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# HIV Associated Cell Death by TNF Derived Peptides: Apoptosis Restricts Viral Transmission

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Research Article

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## ABSTRACT

**The human immunodeficiency virus (HIV) is still a global pandemic and despite successful use of anti-retroviral therapy, a well-established cure remains to be identified. Viral modulation of cell death has a significant role in HIV pathogenesis. Here we sought to understand the major mechanisms of HIV-induced death of lymphocytes and the effects on viral transmission.**

**Flow cytometry analysis of lymphocytes from acute HIV infected patients, and HIV IIIB infected MT2 cells demonstrated both necrosis and apoptosis to be the major mechanisms of cell death in CD4+and CD4-/CD8- lymphocytes. Significantly, pro-apoptotic tumor necrosis factor (TNF) peptide was found to inhibit HIV-related cell death and reduced viral transmission. Whereas pro necrotic TNF peptide (P16) had little effect on HIV-related cell death and viral transmission. Understanding mechanisms by which cell death can be manipulated may provide additional drug targets to reduce the loss of CD4+ cells and the formation of a viral reservoir in HIV infection.**

## INTRODUCTION

The Human Immunodeficiency Virus (HIV) led to almost one million deaths globally in 2017, with 36.9 million people living with HIV infection in the same year. HIV infection leads to a decline in CD4+ T cell counts and a production of viral reservoirs. Major advances in the treatment of HIV infection have improved overall survival rates and reduced progression to Acquired Immune Deficiency Syndrome (AIDS). However, patients living with HIV under control of highly active antiretroviral therapy (HAART) are at risk of disease-associated infections, chronic inflammation and immune-senescence. HIV infects CD4+ cells *via* the GP120 envelope protein interacting with the CD4+ molecule on T cells as its receptor and CCR5 or CR4 as co-receptors, by M- and T-tropic strains respectively. Following reverse transcription in the cytoplasm, the virus integrates into the DNA where it can replicate and produce a pool of latently infected cells. HIV viral proteins have been well characterized for their pro- and anti-apoptotic functions. HIV viral proteins can lead to survival or apoptosis of infected cells, and apoptosis of uninfected bystander CD4+ T cells, contributing to the viral pathogenesis and formation of a viral reservoir and immunodeficiency [1]. Apoptosis is a well-studied mechanism of cell death in HIV infection, however other types of cell death have been found to be involved in the pathogenesis of HIV. Necrosis has previously been described in

human CD4+ cells infected with HIV according to morphological and DNA fragmentation patterns. Pyroptosis *via* caspase-1 was also found to have major involvement in the loss of non-productively infected CD4+ cells in HIV infection of human lymphoid tissue. Other cell death pathways with characterized roles in HIV include autophagy and activation-induced cell death. Therapies are being developed to target apoptosis and pyroptosis. Despite this, a treatment that targets CD4+ T cell death (including bystander-killing) in HIV infection remains to be established. Further understanding of HIV-mediated cell death and its impact on viral survival and transmission is important and this may lead to the development of further therapeutic targets. Tumor Necrosis Factor (TNF) which binds to the Tumor Necrosis Factor Receptor (TNFR) causes pro-inflammatory signaling or cell death. We have previously identified two conserved peptide regions of TNF, one which is responsible for inducing apoptosis (P13) and the other necrosis (P16). The two peptides are derived from the same molecule and yet carry out pleiotropic functions. In order to further understand the effect of apoptosis and necrosis on HIV-mediated pathogenesis and transmission, we identified the major types of cell death in vitro and *in vivo* before investigating the effect of TNF peptides on cell death and viral load. We show that CD4+ CD8- and CD4- CD8- lymphocytes die by necrosis and apoptosis upon HIV infection, and we identify P13 induced apoptosis as a mechanism to reduce HIV-associated cell death and viral transmission [2]. These data provide compelling evidence for a therapeutic strategy which drives apoptosis in order to reduce viral transmission and loss of lymphocytes.

## DESCRIPTION

Acute HIV Infection in apoptosis and necrosis of CD4+CD8- and double negative lymphocytes HIV infects T cells *via* its receptor, CD4, together with its co-receptor, CCR5 or CXCR4, ultimately causing cell death. To reveal whether and how T cells of patients die as a result of HIV infection, we investigated the death of lymphocytes from five acute phase patients. Cell death by apoptosis and necrosis was found in both CD4+ CD8- T cells and double negative (CD4-, CD8-) lymphocytes. A summary of flow cytometer analysis of cell death in five patients shows a decrease in the percentage of live CD4+ and double negative T cells in HIV infected individuals compared to controls. The mechanism and dynamics of HIV-induced lymphocyte death, was further investigated by infection of an MT2 CD4+ T cell line with HIV IIIB over four days. In the presence of HIV, cells died by apoptosis and necrosis. Apoptosis peaked at day three (28.7%), whereas necrosis peaked at day four (54.9%) in HIV infected cells. Specific caspase inhibitor z-VAD was used to induce cell death by necrosis as a control. The percentage of P24 positive cells (used as a measure of viral load) was found to increase rapidly over the course of the four-day infection.

