**Grant Application**

**Title:** HIV, Aging & MDSC

**Program Director/Principal Investigator:**
- **Name:** Helen Horton
- **Position Title:** Associate Professor
- **Department, Service, Laboratory, or Equivalent:** 307 Westlake Ave N, Suite 500
- **Mailing Address:** Seattle, WA 98109-5219

**Subject:**
- **Research Exempt:** Yes
- **NIH-defined Phase III Clinical Trial:** No

**Support Period:**
- **From:** 10/01/12
- **To:** 09/30/13
- **Initial Budget Period:**
  - **Direct Costs:** $25,000
  - **Total Costs:** $25,000

**Type of Organization:**
- **Public:** Nonprofit
- **Private:** Nonprofit
- **For-profit:** Nonprofit

**Entity Identification Number:** 910961784

**Signature:**
- **Date:** 08/14/12
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<td>Nick Lejarcegui</td>
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CONSULTANT COSTS

EQUIPMENT (Itemize)

SUPPLIES (Itemize by category)

PBMC isolation, Antibodies, Luminex

16,367

TRAVEL

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS (Itemize by category)

OTHER EXPENSES (Itemize by category)

Flow Core

1,440

CONSORTIUM/CONTRACTUAL COSTS

DIRECT COSTS

SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD (Item 7a, Face Page) $25,000

CONSORTIUM/CONTRACTUAL COSTS

FACILITIES AND ADMINISTRATIVE COSTS

TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD $25,000
BUDGET JUSTIFICATION

PERSONNEL
Nicholas Lejarcegui; Technician (1.2 calendar months):
Nick is an experienced technician and manager of the flow cytometry core at Seattle BioMed. As such, he has extensive experience with optimization and performance of flow-based assays that will be used in this proposal. He will be responsible for performing the PBMC isolation and flow cytometry experiments to assess MDSC frequencies. He will be supervised by Helen Horton. He will assist in data analysis, presentation, and manuscript preparation.

FRINGE BENEFITS
Fringe Benefits are calculated at 26.0% of salaries in accord with Seattle BioMed’s nonprofit rate agreement dated 02/10/2012.

SUPPLIES
PBMC Isolation - $3,000 is requested. PBMC isolation is estimated at $10 per participant. Thus for 300 participants (150 seronegatives and 150 seropositives) we estimate $3,000.

MDSC frequencies - $9,000 is requested. The principle costs are associated with the relatively high cost of monoclonal antibodies for the flow cytometry. The estimated cost of MDSC staining per sample is $30. Thus for 300 samples we estimate $9,000.

Pro-inflammatory cytokines - $4,367 is requested. Luminex reagents are requested to assess plasma cytokines.

OTHER EXPENSES
Seattle BioMed Shared Flow Cytometry Resources – Funds are requested to cover the costs of LSR II flow cytometer ($72 per hour; estimated 2 hrs per week for 20 weeks). Total flow core costs are requested at $1,440.
A. Personal Statement

The goal of the proposed research is to investigate whether myeloid-derived suppressor cells (MDSC) increase with age in healthy uninfected individuals and whether HIV infection accelerates and exacerbates accumulation of MDSC. Specifically, we plan to phenotypically characterize MDSC in peripheral blood of healthy uninfected and HIV+ adults. We will also correlate the frequency of MDSC with the concentration of pro-inflammatory mediators in plasma. We hypothesize that increased frequencies of MDSC contribute to many of the co-morbidities (e.g. the extremely high cancer risk) seen in HIV-infected people since MDSC are highly immunosuppressive. If we show that MDSC are increased with age and that this is accelerated in HIV+ individuals, we will have sufficient preliminary data to apply for the recent NIH FOA on "Multidisciplinary Studies of HIV/AIDS and Aging", which is aimed at understanding the interface between aging, HIV and co-morbid conditions. I will oversee all of the analyses associated with this proposal. I have a broad background in cellular immunology, with specific training and expertise in T cell immunology. I co-directed the Research & Development lab of the HIV Vaccine Trials Network (HVTN) at Fred Hutchinson Cancer Research Institute as a Research Scientist. Here I was responsible for development, optimization, standardization and validation of immunoassays to functionally quantify and characterize HIV-specific cellular immune responses induced by vaccination in healthy seronegatives and after HIV-1 infection. I am also Director of the Specialized Cellular Immunology sub-core of the UW CFAR (Holmes, PI). In addition, I have successfully administered several NIH-funded projects (e.g. staffing, research protections, budget), collaborated with other researchers, and produced several peer-reviewed publications. I have worked with Dr. Frenkel for a number of years and we already have NIH funding to work on defining Immunological and Virological Events in Early HIV-1 Infection (P01 grant, Mullins). The current application builds logically on our prior work. Dr. Horton has not worked in elderly populations previously and has no funding to look at MDSC in HIV+ individuals either. The current R01 in the Horton lab on MDSC is to assess the role of these cells in healthy HIV-seronegative neonates. Thus, the areas of MDSC and HIV infection, and the area of Aging and HIV are new to the PI. Thus, the PI is applying for Emerging Opportunity Funding to accrue preliminary data to enable submission of a grant to the NIH in response to this FOA for this new emerging opportunity in December 2012.

