that CP did not significantly influence the structure of DMPC membranes, but the small spectral changes that were caused by CP showed that at least part of the peptide population associates with the membranes, most likely with the lipid head groups. In negatively charged model membranes it was found that CP was capable of disrupting membrane vesicles, but only at very high concentrations. Electron microscopy and flow cytometry studies also had shown that cell membranes remained intact in the presence of CP. Hence it appears that CP exerts its biological action by a more complex mechanism than simply perturbing the lipid bilayer membrane.

To study self-association of CP in solution, self-diffusion measurements were performed on 0.05, 1 and 5 mM CP in D2O and 0.05 mM CP in D2O with 0.2% DMSO (pH = 3–4). A constant apparent diffusion coefficient of ~2 x 10−10 m2 s−1 was determined, indicating that CP was monomeric at low pH. To mimic physiological conditions, CP was incorporated in a DMPG bilayer in PBS buffer and significant aggregation was observed via NMR diffusion measurements. The secondary structure of CP in different solvent environments was also examined using multi-dimensional NMR experiments such as total correlation spectroscopy (TOCSY) and nuclear overhauser effect spectroscopy (NOESY).67 These experiments indicated the formation of β-strands in certain environments.

Taken together the biophysical results suggest that the initial binding of CP to lipid membranes may be mediated by electrostatic interactions between the peptide and anionic lipids. Through hydrophobic interactions, CP may change conformation and insert into the membrane bilayer. Once within the membrane, CP may then be free to associate with membrane resident TCR proteins.

Core Peptide Cellular Specificity and Mode of Action

A wider significance of the assembly process similar to that seen with the pairing of opposing charges within the TM of the TCR is relevant to other immune cell receptors including B cell receptor complex; Natural killer (NK) cell receptors [KIR, NKG2D, NKG2C/CD94, Nkp30, Nkp44, Nkp46 receptors], Fc receptors [including the FcεRI, FcγRI, FcγRI and FcγRIII receptors];68–73 immunoglobulin-like transcripts and leukocyte immunoglobulin-like receptors; signal regulatory proteins; dendritic cell immunooactivating receptor; myeloid DNA receptor of 12 kD (DAP-12); platelet aggregating receptor; and glycoprotein VI. The striking feature in the assembly of these immune receptors is the high degree of specificity and position of the charged TM residues which lend themselves to peptide inhibition.

The specificity of CP for T cells is dependent on both amino acid composition and the need for two critically placed positive charges within its sequence.74 Initial experiments aimed at examining CP-TCR interactions within the plasma membrane have shown that CP localizes within membranes and associates with the TCR upon T-cell activation (Fig. 3). This is a specific interaction, not noted with other TM proteins such as CD45, transferrin receptor, IL-2 receptor β chain, or the GPI-anchored protein CD14. This association with the TCR during activation prevents the TCR-ζ chain phosphorylation and results in subsequent decrease in IL-2 production (Fig. 4).75,76
CP had minimal effects on B cell proliferative responses when used at low concentrations (<50 mM) known to affect T-cell function. To determine the effect of the TCR transmembrane peptides on B-cell activation, a number of different B-cell mitogens, CD40L, lipopolysaccharide and anti-IgM and anti-IgD, similar to that used for T-cell activation were analyzed. These three mitogens act at different surface receptors and on different sites of B-cell activation cascade leading to proliferation. Experiments showed that TCR peptides had a differential inhibitory effect on B-cell receptor (BCR) function.

TCR peptides were assessed for their effect on direct NK cytotoxicity using a standard NK cytotoxicity assay. The target cells used were the NK sensitive K562 (human leukemic cell line), which were labeled with radioactive chromium isotope and incubated with freshly isolated peripheral blood leukocytes containing fresh NK cells. The amount of chromium released reflected the magnitude of direct NK cytotoxicity. Both CP and analogues showed a dose-dependent inhibition of direct NK cytotoxicity. These peptides inhibited direct NK cytotoxicity by an average of 47–59%. Other peptides with negative and neutral TM charge substitutions had minimum or no effect on direct NK cytotoxicity. Short peptides derived from the TM sequence of NK activating receptors and associated molecules (NKp46, NKp30, NKG2D and TCR-ζ) have been tested in vitro without any significant inhibition of NK cell cytotoxicity.

