Cigarette Smoke Exposure and HIV-Related Neurologic Disease Progression – Basic Mechanisms and Clinical Consequences

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Cigarette Smoking and Neurologic Disease Risk

• Alzheimer’s disease ↑
• Stroke ↑
• Multiple sclerosis ↑
• Parkinson’s disease ↓
Cigarette Smoking and Multiple Sclerosis

- Increased risk of disease
- More rapid disease progression
- Greater likelihood of treatment failure
- Increased risk of MS among children of cigarette smokers
Cigarette Smoking in HIV Infection

• Over 40% of HIV infected individuals are smokers
  – Twice the estimated prevalence in the general population

• Smoking increases HIV-related morbidity and mortality
  – Infectious complications and lung diseases
  – Increases risk of development of neurocognitive impairment
Immune Effect of Cigarettes Smoking

• Increase levels of soluble immune markers
• Up to 30% increase in peripheral white blood cell counts
  – Correlate with levels of nicotine and carboxyhemoglobin
• Results in the generation of increased levels of reactive oxygen species (ROS) and oxidative stress
Smoking Effects on Immune Cell Subsets: Human Studies

• Light to moderate smokers (<50 pack-years history)
  – Increased CD4 and CD8 T cell numbers

• Heavy smokers (>50 pack year history)
  – Decreased CD4+ and increased CD8+ T cell numbers

• Changes can reverse with cessation
  – Reversion may be delayed by 2-4 years
Immune Effects of Nicotine

- Suppresses PHA-induced lymphocyte proliferation and proinflammatory cytokine production (TNF-α, IL-12, IFN-γ, MIP-1α; I-κB phosphorylation)
- Induces T suppressor cell activity (Treg cells, etc.)
- Suppresses thymic T cell development
- Decreases antibody response
- Reduces T cell priming by dendritic cells
- Increases expression of co-stimulatory molecules, CD40 and CD11b, and chemokine receptor
- Chronic effect: increase in oxidative stress and ROS
ROS Mediated Activation of Cell Signaling Pathways

NADPH Oxidase (NOX)
NOX 1-5

Dual Oxidase (DUOX)
DUOX 1-2

Generation of Superoxide from NADPH Oxidase (NOX) Enzymes

Phagocyte NOX (Phox)

O₂
Molecular oxygen

O₂^•−
Superoxide

OH^*
Hydroxyl radical

Fe³⁺

H₂O₂
Hydrogen peroxide

MPO
Myeloperoxidase

HOCl
Hypochlorous acid
AMP-activated protein kinase (AMPK) senses low ATP and stimulates ATP production.

NAD* suppresses oxidative stress.

*Nicotinamide adenine dinucleotide
Cognitive Effects of Nicotine

• Improvement in attentional performance
  – Improved reaction time
  – Dose-dependent effects on recognition memory tests
  – Abstinence results in worsening

• Opposite effect may occur in children and fetuses
  – Free radical production, antioxidant depletion, increased oxidative stress
Components in Cigarette Smoke

Over 4,000 chemical components, including:

- Butane Lighter Fluid
- Cadmium Batteries
- Stearic Acid Candle Wax
- Hexamine Barbecue Lighter
- Toluene Industrial Solvent
- Nicotine Insecticide
- Acetic Acid Vinegar
- Methane Sewer Gas
- Arsenic Poison
- Carbon Monoxide
- Methanol Rocket Fuel
- Ammonia Toilet Cleaner
Brain Involvement by HIV-1

- Multinucleated Giant Cell
- White Matter Pallor
- Microglial Nodule

Soluble inflammatory mediators, HIV proteins, other factors.
Neurocognitive Impairment (NCI) in HIV Infection

• Results from direct or indirect effects of HIV and related factors on the CNS
• Impairs ability to function in society
• Untreated, results in increased HIV-related morbidity and mortality
• Overall prevalence increased with treatment
HIV-Related Neurocognitive Disorders (HAND)

- A categorization scheme developed to define NCI severity
  - Asymptomatic Neurocognitive Impairment (ANI)
  - Minor Neurocognitive Disorder (MND)
  - HIV-Associated Dementia (HAD)

- Overall prevalence of up to 50% in US, estimates between 3-60% in Sub-Saharan Africa
HIV-1 Transgenic Rat
Inserted Provirus Construct
Behavioral Abnormalities in the HIV-1 Transgenic Rat: Open Field Testing

