Age-independent immunosenescence: an antigen-activated, sexually dimorphic program predicts inflammation of aging and HIV-1 susceptibility

Sunil, K. Ahuja, M.D.[1,2,3,4,¶], Jason F. Okulicz, M.D.[5,6,*§], Lyle R. McKinnon, M.D.[7,8,*,§], Maristella Steri, Ph.D.[9,*], Nathan Harper, M.S.[1,4,*], Scott Letendre, M.D.[10,11,§], Matthew J. Bottomley, Ph.D.[12,13,§], Valeria Orrù, Ph.D.[9], Edoardo Fiorillo, M.D.,Ph.D.[9], Fabio Jimenez[1,4], Muthu Saravanan Manoharan, M.S.[1,2], Anne Branum[1,4], Alisha M. Smith[1,3,4], Justin A. Meunier[1,4], Andrew Carrillo[1,2,4], Lavanya Pandranki, M.S.[1,2], Elizabeth A. Walter, M.D.[1,2], Robert Root-Bernstein, Ph. D.[14], Guido Silvestri, M.D.[15,16,§], Christopher King, M.D., Ph.D.[17,§], Yong-Tang Zheng, M.D.[18,19,§], Hong-Yi Zheng[18,19,§], Tomas M. Ferguson, M.D.[20], Joshua Kimani, M.D.[8,21], T. Blake Ball, M.D.[8,21], Francis A. Plummer, M.D.[8,21,§], Keith R. Fowke, Ph.D.[21], Paul N. Harden, M.D.[13], Kathryn J. Wood, Ph.D.[12], Martin Ferris, Ph.D.[22], Jennifer Lund, Ph.D.[23,24,§], Mark T Heise, Ph.D.[25], Nigel Garrett, M.D.[7], Edwina J. Wright, M.D.[26], Susan J. Little, M.D.[10,§], Davey M. Smith, M.D.[10,27], Francesco Cucca, M.D.[9,28,§], Douglas D. Richman, M.D.[10,27,29], Nu Zhang, Ph.D.[1,3], Brian K. Agan, M.D.[30,31,§], Robert A. Clark, M.D.[1,2,3], Grace C. Lee, Ph.D.[32,33], Weijing He, M.D.[1,4]

[1]Veterans Administration Research Center for AIDS and HIV-1 Infection and Center for Personalized Medicine, South Texas Veterans Health Care System, San Antonio, TX, USA 78229

[2]Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA 78229

[3]Department of Microbiology, Immunology & Molecular Genetics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA 78229

[4]The Foundation for Advancing Veterans' Health Research, San Antonio, TX, USA 78229

[5]Infectious Disease Clinical Research Program, Uniformed Services University of Health Sciences, Bethesda, MD, USA 20814

[6]Infectious Disease Service, San Antonio Military Medical Center, Fort Sam Houston, TX, USA 78234

[7]Centre for the AIDS Program of Research in South Africa (CAPRISA), Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa 4041
[8]Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya 30197
[9]Istituto di Ricerca Genetica e Biomedica (IRGB), CNR, Monserrato, Italy 09042
[10]Department of Medicine, University of California, San Diego, CA 92903.
[11]HIV Neurobehavioral Research Center Antiviral Research Center, University of California, San Diego, CA, USA 92903.
[12] Transplantation Research Immunology Group, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom OX1 2JD
[13]Oxford Kidney Unit, Churchill Hospital, Oxford, United Kingdom OX3 7LE
[14]Department of Physiology, Michigan State University, East Lansing, MI, USA 48824,
[15]Emory Vaccine Center and Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA. 30329
[16]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA 30322
[17]Center for Global Health and Diseases, Case Western Reserve University, Cleveland, Ohio, USA 44106
[18]Laboratory of Molecular Immunopharmacology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223
[19]Key Laboratory of Animal Models and Human Disease Mechanisms, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223
[20]Infectious Disease Service, Tripler Army Medical Center, Honolulu, HI 96859
[21]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, MB, Canada R3T 2N2
[22] Department of Genetics, University of North Carolina, Chapel Hill, North Carolina, USA 27599.
[23]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA 98109
[24]Department of Global Health, University of Washington, Seattle, Washington, USA 98195

[25] Carolina Vaccine Institute, department of Genetics, and Microbiology and Immunology, The University of North Carolina School of Medicine Chapel Hill, NC, USA 27599
[26]Department of Infectious Diseases, Alfred Health, Central Clinical School, Monash University, Melbourne, Victoria 3000, Australia
[27]Veterans Affairs San Diego Healthcare System, San Diego, CA, USA 92161
[28]Dipartimento di Scienze Biomediche, Universita di Sassari, Sassari, Italy 07100
[29]Department of Pathology, University of California San Diego, CA, USA 92903.
[30]Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA, 20814
[31]The Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, Maryland, USA 20817
[32]College of Pharmacy, The University of Texas at Austin, Austin TX, USA 78712
[33]Pharmacotherapy Education and Research Center, School of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA 78229

*Contributed as second authors
§Key cohort contributors
¶Address correspondence to: ahujas@uthscsa.edu

Abstract

In epidemiologic, immunologic, and genetic analyses of 16,217 people, 279 non-human primates, and 334 mice, we identified age-independent immunosenescence (AIIS), a process concurrent with but distinct from age-dependent immunosenescence (ADIS). AIIS is the consequence of an evolutionarily conserved response to antigenic stimulation characterized by unrestrained expansion of CD8+ relative to CD4+ T-cells. At any age, AIIS correlates with lower immunologic integrity, and its immunologic hallmarks (e.g., CD28−CD8+ T-cells) have been misattributed to ADIS. AIIS is potentially reversible with attenuation of antigenic stimulation, including that associated with risk factors for HIV-1 infection, as well as HIV viremia. AIIS precedes and contributes to some age-related conditions (e.g., inflammaging, cancer) as well as HIV and possibly cytomegalovirus (CMV) seroconversion. Persons vary in their proclivity to develop AIIS; hence, among individuals at risk for HIV and CMV seroconversion, persistent seronegativity for these viruses is an indicator of AIIS resistance. A polymorphism in the major histocompatibility locus correlates with AIIS resistance. At all ages, most females resist AIIS; in older persons, ADIS is more prevalent in females. Thus, we have defined a novel framework for conceptualizing (1) immunosenescence, as discriminating between AIIS and ADIS may help to elucidate proximate determinants of lifespan and disease risks, as well as sex-specific differences in these outcomes, and (2) cause/effect relationships that shape risk/severity continua, as it is not risk factors for HIV infection *per se* that drive the epidemic; rather, persons with lower immunologic integrity attributable to AIIS susceptibility are the primary candidates for HIV acquisition and worse outcomes post-infection.

