A GxxxA-like Motif within HIV-1 Fusion Peptide Is Critical to Its Immunosuppressant Activity, Structure, and Interaction with the Transmembrane Domain of the T-cell Receptor*

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Background: HIV utilizes its fusion peptide (FP) to both fuse and immunosuppress T-cells.

Results: A conserved GxxxA-like motif within FP is critical for its function and structure.

Conclusion: The GxxxA-like motif exerts its role via interaction with the transmembrane domain of TCRα.

Significance: We shed light on the molecular mechanism of FP immunosuppressant activity and on the molecular recognition within the membrane milieu.

To thrive in the human body, HIV fuses to its target cell and evades the immune response via several mechanisms. The fusion cascade is initiated by the fusion peptide (FP), which is located at the N-terminal of gp41, the transmembrane protein of HIV. Recently, it has been shown that the HIV-1 FP, particularly its 5–13 amino acid region (FP5–13), suppresses T-cell activation and interacts with the transmembrane domain (TMD) of the T-cell receptor (TCR) complex. Specific amino acid motifs often contribute to such interactions in TMDs of membrane proteins. Using bioinformatics and experimental studies, we report on a GxxxA-like motif (AxxxA), which is conserved in the FP throughout different clades and strains of HIV-1. Biological activity studies and FTIR spectroscopy revealed that HIV FP5–13-derived peptides, in which the motif was altered either by randomization or by a single amino acid shift, lost their immunosuppressive activity concomitant with a loss of the β-sheet structure in a membranous environment. Furthermore, fluorescence studies revealed that the inactive mutants lost their ability to interact with their target site, namely, the TMD of TCRα, designated CP. Importantly, lipoteichoic acid activated macrophages (lacking TCR) were not affected by FP, further demonstrating the specificity of the immunosuppressant activity of CP. Finally, although the AxxxA WT and the GxxxA analog both associated with the CP and immunosuppressed T-cells, the AxxxA WT but not the GxxxA analog induced lipid mixing. Overall, the data support an important role for the AxxxA motif in the function of FP and might explain the natural selection of the AxxxA motif rather than the classical GxxxA motif in FP.

Human immunodeficiency virus (HIV) is the etiological agent for acquired immunodeficiency syndrome (AIDS). Since it was first reported, ~60 million people have been infected with HIV (1–3). Effective infection of HIV is accomplished by successful fusion between its membrane and the membrane of the T-cell, as well as by its ability to evade the immune response against it. To fuse with the target cell, HIV virions express the envelope glycoprotein gp160, which is composed of gp120-gp41 subunits. The gp120 glycoprotein binds its cell receptor (CD4) and a co-receptor (CXCR4 or CCR5) (4, 5). Following the attachment of gp120, gp41 undergoes conformational changes that enable it to catalyze the fusion between the viral and the cellular membranes or between infected and native cells (6–8). Gp41 is composed of several functional domains including the fusion peptide (FP),3 the N-terminal heptad repeat, the loop, the C-terminal heptad repeat, and the transmembrane domain (TMD) (see Fig. 1) (9, 10). A proposed role of the FP is to reduce the energy barrier for the fusion by binding and dehydrating the outer bilayer at a localized site (11). The fusion itself starts with the insertion of the FP into the host cell membrane (12–14). Only then can N-terminal heptad repeat and C-terminal heptad repeat form a six-helix bundle that bridges the gap between the opposing membranes, enabling the actual membrane fusion (Fig. 1) (11, 13–16).

HIV also suppresses the immune response via several mechanisms. Examples include the following: (i) interfering with the expression of the costimulatory molecules CD40 ligand and CD80 (B71) (17); (ii) inducing anergy in naive T lymphocytes through CD4-independent protein kinase A-mediated signaling (18); (iii) inducing dysfunction of uninfected bystander CD4+ T-cells via interaction with both CD4 and co-receptors (19); (iv) exposing CD4+ T-cells during stimulation to non-infectious HIV expressing functional envelope glycoproteins, which fail to provide activation signals to autologous dendritic cells (20); and (v) impairing the ability of dendritic cells to stimulate T-cell proliferation during HIV-1 infection (21).