B. Positions and Honors

Positions and Employment

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<tr>
<th>Year</th>
<th>Position and Institution</th>
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<tr>
<td>1997-1999</td>
<td>Research Associate, Immunogenetics, Wisconsin Regional Primate Research Ctr</td>
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<td>1999-2001</td>
<td>Assistant Researcher, Immunogenetics, Wisconsin Regional Primate Research Ctr</td>
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<tr>
<td>2001-2005</td>
<td>Scientist, Program in Infectious Diseases, FHCRC</td>
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<td>2005-2008</td>
<td>Associate in Clinical Research, Program in Infectious Diseases, FHCRC</td>
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<tr>
<td>2004-2008</td>
<td>Research Assistant Professor, Department of Medicine, University of Washington</td>
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2008-present  Affiliate Associate Professor, Departments of Medicine and Global Health, UW
2008-Present  Associate Member/Professor, Seattle Biomedical Research Institute.

Other Experience and Professional Memberships
2008-Present  Member, American Association of Immunologists
2011-Present  Member, NIH AIP study section

C. Selected Peer-reviewed Publications (Selected from 39 peer-reviewed publications)

Most relevant to the current application


Additional recent publications of importance to the field (in chronological order)


D. Research Support

Ongoing Research Support

NIH P30 AI027757 (Holmes) 06/01/08-05/31/13

Centers for AIDS Research: D-CFAR

The mission of the Molecular Immunology Core is to develop, refine, and provide by training and technology transfer state-of-the-art immunological assays to evaluate and quantify humoral and cellular responses to support NIH-sponsored AIDS research and training. The assays, reagents, and training offered by the Core are designed to support basic, clinical, and translational research in the prevention, detection, and treatment of HIV infection and AIDS.

Role: Core Director of SBRI subcontract

NIH R01 AI090783 (Horton) 06/15/10-05/31/14

Identifying, Characterizing, and Inducing Effective Anti HIV T Cell Response.

This proposal will investigate whether HIV-specific CTL that target conserved regions of HIV-1 are superior at controlling HIV replication than HIV-specific CTL that target variable regions. The proposal will also optimize a human in vitro vaccination assay to screen potential HIV immunogens before they enter human clinical trials.

Role: PI

NIH P01 AI057005 (Mullins) 07/15/10-06/30/15

Immunological and Virological Events in Early HIV-1 Infection.

This proposal will investigate whether proliferative capacity of HIV-specific CD8+ T cells determines their effectiveness at controlling viral replication.

Role: Co-Director of Project 1.

R01 AI100018 (Horton) 04/01/12-03/31/17

NIH/NIAID

Myeloid-derived Suppressor cells (MDSC) suppress infant immune responses

The Horton lab has identified that MDSC are present in cord blood and young infants. These are highly immunosuppressive cells. This proposal will perform in depth characterization of the suppressive effects of these cells on multiple cell types and will determine if their presence limits the ability of infants to respond to childhood vaccinations and infections.

Role: PI

Completed Research Support

U01 A1 068618-01 (McElrath, H.Horton) 6/29/06-05/31/13

NIH/NIAID

HVTN Laboratory Program

The HVTN Laboratory Program conducted state-of-the-art laboratory-based clinical research and evaluation through the HIV Vaccine Trials Network that could lead to the development of a safe and efficacious HIV vaccine.