Introduction

From birth, persons are subject to repetitive, low-grade, transient antigenic challenges (e.g., viral infections). A potential long-term immunologic consequence of these challenges could be a progressive erosion of immunologic integrity over time (immunosenescence). However, lower immunologic integrity is also observed in younger individuals experiencing chronic, high-grade antigenic stimulation (e.g., HIV infection). Additionally, many younger HIV-seropositive (HIV+) persons manifest diseases/conditions commonly observed in older, HIV-seronegative (HIV–) adults (e.g., cancers, atherosclerosis) (1, 2). These observations suggest that chronic antigenic stimulation associated with HIV infection and repetitive, transient antigenic stimulation during aging may share an immunosenescence-mediating pathway independent of age. We therefore hypothesized that immunosenescence may be attributable to two distinct, but concurrent, processes (**Figure 1A**): (i) an age-dependent process, henceforth termed *age-dependent immunosenescence* (ADIS), and (ii) an immunologic program activated in response to antigenic stimulation, mediating a form of immunosenescence independent of age, henceforth termed *age-independent immunosenescence* (AIIS). We posited that ADIS is irreversible, whereas AIIS is potentially reversible with cause-specific cessation of antigenic stimulation and, because the processes are concomitant, some disease and mechanistic correlates of AIIS have been misattributed to ADIS.

We developed the concept of AIIS based on the principle of allostasis, defined as maintaining homeostasis through adaptive changes in response to stress and environmental influences (3, 4). Because humans experience repetitive antigenic

challenges, successful recalibration of immune function (immune allostasis) in response to antigenic stimuli is crucial. AIIS was considered as an immunologic manifestation of increasing accumulation of host antigenic burden resulting from unsuccessful immune allostasis and therefore correlated with lower immunologic integrity (**Figure 1B**). Conversely, we defined successful immune allostasis as the ability to adjust to antigenic stimulation by limiting accumulation of host antigenic burden and preserving immunologic integrity (**Figure 1B**). Throughout life, individuals may toggle between successful and unsuccessful allostasis (**Figure 1B**), predicated on (i) extrinsic factors, i.e., causes, intensity, and chronicity of antigenic stimulation, and (ii) intrinsic factors, i.e., host characteristics (sex, genetics) that may influence susceptibility to activation of the AIIS program.

We envisaged that the concept of immune allostasis is a parsimonious explanation of three immunologic constructs: (i) the female survival advantage (5-7); (ii) inflammaging, or low-grade, sterile inflammation in older persons (1, 8-11); and (iii) accelerated or premature aging, reflecting age-associated conditions/traits in younger persons experiencing increased antigenic stimulation (1, 2). Females usually manifest traits indicative of greater immunocompetence, including longer lifespan, better vaccine responses, and resistance to coronary artery disease and certain cancers (5-7, 12-19). We posited that these traits reflect successful immune allostasis. In this model, despite experiencing similar levels of antigenic stimulation, females are more likely than males to resist development of AIIS. This resistance may reflect an evolutionary survival strategy,

both enabling women to reach childbearing age with greater immunocompetence and mitigating the risks of lower immunologic integrity on fetal health.

Inflammaging is widely viewed as a key determinant of age-associated conditions and mortality (1, 2, 8-11). We posited that inflammaging reflects the additive effects of impaired immune allostasis over a lifetime, manifesting as AIIS in older persons, whereas premature aging reflects development of AIIS at a younger age. Thus, the principles of immune allostasis predict that: (i) chronologic age is an imperfect proxy for antigenic experience and (ii) those with a proclivity to develop AIIS (AIIS susceptibility) may have a heightened risk of diseases and conditions associated with lower immunologic integrity. In this scenario, the disease/condition (effect) is a proxy or indicator/biomarker for persons with this proclivity (cause). However, if cause and effect are inappropriately reversed, the disease/condition may be mistakenly considered the cause of unsuccessful immune allostasis.

As primary examples in which reversal of cause and effect may confound our understanding of disease pathogenesis, lower immunologic integrity tracked by AIIS may precede and contribute to cancer development and cytomegalovirus (CMV) or HIV seroconversion. Hence, in some instances, cancer development may be an indicator of AIIS susceptibility. Similarly, while CMV and HIV seropositivity are viewed as proximate contributors to inflammaging (11, 20-22) and premature aging (1, 2), respectively, CMV and/or HIV seropositivity vs. persistent seronegativity may be indicators/biomarkers of individuals who are more likely to have AIIS susceptibility vs. resistance, respectively

(**Figure 1C**). In HIV– individuals, CMV seropositivity has been associated with inflammaging (1, 11, 20-22), many age-associated diseases/conditions (e.g., cardiovascular diseases), adverse outcomes in critically-ill individuals, and increased mortality (20-32); with reversal of cause and effect, these associations may be attributable to a subset of CMV+ persons with AIIS susceptibility (**Figure 1C**).

To test our hypothesis, we developed laboratory metrics of immunologic integrity that distinguish AIIS and ADIS. We applied them to studies of humans from worldwide populations (age 6-102 years) with and without HIV or CMV seropositivity, non-human primates (age 2-28 years) with and without seropositivity for simian immunodeficiency virus (SIV), and mice from the Collaboratory Cross panel (33) with and without Ebola virus infection (34) (**Figure 1D, Table S1**). We coupled this investigation with large-scale immunophenotyping studies to identify immunologic traits that track AIIS vs. ADIS.

