HIV-1 Exposed Seronegative Persons (HESN) Have Lower Mucosal Innate Immune Reactivity

Jennifer A. Fulcher[1], Jennifer C. Hoffman[1], Laura M. Romas[3], Karen Tanner[2], Terry Saunders[2], Julie Elliott[2], Adam D. Burgener[3], Peter A. Anton[2], Otto O. Yang[1]

[1]Division of Infectious Diseases, [2]Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA
[3]Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada

Background

- Risk of HIV acquisition varies, and some individuals appear to remain uninfected despite repeated high-risk sexual exposures, termed HIV-Exposed Seronegative (HESN).
- HIV-specific immune correlates (CTLs, mucosal immunoglobulin) have been identified in HESN, suggesting prior mucosal exposure to HIV-1 without transmission.
- Immune activation contributes to the transmission and progression of HIV-1 infection. For example, infections that trigger mucosal inflammation (e.g. HSV-2) are associated with increased risk of transmission.

Hypothesis

- Innate immune reactivity may increase vulnerability to HIV infection via disruption of mucosal function, activation of dendritic cells/macrophages, and recruitment of HIV target cells (activated CD4+ T cells). **Therefore, we hypothesize that HESN individuals have overall lower mucosal immune activation and reactivity.**

Methods

Subjects:

- HESN men previously identified as high-risk were recruited from Multicenter AIDS Cohort Study (MACS) cohort
- Healthy control men (age-matched) were recruited from UCLA Mucosal Immunology Core registry
- Two study visits with: sigmoidoscopy (30 rectal mucosal biopsies per visit), STI screen and plasma collection, saliva and rectal sponge for proteomics studies

Experiments:

- Biopsies were used in an *ex vivo* explant model then stimulated with select innate immune ligands
- After stimulation, explants assayed for:
  - cytokine expression
  - proteomic studies
  - gene expression
  - HIV infection
  - immunohistochemistry

Results

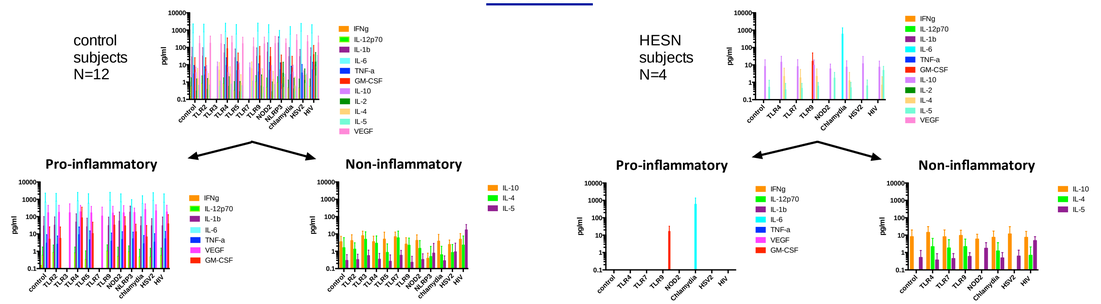


Figure 1. Cytokine expression following stimulation with select innate immune ligands. Explants from control or HESN subjects were exposed to innate immune stimuli for 18-20 hours then supernatant collected and cytokine expression detected using Luminex. As shown, similar amounts of non-inflammatory cytokines were produced by all subjects. In contrast, large amounts of pro-inflammatory cytokines were produced by control subjects while no HESN subjects had detectable pro-inflammatory cytokine production. Data shown as mean from all subjects ± SD.

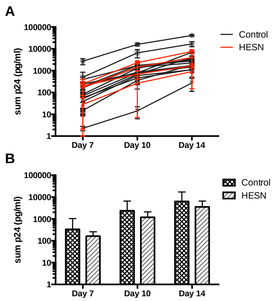


Figure 2. HIV-1 replication in control vs HESN explants. Explants from control or HESN subjects were infected with HIV-1_{BaL} ($3x10^3$ $TCID_{50}$) then p24 detected by ELISA at time points indicated. Data shown as **(A)** mean of triplicate explants ± SD for each subject and **(B)** mean from all subjects ± SD. While data did not reach statistical significance, there was a trend toward lower replication in HESN explants.