In addition, studies have shown that elements in gp41 can inhibit T-cell proliferation. These include the following: (i) a
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FIGURE 1. A schematic illustrating the HIV-I-HXB2 fusion process and denotes the gp41 extracellular domain. A, HIV fusion cascade. i, naïve state in which gp120 shields gp41. ii, prehairpin intermediate state in which gp120 is attached to its receptor and co-receptor, causing a conformational change. iii, extended prehairpin, in which gp41 is released and the FP is inserted into the host membrane. iv, post-fusion; NHP and CHP formed a six-helix bundle and the fusion progressed. (61). B, A scheme showing the functional regions within the extracellular portion of HIV HXB2 gp41 (amino acids 512–856) (9, 62). CHR, C-terminal heptad repeat; NHR, N-terminal heptad repeat.

peptide derived from the interface between the N-terminal heptad repeat and the loop regions of gp41 (amino acids 583–599), termed the immunosuppressive unit (22), which interferes with calcium influx and the function of a protein kinase C (PKC) (23, 24); (ii) the FP, which specifically binds the TMD of TCRα (termed CP) and as a result interferes with the assembly of the TCR complex (25–29); and (iii) the TMD of gp41, which shares a nine-amino acid motif with the TMD of the α-subunit of the T-cell receptor (TCRα). As a result, the TMD also interferes with the assembly of the TCR complex (30). Importantly, each of these three regions has a different mode of action. Molecular dynamic simulation was further utilized to identify the TCR/FP interacting region at the molecular level, providing a three-dimensional model of the TCR-FP assembly structure. The simulation data revealed that FP5–13 interacts with CP within the membrane in a β-sheet/α-helix interaction (27). Experimentally, attenuated total reflectance (ATR)-FTIR studies revealed a β-sheet structure of the FP5–13. A synthetic peptide mimicking FP5–13 indeed preserved the potent inhibitory activity of FP both in vitro and in vivo. The FP5–13 was found to colocalize and immunoprecipitate specifically with the TCR (26–28).

Interactions between TMDs are usually driven by amino acid motifs, among which the GxxxxG motif, originally identified in the TMD of glycoporphin A, is the most studied (31–33). Other than stabilizing interactions within the membrane, glycine residues have also been shown to facilitate conversion to a β-sheet secondary structure and to mediate sheet-to-sheet packing within the membrane (34). The glycine residues in the GxxxxG motif can also be replaced by the small residue Ala (32, 35, 36). Furthermore, the GxxxxG dimerization is reinforced by the presence of phenylalanines (37).

In this study, we combined bioinformatics with experimental studies and characterized an AxxxA motif within the FP5–13 region (GAFLGFLG) of HIV-1, which is crucial for the immunosuppressant activity of FP. Mode of action studies using several analogs revealed that the motif controls the heteroassociation between FP and TCRα-TMD within the membrane and contributes to the β-sheet secondary structure of FP. Interestingly, the AxxxA WT and the GxxxxG analog both associate with the CP and immunosuppress T-cells, whereas the AxxxA WT but not the GxxxxG analog induced lipid mixing of zwitterionic membranes. The results are discussed regarding the mechanism of the immunosuppressant activity of FP as well as the possible reason for the natural selection of the AxxxA motif rather than the classical GxxxxG motif.

**EXPERIMENTAL PROCEDURES**

**Materials**—Rink amide 4-methyl-benzhydrylamine resin and 9-fluorenylmethoxy carbonyl (Fmoc) amino acids were purchased from Calbiochem-Novabiochem AG. Other reagents used for peptide synthesis include N,N-disopropylamine (Sigma-Aldrich), dimethylformamide, dichloromethane, and piperidine. Egg phosphatidylcholine (PC) was purchased from Lipid Products (South Nutfield, UK). 4-Chloro-7-nitrobenz-2-oxa-1,3-diazole fluoride (NBD-F) and rhodamine-N-hydroxysuccinimide (Rho-N) were purchased from Molecular Probes (Junction City, OR). The myelin oligodendrocyte glycoprotein (MOG) p35–55 antigen used for the specific activation of the T-cell line was synthesized using the Fmoc technique with an automatic multiple peptide synthesizer (AMS 422, ABIMED, Langenfeld, Germany). Primary CD4 T-cells specific to MOG p35–55 were a kind gift from Professor Avraham Ben-Nun from the Weizmann Institute of Science.