RESULTS

Immune Health Grade (IHG): characteristics

We considered the equilibrium between levels of peripheral blood absolute CD8+ and CD4+ T-cell counts (CD8-CD4 equilibrium; **Figure 1E**) as a critical metric of immune allostasis because: (i) T-cells are essential for immune function (35-37) and (ii) CD8+ T-cell expansion, with or without a decline in CD4+ T-cells, characterizes immune response to varied antigenic challenges, especially viral infections (24, 38-41). We considered preservation of CD8-CD4 equilibrium vs. progression to CD8-CD4 disequilibrium as signs of successful vs. unsuccessful immune allostasis; in turn, equilibrium and disequilibrium

reflected lower vs. higher accumulation of host antigenic burden accumulated, correlating with higher and lower immunologic integrity, respectively (**Figure 1E**).

To gauge CD8-CD4 equilibrium, we co-indexed concomitant measures of the CD4:CD8 T-cell ratio and CD4+ counts and derived IHGs (**Figure 1F**). The cutoff levels (CD4:CD8 ratio ≥1.0 vs. <1.0; CD4+ count ≥800 vs <800 cells/mm^3) were selected *a priori* based on the following rationale. An inverted CD4:CD8 T-cell ratio was used as a cutoff parameter because it reflects CD8+ expansion to levels that, unless compensated for by a concomitant increase in CD4+ levels, correlate with impaired immune health in HIV– persons (42-50). Most HIV+ persons present with an inverted ratio (50-52). The CD4+ cutoff level was based on our finding that the lower interquartile bounds of the median CD4+ count in nearly 16,126 HIV– persons was approximately 800 cells/mm^3 (median [interquartile range, IQR]: 952 [840-1,036 cells/mm^3]; **Supplementary Methods** and **Table S2**) (53, 54).

Figure 2A lists IHG features in the 3896-person, community-based cohort from Sardinia (SardiNIA cohort: age 18-102 years, 42.8% males) (55). IHG-I and IHG-II reflect CD8-CD4 equilibrium; they correlated with lower levels of CD8+ T-cells in persons with higher (IHG-I) or lower (IHG-II) CD4+ counts (**Figure 1E,F** and **2A**). In contrast, IHG-III and IHG-IV reflect CD8-CD4 disequilibrium; they correlated with higher levels of CD8+ T-cells in persons with higher (IHG-III) or lower (IHG-IV) CD4+ T-cells (**Figure 1E,F** and **2A**). Categorization to IHG-III or IHG-IV indicated unsuccessful immune allostasis expressed as AIIS (**Figure 1E,F** and **2A**).

While both the CD4+ count and ratio are commonly used to gauge immune health (49, 50, 52, 56), their use vs. IHGs may result in confounding (**Figure 2A**; **Table S3)**. Despite similar median ratios in IHG-III and IHG-IV (median 0.87 and 0.82, respectively), these grades tracked significantly different levels of CD8+ and CD4+ counts (**Figure 2A**). Both IHG-I and IHG-III had higher CD4+ counts, and the modest differences between them (median 1154 vs. 988 cells/mm^3) would not be considered clinically meaningful. However, IHG-III was characterized by nearly twice as many CD8+ T-cells as IHG-I (median 1195 and 525 cells/mm^3, respectively; **Figure 2A**). Thus, we posited that CD8-CD4 equilibrium status may provide a more precise gauge of immune health.

CD8-CD4 equilibrium shifts with age

In each age stratum of the SardiNIA cohort until 80 years, IHG-I was the most common grade (**Figure 2B**, top), suggesting that other IHGs emerge from this primary level. IHG-II was the second most common grade. Three CD8-CD4 equilibrium features were observed with age (**Figure 2B**).

Feature 1. With age, the prevalence of IHG-I declined, with a reciprocal increase in IHG-II (**Figure 2B,** top; **Figure 2C**, left). IHG-II was marked by the lowest levels of CD8+ T-cells and lower levels of CD4+ T-cells (**Figure 2A**). Thus, a switch from IHG-I to IHG-II may reflect an adaptive response aimed at achieving successful immune allostasis, with proportionate declines in levels of CD8+ and CD4+ T-cells to preserve CD8-CD4 equilibrium. Hence, an IHG-I to IHG-II switch reflects suppression of CD8+ expansion in

the face of lower CD4+ T-cell counts (CD8+ suppressors), whereas preservation of IHG-I reflects restrained expansion of CD8+ T-cells in the face of higher CD4+ counts (CD8+ restrainers; **Figure 2A**).

Feature 2. With age, the prevalence of IHG-III and IHG-IV increased (**Figure 2B,** top; **Figure 2C**, right). Within each age stratum, these two IHGs were significantly more common in males (**Figure 2B**, top; **Figure 2D**; **Figure S1**).

Feature 3. A comparison of older and younger persons with IHG-I and IHG-II revealed that age associated with a disproportionately greater loss of CD8+ vs. CD4+ T-cells (**Figure 2B**, bottom left; **Figure S1**). This disproportionality accounted for the higher ratio values in older persons preserving IHG-I or IHG-II status compared with their younger counterparts (**Figure 2B**, bottom; **Figure S1**).

The age-associated shifts in CD8-CD4 equilibrium indicate that comparing older and younger persons without regard to equilibrium status is prone to confounding. First, such a comparison conflates four IHGs whose distributions vary significantly by age (**Figure 2B,C**). Second, IHG-III and IHG-IV are more prevalent in males (**Figure 2B**,**D**; **Figure S1**). Third, because the prevalence of IHGs with an inverted ratio (IHG-III or IHG-IV) also increases with age, the aggregated ratio values of all IHGs result in a lower CD4:CD8 ratio value in older persons, giving the impression that a lower ratio is a feature of aging (**Figure 2E**). This age-associated decline in aggregated ratio values has been attributed to inflammaging or the immune risk profile observed in some older persons (28, 42-44,

47, 50). However, this is a misattribution, as the ratio is higher in most older persons attributable to an asymmetrically greater decline in CD8+ than CD4+ T-cells with age (**Figure 2B**, bottom; **Figure S1**). Collectively, our results (**Figure 2**) suggest three points: (i) prominent shifts in CD8-CD4 equilibrium occur with age, (ii) at all ages, males appear to be more susceptible to manifest AIIS (IHG-III or IHG-IV), and (iii) assessment of CD8-CD4 equilibrium metrics may serve as a more precise metric to map the trajectory of immune status than absolute CD4+ and CD8+ T-cell counts or ratio values alone.

