Prothymosin α Variants Isolated From CD8+ T Cells and Cervicovaginal Fluid Suppress HIV-1 Replication Through Type I Interferon Induction

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Soluble factors from CD8+ T cells and cervicovaginal mucosa of women are recognized as important in controlling human immunodeficiency virus type 1 (HIV-1) infection and transmission. Previously, we have shown the strong anti-HIV-1 activity of prothymosin α (ProTα) derived from CD8+ T cells. ProTα is a small acidic protein with wide cell distribution, to which several functions have been ascribed, depending on its intracellular or extracellular localization. To date, activities of ProTα have been attributed to a single protein known as isoform 2. Here we report the isolation and identification of 2 new ProTα variants from CD8+ T cells and cervicovaginal lavage with potent anti-HIV-1 activity. The first is a splice variant of the ProTα gene, known as isoform CRA_b, and the second is the product of a ProTα gene, thus far classified as a pseudogene 7. Native or recombinant ProTα variants potently restrict HIV-1 replication in macrophages through the induction of type I interferon. The baseline expression of interferon-responsive genes in primary human cervical tissues positively correlate with high levels of intracellular ProTα, and the knockdown of ProTα variants by small interfering RNA leads to downregulation of interferon target genes. Overall, these findings suggest that ProTα variants are innate immune mediators involved in immune surveillance.

Keywords. prothymosin alpha; macrophages; cervicovaginal lavage; HIV-1; CD8+ T cells.
to cell proliferation and apoptosis [5, 12]. iso2 is not only found in supernatants of cultured cells but also in blood, where it is involved in cell-mediated immunity as well as antiischemic, anticancer, antibacterial, and antiviral functions [4, 5, 13–19].

Exposure of cervicovaginal mucosal surfaces to HIV-1 in women without any preexisting sexually transmitted diseases rarely results in productive HIV-1 infection, suggesting protective integrity of the cervicovaginal mucosal surface and the presence of innate immune defenses [20–23]. Various groups have shown a correlation between high levels of RANTES (CCL5) and IFN-α in cervicovaginal mucosa and protection against HIV-1 transmission [3, 24]. However, the mechanisms regulating these responses are not known.

We previously showed that ProTα stimulates type I IFN [4]. Therefore, we asked whether ProTα might contribute to the protection of cervicovaginal mucosa against HIV-1 by inducing innate inhibitors of viral replication and did a thorough investigation of proteins from cervicovaginal lavages of healthy women. We identified 2 novel variants of ProTα with potent anti–HIV-1 activity in cervicovaginal lavage and active fractions derived from CD8α T-cell supernatant. One is a splice variant of the ProTα gene (isoform CRA_b or isoB ProTα), and the second is a protein coded by ProTα pseudogene 7 (p7 ProTα). We cloned and expressed the isoB and p7 ProTα variants and demonstrated that they have potent type I and type III IFN-inducing activities. These data suggest that isoB and p7 could contribute to the protection of the cervicovaginal mucosa against HIV-1 infection via stimulation of innate inhibitors of HIV-1 replication. We also showed that the anti-HIV-1 activity of ProTα variants is dependent on type I IFN. Understanding the mechanism of ProTα release and stimulation of type I and type III IFN–inducing activities by ProTα variants could provide promising therapeutic compounds for IFN-sensitive viral infections.

METHODS

For more-detailed protocol on each method, please see the Supplementary Materials.

Collection of Cervicovaginal Lavage Fluids and CD8α T-Cell Supernatants

Cervicovaginal lavage fluids were collected from healthy participants following the study protocol approved by the Mount Sinai School of Medicine Institutional Review Board. Supernatants were collected from CD8α T cells derived from different healthy donors. All participants provided written informed consent.

Inhibition of HIV-1 Replication by ProTα

To screen for HIV-1 entry-inhibitors, macrophages were incubated with the R5 isolate of HIV-1BaL, and cultured in the presence of different chromatography fractions and HIV-1. HIV-1 p24 Enzyme-linked immunosorbent assay (p24-ELISA) was performed on day 7.