**Peptide Synthesis and Fluorescent Labeling**—Peptides were synthesized using the Fmoc solid phase method on a Rink amide 4-methyl-benzhydrylamine resin (0.68 meq/gm), as described previously (38). The synthetic peptides were purified (>98% homogeneity) by reverse phase high performance liquid chromatography on a C4 or C18 column using a linear gradient of 30–70% acetonitrile.
trile in 0.1% trifluoroacetic acid (TFA) for 40 min. The peptides were subjected to amino acids and mass spectrometry analysis to confirm their composition. To avoid aggregation of the peptides prior to their use in the cell culture assays, the stock solutions of the concentrated peptides were maintained in dimethyl sulfoxide (DMSO). The final concentration of DMSO in each experiment was <0.25% v/v and had no effect on the system under investigation. For NBD-fluorescent labeling, resin-bound peptides were treated with NBD-F (2-fold excess) dissolved in dimethyl formamide (DMF), leading to the formation of resin-bound N-terminal NBD peptides (39). After 1 h, the resins were washed thoroughly with DMF and then with methylene chloride, dried under nitrogen flow, and then cleaved for 3 h with 95% TFA, 2.5% H2O, and 2.5% triethylsilane. For Rho-N fluorescent labeling, the Fmoc protecting group was removed from the N terminus of the resin-bound peptides by incubation with piperidine (20% in DMF) for 12 min, whereas all of the other reactive amine groups of the attached peptides were kept protected. The resin-bound peptides were washed twice with DMF and then treated with rhodamine-N-hydroxysuccinimide (2-fold excess), in anhydrous DMF containing 2% N,N-diisopropylethylamine, leading to the formation of a resin-bound N-rhodamine peptide. After 24 h, the resin was washed thoroughly with DMF and then with methylene chloride, dried under nitrogen flow, and then cleaved for 3 h with 95% TFA, 2.5% H2O, and 2.5% triethylsilane. The labeled peptides were purified on a reverse phase HPLC C4 or C18 column as described above. Unless stated otherwise, stock solutions of concentrated peptides were maintained in DMSO to avoid aggregation of the peptides prior to use.

Preparation of Large Unilamellar Vesicles (LUV)—Thin films of PC were generated after dissolving the lipids in a 2:1 (v/v) mixture of CHCL3/MeOH and drying them under a stream of nitrogen gas while rotating them. The films were lyophilized overnight, sealed with argon gas to prevent oxidation of the lipids, and stored at −20 °C. Before the experiments, films were suspended in the appropriate buffer and vortexed for 1.5 min. The lipid suspension underwent five cycles of freezing-thawing and extrusion through polycarbonate membranes with 1- and 0.1-μm diameter pores to create large unilamellar vesicles.

Lipid Mixing—Lipid mixing of LUVs was measured using a fluorescence probe dilution assay (40). LUVs were prepared with PBS, as described above, from unlabeled and labeled films combined to give a 9:1 ratio of 100 μM total lipid concentration. The basal fluorescence level was measured initially for a 400-μl vesicle mixture. Then, peptides dissolved in 2 μl of DMSO were added to the mixture. Fluorescence was monitored after the addition of the peptide until a steady state was achieved, as indicated by a plateau. The emission of NBD, the energy donor, was monitored at 530 nm with the excitation set at 467 nm. Fluorescence intensity was compared with 2% (v/v) of Triton X-100.

Macrophage Activation by LTA—RAW264.7 cells (2 × 105 per well) were cultured overnight in a 96-well plate. The following day, the medium was replaced by fresh DMEM, including all supplements. Peptides were dissolved in DMSO and added to the cells to reach a 20 μM final concentration. The final concentration of DMSO was 1% for all groups. Cells were incubated with the peptide for 2 h and then washed and incubated with fresh medium containing 500 ng/ml lipotechoic acid (LTA).

Determination of Secreted TNFa—Cells were incubated with the indicated activators for 5 h at 37 °C, after which samples of the medium from each treatment were collected and stored at −20 °C. TNFa levels in each sample were evaluated using a mouse TNFa enzyme-linked immunosorbent assay kit (BIOSOURCE™ ELISA, Invitrogen) according to the manufacturer’s protocol. All experiments were done in triplicate.

Fluorescence Energy Transfer (FRET) Measurements—The FRET experiments were performed by using NBD and Rho-labeled peptides as energy donors and energy acceptors, respectively. Fluorescence spectra were obtained at room temperature, with excitation set at 467 nm (10-nm slit) and emission scan at 500–600 nm (10-nm slits). An NBD-labeled peptide was added first from a stock solution in DMSO (final concentration 0.1 μM and a maximum of 0.25% (v/v) DMSO) to a dispersion of PC LUV (200 μM) in PBS. This was followed by the addition of Rho-labeled peptide (stock in DMSO) in several sequential doses, ranging from 1:20 to 1:7 Rho/NBD molar ratios. Fluorescence spectra were obtained before and after addition of the Rho labeled peptide. The fluorescence values were corrected by subtracting the corresponding blank (buffer with the same vesicles concentration).