CP inhibits TCR-ζ chain phosphorylation following antigen stimulation (Fig. 4). This inhibition may be the result of: disruptive CP TM charge interactions leading to the prevention of higher order TCR complex formation, with either the TCR itself or co-accessory molecules within sphingolipid-rafts, inhibiting signal transduction; steric hindrance preventing receptor assembly/disassembly; or steric hindrance in the formation of CD3-ε/ζζ homodimers/oligomers required for proper receptor signaling (SCHOOL theory); discussed below. The exact molecular mechanism has not been defined and could involve:

**Figure 3.** CP localizes with TCR in the cell membrane of human T cells. Peripheral blood mononuclear cells were incubated with FITC-conjugated mouse anti-human immunoglobulin and streptavidin-Texas Red following anti-CD3 and CP-biotin incubation. After incubation cells were paraformaldehyde-fixed (A–C) or were held at 37°C to allow TCR clustering into patches or caps before fixing (D–I). The samples were viewed by confocal microscopy. Reproduced with permission.

**Figure 4.** Treatment of T cells with CP inhibits TCR-ζ chain phosphorylation. (A) 2B4 cells were incubated with LK cells and moth cytochrome C (MCC) for the times indicated (lanes 3–7). TCR-ζ chain, immune-captured from each cell lysate (lanes 3–7) and from 2B4 and LK cells alone (lanes 1 and 2, respectively), was subjected to SDS-PAGE and immunoblotting with anti-phosphotyrosine (4G10). Lane 8 is a negative control without cells necessary to establish which bands in the western blot are specific to the immune-capturing antibody. (B) 2B4 cells which had been pretreated with or without CP or LH-RH for 30 min at 37°C as indicated were incubated with LK cells and MCC for 15 min (lanes 2–6). Cells in lane 1 were not stimulated with MCC and lane 7 is a negative control without cells. TCR-ζ chain was immune-captured from each cell lysate and subjected to SDS-PAGE and western blotting with anti-phosphotyrosine (4G10).
(1) Prevention of higher order TCR oligomerisation complexes in sphingolipid-rafts required for signal transduction. Coupling between TCR and CP is specific (Fig. 3). No co-localization between CP and other plasma bond receptors has been noted and no charge-modified CP analogues co-localize with TCR. TCR cell surface expression after 4 days incubation with CP (1–100 uM) did not show any difference from controls when analyzed by FACS. In addition, co-transfection of TCR-α with CD3-δ in the presence of CP using a COS-7 cell system did not influence dimerisation of these subunits (unpublished data). These experiments raise doubt on the ability of CP to sterically inhibit assembly of the TCR. It is more feasible that TM lipid-peptide charge interactions that occur during T-cell activation leads to the prevention of higher order TCR oligomerisation complexes in sphingolipid rafts inhibiting signal transduction. It is predicted that peptides from corresponding signaling subunits would have a similar mechanism of action.

(2) SCHOOL model. The basic tenet of this model is that MIRR triggering is the result of ligand-induced interplay between intra-receptor TM interactions and signaling subunits and inter-receptor homo-interactions between MIRR signaling subunits that leads to formation of oligomeric signaling structures, thus triggering the receptors and initiating signaling cascade (reviewed in ref. 79). Within this model CP via electrostatic interactions compete with the TCR-α chain for binding to CD3 δε heterodimer and the ζζ homodimer resulting in disconnection and pre-dissociation of the signaling subunits from the remaining receptor complex (Fig. 5).