Protein Fractionations

Samples were loaded onto a MonoQ (GE Healthcare) column by using an AKTA explorer 100 (GE Healthcare, Piscataway, New Jersey). Aliquots of each fraction were assayed for HIV-1–suppressing activity. HIV-1–suppressing fractions were further separated by reverse phase chromatography (C18).

Protein Sequencing

Active fractions were digested with trypsin and sequenced by liquid chromatography–mass spectrometry (LC/MS/MS) on a linear ion-trap quadrupole mass spectrometer (LTQ, Thermofisher, California). Spectra were searched against the nonredundant database (nr, NCBI, Bethesda, Maryland).

Depletion of ProTα Variants From Active Fractions, Using an Antibody-Affinity Column

Monoclonal antibodies to ProTα (Alexis Biochemicals) were immobilized to agarose beads, using an AminoLink Immobilization kit (Pierce). Fractions containing ProTα were applied to the column. Bound protein was eluted and tested for activity.

Cloning of ProTα Variants, Preparation of Recombinant Protein, and Proteinase Digestion

Total RNA was extracted from CD8α T cells, using the RNaseasy kit (Qiagen). For preparation of recombinant protein, complementary DNA (cDNA) was cloned into pRSET A (Invitrogen). V8 proteinase was used to digest recombinant proteins (Pierce).

RNA Extraction and Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis

Total RNA was extracted as described before [4]. EcoDry Premix kit from Clontech (Mountain View, California) was used for cDNA preparation. In all RT-qPCR studies, numbers indicate the measure of the expression of a particular messenger RNA (mRNA) relative to the expression levels of a reference gene encoding ribosomal protein S11 (RPS11).

Western Blotting

Recombinant proteins were separated by 4%–10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to a polyvinylidene fluoride membrane (Invitrogen), and probed with monoclonal antibodies from Alexis Biochemicals (San Diego, California).

Bioinformatics Data Analysis

Genome-wide gene-expression profiles of 24 normal human cervical tissues samples were analyzed from the NCBI Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo; accession number GSE9750).

Transfection of HeLa Cells With Small Interfering RNA (siRNA) to ProTα

HeLa cells were transfected with siRNA (Qiagen and Integrated DNA Technologies) to ProTα using Lipofectamine 2000 (Life
Technologies). Forty-eight hours after transfection, RT-qPCR was performed.

Ethics Statement
Collection of cervicovaginal lavage specimens was approved by Institutional Review Board of the Icahn School of Medicine at Mount Sinai in New York. All participants provided written informed consent.

Statistical Analysis
All experiments were performed at least 3 times and analyzed using a 2-tailed, unpaired t test by Prism 5 (GraphPad Software).

RESULTS
Purification and Identification of ProTα Variants From Cervicovaginal Lavage Specimens and CD8+ T-Cell Supernatants
Cervicovaginal lavage specimens collected from 3 healthy donors in 10 mL of water contained ProTα at concentrations of 90 ng/mL, 63 ng/mL, and 40 ng/mL, as measured by ELISA. Proteomic studies were performed on cervicovaginal lavage specimens and supernatants of CD8+ T cells to purify and identify proteins with potent anti-HIV-1 activity. Anion-exchange fractions with HIV-1-suppressing activity were fractionated further by C18 chromatography. One active anion-exchange fraction yielded 2 active peaks (fractions 1 and 2; Figure 1A).

Figure 1. Isolation and identification of prothymosin α (ProTα) variants isoB and p7 from CD8+ T-cell supernatant and cervicovaginal lavage (CVL) specimens. A, C18 chromatogram of active fraction (Fr) derived from CD8+ T-cell supernatant (CD8 sup) that yielded the ProTα p7 (Fr1) and isoB (Fr2) proteins. B, Unique peptide sequences of ProTα variants identified by mass spectrometry (MS), and number of peptide hits in active Frs from CD8 sup and CVL. C, Protein sequence alignment of ProTα variants (p7 and isoB) and a ProTα N-terminal peptide (thymosin α1). Amino acid sequences of peptides identified by MS are in blocks, and nuclear localization signal is in bold and underlined. D, Silver staining of sodium dodecyl sulfate polyacrylamide gel containing 3 ng of electrophoresed protein from Fr1 and Fr2. Abbreviations: aa, amino acid; AU, absorption units; MW, molecular weight; STD, standard.
versus control samples. *P \leq 0.05.

transcript is an alternatively spliced form of the ProTα gene located on chromosome 2 (accession number EAW70973; Figure 1C) [26]. Polyacrylamide gel electrophoresis of aliquots of protein chromatography peaks 1 (p7) and 2 (isoB) showed the predicted proteins’ molecular weights of 8.2 kDa and 9.9 kDa, respectively (Figure 1D).