Role: Co-investigator
Immunization of Oral Mucosa for Induction of Rectal and Genital Mucosal Immunity
This proposal will investigate whether specific T cells can be induced in the genital mucosa following oral immunization.
Role: Co-Investigator

The Role of Immune Activation and Natural Killer Cells in Acquisition and Control of Infant HIV-1.
This proposal will determine if NK cells from HIV-exposed uninfected infants have superior cytolytic activity than NK cells from HIV-exposed infants who become infected. The Horton lab is responsible for performing the NK cell in vitro viral suppression assays.
Role: Co-investigator of sub-contract

Activation-induced non-responsiveness causes HIV Prog.
This study will characterize an immune correlate of protection from HIV-1 disease progression and will have far reaching implications on therapeutic intervention in chronically-infected individuals and rationale for design of HIV vaccines.
Role: PI

Tregs Differentially Suppress HIV-specific CD8+ T Cells.
This proposal will determine if HIV-specific CTL restricted by “protective” HLA alleles (HLA-B27 and B57) are differentially suppressed by Tregs during chronic HIV infection.
Role: PI
ABSTRACT:

Anti-retroviral therapy (ART) has dramatically increased longevity of HIV-infected individuals. However, despite the enormous success of ART in increasing longevity, HIV+ individuals suffer disproportionately from many medical conditions that are normally attributed to old age (e.g. cancer, cardiovascular disease, neurocognitive impairment, etc). Whether HIV infection itself is causing “accelerated aging” is unclear because the number of older individuals living with HIV/AIDS has risen dramatically in recent years. Indeed from 2001-2008 the number of HIV+ individuals over 50 years old in the US has almost doubled, such that by the year 2015 it is estimated that half of all HIV+ Americans will be over 50 years old. The NIH has recently announced a R01 Funding Opportunity entitled “Multidisciplinary Studies of HIV/AIDS and Aging”, which is aimed at understanding the interface between aging, HIV and co-morbid conditions.

Chronic immune activation is a hallmark of HIV-1 infection. Myeloid-derived suppressor cells, or MDSCs, are a heterogeneous immunosuppressive cell type described previously in cancer research and are thought to accumulate due to a prolonged pro-inflammatory environment. MDSCs suppress anticancer immunity and are usually absent or present at very low frequencies in healthy adults. Recent evidence has shown that MDSCs are also present in patients with chronic HIV-1 disease. They correlated with increased HIV-1 viral loads, decreased CD4 counts, and have been shown to suppress cytotoxic T cells (CTLs) in vitro.

Certain inflammatory cytokines including IL-6 activate and expand MDSC populations. Chronic HIV-1 infection is known to increase IL-6 and other inflammatory cytokines. Notably, many pathologies associated with advanced age are also thought to be attributed to an enhanced pro-inflammatory environment. A single study has shown elevated levels of MDSC in healthy mice as they age. However, no studies have looked at MDSC frequencies in aging humans.

We hypothesize that MDSC frequencies increase with age in healthy individuals but that HIV infection accelerates the accumulation of these immunosuppressive cells. In addition, we hypothesize that increased MDSC frequency may be responsible, at least in part, for the increased co-morbidities associated with prolonged HIV infection.

The PI has not worked in elderly populations previously and has no funding to look at MDSC in HIV+ individuals either. The current R01 in the Horton lab on MDSC is to assess the role of these cells in healthy HIV-seronegative neonates. Thus, the areas of MDSC and HIV infection, and the area of Aging and HIV are new to the PI. Thus, the PI is applying for Emerging Opportunity Funding to accrue preliminary data to enable submission of a grant to the NIH in response to this FOA for this new emerging opportunity in December 2012. The aims of this Emerging Opportunity Grant are:

**Aim 1: Compare frequency of MDSCs in HIV-1-positive and HIV-1-negative age- and gender-matched participants.** Using peripheral blood from HIV-1 infected participants from the Madison clinic (in collaboration with the CFAR Clinical Core) and Frenkel clinic (in collaboration with Dr. Lisa Frenkel), we will quantify MDSCs by flow cytometry and compare to age- and gender-matched HIV-seronegative participants (in collaboration with Dr. LaCroix at FHCRC).

**Aim 2: Determine if MDSC frequency in HIV-1-positive and HIV-1-negative age- and gender-matched participants is correlated with increased concentrations of pro-inflammatory mediators.** Using blood plasma we will measure inflammatory cytokine levels and determine if levels correlate with MDSC frequency. We will measure IL-6, IL-10, PGE2, SAA, hsCRP, and TNFα levels in age- and gender-matched participants with and without HIV infection.