**Horizontal activity**

- WT: 5000
- TG: 3000
- Difference: 1000
- p-value: 0.0002

**Total distance**

- WT: 3000
- TG: 1000
- Difference: 2000
- p-value: 0.0007

**Vertical activity**

- WT: 250
- TG: 150
- Difference: 100
- p-value: 0.014

**Rearing**

- WT: 40
- TG: 20
- Difference: 20
- p-value: 0.0002
Proliferative Responses by Activated TG and WT Rat Splenic T Cells and Monocyte/Macrophages

T cell stimulation

Monocyte/Macrophage cell stimulation
Cytokine Secretion by Activated TG and WT Rat Splenic T Cells

IFN-γ

TNF-α

IL-1β

48 hrs

72 hrs
Cytokine Secretion by Activated TG and WT Rat Splenic Macrophages
Inflammation and Astrocytosis in TG and WT Rat Brain White Matter

Inflammatory Cell Activation in TG and WT Rat Brain White Matter

Cytokine Levels in Transgenic and Wild-type Rat Brain Tissue Lysates

**INF-γ**
- WT: 30
- TG: 120
- p < 0.0001

**TNF-α**
- WT: 20
- TG: 70
- p = 0.002

**IL-β**
- WT: 200
- TG: 530
- p < 0.0063
Astrocytes and Mononuclear Phagocytes Labeled for HIV Vif and Nef in TG and WT Rat Brain Tissue Sections

GFAP: astrocytes
Iba1: activated macrophages + microglial cells

## Brain Astrocytes and Mononuclear Phagocytes
### Labeled for HIV Tat and gp160 in TG and WT Rats

<table>
<thead>
<tr>
<th>Transgenic</th>
<th>Wild-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tat GFAP</td>
<td>Tat GFAP</td>
</tr>
<tr>
<td>gp160 GFAP</td>
<td>gp160 GFAP</td>
</tr>
<tr>
<td>Tat Iba1</td>
<td>Tat Iba1</td>
</tr>
<tr>
<td>gp160 Iba1</td>
<td>gp160 Iba1</td>
</tr>
</tbody>
</table>

**GFAP:** astrocytes  
**Iba1:** activated macrophages + microglial cells

Activation of MAP Kinases in TG and WT Rat Brains

Activation of p38, JNK, Erk1/2 and Erk5 in TG and WT rat brains.
Assessment of Molecular Markers of Cellular Activation and Stress

- p38
- pp38
- JNK/SAPK
- pJNK/SAPK
- Erk1/2
- pErk1/2
- Erk5
- pErk5
Detection of DUOX1 in TG and WT Rat Brains

Fig 5: (a) Western blots detecting DUOX1 levels in brain tissue lysates from WT and TG rats; and (b) with quantitation of DUOX1/β-actin relative band intensity (N=3 rats per group).
Detection of Activated AMPK (pAMPK) in TG and WT Rat Brains

(a) Western blot of phosphorylated AMPK (phospho-AMPK) and total AMPK in brain tissue lysates from WT and TG rats. (b) Levels of phospho-AMPK were higher in brain from TG animals (N=3 rats per group).
Rat Model of Cigarette Smoking

Brain Inflammation Induced by Cigarette Smoke in Lewis Rats

Smoke (-)  Smoke (+)

Staining for class II MHC

PCR Analysis of Cytokine Responses Induced by Cigarette Smoke in Lewis Rat Brain

**IFN-γ**

- CS- vs. CS+:
  - Expression
  - p = 0.0003

**IL-18**

- CS- vs. CS+:
  - Expression
  - p = 0.0022

**TNFα**

- CS- vs. CS+:
  - Expression
  - p = 0.0022

**IL-1α**

- CS- vs. CS+:
  - Expression
  - p = 0.0207

**IL-1R2**

- CS- vs. CS+:
  - Expression
  - p = 0.0281

**IL-1β**

- CS- vs. CS+:
  - Expression
  - p = 0.1949

PCR Analysis of Cytokine Responses Induced by Cigarette Smoke in Lewis Rat Brain

- **IL-23**:
  - Expression:
  - CS-: Lower expression
  - CS+: Higher expression
  - p = 0.0002

- **IL-17**:
  - Expression:
  - CS-: Lower expression
  - CS+: Higher expression
  - p = 0.0002