**Fractions Containing Native Variants of ProTα IsoB and p7 Suppress HIV-1 Replication and Induce IFN-β and IFN-λ1 mRNA in Primary Human Macrophages**

To investigate the antiviral activity of the fractions containing ProTα variants, primary human macrophages were infected with HIV-1BaL and then treated with the fractions containing purified native proteins. HIV-1 replication was suppressed by >95% of control (HIV-1 p24 antigen level ranged from 68 ng/mL in the medium to 1–3.5 ng/mL in the presence of fractions; Figure 2A). Fractions containing native ProTα variants exhibited no cellular toxicity, ruling out that the viral inhibition was accounted for by reduced cell viability, whereas the positive control (2 \ \mu M of etoposide) showed statistically significant reduction in cell viability (Figure 2B).

In our previous work, we showed that iso2 ProTα stimulated IFN-β. To study whether ProTα variants (isoB and p7) activate IFN-β, we incubated human macrophages with fractions containing native isoB and p7 (3 ng/mL) or LPS as a positive control (100 ng/mL) and measured IFN-β and IFN-λ1 mRNA expression by real-time PCR (Figure 2C). In all our RT-qPCR studies, numbers indicate the level of expression of a particular mRNA relative to the expression level of the reference gene RPS11. Both fractions proved very potent inducers of IFN-β and IFN-λ1 mRNAs, with mean relative levels of induction (± standard deviation [SD]) by fraction 1 (containing p7) of 1914 ± 191 and 457 ± 48, respectively, and mean relative levels of induction by fraction 2 (containing isoB) of 150 ± 5.7 and 117 ± 13, respectively. An unrelated protein fraction lacking ProTα protein and anti-HIV-1 activity (nonsuppressing fraction) but containing the same amount of protein had no effect on IFN-β or IFN-λ1 mRNA (Figure 2C).

Next, we asked whether the fractions containing either isoB or p7 also induced proinflammatory cytokines, such as TNF-α and IL-6. Relative stimulation of mRNA expression (±SD) of TNF-α and IL-6 by fraction 1 (isoB) was 241 ± 26 and 490 ± 50, respectively, while fraction 2 (p7) induced a similar level of TNF-α (242 ± 25) and slightly more IL-6 (643 ± 70; Figure 2D). Again the same concentration of nonsuppressing fraction lacking ProTα had no effect (Figure 2D).

To rule out the effect of any other factors in anti-HIV-1 and IFN-inducing activities in the fraction containing native ProTα variants, an affinity column was used to deplete ProTα protein from the parent fraction of fractions 1 and 2. Depletion was confirmed by determining the ProTα concentration by ELISA (60 ng/mL in the parent fraction and below level of detection).
Dose-dependent anti-human immunodeficiency virus type 1 (HIV-1) activity of recombinant prothymosin α (ProTα) variants. A, Western blotting analyses of recombinant variants of ProTα. Dose-dependent anti-HIV-1 activity of recombinant variants of iso2 ProTα (B), isoB (C), p7 of ProTα (D), and thymosin α1 (E) in primary human macrophages. Primary human macrophages were infected with HIV-1BaL at a multiplicity of infection 0.1 for 2 hours, unbound virus was washed out, and cells were treated either with medium or different concentrations of the indicated ProTα variants or as a negative control thymosin α1 recombinant protein. Enzyme-linked immunosorbent assay for the presence of HIV-1 p24 antigen was performed at day 7 after infection. F, An MTS cytotoxicity assay was performed on primary human macrophages treated with ProTα variants (200 ng/mL) or, as a positive control, 2 µM etoposide (a DNA-damaging agent) at day 7. The MTS assay was performed according to the manufacturers’ instructions (Promega, Madison, Wisconsin). Each experiment was done at least 3 times. Data represent mean ± SD of the expression levels of the indicated messenger RNA (mRNAs) relative to the expression of ribosomal protein S11 (encoded by RPS11) control mRNA. The Student t test was used to compare means of treated versus control samples. *P < .05.