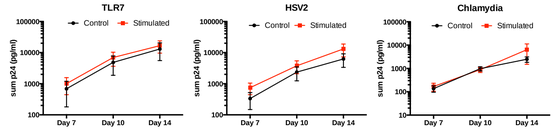


Figure 3. HIV-1 replication in explants following innate immune stimulation. Control subject explants were stimulated with select innate immune ligands then infected with HIV-1_{BaL} ($3x10^3$ $TCID_{50}$). Data shown as mean from all subjects ± SD. No statistically significant difference was seen in any of the tested conditions. Of note, these initial experiments were done using a saturating infectious dose which may limit ability to detect differences.

Accession	Description	Function	p-value	Avg Log2 FC HESN
H2A1D_HUMAN	Histone H2A type 1-D	Nucleosome assembly	0.037	0.20
IGHM_HUMAN	Ig mu chain C region (IGM)	Mucosal Immunoglobulin	0.022	0.19
FIBB_HUMAN	Fibrinogen beta chain	Blood coagulation; platlet activation/degranulation, promote protein secretion, promote hormone secretion, signal transduction	0.008	0.19
TTHY_HUMAN	Transthyretin	Extracellular matrix organization, hormone binding, protein binding	0.004	0.17
CATB_HUMAN	Cathepsin B	Epithelial cell differentiation, Extracellular matrix dissasembly, proteolysis, Trafficking and processing of endosomal TLR	0.007	0.16
CO4A_HUMAN	Complement C4-A	Classical complement pathway, inflammation, apoptotic cell clearance	0.012	-0.24
TRFL_HUMAN	Lactotransferrin	Iron binding, antibacterial	0.009	-0.32
LEG4_HUMAN	Galectin-4	Cell Adhesion	0.037	-0.39
CLCA1_HUMAN	Calcium-activated chloride channel regulator 1	Calcium transport	0.048	-0.39
ITLN1_HUMAN	Intelectin-1	Regulation of glucose uptake, antimicrobial defense	0.014	-0.61
PDIA3_HUMAN	Protein disulfide-isomerase A3	Antigen processing and presentation via MHC class I, Redox homoestasis, Protein folding, Positive regulation of apoptotic signaling, signal transduction	0.012	-0.66
CH10_HUMAN	10 kDa heat shock protein, mitochondrial	Protein folding, endopeptidase activity	0.008	-0.95
SYWC_HUMAN	Tryptophan--tRNA ligase, cytoplasmic	Negative regulation of cell proliferation, protein production, gene expression	0.033	-1.25
MDHM_HUMAN	Malate dehydrogenase, mitochondrial	Carbohydrate metabolsim	0.044	-1.28

Table 1. Differential protein expression in HIV-stimulated HESN explants. Following stimulation with inactivated HIV-1, protein expression in supernatant from control or HESN explants was quantified using label-free tandem MS. Table shows differentially expressed proteins using cutoff $p<0.05$. Red and green shading denotes upregulated and downregulated proteins, respectively. Notably, downregulated proteins included those involved in immune response, consistent with our findings in Fig. 1.

Summary

- Gut mucosal explants from both control and HESN subjects produced similar amounts of non-inflammatory cytokines following innate immune stimulation
- In striking contrast (especially given the small N=4), HESN explants produced no detectable pro-inflammatory cytokines following innate immune stimulation, while control explants produced the expected high levels
- Several immune proteins were differentially expressed between HIV-stimulated HESN and control explants, including downregulation of those involved in defense and immune response
- Gut mucosal explants from all subjects support productive HIV-1 replication at saturating doses of infection; this is in contrast to clinical observation that HESN individuals remain uninfected despite repeated exposure, suggesting that these individuals are not "resistant" but rather can "control" HIV-1 infection to prevent dissemination

Conclusions

- In this intensive pilot study, mucosal innate immune reactivity appears selectively dampened in HESN subjects as evidenced by absence of pro-inflammatory cytokine production following innate immune stimulation and down-regulation of immune response proteins.
- While requiring further investigation, this finding in the physiologically-inflamed gut mucosa in HESN subjects may help identify additional targets for prevention efforts.

Acknowledgments

- We would like to thank all subjects in the study for their generous participation. We also acknowledge the support of the UCLA CFAR, the MACS study and Dr. Roger Detels.