By completing the aims outlined above, we will accrue preliminary data showing (i) whether MDSC frequencies increase with age in healthy adults, (ii) whether HIV infection accelerates accumulation of MDSC, and (iii) whether the frequencies of MDSC are correlated with increased pro-inflammatory mediators.
RESEARCH PLAN

SIGNIFICANCE

Declining immune function is a hallmark of aging. Indeed, 90% of yearly Influenza deaths in the US are in persons 65yrs or older\(^1\) likely compounded by the fact that routine vaccinations, for example influenza vaccination, are less efficacious in the elderly\(^2\)\(^-\)\(^4\). Declining immune function is also a hallmark of HIV infection and cannot be solely attributed to declining CD4 counts, as even individuals with CD4 reconstitution on ART have reduced ability to control infections. Notably, influenza vaccination is also less efficacious in HIV-infected individuals with fully suppressed viral load on ART\(^5\).

Many of the pathological conditions associated with aging have been linked to increased levels of pro-inflammatory mediators\(^4\)\(^,\)\(^6\)\(^,\)\(^7\). Chronic immune activation and high levels of pro-inflammatory cytokines are also profoundly increased in HIV-1 infection\(^8\) raising the intriguing hypothesis that a common mechanism may account for the declining immune function seen in both healthy elderly individuals and individuals with HIV infection.

Myeloid-Derived Suppressor Cells (MDSC) are a heterogeneous population of immunosuppressive cells that are thought to accumulate as a result of prolonged inflammation. MDSC are usually present at very low frequency in peripheral blood of healthy adults, but in cancer patients these cells have been shown to be present in high numbers and suppress beneficial antitumor immune responses. Pro-inflammatory cytokines including interleukin (IL)-6 activate and expand MDSC populations (reviewed in \(^9\)). Given that chronic immune activation and high levels of pro-inflammatory cytokines are profoundly increased in HIV-1 infection (reviewed in \(^10\),\(^11\)), it is not surprising that MDSC have recently been found in HIV-infected individuals, where they can be shown to potently suppress cytotoxic T cell (CTL) responses\(^12\). These findings suggest that MDSC may contribute to many of the co-morbidities (e.g. the extremely high cancer risk) seen in HIV-infected people\(^13\),\(^14\).

Our overall hypothesis is that MDSC accumulate as part of the natural aging process due to an increased pro-inflammatory environment. HIV-1 infection, and the concomitant enhanced inflammatory milieu induced by HIV infection, accelerates and exacerbates accumulation of MDSC. High levels of MDSC contribute to declining immune function and increased co-morbidities associated with HIV-1 infection. Note that increased co-morbidities are seen in HIV-infected individuals even when fully virally-suppressed on ART\(^15\)\(^-\)\(^18\). This is thought to be due to the fact that inflammation is not fully resolved upon ART administration in many infected individuals\(^16\),\(^19\)-\(^21\). Thus, this pilot project will use peripheral blood from HIV-infected individuals on ART.

INNOVATION

MDSC have never been studied in aging individuals regardless of HIV status, thus, the project itself is highly innovative. In addition, the new collaboration between a cellular immunologist (Dr. Horton) specializing in infectious disease immunology and an epidemiologist specializing in the effects of aging in women (Dr. LaCroix) is also innovative. Should we show that MDSC are increased during the natural aging process we may be able to shed light on the “accelerated aging” seen in individuals with chronic infections like HIV-1.

Time-sensitive nature of proposal: (i) NIH has recently announced a Funding Opportunity entitled “Multidisciplinary Studies of HIV/AIDS and Aging”, aimed at understanding the interface between aging, HIV and co-morbid conditions (next due date for proposals is Dec 2012); (ii) The “Long Life Study” sub-study of the Women’s Health Initiative (see below) will be used to obtain peripheral blood from elderly HIV-uninfected individuals (>65yrs). This sub-study will complete recruitment in 2013. MDSC need to be assessed in fresh blood and thus it is imperative that we obtain these samples before recruitment ends.

EXPERIMENTAL PLAN

Study populations and samples. HIV-uninfected: Seattle Biomed already has approved protocols for blood collection from healthy HIV-uninfected individuals working at the Institute. These will be used for...
individuals under 65yrs of age. For older participants we plan to collaborate with Dr. Andrea LaCroix, Co-Project Director of the Clinical Coordinating Center for the *Women’s Health Initiative* (WHI) at FHRC (see letter of support). The “Long Life Study” is a sub-study of this Initiative that plans to enroll 8,000 WHI Extension Study participants who are at least 63 years old. Recruitment started this year and should be completed by the end of 2013. Almost 3000 women have already consented and we plan to use this population for our pilot study.