Dose-Dependent Antiviral Activity of Recombinant ProTα Variants

To rule out the effect of any other factors in the fraction and to determine whether anti-HIV-1 and IFN-β- and IFN-λ-1-inducing activities could be solely attributed to ProTα variants, we cloned and expressed cDNAs of iso2, isoB, p7, and the N-terminal peptide of ProTα, thymosin α1 (To1), which is identical in all variants in the pRSET A bacterial expression vector [4]. Western blotting analyses using anti-N-terminal and anti-C-terminal antibodies to iso2 ProTα showed that all of the recombinant ProTα variants of predicted size were expressed (Figure 3A).

Dose-dependent anti-HIV-1 activity of recombinant ProTα proteins was determined in primary human macrophages. In agreement with our previous report, we showed dose-dependent inhibition of HIV-1BaL replication by recombinant iso2 ProTα protein (Figure 3B) [4]. We found dose-dependent inhibition of HIV-1BaL replication by isoB and p7 variants of ProTα in primary human macrophages (Figure 3C and 3D). We confirmed that recombinant To1 purified by using the same method did not have anti-HIV-1 activity (Figure 3E). In the following experiments, we used 200 ng/mL of recombinant protein that suppressed HIV-1 replication by >95%, compared with control (from 75 ng/mL to 3–5 ng/mL of HIV-1 p24). At the concentrations of 200 ng/mL of recombinant ProTα variants, there was no cellular toxicity observed, whereas the positive control cells treated with 2 µM of etoposide resulted in a statistically significant reduction (Figure 3F).

Recombinant ProTα Variants Induce IFN-β and IFN-λ-1 in Primary Human Macrophages

To test whether the different recombinant ProTα proteins maintained comparable cytokine induction and antiviral activity of native proteins (Figure 2C and 2D), we measured the mRNA expression level of IFN-β, IFN-λ-1, TNF-α, and IL-6 in primary human macrophages treated with recombinant proteins. Similar to the native variants, all recombinant ProTα proteins were strong inducers of mRNA expression of IFN-β, IFN-λ-1, TNF-α, and IL-6 (Figure 4A and 4B).

Consistent with our prior work, recombinant iso2 ProTα induced relative expression of IFN-β by a mean (±SD) of 68 ± 25 over untreated macrophages and of IFN-λ-1 by 12 ± 2 (Figure 4A). Recombinant isoB induced relative expression of IFN-β mRNA by a mean (±SD) of 17 ± 4 and of IFN-λ-1 mRNA by 5 ± 1. Compared to isoB, p7 ProTα induced >14 times the amount of IFN-β (mean ± SD, 247 ± 57) and almost double the amount of IFN-λ-1 (10 ± 2) mRNAs (Figure 4A).

Recombinant iso2 ProTα induced TNF-α and IL-6 (mean ±SD, 16 ± 3 and 41 ± 5 respectively; Figure 4B). Recombinant isoB induced TNF-α mRNA (mean ± SD, 7 ± 0.6) and IL-6 mRNA (3 ± 0.9), while recombinant To1 purified by the same method as isoB and p7 did not induce IFN-β or IFN-λ-1 but induced similar levels of TNF-α (7 ± 3). Recombinant p7 induced TNF-α (mean ± SD, 28 ± 3) and IL-6 (26 ± 3) mRNA expression (Figure 4B).