CD8-CD4 equilibrium states: a continuum

Declines in CD8+ and CD4+ T-cell counts are not a prominent feature of healthy, younger adults (**Figure S1**). Hence, a higher CD4:CD8 ratio value has different connotations depending on age. In a younger person (e.g., <40 years) with IHG-I or IHG-II, a higher ratio may reflect preservation of the capacity to suppress or restrain CD8+ T-cell expansion in response to antigenic exposure, signifying elite CD8+ suppressor/restrainer status (henceforth, elite CD8+ status). In older persons, a higher ratio reflects a disproportionately greater loss of CD8+ than CD4+ T-cells, signifying an “aged” CD8-CD4 equilibrium or ADIS. Hence, to mitigate confounding, we developed a strategy to categorize the spectrum of CD8-CD4 equilibrium across the lifespan (**Figure 3A**).

In the SardiNIA cohort, the median ratio for IHG-I was 2.51 vs. 2.10 in persons ≥70 vs. <40 years of age, respectively (**Figure 2B**, bottom left). For stringency, we considered a CD4:CD8 T-cell ratio ≥2.50 in persons older than 40 years with IHG-I and IHG-II as an indicator of ADIS (**Figure 3A**). In our literature survey of 13,703 HIV− persons worldwide,

the median CD4:CD8 ratio was 1.76 (IQR: 1.57-2.04; **Table S2**). Using 1.75 as a focal point, we defined IHG-I or IHG-II with a ratio between 1.0 and <1.75 as incipient AIIS, irrespective of age (**Figure 3A**). Thus, we categorized younger individuals (<40 years) into three equilibrium states: (i) full-scale AIIS (IHG-III or IHG-IV); (ii) incipient AIIS (IHG-I or IHG-II and ratio values between 1.0 and <1.75); and (iii) elite CD8+ status (IHG-I or IHG-II with greater suppression/restraint of CD8+ T-cell expansion, resulting in ratio values ≥1.75) (**Figure 3A**). However, older individuals fall into 4 equilibrium states: (i) full-scale AIIS, (ii) incipient AIIS, (iii) elite CD8+ status (individuals preserving IHG-I or IHG-II with ratio values between 1.75 and <2.50), and (iv) ADIS, i.e., preservation of IHG-I or IHG-II but with ratio values ≥2.50 (**Figure 3A**). Hence, in all age ranges, preservation of elite CD8+ status may track individuals with superior immunologic integrity.

We then computed density plots to illustrate these equilibrium states, with the abscissa signifying CD8-CD4 equilibrium states (full-scale and incipient AIIS, elite CD8+ status, and ADIS depending on age) and the ordinate signifying the relative proportions of these states. In the SardiNIA cohort (**Figure S2**) and for replication, in a separate HIV– population from the University of California, San Diego (HIV– UCSD) (n = 759; 66.4% males; median age: 39 years, IQR: 30-48), the contours of the density plots were similar and indicate that the distribution of equilibrium states in younger (<40 years) and older individuals differed (**Figure 3B**)**.** The plots overlapped in persons with ratio values less than 1.0 and intersected at a ratio cutoff of 2.5. The intersection of the density plots at a ratio of 2.5 supports our use of this cutoff in older persons preserving IHG-I and IHG-II as an indicator of ADIS. Paralleling the density plots, in both cohorts, the number of

individuals older than 40 years with IHG-I or IHG-II with ratio values ≥2.50 doubled (**Figures S2,S3**). Both younger and older persons could have full-scale AIIS, whereas incipient or elite CD8+ status is more common in younger persons. Among older persons, the density plot was skewed right for higher ratios (≥2.5) indicating ADIS. (**Figure 3B**; **Figures S2,S3**).

Our study hypothesis predicts that equilibrium states are conditional on historical and ongoing antigenic experiences and susceptibility to develop AIIS, irrespective of age and CMV and/or HIV serostatus. To test this concept, we evaluated data from primary and early HIV infection cohorts, before and after HIV viremia-associated antigenic stimulation was suppressed with antiretroviral therapy (ART) (**Figure 3C-E**; **Table S4**). HIV was a potent inducer of AIIS. Leftward skewing of the density plots, signifying a shift in CD8-CD4 equilibrium toward full-scale AIIS, was apparent within 2 weeks of the estimated date of infection and became progressively worse as the duration of infection increased (**Figure 3C**). However, in persons whose HIV viral load was suppressed by ART for at least 8 years, there was a step-wise shift toward incipient AIIS with each successive 2-year window of ART (**Figure 3D**).

We found a significant overlap in equilibrium profiles of HIV– and some aviremic HIV+ persons, supporting our proposition that equilibrium states are predicated on historical and ongoing antigenic stimulation rather than age and HIV and/or CMV serostatus (**Figure 3E**). The greatest overlap between HIV– and aviremic HIV+ persons occurred in the category of incipient AIIS.

These findings support our study hypothesis, as they showed that AIIS is (i) an inducible (**Figure 3C**) and potentially reversible (**Figure 3D,E**) trait correlating with severity and chronicity of antigenic stimulation and (ii) a manifestation of accumulated host antigenic burden. While AIIS is reversible, depending on the severity and chronicity of the original source(s) of antigenic stimulation, it may take several years to reduce the levels of host antigenic burden indexed to level of CD8-CD4 disequilibrium (**Figure 3D**).

Figure 3F highlights two points. First, equilibrium states show sexual dimorphism: (i) akin to full-scale AIIS (**Figure 2B,2D**), incipient AIIS is more common in males, (ii) females appear to be more successful in resisting both incipient and full-scale AIIS and therefore preserving elite CD8+ status, and (iii) the marker of ADIS is more common in females. Second, equilibrium status in older individuals may be skewed toward ADIS or full-scale AIIS.

