SAFE WORK PRACTICES INFORMATION PAGE

Working with Tat and other Potentially Hazardous Proteins

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I. PURPOSE: The potential immunosuppressive, cytotoxic, and carcinogenic properties which have been associated with Tat protein and related viral proteins and peptides may present a potential health threat to laboratory staff, animal handlers and other personnel who may accidentally come into contact with these agents. This page is intended to provide the research community with sufficient information regarding toxicology of Tat and other potentially hazardous proteins to assist in the development of research protocols that include measures for effectively reducing risk of occupational exposure.

II. BACKGROUND

A. Tat Protein: Trans-activating transduction (Tat) proteins are currently receiving much interest due to associated qualities which make them promising for use in a number therapeutic applications. Tat is a regulatory protein of HIV-1 produced very early after infection and essential for HIV-1 gene expression, replication, and infectivity (Gavioli et al., 2004). Tat proteins are small (14 kDa) 86 – 1001 amino acid nuclear proteins transcribed from complex spliced mRNAs which function as transacting transcriptional activators and are involved in array of viral functions (Moreau et al. 2004). Tat protein, functions intracellularly as a *trans*-activating factor of the human immunodeficiency virus type 1 (HIV-1), and also acts as an extracellular molecule modulating gene expression, cell survival, growth, transformation, and angiogenesis (Ruznati et al., 2000). Recent research has focused on the potential for the use of Tat protein derived "Tat toxoids" as possible candidates for the development of an HIV-1 vaccine (Gallo, 1999). The ability of the Tat protein to readily permeate cell membranes and accept tags has also led to research of the feasibility of use of the Tat protein as a delivery vehicle for a wide range of therapeutic agents. (Moreau E, et. al, 2004) The key role of Tat protein in HIV-1-induced immune pathogenesis has been firmly established through a number of in vitro and in vivo studies (Gallo RC 1999, Moreau E 2004). Recent studies have indicated that exposure to Tat protein, even in the absence of HIV infection, can lead to serious health consequences (Kim et al., 2003).

B Other Potentially Hazardous Regulatory and Accessory Proteins: In addition to Tat Protein research involving other proteins related to HIV-1 and other retroviruses has also been widely undertaken. Deletion of the Nef protein (Negative Regulatory Factor) which serves as a viral infection promoter has shown mixed results in several HIV-1 vaccine studies (Verity et al. 2007). Immunosuppressive proteins Vif (Viral Infectivity Factor) and Vpr (Viral Infectivity factor), regulatory proteins such as Vpu (Progeny Virion Production) and Env (Envelope Glycoprotein), and other accessory proteins are now none to also be key components to HIV pathogenesis (Seelamgari et. al, 2004, Bour 2003, Ndolo et. al 2003). Cytotoxic and/carcinogenic properties have been identified in several of these regulatory and accessory proteins.

III. POLICY:

A. Tat Protein: The Institutional Biosafety Committee (IBC) has classified Tat protein as a reportable biological hazard which must be handled under Biosafety Level 2 (BSL-2) precautions. All use of Tat protein must be registered with the IBC via completion of a Memorandum of Understanding and Agreement (MUA). *in vivo* use of Tat protein must be reported via completion of Appendix C of Institutional Animal Care and Use Committee IACUC protocol.

B Other Potentially Hazardous Proteins and Peptides: Though not reportable at this time, Principal Investigators should conduct thorough hazard assessments, develop standard operating procedures, and train staff fully prior to undertaking research involving other potentially hazardous proteins (Nef, Vpu, Vpr, Vif, Vpx, Env, etc.). The IBC strongly recommends that all HIV and other retroviral proteins/peptides be handled under BSL-2 conditions.

IV. OCCUPATIONAL EXPOSURE HAZARDS: Extensive scientific literature is available regarding potential health threats associated with Tat protein exposure. Information regarding potential health threat posed by other regulatory and accessory proteins HIV and other retroviruses is somewhat limited. The section below focuses on the toxicologic effects of Tat protein with limited reference to other regulatory/accessory proteins**:**

A. Cytotoxic Effects:

1. Tat protein: Tat protein is secreted actively by infected cells through a leaderless secretion pathway and is free in the plasma, where it can interact and be taken up by infected and noninfected cells. Numerous deleterious effects have been described in scientific literature:

a. Tat protein-related depression of the immune system is a result of several complex interactions (Ibelgauft, 2007). Tat protein induces production of the highly immunosuppressive cytokine IL10 by peripheral blood monocytes (Badou et al, 2000). Inhibition and/or destruction of natural killer cells, T cell lymphocytes, monocytes, and other immune cells have been widely reported. (Travis, 1999, Kim, 2003, Gallo 1999).