Because of the similarities in responses mediated by ProTα and LPS, we were careful to exclude potential contamination of recombinant ProTα with residual levels of LPS. Endotoxin-removing gel was used to exclude any LPS in the ProTα protein preparations. To further show that the observed effects are
caused by ProTα proteins and not by LPS, we digested proteins with V8 proteinase and tested their IFN-β mRNA-inducing activities in macrophages. Proteinase digestion of recombinant variants completely abrogated IFN-β induction (Figure 4C), whereas proteinase treatment had no effect on corresponding LPS-mediated activity. In addition, recombinant protein Tα1 purified by the same method did not show anti-HIV-1 and type I IFN-inducing activities (Figure 3B and 3C and Figure 4A, respectively). These results collectively exclude LPS contamination from recombinant protein preparations and underscore that the anti-HIV-1 responses in macrophages we observed were mediated through ProTα proteins.

Anti-HIV-1 Activity of iso2, isoB, and p7 Is Dependent on Type I IFN
To determine whether the anti-HIV-1 activity of the proteins is fully accounted for by their abilities to induce type I IFN, macrophages derived from wild-type (WT) and type I IFN receptor 1-deficient (Ifnar1−/−) mice were infected with vesicle stomatitis virus envelope-pseudotyped HIV-1 with a LTR-luciferase reporter gene, followed by treatment with different recombinant ProTα variants or LPS. Suppression of HIV-1 LTR-luciferase gene expression by recombinant ProTα variants was completely abolished in Ifnar1−/− macrophages (Figure 4D), suggesting that type I IFN induction by ProTα is essential for HIV-1 suppression in this model. Suppression by ProTα variants and LPS was not only lost in the Ifnar1−/− macrophages, but stimulation of HIV-1 LTR luciferase reporter gene expression was above the control (from 15% in WT to 164% in Ifnar1−/−, compared with macrophages treated with control medium [100%]), likely because of the stronger unopposed proinflammatory response (Figure 4D). Importantly, recombinant Tα1 isolated with the same method had no effect on HIV-1 gene expression in WT macrophages.

Regulation of Different Variants of ProTα mRNA Expression With Different Stimuli
Several reports have showed that anti-HIV-1 soluble factors produced by CD8+ T cells could be induced by stimulation of T-cell antigen receptor (TCR) [2, 27]. We asked whether stimulation of TCR in CD8+ T cells could activate transcription of different variants of ProTα. Primary CD8+ T cells were stimulated with immobilized anti-CD28 and anti-CD3 antibodies. Stimulation of TCR in primary human CD8+ T cells had no effect in ProTα mRNA expression, while strong stimulation of IFN-γ mRNA used as a positive control was observed in all 3 donor cells, suggesting that stimulation of TCR has no effect on ProTα mRNA expression (Supplementary Figure 1A).

There are conflicting data in the literature linking the level of iso2 ProTα expression with either proliferation or the differentiation state of the cells [28-33]. We asked whether monocyte differentiation into dendritic cells would alter mRNA expression of different variants of ProTα. RNA was extracted from primary human monocytes before and after differentiation into dendritic cells. RT-qPCR was performed for the presence of 3 different variants of ProTα. Only isoB ProTα mRNA expression was significantly reduced in dendritic cells, compared with monocytes (mean ± SD), from 491 ± 50 to 18 ± 1.7, while expression of iso2 and p7 remained constant (Supplementary Figure 1B), suggesting involvement of ProTα variants in cell differentiation.
Positive Correlation of ProTα mRNA Expression With IFN Target Genes in Primary Human Cervical Tissues

Different studies have reported that high levels of IFN-α in cervicovaginal mucosa correlate with the protection against HIV-1 transmission [24]. Because of the presence of ProTα proteins in cervicovaginal lavage specimens, we asked whether there is correlation of intracellular ProTα mRNA expression level with the baseline expression of IFN-stimulated genes. We analyzed publicly available genome-wide expression profiles of 24 normal human cervical tissues samples (see “Methods” section for details). Multiple experimentally defined IFN target gene sets were induced in human cervical tissues, with significantly higher ProTα mRNA expression (Supplementary Figure 2). Next, we asked whether downregulation of ProTα gene expression by different siRNAs would correlate with reduction of baseline IFN target gene sets. We transfected HeLa cells with control siRNA and 3 different siRNAs to ProTα, which target all of the variants. All 3 siRNAs reduced expression of different variants of ProTα to different extents, while control siRNA did not have any effect on ProTα mRNA expression (Figure 5A). The knockdown of ProTα variants correlated with a dramatic reduction of RIG-I and ISG-20 gene expression, while MDA-5 mRNA was reduced to a lesser extent (Figure 5B). Interestingly, there was no effect of ProTα mRNA reduction on PKR gene expression, suggesting selective decrease of IFN target genes by downregulated ProTα.