T-cell Activation and Proliferation—Primary CD4 T-cells specific to MOG p35–55 were plated onto round 96-well plates in medium containing RPMI 1640 supplemented with 2.5% fetal calf serum (FCS), 100 units/ml penicillin, 100 μg/ml streptomycin, 50 μM β-mercaptoethanol, and 2 mM l-glutamine. Each of the 96 wells had a final volume of 200 μl and contained 2 × 10^4 T-cells, 5 × 10^5 irradiated (25 gray) antigen presenting cells from spleen, and 5 μg/ml of MOG p35–55. In addition, the relevant peptide was added at a final 50 μg/ml concentration. Each read out was made with minimum of four repeats. To exclude interaction between the examined peptides and the MOG p35–55 antigen, we initially added the MOG p35–55 antigen to the antigen presenting cells in a test tube, and in a second test tube, we added the examined peptides to the T-cells. After 1 h, we mixed the antigen presenting cells with the T-cells and incubated them for 48 h in a 96-well round bottomed plate. The T-cells were pulsed with 1 μCi (H^3) thymidine, with a specific activity of 5.0 Ci/mmol for 24 h, and [H^3] thymidine incorporation was measured using a 96-well plate β-counter. The mean cpm ± S.D. was calculated for each quadruplicate or more. The results of T-cell proliferation experiments are shown as the percentage of T-cell proliferation inhibition triggered by the antigen in the absence of any peptide.

Mice—C57BL/6] mice were purchased from Harlan Olac (Bicester UK). The mice were maintained in a specific pathogen-free facility and were used according to the guidelines and under the supervision of the animal welfare committee.

ATR-FTIR Spectroscopy—Spectra were obtained with a Bruker equinox 55 FTIR spectrometer, equipped with a deuterated triglyceride sulfate detector, coupled with an ATR device. For each spectrum, 150 scans were collected, with a resolution of 4 cm⁻¹. Samples were prepared as described (41). Briefly, lipids alone or with a peptide were deposited on a ZnSe hori-
zontal ATR prism (80 × 7 mm). Before the sample was prepared, the trifluoroacetate (CF₃COO⁻) counterions, which strongly associate with the peptide, were replaced by chloride ions through several washings in 0.1 M HCl and lyophilization. This eliminated the strong stretching absorption band of the water near 1673 cm⁻¹ (42). Peptides were dissolved in MeOH, which was chosen over DMSO, used in the lipid mixing experiments, as MeOH evaporates readily and is a suitable solvent for lipids. Lipids were dissolved in a 1:2 MeOH/CHCl₃ mixture. Lipid-peptide mixtures at a 200:1 molar ratio or lipids alone with the corresponding volume of methanol were spread on the ZnSe prism. The solvents were eliminated by drying under vacuum for 15 min. Pure phospholipid spectra were subtracted to yield the different spectra. The background for each spectrum was a clean ZnSe prism. The samples were hydrated by introducing an excess of deuterium oxide (2H₂O) into a chamber placed on top of the ZnSe prism in the ATR casting and incubating for 5 min before acquiring the spectra. Hydrogen/deuterium exchange was considered complete if the complete shift of the amide II band was achieved. Any contribution of 2H₂O vapor to the absorbance spectra near the amide I peak region was eliminated by subtracting the spectra of pure lipids equilibrated with 2H₂O under the same conditions.

**ATR-FTIR Data Analysis**—To resolve overlapping bands, we processed spectra using PEAKFIT (Jandel Scientific, San Rafael, CA) software. Fourth-derivative spectra were calculated to identify the positions of the component bands in the spectra. These wave numbers were used as initial parameters for curve fitting with Gaussian component peaks. Positions, bandwidths, and amplitudes of the peaks were varied until a good agreement between the calculated sum of all components and the experimental spectra was achieved (r² > 0.997) under the following constraints: (i) the resulting bands shifted by no more than 2 cm⁻¹ from the initial parameters, and (ii) all of the peaks had reasonable half-widths (<20–25 cm⁻¹). The relative amounts of the different secondary structure elements were estimated by dividing the areas of individual peaks assigned to a particular secondary structure by the whole area of the resulting amide I band (43).