Immunologic traits differentiating AIIS vs. ADIS

The abovementioned findings (**Figure 3**) indicate that age can be used as a proxy for ADIS, whereas it is an imprecise proxy for AIIS. With this viewpoint, we next sought to identify immunologic traits that track AIIS vs. age by comparing the levels of 75 immunologic traits in SardiNIA participants (55) according to age (i.e., <40 vs. ≥70 years in persons with IHG-I or IHG-II, adjusting for sex) and IHG status (IHG-III vs. IHG-I and IHG-IV vs. IHG-II) (**Figure 4A**, **Figures S4-S7, Table S5**). Traits that varied to a greater extent by age in persons preserving IHG-I and IHG-II but did not differ by IHG status for

both comparisons were categorized as representing age-related or ADIS traits, whereas traits that varied to a greater extent by IHG status but did not differ by age (<40 vs. ≥70 years) in persons with IHG-I or IHG-II were categorized as being related to AIIS (**Figure 4A**, top). To identify the AIIS-specific traits, we mitigated confounding by CD4+ count, sex, and age, by comparing IHGs with similar levels of CD4+ T-cells (i.e., IHG-III vs. IHG-I and IHG-IV vs. IHG-II) and adjusting for sex and age.

Depending on whether the immunologic traits differed by age and/or IHG status, four groups emerged (**Figure 4A**, top; **Figure S8**; **Table S5**). Within each group, predicated on directionality of the traits (greater or lower proportions by age and/or IHG status), the traits subgrouped into signatures: (Group i) 13 traits, subgrouped into signatures 1-4, classified as AIIS; (Group ii) 22 traits, subgrouped into signatures 5-8, representing ADIS; (Group iii) 10 traits, subgrouped into signatures 9-12, showed associations with both AIIS and age, albeit in some instances, the directionality of the trait levels showed opposite patterns in AIIS vs. age; and (Group iv) 30 traits, subgrouped into signatures 13-19, did not differ by age or IHG status and were classified as neutral (**Figure 4A,** top). All signatures/traits are provided in **Table S5** and representative signatures are depicted in **Figure 4A (**bottom).

AIIS-specific traits higher in IHG-III or IHG-IV included natural killer (NK) T-cells, CD8+NKT-like cells, $CD127^{-}CD8^{bright}$ T-cells (effector-memory), $CD25^{++}CD8^{bright}$ (activated/proliferating) T-cells, and $CD28^{-}CD8^{dim}$ (senescent/terminally differentiated) T-cells (e.g., **Figure 4A**, signature 1; **Table S5**). The only AIIS trait lower in IHG-III and IHG-

IV was naïve-transitioning T-cells ($CD4^{+}CD45RA^{+}CD25^{hi}$, not Tregs) (signature 2; **Table S5**).

Traits representing ADIS (e.g., signatures 5-8 and lower levels of signature 10 traits) included increased proportions of memory subsets within the CD4+ T-cell compartment (except for the central memory subset) and decreased proportions of naïve CD8+ T cells (signature 6; **Figure 4A**), B cells (CD19+), and plasmacytoid DC (CD123+CD11c-). While some traits showed similar directionality with AIIS and age, others had opposite patterns. $CD28^{-}CD8^{bright}$ T-cells are viewed as a hallmark of aging (57, 58); while levels of these cells were higher with both age and AIIS status, they were disproportionately higher with AIIS (**Figure 4A**, signature 9). Naïve CD4+ T-cells were lower with both age and AIIS status (**Table S5**). However, levels of $CD28^{-}CD25^{++}CD127^{-}CD8^{bright}$ T-cells, likely representing regulatory CD8+ T-cells (59, 60), were higher with AIIS but lower with age (**Figure 4A**, signature 10); a similar discordant pattern was observed with $CD28^{-}CD25^{++}CD8^{bright}$ T-cells (**Table S5**). Neutral traits included mature dendritic cell (CD86+), monocytes, and some Treg subsets (e.g., **Figure 4A**, signature 13; **Table S5**).

Thus, traits representing ADIS (e.g., signatures 5-8 and lower levels of signature 10 traits) may reflect age-associated physiologic involution of the immune system (e.g., loss of naïve CD8+ T-cells). By contrast, AIIS is characterized by expansion of regulatory-like, effector, and terminally differentiated CD8+ T-cells, senescent CD8+ T-cells, CD8+ Tregs, CD8+NKT-like cells, and NK T-cells (e.g., signatures 1-4 and higher levels of signature 10 traits). Irrespective of age, expansion of these cell populations is common in

individuals experiencing antigenic stimulation and manifesting lower immunologic integrity, e.g., during chronic viral infections, transplant rejection, and some cancers (39, 41, 57, 58, 61-63). These findings support our hypothesis that immunologic traits of AIIS reflect the consequences of increased antigenic experience. However, since age is an imperfect proxy for antigenic experience, AIIS traits are also commonly observed in older persons and misattributed to age-specific processes.

Evolutionary conservation of AIIS

In sooty mangabeys (64) and Chinese rhesus macaques (65), we found that AIIS and its immunologic traits were evolutionarily conserved (**Figure 4B**; **Figure S9**). The median (IQR) ages of SIV− and SIV+ sooty mangabeys and SIV− rhesus macaques were 10 (6-13), 13 (10-16), and 11 (6-18) years, respectively. In sooty mangabeys, natural infection with SIV is an additional cause of AIIS; thus, they are an ideal experimental model to evaluate outcomes (AIIS prevalence) associated with a single source (environmental influences other than SIV) vs. two sources (non-SIV plus SIV) of antigenic stimulation.

Two sources of antigenic stimulation had additive effects on AIIS rates (**Figure 4B**, leftmost): IHG-III or IHG-IV was present in 69% of SIV+ vs 42% of SIV– sooty mangabeys (odds ratio [OR]=3.09; 95% CI=1.55-6.17; *P*=0.001); nearly 50% of SIV– rhesus macaques had IHG-III or IHG-IV (**Figure S9**). The higher rates of AIIS in SIV– animals than HIV– humans likely relate to the animals' greater exposure to antigenic challenges (64). In SIV+ monkeys, SIV viral loads were higher in the AIIS compared to the AIIS-free group (**Figure 4B**, left). Thus, while viremia-associated antigenic stimulation contributes

to development of AIIS, some animals manifested high-grade AIIS resistance, remaining AIIS-free both before and after SIV infection (**Figure 4B**, leftmost).