TM Peptides as Therapeutic Agents for Infections

The rapid emergence of pathogenic infections that are resistant to conventional drugs has escalated the interest in finding new therapeutic agents with increasing focus on peptides as antimicrobial agents. These peptides can be divided into different groups based on their structure and include linear peptides with amphipathic and hydrophobic helices, cyclic peptides and small proteins which form β-sheet structures. Their mechanism of action is diverse, not necessarily based on electrostatic interaction, and outside the scope of this review. It is interesting that the structural features of many TM peptides because of their hydrophobic nature resemble those of antimicrobial peptides giving them the potential to be evaluated as anti-microbial agents. We tested CP as an antimicrobial agent against Escherichia coli and Staphylococcus aureus and noted that the minimum inhibitory concentration (MIC) was too high for this compound to be used clinically (data unpublished). Recently however, C-terminal amidation of CP showed a broad-spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria with the MIC values between 3 and 77 μM. Unlike native CP, amidated CP was found to kill Escherichia coli without lysing the cell membrane or forming pores. It was reported that CP could penetrate the bacterial cell membranes and accumulate in the cytoplasm of both Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli. These results indicated that peptide modification, of TM hydrophobic peptides such as amidation of C-terminus may enhance structural stability and anti-microbial activity.80

Viruses have also evolved sophisticated strategies to infect, replicate and persist in the host. One interesting strategy may involve targeting TM signal transduction of immune receptors. The downregulation of TCR via TM charge interactions between viruses and TCR/co-accessory molecules may be one way by which viruses escape immune surveillance. Consequently these sites or points of contact can be the focus to design peptides which target membrane embedded interactions allowing immune recognition or preventing viral entry. Various unrelated viruses such as HIV, cytomegalovirus (CMV), severe acute respiratory syndrome coronavirus (SARS-CoV), herpesvirus saimiri (HVS), and human herpesvirus 6 (HHV-6) may inhibit TCR signaling via TM charge interactions to disarm the receptor and allow entry and persistence into the cells until it re-activates to form infectious particles.81 Examples include: (a) the recognition of the human CMV tegument protein pp65 by NKp30 that results in a general inhibition mediated by the dissociation of the NKp30-ζ complex and loss in the ability of cells to kill virus-infected cells;82 (b) human herpesvirus-6 U24 protein down-regulating TCR surface expression and U24-expressing T-cells becoming resistant to activation by antigen-presenting cells;83 and (c) TCR downregulation activity by the highly conserved membrane-proximal sequence of the tyrosine kinase-interacting protein (Tip) of HVS.84,85 The positioning of the TM electropositive residues is remarkably conserved in HVS Tip213-228, the relevant domain of the two-in-one (Tio) protein of herpesvirus ates, and human T lymphotropic virus type 1 gp21 (Fig. 5; reviewed in ref. 86). This concept of infectivity could be useful in the prevention of viral attack and sequestration of viral particles by means of peptides, either at the TM or extracellular region. For example, it may be possible that a sulfotyrosine binding site of the HIV envelope gp120,87 can be exploited to prevent binding to CCR5.

HIV infection of target cells requires fusion of the viral membrane with the cellular membrane catalysed by the HIV envelope glycoprotein gp160. This in turn is composed of two subunits, gp120 and gp41, the latter playing a critical role in virus entry into the host cell. Molecular dynamic studies of the gp41 TM domain suggest that arginine residues play an essential role in maintaining the integrity of the three-helix bundle thought to be important for the oligomerisation of gp41 and virus-cell membrane fusion process.88 In the early 1990s, a number of highly potent anti-human HIV-1 peptides were derived from the C-heptad repeat (CHR) domain of the HIV-1 envelope glycoprotein TM subunit gp41.89-91 Biophysical and biochemical analyses suggested that the CHR peptides inhibited HIV-1 env-mediated membrane fusion by interacting with the viral gp41 N-heptad repeat (HR) domain to form heterologous trimers-of-heterodimer complexes, thus blocking gp41 six-helix bundle (6-HB) core formation, a critical step in virus-cell fusion.92,93 T20 (generic name, enfuvirtide: brand name, Fuzeon), is a new class of anti-viral medications composed of a peptide with 36 L-amino acids (amino acids 638 to 673) containing a HR sequence-binding domain and a tryptophan-rich domain that inhibits viral entry
reported developing T20 resistance and new peptidomimetics and new approaches to prevent HIV replication are therefore required.