b. In additional to inducing destruction of T cells/other immune cells, Tat protein plays a major role in damaging and/or causing apoptosis of other bystander cells and in the killing of cells which are actively infected with HIV. (Campbell et al., 2004, Gallo et al., 1999).

c. Neuropathological effects including breakdown of the cerebellum and cortex, brain edema, astrocytosis, neuronal dendrite degeneration, neuronal apoptosis have been observed in Tat protein studies. The related symptomology may include

tremor, ataxia, reduced cognitive and motor ability, seizures, and premature death. (Kim, 2003).

c. Exposure to Tat protein may lead to activation of quiescent proviruses resulting in latent infection (Gallo et al., 1999).

d. Other cytotoxic effects: The full effects rendered by extracellular Tat protein all still not completely understood. Concern has been raised that concentrations used in research applications may be several times more concentrated than would be experienced in natural infection (Travis, 1999).

2. Nef Protein:

a. In vivo studies (mice) involving exposure to solely the Nef protein indicated that the animals developed an AIDs-like disease exhibiting depletion of CD4 T-cells, alteration of T-cell activation and differentiation, and lymphoid organ disorders. Other studies have indicated that Nef exposure may lead to apoptosis of uninfected T-cells, disruption of cell signaling pathways, increase viral loads and disease progression, dendritic cell maturation, and interruption of B cells function (Ndolo et al., 2004, Mangino et al., 2007).

b. Research has also indicated that exposure to Nef protein may lead to a compromise of neuronal health due to cell apoptosis and other potential deleterious effects (Trill-Pazos et al., 1998).

3. Vpu Protein:. *in vitro* experimentation has indicated that Vpu induces apoptosis of bystander cells through disruption of antiapoptotic genes (Akari et al., 2001).

B. Carcinogenic/Mutagenic Effects:

1. Indications of carcinogenic properties in association with Tat Protein have been widely demonstrated:

a. Studies have indicated that Tat expression in the liver predisposes to both initiation (promotion) of hepatocarcinogenesis and malignant progression of liver tumors (Altavilla et al., 2000, 2004).

b. In vitro studies have also implicated Tat protein as a major factor in the development of Kaposi Sarcoma in human Acquired Immune Deficiency Virus (AIDs) (Ensoli et al., 1990, 1994).

c. Angiogenic properties and enhancement of the effect of endogenous and exogenous carcinogens in human immunodeficiency virus-1-infected patients have been described (Analini, 1995).

d. *in vitro* and *in vivo* studies have indicated that Tat protein exposure may result in reduction of oncosuppressive properties and the induction of genes needed to proceed through the cell cycle including p107, cyclin A, and cyclin B (Lazzi et al., 2002).

2. Vdu Protein: Research has indicated a possible association between occurrence of AIDs-related B-cell non-Hodgkin's Lymphoma and exposure to Vpu (Henderson et al., 2004).

3. Nef Protein: The Nef protein is suspected of disabling the tumor suppressing protein p53 through active binding (Greenway et al., 2002). This finding may indicate potential oncogenic properties.

IV. SAFE WORK PRACTICES: The list of potential Tat protein-related health hazards identified above make it imperative that PIs conduct thorough risk assessments and prepare protocols which include standard operating procedures (SOPs) identifying appropriate administrative controls, personal protective equipment (PPE), work methods, engineering controls, and waste disposal procedures for eliminating or sufficiently reducing exposure threat to all staff involved in the affected research.

A. Administrative Controls

1. All use of Tat Protein must be registered with the IBC via submission of an MUA.

2. IACUC protocols involving *in vivo* use of Tat protein must include a completed "Research Involving Biohazards" form in Appendix C.

3. Principal investigators will develop and implement standard operating procedures (SOPs) by which laboratory staff and animal care workers will prepare/administer Tat protein and conduct animal husbandry with minimal potential for exposure. These SOPs will be incorporated into MUAs and IACUC protocols.