**HIV-infected:** In collaboration with the CFAR Clinical Core, we plan to enroll HIV+ individuals (age- and gender-matched to our uninfected population) from the Madison Clinic at Harborview Medical Center. Approximately 25% of the HIV+ individuals at Madison Clinic are women (around n=825), however, many have language barriers that would make consent difficult. To increase the number of HIV+ women eligible for this study we will also collaborate with Dr. Lisa Frenkel and her clinic (see letter of support).

We plan to assess MDSC frequency in PBMC of 150 HIV-uninfected (50 individuals per age group; 3 age groups: 18-30yrs, 30-60yrs and >65yrs) and 150 age- and gender-matched HIV-infected individuals on ART, which gives us over 80% power to detect a 2% difference in MDSC frequency between the age-matched groups (2-sided, alpha = 0.05).

**Aim 1: Compare frequency of MDSCs in HIV-1-positive and HIV-1-negative age- and gender-matched participants.**

**Hypothesis:** MDSC frequency is increased with age in healthy individuals but accumulation is accelerated in individuals with HIV.

**Rationale:** MDSC have recently been described in HIV-infected individuals. In the cancer literature the frequency of MDSC correlates with pro-inflammatory mediators in the plasma and we know that similar pro-inflammatory mediators are increased in plasma of healthy HIV-uninfected individuals as they age. Furthermore, a single study has shown elevated levels of MDSC in healthy mice as they age. Thus it is highly feasible that we will find increased levels of MDSC in peripheral blood of elderly HIV-uninfected individuals.

**Methods:** Using peripheral blood from HIV-1-uninfected cohorts in different age groups from Seattle BioMed and the WHI and from age- and gender-matched HIV-1-infected individuals from the Madison and Frenkel clinics, we will quantify MDSCs using a 7-color flow cytometric phenotyping panel.

**Aim 2: Determine if MDSC frequency in HIV-1-positive and HIV-1-negative age-/gender-matched participants is correlated with increased concentrations of pro-inflammatory mediators.**

**Hypothesis:** MDSC frequency in both elderly HIV-uninfected and HIV+ individuals will be directly correlated with increased concentrations of pro-inflammatory factors in plasma.

**Rationale:** MDSC are not normally present at high levels in healthy adults. Cytokines implicated in the expansion and activation of MDSCs include VEGF, IL-6, IL-10, and prostaglandins (PGE2). These inflammatory mediators are increased in serum of HIV+ individuals. We expect to find that high levels of these cytokines will correlate with MDSC frequencies in both elderly HIV-uninfected individuals and in HIV-1-infected individuals.

**Methods:** Levels of VEGF, IL-6, IL-10, PGE2, SAA, hsCRP, and TNFalpha in plasma will be measured by Luminex, a flow-cytometry-based assay for detection of up to 100 different soluble mediators via fluorescent-bead-linked antibodies.

**Statistical Considerations:** Regression models that include age, gender and other potential confounders will test potential associations. We acknowledge that in the oldest age group (>65yrs), all of our HIV-uninfected samples will be from women. There are no published data to show that there is a difference in MDSC frequency based on gender – but we will look at this question with the data generated in this pilot.

**FUTURE DIRECTIONS:** Should we find a correlation between MDSC, aging and HIV, we will submit a R01 proposal in December for the NIH FOA on this topic. Our future plans will assess potential therapeutic interventions to reduce/eliminate MDSC. For example, All-Trans Retinoic Acid, which forces maturation of MDSC into non-suppressive monocytes and dendritic cells, is one such promising therapeutic.


14 August 2012

Helen Horton, PhD
Associate Professor, Seattle Biomedical Research Institute
Affiliate Associate Professor, Departments of Medicine and Global Health, UW

Re: Horton CFAR proposal, “HIV, Aging & MDSC”

Dear Helen:

I look forward to collaborating with you to study Myeloid-Derived Suppressor Cells in HIV-1 infected women. These cells may have a central role in HIV-1 disease progression, and learning more about their role could lead to a better outcome.

In our clinic we follow nearly 100 HIV-1 infected children, ranging from 2 to 23 years of age. About 40% of these children live with their HIV-1 infected mother, the others with adoptive or foster parents. As your collaborator I will gladly approach the infected children and mothers to enroll into your study.

Wishing you a successful application, sincerely,

Lisa

Lisa M. Frenkel, MD
Professor, Pediatrics, Laboratory Medicine and Global Health
lfrenkel@uw.edu