**RESULTS**

The AxxxG Motif Is Conserved in FP of Various HIV-1 Strains—HIV-1 FP₅₋₁₃ (GALFLGFGL) adopts a β-sheet conformation and interacts with a nine-amino acid fragment of the TMD of the TCR to exert its immunosuppressant activity (27, 28). We identified an AxxxG motif within this region, a known amino acid sequence that drives TMDs association (44). Using bioinformatics, we found that it is highly conserved within various HIV-1 clades and strains (Fig. 2). Additionally, we identified this motif in several SIV strains. HIV-1 has a high mutation rate, which is 1 in 10⁴ to 1 in 10⁵ nucleotide mutations per replication cycle or about one mutation per newly produced viral genome (45, 46). Therefore, the conservation of this motif suggests its significance for the various properties of the FP.

The AxxxG Motif Is Important for the Biological Function and the Biophysical Properties of FP₅₋₁₃—The GxxxG motif is mainly known as a mediator of helix-helix interaction. However, its glycine residues have been shown to facilitate the conversion to β-strand secondary structure and to mediate sheet-to-sheet packing (34). To investigate whether the AxxxG motif contributes to the FP immunosuppressant activity, we synthesized several peptides. The list, shown in Table 1, includes the following: (i) FP₅₋₁₃, the wild-type peptide; (ii) FP₅₋₁₃(A1G2), in which the classical GxxxG motif was created by switching the position of the first two amino acids; (iii) FP₅₋₁₃(L2A3), in which the AxxxG motif was altered by switching the position of the second and third amino acids; (iv) FP₅₋₁₃(scrambled), in which the sequence was scrambled; and (v) FP₅₋₁₃(Rsc), in which the GxxxG motif was recreated...
The peptides were then tested for their ability to inhibit T-cell proliferation. The data shown in Fig. 3 reveal, as expected, that the WT peptide is active. In contrast, a peptide analog, FP5–13(L2A3), in which the positions of only two amino acids were replaced to disrupt the GxxG motif, lost its proliferation inhibitory activity (Fig. 3). Similarly, a scrambled peptide also lost its inhibitory effect, pointing to the specificity of the interaction. To further validate the importance of the GxxG motif, two more analogs were synthesized. In the first analog, FP5–13 (rescued) L7G8, the glycine at position 7, and leucine at position 8 of the scrambled peptide, replaced positions, thus recreating a GxxG motif, while leaving the rest of the sequence unchanged. Interestingly, the rescued analog became highly active. In the second analog, FP5–13(A1G2), the AxxG motif was changed to a GxxG motif by switching the positions of only Ala and Gly. This analog was ~2-fold more active than the WT. In addition, we found that the FP of the measles virus had no immunosuppressant activity on T-cells (data not shown), further supporting the specific activity of HIV-1-FP.

In addition to inhibition of T-cell activation, the ability of the active peptides to inhibit cells completely lacking TCR was also examined. The peptides were added to macrophages which were then activated by LTA. Fig. 4 demonstrates that the peptides had no suppressing effect on macrophages. In fact, a slight increase in activation was observed, perhaps due to the ability of macrophages to recognize foreign antigens. This result, added to previous studies of colocalization and immunoprecipitation (25–29), further demonstrates the specificity of the FP to the TCR. These results might suggest that other peptides having the GxxG motif would be active as well. However, analogs of FP5–13 in which the motif was preserved but the two phenylalanines were replaced by glycines or leucines were also practically inactive (28). This suggests that the GxxG motif alone is crucial but insufficient to restore the inhibitory effect of T-cells and consequently, other amino acids are required.

Secondary Structure of the Peptides in PC Membranes Determined by FTIR Spectroscopy—FTIR spectroscopy was used to determine the secondary structure of the peptides within PC membranes after complete deuteration. The amide I region spectra, as well as the fitted band components of the peptides bound to PC multibilayers, were determined and are shown in Fig. 5. Assignment of the different secondary structures to the various amide I regions was calculated according to the values taken from earlier studies (43, 47, 48). The relative areas of the major component peaks are summarized in Table 1. The data reveal a strong band typical for a β-sheet structure at 1620 cm⁻¹. These results are supported by previous studies indicating that FP interacts as a β-sheet within the membrane (27, 28). Here, we show that only the three active peptides: the WT, A1G, and the rescued peptides adopt a β-sheet structure. In comparison, the non-active peptides, L2A, and the scrambled peptide, lost their β-sheet structure.