As in humans, (i) in sooty mangabeys and rhesus macaques, IHG-III and IHG-IV were more common in males, irrespective of SIV serostatus; (ii) emergence of AIIS was not dependent on aging, as it was evident in nearly 25% of younger SIV– sooty mangabeys (age 3-5 years); (iii) AIIS rates increased progressively with age in a proportionately similar manner in both SIV– and SIV+ animals; and (iv) IHG-III and IHG-IV in sooty mangabeys was associated with immunologic traits akin to those found in HIV– humans with AIIS, i.e., lower levels of $CD127^{+}CD8^{+}$ and higher levels of $CD28^{-}CD95^{+}CD8^{+}$ effector T-cells (**Figure 4B**; **Table S6**). Proportions of these cell types differed to a greater extent by AIIS status than age (**Figure S9**; **Table S6**). In sooty mangabeys, IHG-III and IHG-IV were also associated with other traits noted with reduced immunologic integrity (lower levels of CCR7+ and CXCR4+ bearing CD8+ T-cells (66); **Figure S9**). In rhesus macaques, expression of programmed cell death protein 1 (PD-1) on T-cells, a marker of T-cell dysfunction (67), was progressively higher with worsening IHG status (**Figure S9**). We observed similar traits of AIIS in treated or untreated HIV+ persons with IHG-III or IHG-IV (Okulicz et al, submitted). Thus, immunologic traits of AIIS appeared to be conserved in humans and non-human primates, irrespective of HIV and SIV serostatus.

AIIS inducers and outcomes

We next sought to identify inducers of and clinical outcomes associated with AIIS in HIV– humans, both in our cohorts and in the literature (**Figure 5A**; complete reference list in

Table S7). Because individual measures of CD8+ and CD4+ levels were infrequently reported, we used a CD4:CD8 T-cell ratio <1.0 as an indicator of IHG-III or IHG-IV (AIIS; **Figure 1F**) in analyzing previously published data.

In HIV– persons, non-infectious sources of antigenic stimuli (e.g. impure clotting factors) were associated with higher rates of AIIS (**Figure 5A**). AIIS rates were elevated (≥20%) in settings with higher microbial exposure (e.g., pediatric hospital, Pakistan, Ethiopia (68-71)) and in HIV– persons with behavioral (72-78) and nonbehavioral (70, 71, 79-84) risk factors for HIV infection. Tetanus toxoid vaccination was associated with a trend for temporary induction of AIIS in approximately 36% of otherwise healthy persons (85).

These data also support our hypothesis that AIIS rates relate to antigenic exposure: in developed countries with lower microbial burdens, rates increased gradually with age, whereas in regions with greater microbial burdens, rates were higher and AIIS occurred at an earlier age (**Figure 2B,5A**). Again, within each age stratum, the prevalence of AIIS was significantly higher in males than females (**Figure 2B,5A**). In HIV– persons, AIIS associated with increased mortality (42, 43), a trend for reduced cognitive function (28, 42), rapid progression of leukemia (45, 46), and a trend for lower influenza vaccine responsiveness, including in younger adults (44, 48) (**Figure 5A**). Our hypothesis of evolutionary conservation was again supported, as 22% of SIV– chimpanzees had evidence of AIIS (**Figure 5A**) (86).

Infectious diseases/schistosomiasis and AIIS

Helminthic infections appeared to be a potent inducer of AIIS (**Figure 5A**). In HIV– children from Kenya (87), higher egg counts of *Schistosoma haematobium* in the urine were associated with greater AIIS prevalence rates and peripheral blood CD25+CD127– CD4+ T-cells (**Figure 5B**). CD8+ T-cells with similar markers correlated with AIIS (**Figure 4A,B**; **Table S5**). Nearly 86% of Kenyan children with higher urine egg counts had AIIS (**Figure 5B**). Other infectious diseases (e.g., tuberculosis, malaria) also correlate with a lower CD4:CD8 ratio (88, 89) suggestive of incipient AIIS.

AIIS and immunologic integrity: cancer risk

Solid organ transplant recipients have a nearly 65- to 100-fold increased risk for recurrent cutaneous squamous cell carcinoma (CSCC) (90). Previously, we evaluated an HIV– cohort of long-term renal transplant recipients to identify immune correlates of CSCC (91). Herein, we evaluated whether AIIS contributes to rate of cancer recurrence. About 18% of this cohort had developed AIIS: IHG-II was the most prevalent IHG and female renal transplant recipients were more successful in resisting AIIS (**Figure 5C**, upper left). Thus, we observed parallels in the adaptation to antigenic stimuli in renal transplant recipients and older persons, i.e., preservation of IHG-II status (compare **Figure 2B**, top vs. **5C**, upper left). This adaptation likely involves restricting CD8+ expansion to maintain CD8-CD4 equilibrium when CD4+ T-cells are declining.

When we dichotomized renal transplant recipients by AIIS status at baseline, second occurrence of CSCC was greater in those with baseline AIIS (**Figure 5C**, upper right). Median age and duration of immunosuppression did not differ by AIIS status (**Table S8**).

We previously identified elevated levels of CD57+CD8+ T-cells as an independent determinant of recurrent CSCC (91). This immunologic trait commonly correlates with markers in primates with AIIS (58) (e.g., **Figure 4B**). CD57+CD8+ T-cells showed a gradient pattern, with the highest levels in those with full-scale AIIS (**Figure 5C**, middle left). CMV seropositivity rates mirrored the distribution of %CD57+CD8+ T-cells (**Figure 5C**, middle right; **Figure S10**). Hence, a possible interpretation of our findings is that renal transplant recipients resisting full-scale AIIS were also more successful in resisting (i) recurrent CSCC, (ii) CMV seroconversion, and (iii) expression of immunologic traits more prevalent in persons with CSCC (model, **Figure 5C**, bottom). In this interpretation, lower immunologic integrity linked to AIIS may antedate and possibly contribute to cancer development. To further support this link, we evaluated the association of AIIS with Ebola virus outcomes in mice (**Figure 5D**) and AIDS in HIV+ persons (**Figure 5E**) discussed below.