DISCUSSION

We isolated and identified 2 novel ProTα variants, isoB and p7, with strong anti-HIV-1 activities from the CD8+ T-cell secretome and the cervicovaginal lavage fluid of healthy donors. Our results demonstrate that isoB and p7 variants possess potent anti-HIV-1 activity through the induction of type I and type III IFNs. These functions were confirmed using recombinant proteins. The reduced potency of the bacteria-derived recombinant proteins suggests that posttranscriptional modifications of the native forms are important for the full activity, and further experiments are required to determine specific modifications.

Unlike iso2 ProTα, which contains a bipartite nuclear localization signal at the carboxy terminus, both p7 and isoB lack this nuclear localization signal (Figure 1C), suggesting that they represent cytoplasmic forms of ProTα possibly destined for export via a nonclassical endoplasmic reticulum Golgi-independent secretory pathway, since they are found in the cell supernatants [34, 35].

It is presently impossible to distinguish whether the previously described activity of iso2 ProTα is indeed accounted for by its isoB and p7 variants. In fact, given the small size and the high homology of the transcripts for iso2, isoB, and p7, it is difficult to selectively knockdown either form independently, and no neutralizing antibody is available to specific variants.

Previous reports showed that ProTα is rapidly released from Pichinde virus–infected cells [13]. This finding combined with the data from our laboratory suggest that ProTα may serve as an early line of defense in response to viral or microbial infections [4, 14, 36]. For example, ProTα was reported to be released as early as 15 minutes from cells infected with attenuated, but not WT, Pichinde virus, thus suggesting that the WT virus has co-evolved molecular mechanisms to block ProTα release to prevent the activation of innate viral inhibitors, such as type I and type III IFN, that would decrease its fitness. Intriguingly, HIV-1 infection also downregulates expression of ProTα in T cells [37–39].

The first line of defense in response to viral infection is accompanied not only by induction of type I IFN, but also by
the release of other proinflammatory cytokines, such as TNFα, IL-6, and IL-1 [40]. The synergism of proinflammatory cytokines in antiviral effects has been underappreciated, and only recently it is becoming clear that the synergic response elicits multiple mechanisms that contribute to the restriction of viral replication [40]. Therefore, it is possible that proinflammatory cytokines in addition to IFNs, such as TNF-α and IL-6, participate in the antiviral activity of ProTα variants.

Nevertheless, the activation of the IFN pathway seems to be an important mediator of the variants’ functions. There is a positive correlation between increased IFN target genes and high levels of intracellular ProTα expression in primary human cervical tissues, but more work is required to evaluate contribution of each ProTα variant in this activity. We show that the knockdown of all ProTα variants lowers the baseline expression of IFN target genes, suggesting that under normal conditions ProTα variants are involved in the stimulation of antiviral IFN pathway(s).

Stimulation of IFN target genes and the release of IFN have been shown to be p53 dependent and that p53 acetylation is indispensable for this antiviral activity [41, 42]. Since the overexpression of iso2 ProTα upregulates only target genes of acetylated p53 [43], it is possible that the intracellular stimulation of the type I IFN pathway by ProTα is p53 dependent. However, more experiments are necessary to test this hypothesis.

The IFN stimulatory activity of the ProTα variants may regulate the activation of other anti–HIV-1 restriction factors, such as the APOBEC 3 family of proteins and tetherin, which are sensitive to IFN, thus suggesting a protective role of the ProTα variants against HIV-1 infection at the cervicovaginal mucosa [44, 45]. It will be important to evaluate the correlation between the level of ProTα variants and protection against HIV-1 transmission. A better understanding of the mechanisms of HIV-1 suppression by ProTα variants could lead to the development of novel antiviral therapies.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

Supplementary Data

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References