The Trade Off of the Motif, Fusion versus immunosuppression—Mutations in HIV-1 are very common (45, 46). Our results indicate that the GxxG motif is more potent than the AxxG motif in immunosuppressant activity. This leads to the
question why, given natural selection, a mutant such as A1G2 does not thrive in the population. Because the main known purpose of FP is to fuse to the target cell membrane, we examined the ability of all the peptides to induce lipid mixing (Fig. 6). The results clearly demonstrate that the WT peptide, although not the best immunosuppressant, is highly active in lipid mixing compared with the A1G2 analog, which is practically inactive. This result might explain why nature has selected the WT sequence AxxxG over GxxxG. Overall, the lipid mixing data reveal that there is no direct correlation between the immunosuppressant activity of a particular peptide and its ability to induce lipid mixing.

**DISCUSSION**

To effectively infect the human CD4 T-cells, HIV-1 must both successfully fuse to the T-cells and escape the immune response. One possible mechanism involves a particular region in gp41, the FP (Fig. 1). The FP is known to initiate the fusion cascade of HIV, and a peptide derived from this region possesses immunosuppressant activity in cell culture and in animal models (26, 27). This activity is primarily due to the HIV-1 FP5–13 region (GALFLFLG), which was shown to interact with the CP, a nine-amino acid TMD peptide derived from TCRα (27, 49).

In the present study, we identified a GxxxG-like motif (AxxxG) within FP5–13 by aligning FPs from different HIV-1 clades and strains. This region is highly conserved within all of these strains (Fig. 2). Note that the GxxxG-like motif is well known for its ability to drive TMD association (44). Because HIV-1 has about one mutation for each newly produced viral genome (45, 46), the conservation of the motif suggests that it plays a role in the function of the FP. Indeed, this study provides experimental evidence for the contribution of the GxxxG-like motif to the immunosuppressant activity of the HIV FP5–13. For that purpose, we synthesized the HIV FP5–13 and its analogs, in which the GxxxG-like motif was altered (32, 35, 36). The structures of the peptides in the membrane were determined by using FTIR spectroscopy, and their function was examined using the CD4 T-cell proliferation assay. In addition, the FP of the measles virus was used as a control of an unrelated FP, and

**FIGURE 6.** Lipid mixing of PC:choline (9:1) vesicles induced by the peptides. Peptides were added to 200 μL LUV of PC:Choline (9:1) that contained unlabeled and labeled LUV at 9:1 molar ratio, respectively. Lipid mixing was monitored by measuring the increase of NBD fluorescence intensity at 0.06 [peptide]:[lipid] ratio. The ability of the WT and its analogs to induce lipid mixing was measured and compared with 2% Triton which was taken as 100%.
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Although the GxxxG motif is crucial for the function of FP, other amino acids are also required. For example, it has been previously demonstrated that FPs in which the aromatic amino acids were mutated to non-aromatic residues significantly reduced their immunosuppressant activity (28).

Recent studies have shown that FP is not the only region within gp41 that contains a functional GxxxG motif. gp41 TMD (FIMVGGGLVLRIFVAVLSIV) is also highly conserved within different strains of HIV (53) and was suggested to play several roles during HIV-1 cell fusion, including anchoring the envelope glycoprotein to both viral and cellular membranes (54), assisting in the oligomerization of gp41 (55), inducing phospholipid vesicle fusion (55, 56), promoting cell-cell fusion (57, 58), and immunosuppressing T-cell activation (30). Importantly, experiments performed with the intact virus demonstrated that a mutant in which the GxxxG motif within the TMD in the HXB2 envelope was altered, was defective in fusion (58). Furthermore, the FP and the TMD of HIV-1 heteroassembled in the membrane and synergize in inducing membrane fusion (55). A similar observation was previously demonstrated in the influenza virus, in which TMD-FP interactions were shown to play a key role in the fusion cascade (59). In HIV-1, in addition to their role in membrane fusion, both the TMD and the FP domains possess immunosuppressant activity (26, 30).

In summary, combining bioinformatics and experimental studies, we identified a conserved GxxxG-like motif (AxxxG) within HIV-1 FP, which is crucial for its immunosuppressive activity possibly via interaction with the TMD of TCRs. In contrast, LTA activated macrophages (lacking TCR) were not affected by FP. Furthermore, although the AxxxG WT and the GxxxG analog both associated with the TMD of TCR and immunosuppressed T-cells, the AxxxG WT but not the GxxxG analog induced lipid mixing. This might explain the natural selection of the AxxxG motif rather than the classical GxxxG motif in FP. Besides giving us important mechanistic information, such short peptides can serve as templates for the design of immunosuppressive drugs urgently needed for various autoimmune diseases.

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