AIIS and immunologic integrity: Ebola infection in mice

We focused on the Collaborative Cross-RIX mice, a large panel of recombinant, inbred intercrosses (RIX) designed for complex trait analysis (33). Based on median values of splenic CD4+ and CD8+ counts, we derived IHGs for 334 mice. At baseline, 48.5% (*n*=162), 38.3% (*n*=128), 1.5% (*n*=5), and 11.7% (*n*=39) were IHG-I, IHG-II, IHG-III, and IHG-IV, respectively. We evaluated the subset of the Collaborative Cross-RIX mice infected with Ebola virus (*n*=99) (34); post-infection, mice strains were classified as resistant, partly resistant, and lethal. Among the strains of mice with lethal infection, there

was an overrepresentation of strains whose uninfected counterparts had IHG-III or IHG-IV (AIIS) (**Figure 5D**).

IHGs and immunologic integrity: AIDS risk and summary

Preservation of IHG-I is likely an indicator of superior immunologic integrity, as there was a progressive, age-associated increase in prevalence of other grades (**Figure 2B**). Congruently, analysis of the early HIV infection cohort revealed that the hazard of progressing to acquired immunodeficiency syndrome was lowest in those preserving IHG-I after HIV infection (**Figure 5E**). Together, the features of the AIIS-specific immunologic traits as well as the association of full-scale AIIS with proxies for lower immunologic integrity (e.g., cancer, AIDS, vaccine nonresponsiveness, mortality; **Figure 5A,C**) suggested that, at any age, AIIS is an indicator of lower immunologic integrity.

CD8-CD4 disequilibrium and HIV seroconversion risk

HIV seropositivity rates are higher in persons with specific behavioral (e.g., intravenous drug use, condomless sex) and non-behavioral [e.g., helminthic infections (81, 82)] factors. Also, despite documented exposure to HIV, persons without risk factors infrequently seroconvert. This unique restriction of HIV-1 infection to groups of individuals with risk factors posed an epidemiologic conundrum. Since a feature common to these risk factors was increased antigenic stimulation, we posited that this conundrum was resolvable if individuals in these groups who acquire HIV shared a cause (increased susceptibility to develop AIIS/lower immunologic integrity)-effect (increased HIV risk) relationship (**Figures 1C, 5F**). Thus, in the posited cause-effect relationship, AIIS

susceptibility leading to development of incipient/full-scale AIIS correlates with an elevated risk of HIV seroconversion, whereas AIIS resistance leading to preservation of elite CD8+ status is a protective factor, affording resistance to HIV seroconversion. In support of this posit, AIIS rates were ≥12% in younger individuals with risk factors (e.g. ~27% in men who have sex with men and 90% of children with a severe helminthic infection) (**Figure 5A,B**). Additionally, there is extensive co-distribution of schistosomiasis and HIV in Africa (82, 83) (**Figure 5F**). In contrast, only older HIV– persons without risk factors manifested AIIS rates that approximated those in younger persons with risk factors (**Figure 5A**). For example, among older HIV– individuals (>60 years; n=2,561), AIIS rates were ~11% (**Figure 5A**).

To further support the posited cause-effect relationship, we analyzed data from 1,050 female sex workers (FSWs) from Kenya who were HIV– at study entry (baseline) (median [IQR] age, 31 [27-37] years) (92). During prospective follow-up, 127 women seroconverted. Behavioral risk factors and laboratory data to compute IHGs were available for 762 FSWs. To evaluate the association of baseline CD8-CD4 equilibrium status and future HIV seroconversion risk, we restricted analysis to 449 FSWs with at least two HIV seronegative tests performed at least 3 months apart (**Figure S11**; **Table S9**). Of these, 53 FSWs subsequently seroconverted (**Figure S11**). They were followed for 259.6 person-years before HIV seroconversion; 396 women who remained HIV– were followed for 1856.8 person-years. The median interval between enrollment (baseline) and subsequent HIV seroconversion was 3.26 (IQR, 0.89-5.74) years.

We examined the association of factors that correlate with behavioral risk (i.e., sexual behaviors) and biologic risk (i.e., risk markers for HIV acquisition) with (i) CD8-CD4 equilibrium states at baseline and (ii) rates of future HIV seroconversion (**Figure 6A**, top and bottom, respectively). At baseline, 15% of FSWs manifested AIIS. Neither AIIS nor HIV infection rates differed by duration of sex work (**Figure 6A**, top - far left). However, three proxies for behavioral risk factors (fewer condoms, more clients, more clients than condoms used) were associated with progressively higher AIIS rates (**Figure 6A**). For example, in FSWs reporting more clients than condoms used [Δclients-condoms (clients/per week – condoms/week) ≥6], nearly 30% had AIIS (**Figure 6A**). To derive a quantitative measure of behavioral risk, we computed a behavioral activity score (BAS, Supplementary Methods), which is scaled from –4 to 3+; in HIV– FSWs, a higher BAS was associated with progressively higher AIIS prevalence at baseline (**Figure 6A**, top).

Sexually transmitted infections (STIs) are biologic markers for risk of HIV acquisition. We evaluated direct (gonorrhea and syphilis) and indirect (vaginal discharge, abdominal pain, genital ulcer, dysuria, and vulvar itch) indicators of STIs (93). To derive a quantitative measure of biologic risk, we computed direct, indirect, and total STI scores based on the presence or absence of these indicators at baseline (**Figure 6A**, top far right; Supplementary methods). Higher baseline STI scores also correlated with higher baseline AIIS rates. Thus, reflecting that BAS and STI scores were proxies for level of antigenic stimulation as well as HIV exposure, higher scores associated with higher rates of both AIIS at baseline and subsequent HIV seroconversion (**Figure 6A**).