- **IL-6**:
  - Expression:
  - CS-: Lower expression
  - CS+: Higher expression
  - p = 0.0002

- **FoxP3**:
  - Expression:
  - CS-: Lower expression
  - CS+: Higher expression
  - p = 0.0002

- **TGF-β**:
  - Expression:
  - CS-: Lower expression
  - CS+: Higher expression
  - p = 0.0019

- **IL-10**:
  - Expression:
  - CS-: Lower expression
  - CS+: Higher expression
  - p = 0.0002

- **IL-10**:
  - Expression:
  - CS-: Lower expression
  - CS+: Higher expression
  - p = 0.0002
Brain Pro-Inflammatory Responses Induced by Cigarette Smoke in Lewis Rats: ELISA

![Graph showing cytokine levels](image)

- IFN-γ: p = 0.009
- TNF-α: p = 0.002

Pro-oxidant and Antioxidant Gen Expression Induced by Cigarette Smoke: PCR Analysis

- **iNOS**
  - Expression: 0 to 25
  - CS- vs. CS+: p = 0.0002

- **SOD**
  - Expression: 0 to 35
  - CS- vs. CS+: p = 0.1605

- **TXN**
  - Expression: 0 to 25
  - CS- vs. CS+: p = 0.0002

- **Arylhydrocarbon Receptor**
  - Expression: 0 to 50
  - CS- vs. CS+: p = 0.0207
Brain Nrf2 Activation by Cigarette Smoke

CS-

CS+

GFAP+ → Astrocytes
Immune Cell (Splenocyte T Cell) Proliferative Responses Induced by in WT Fischer 344 Rats

Prominent early proliferative response
LPS-Induced Splenocyte Proliferative Responses in Normal Fischer 344 Rats

Pronounced proliferative response are delayed
T Cell Proinflammatory Responses to Cigarette Smoke in HIV TG and WT Rats: IFN-γ

CD3/CD28 Activation

WT = Wild-type
TG = Transgenic
CS- = no cigarette smoke
CS+ = cigarette smoke exposed
T Cell Proinflammatory Responses to Cigarette Smoke in HIV TG and WT Rats: TNF-α

CD3/CD28 Activation

WT = Wild-type
TG = Transgenic
CS- = no cigarette smoke
CS+ = cigarette smoke exposed
Pathogenesis of HIV Infection of the CNS

Adapted from: Bell JE. Histopathology 2004;45:549.
Brain VEGF, MIP-α/CCL3 and MCP-1/CCL2 Gene Expression: Effects of 2 Wk Cigarette Smoke Exposure
Brain Cytokine Gene Expression: Effects of 2 Wk Cigarette Smoke Exposure
Brain Pro- and Antioxidant Gene Expression: 2 Wk Cigarette Smoke Exposure

**Pro-oxidant: DUOX1**

**Antioxidants: SOD (superoxide dismutase) and TXN (thioredoxin)**
NAD: A Regulator of Oxidative and Pro-inflammatory Responses

Nicotinamide

Nicotinamide phosphoribosyltransferase

Nicotinamide mononucleotide

Nicotinamide/nicotinic acid mononucleotide adenyllyltransferase

SIRT1

O-acetyl-ADP-ribose

PRPP

ATP

Nicotinic acid

Tryptophan

Nicotinamide riboside
NMN Suppression of IFN-γ Secretion by Activated PBMCs

**CD3/CD28**

**LPS**

**WT**

**TG**

WT-Smoking

WT-Nonsmoking

TG-Smoking

TG-Nonsmoking
NMN Suppression of TNF-α Secretion by Activated PBMCs

CD3/CD28

LPS

WT

TG

WT-Smoking
WT-Nonsmoking

TG-Smoking
TG-Nonsmoking
Conclusions

• Cigarette smoke and smoking can be associated with significant morbidity and mortality in HIV infection
• Animal models of HIV infection can provide valuable insight into mechanisms that underlie these effects.
• Approaches that target mechanisms that underlie effects from smoke exposure may prove to be beneficial in complications that occur in HIV infection that result from exposure.
Collaborators

HIV Animal Models
• Joseph Bryant
• Odell Jones
• Harry Davis

Smoking Model
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• Mandeep Mehra, MD

Royal Lab
• Ming Gou
• Li Zhan

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