Figure 5. For figure legend, see page 281.

into cells. T20 was approved by the US FDA in March 2003 for the treatment of HIV-1 infection. Even though T20 is very effective in inhibiting infection by HIV-1, recently many patients have reported developing T20 resistance and new peptidomimetics and new approaches to prevent HIV replication are therefore required.
Fusion peptide (FP). Quintana et al. reported a HIV-1 fusion peptide (FP) that targets the TCR and also inhibits specific T-cell activation. The N-terminus of this peptide inserts in the target membrane and the more hydrophilic C-terminus domain lies parallel to the cell surface. Thus FP 1-16 anchors the virus to the cell. In addition to this role FP has also been shown to interact with the TCR and interfere with T-cell activation. Quintana et al. have shown that FP plays a double role in HIV infection, mediating membrane fusion and downregulating T-cell responses to virus that could block infection. They demonstrated that FP was able to inhibit the arthritogenic T cells in an adjuvant arthritis model and also reduce disease associated IFN response. The authors suggested that HIV might have naturally developed a strategy to interfere with the TCR/CD3 interaction in order to stop the virus specific immune response to gp41.

Peptide V. To date, we have tested peptides for their effectiveness in inhibiting HIV-1 replication and infectivity and noted that based on the principles of TM charge interactions HIV entry into T cells and viral replication can be prevented by peptides with appropriately placed charges (unpublished data). In an infection assay where lymphocytes are treated with peptide either 1 h before (“pre”), simultaneously (“sim”), or 4 h after (“post”) HIV-1 infection, peptide “V” derived from the TM region of one of the CD3 invariant chains, demonstrated good results with HIV-1 inhibition observed in “sim” and “post” conditions. Results showed no HIV DNA was present in the “sim” and “post” combined treatment of PBMC’s for the duration of the experiment (10 d) as determined by PCR/agarose gel electrophoresis to detect the presence of HIV DNA.

In studying the membrane distribution of FP in T-cells Quintana et al. demonstrated that FP colocalized with TCR and CD4 molecules. T-cell activation in vitro and in vivo suggested a role of FP in the downregulation of HIV specific immunity and was suggested to block the TCR/CD3 interaction needed for antigen induced T-cell activation. It may be that the similar polar amino acid residue distribution and experimental findings between TCR CP and HIV FP suggest an analogous mechanism of action, although this view has been contested. Primary sequence analysis of proven and predicted immunomodulatory sequences of viral fusion protein regions and other domains shows a similarity in the charge distribution pattern with two essential positively charged residues spaced apart by three to four or seven to eight amino acids, suggesting a similarity of mechanisms used by diverse viruses in their pathogenesis to modulate the host immune response. It is envisaged that CP and FP compete with TCR-α for binding to CD3-δε and ζζ, resulting in functional disconnection of these subunits (Fig. 5). This prevents TCR subunit formation inhibiting T-cell activation upon stimulation with antigen. Similar viral TM sequences have been shown to affect TCR-TM interactions leading to immuno-modulation/suppression.

Equally plausible is the possibility that because these peptides are closely associated with the TCR, HIV infection is inhibited indirectly by the prevention of T-cell activation required for full reverse transcription and integration of the HIV genome. A third possibility which may not be mutually exclusive is that these peptides, known to be very hydrophobic and able to reach organelles such as ER preventing the correct assembly of the envelope glycoprotein and thereby packaging of the virus, through electrostatic membrane interactions within the ER membrane.

Summary

The membrane provides a unique environment for specific protein-protein/protein-lipid interactions that plays an important role in assembly and function of key receptors. These interactions are based on biophysical forces which are restrained by electrostatic charge and lateral TM helix associations as a result of membrane-protein folding. Targeting these very specific sites with designer peptides to prevent homo- or heterodimerisation opens a new paradigm in drug design and therapeutics. Novel strategies for drug discovery based on selective disruption of protein-protein interactions, using high throughput discovery tools e.g., peptide chips, plasmon resonance, fluorescence and phospho-imaging offer the development of a new generation of molecular therapeutic agents. The TM inhibitory peptide CP serves as only one early example of what is possible, and using the same principles/mechanisms of inhibitory action, opens the field of drug design in many different areas of medicine and therapeutics.

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