4. All tasks having potential for occupational exposure to Tat and other potentially hazardous proteins/peptides (via mixing of doses, dose preparation, administering of injections, etc.) will only be conducted by competent staff whom have received appropriate training (OSHA: "Worker Right to Know") regarding the related health and safety risks, SOPs, and procedures to be followed in event of an exposure incident.

5. *All staff* working in university and hospital laboratories is required to complete applicable modules of the VCU Laboratory Safety Training Modules. Principal Investigators are required to maintain documentation that staff has completed applicable training modules in their central files.

6. Special consideration should be given to protection of staff who may be HIV positive as exposure to Tat protein and other HIV-1 regulatory/accessory proteins may increase risk of developing AIDs.

B. Personal Protective Equipment: Staff involved with any tasks where potential for urethane exposure exists must don the following PPE:

1. Examination gloves.

2. Safety glasses or safety goggles (ANSI Z-87 approved).

3. Lab coat.

4. Appropriate laboratory attire.

5. If aerosol exposure threat exists suitable respiratory protection must be provided. Prior to instituting respiratory protection to personnel, the laboratory must participate in the university Respiratory Protection Program.

C. Work Methods: Biosafety Level 2 precautions must be employed whenever performing manipulations involving Tat protein. Biosafety Level 2 precautions are strongly advised whenever performing manipulations involving other HIV-1/retroviral proteins and peptides.

1. Whenever feasible, procedures with the potential for producing aerosols should be conducted with a certified biological safety cabinet or certified fume hood.

2. Needles used for Tat protein/other protein injection must be disposed of in approved sharps containers immediately following use.

3. Needles used for Tat protein/other protein injection should never be bent, sheared, or recapped. If recapping is absolutely necessary, a "Needle Recapping Waiver" must be submitted for IBC review/approval prior to proceeding.

4. Areas where Tat protein is prepared and/or administered should be cleaned/decontaminated immediately following completion of each task utilizing a detergent product containing protease enzyme (e. g. Terg-A-Zyme®) or a 10% bleach/water solution (prepare fresh stock as needed).

5. Avoid all contact with Tat and other proteins, immediately wash areas which come in contact with the agents with warm water and soap. Report any exposure incidents to Employee Health as soon as possible.

6. Wash hands thoroughly with soap and warm water immediately upon removing examination gloves.

D. Engineering controls

1. In cases where the recommended level of PPE does not provide sufficient protection (e.g.: splash potential, aerosolization potential) tasks must be conducted within a biological safety cabinet chemical fume hood utilizing sash for added protection**.**

2. It is recommended that syringes used for Tat and other potentially hazardous proteins injection be safety engineered (self-sheathing syringes, luer-lock syringes, etc.).

E. Waste Disposal

1. Surplus Tat protein stocks must be disposed of as a regulated medical waste (RMW) per the instructions provided on the University Bloodborne Pathogens - Infectious Waste Management website. Disposal via autoclaving and/or red bag procedures is deemed acceptable.

2. Waste materials known/suspected to be contaminated with Tat protein must be disposed of as a RMW per the instructions provided on the University Bloodborne Pathogens - Infectious Waste Management website. Disposal via autoclaving and/or red bag procedures is deemed acceptable.

3. Animal carcasses: University policy is to dispose of all animal carcasses used in research as RMW through the Department of Animal Resources.

4. All contaminated sharps waste materials must be placed in proper sharps container and disposed of as RMW.

F. Spills: Large spills of Tat protein and other potentially hazardous proteins/peptides should be reported to OEHS and should not be handled without OEHS assistance. Smaller spills (such as those occurring with a BSC or chemical fume hood) may be managed by the laboratory following the following procedures:

1. Don proper protective clothing: Examination gloves (2x layers), safety goggles (ANSI Z87 approved), laboratory coat, and proper laboratory attire.

2. Mist spill area with a detergent product containing protease enzyme (e. g. Terg-A-Zyme®), last stand for at least 20 minutes, then carefully wipe surface.

3. Mist surface with water and wipe, complete surface cleaning with final misting/wiping utilizing 70% ethyl alcohol solution.

4. Spills involving potential sharps hazards should be addressed utilizing the additional the enhanced safety procedures provided in Section V. of the VCU Bloodborne Pathogens - Infectious Waste Management webpage.

Small spills of urethane should be cleaned with absorbent paper and soap and water. Don appropriate PPE during clean-up, dispose of all waste generated through OEHS. For larger spills of urethane contact the OEHS emergency line (828-9834) for assistance.

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