We have previously identified peptide fragments within TNF that are responsible for its pro-apoptotic and pro-necrotic functions. We then screened TNF peptides for their effect on cell death in MT2 cells. P13 was found to induce apoptosis. P14, P15 and P16 induced necrosis. We then examined the effect of pro-apoptotic P13 and pro-necrotic P16 on viral load in HIV infected MT2 cells. Viral load (detection of HIV P24 antigen) increased in live, necrotic and apoptotic MT2 cells over four days of HIV infection. Incubation of cells with apoptotic peptide P13 slowed viral growth by a factor of two in the live cell group. A similar effect was seen in the necrotic cell and the apoptotic cell groups. Incubation of cells with necrotic peptide P16 had little effect on viral growth. Cells infected with HIV underwent dramatic cell death on day three and day four [3]. Cell viability decreased less rapidly in HIV infected cells incubated with P13. In the presence of P16, cell death occurred earlier, with some recovery of viable cells at day three, before decreasing again at day four in both HIV infected and non-infected cells. TNF was found to have no effect on HIV induced cell death.

HIV causes cell death, leading to viral transmission, producing the early and then chronic phases of infection. A cure for HIV has so far only been achieved by stem cell transplantation in one patient with acute myeloid leukemia. The different mechanisms of cell death in HIV infection and the consequences of this on pathogenesis, for example viral transmission and reservoir formation, provide additional drug targets. Modulation of cell death pathways alongside effective HAART may provide better treatment for HIV infection, by preventing loss of CD4+ T cells in early stages and removing the viral reservoir in late stages. We have divided the peripheral blood mononuclear cells (PBMCs) into live and dead (apoptotic and necrotic) cells. In HIV infected patients, although the total number remains similar to the healthy control donors, the number of CD4+, non-CD4CD8 cells are reduced in the live cells. There are more non-CD4CD8 cell deaths than CD4+ cell deaths. Previously published data has shown that chronic HIV infected cells are resistant to cell death (Cell Death Dis. 2013 July; 4(7): e718). Our findings are in agreement with this. Since HIV infects mainly CD4+ T cells, the reduction of non-CD4CD8 cells may be due to bystander killing.

In the *in vitro* MT2 cell model, HIV infection caused both apoptosis and necrosis on day three and four after infection. Then we have utilized the apoptotic and necrotic peptides to try to intervene in HIV-caused cell death. The pro-apoptotic peptide was shown to limit the viral load and viral transmission, while the pro-necrotic peptide had no effect on HIV-caused cell death. Since the cell membrane in apoptosis remained intact, the apoptosis induced by P13 reduced cellular nutrition for HIV and restricted viral transmission. On the other hand, necrosis induced by P16 resulted in the breaking of cell membranes, which facilitated viral transmission. We identified necrosis and apoptosis as the major mechanisms of cell death in CD4+lymphocytes in the acute phase of HIV infection. We also found that double negative (CD4-/CD8-) lymphocytes died by apoptosis and necrosis. A small percentage of regulatory T cells (Tregs) are known to be CD4/CD8 double negative. The loss of Tregs in chronic HIV infection has been characterized in CD3+CD4+CD25hiFoxP3+ cells. Resting double negative T cells were found to express HIV viral proteins, driving CD4 receptor internalization, and potentially contributing to the viral reservoir. Further work would be needed to characterize the phenotype of the double negative cells identified in this study. In this study, we sought to identify the major types of cell death in HIV infection and the effects of TNF peptides on acute infection. We found that the apoptotic peptide reduced viral load by a factor of two, while the necrotic peptide had no effect on reducing the viral load [4]. Therefore, apoptosis-inducing peptides could potentially be used as therapeutic agents for HIV infection and this could reduce the viral reservoir.

The goal of this study was to understand the type of cell death in HIV and its effects on viral transmission. We recruited five patients with HIV through clinics at the You'an Hospital, Capital Medical University, Beijing, China. They were all studied with informed consent in full compliance with national and institutional ethical requirements. The cohort was tested for HIV at regular screenings and diagnosed using western blot. The diagnosis was then confirmed with a measurement of viral load. Samples were taken within two weeks of diagnosis. All samples were stored at −80°C until analysis. HIV-infected cells can die either by apoptosis (programmed cell death without the cell membrane breaking) or necrosis (cell death which is not programmed and in which the membrane is often broken). Previously there was disagreement about which type of cell death will lead to the spread of the virus to other cells. We have provided evidence that when apoptosis takes place HIV spreading is restricted while when necrosis takes place HIV spreading is facilitated [5]. This may provide some new insight to develop novel treatment.

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