While there was a significant correlation between BAS and STI scores (P<0.001), the correlation coefficient was not high (Spearman r = 0.22, **Figure S12**). We posited that risk factors may induce AIIS through overlapping but distinct mechanisms. STIs may correlate with sporadic increases in antigenic stimulation coincident with infection, whereas the behavioral activities likely associate with more consistent (e.g., daily/weekly) antigenic stimulation related to blood, sperm, and semen exposures; both blood and sperm may induce allogenic immune responses or immunosuppression (94-100). Supporting our posit, behavioral and biologic risk factors had additive effects on leftward skewing of CD8-CD4 equilibrium toward incipient and full-scale AIIS (**Figure 6B**). The leftward skewing was more pronounced with BAS than STI scores, and maximal in FSWs with both higher baseline BAS and total STI scores. This leftward skewing may have contributed to HIV seroconversion, as both incipient and full-scale AIIS at baseline were overrepresented in FSWs who subsequently seroconverted (**Figure 6C**).

In addition to baseline equilibrium characteristics, shifts in equilibrium during prospective follow-up were associated with HIV seroconversion. Among FSWs who were HIV– for at least 6 years, the hazard of subsequent HIV seroconversion was greater in FSWs with a worse equilibrium status at baseline and year 6, or those who manifested a switch from better to worse equilibrium characteristics in this interval (i.e., switched from elite CD8+ status to incipient or full-scale AIIS, or from incipient to full-scale AIIS) (**Figure 6D**).

To support our premise that shifts in equilibrium states were directly attributable to antigenic stimulation associated with risk behaviors vs. stochastic factors, we examined

the reversibility of AIIS in FSWs who remained HIV– during longitudinal follow-up. During prospective follow-up, FSWs were encouraged to practice safe sex. In FSWs who remained HIV– and had both laboratory and risk behavior data available within each two-year interval for 10 years, paralleling a decline in the BAS, AIIS rates decreased from 33% at baseline to zero (**Figure 6E**, left two panels). Reconstitution of AIIS-free status was attributable mainly to lowering in CD8+ (P<0.001) counts as CD4+ counts remained unchanged (**Figure 6E**, right two panels; **Figure S12**), further supporting our hypothesis that AIIS is intrinsically linked to CD8+ T-cell expansion (**Figure 1E**). Substantiating these results, in 73 FSWs who remained HIV– for at least 6 years we observed that, during prospective follow-up: (i) BAS declined in the subset of FSWs for whom these data were available at baseline and in year 6 (n=49) (**Figure 6F**, inset), and (ii) CD8-CD4 equilibrium improved toward elite CD8+ status (**Figure 6F**; **Figure S13**).

AIIS resistance and HIV seronegativity

High-grade resistance to develop AIIS is highlighted by three findings. First, a subset of HIV– FSWs with the worst risk characteristics (e.g., higher BAS and total STI scores) preserved AIIS-free status (**Figure 6A**, top) and elite CD8+ status (**Figure 6B**). Second, in 43 FSWs in whom pre- and post-seroconversion CD8-CD4 equilibrium data were available (median interval between last HIV– and first HIV+ test, 8.3 months [IQR, 5.5-13.4 months]), a subset of FSWs preserved AIIS-free status (**Figure 6G**). Third, HIV+ FSWs and SIV+ sooty mangabeys shared characteristics: (i) pre-existing AIIS, (ii) mix of pre-existing and lentiviral-induced AIIS, and (iii) high-grade resistance to AIIS (compare **Figure 4B**, far left vs. **6G**). We therefore inquired whether preservation of elite CD8+

status despite high-grade antigenic stimulation (termed high-grade AIIS resistance) associated with lower HIV seroconversion risk, after controlling for HIV exposure (both level of antigenic stimulation and HIV exposures are proxied by BAS and STI scores).

HIV seroconversion rates were lowest in FSWs with elite CD8+ status, intermediate in those with incipient AIIS, higher in those with IHG-III, and highest in those with IHG-IV at baseline (**Figure 7A**; **Figure S11**). Additionally, we found that: (i) one cannot intuit CD8-CD4 equilibrium from the CD4:CD8 T-cell ratio because similar ratio values can arise from distinct equilibrium states, an observation consistent with results in other cohorts (e.g., **Figure 2A**) and (ii) equilibrium status, not absolute CD8+ or CD4+ count, associated with HIV seroconversion rates (**Figure 7A**).

In univariate analysis, three baseline characteristics increased the risk of HIV seroconversion by three-fold or greater: (i) higher BAS, (ii) higher STI score, and (iii) incipient or full-scale AIIS (**Figure 7B**, left). However, in multivariate analysis, higher BAS as well as incipient and full-scale AIIS, but not higher STI scores, were independently associated with increased HIV seroconversion rates (**Figure 7B**; **Table S10**). FSWs with a higher BAS had increased odds of acquiring HIV regardless of total STI scores (**Figure 7C**). The heightened influence of BAS on the odds of HIV seroconversion may relate to our finding that BAS was associated with greater skewing of CD8-CD4 equilibrium toward full-scale AIIS than STI scores (**Figure 6B**).

Among FSWs with similar BAS and STI scores, the odds of HIV seroconversion were lower in those who preserved elite CD8+ status vs. those who had incipient or full-scale AIIS at baseline (**Figure 7C**). While the aforementioned associations in FSWs were derived from 449 FSWs selected based on having at least two HIV seronegative tests at least 3 months apart, similar findings were observed in the 762 FSWs with a single HIV seronegative test and for whom BAS and STI data were available (**Figure S11**). Thus, taken together, these data suggest the equilibrium status at the time of HIV exposure associates with an HIV acquisition risk hierarchy: full-scale AIIS > incipient AIIS > elite CD8+ status.

Indicator function of HIV serostatus

Our survey of CD8-CD4 equilibrium and its disease associations (**Figures 2-6**) in varied epidemiologic settings indexed to diverse sources and grades of antigenic stimulation suggest that incipient/full-scale AIIS is an inducible trait that associates with lower immunologic integrity. Hence, elite CD8+ status with IHG-I and IHG-II is more likely to be a primordial equilibrium status that tracks superior immunologic integrity. In this framework, using antigenic stimulation experienced in association with sex work and HIV seroconversion as an outcome, our results support the following CD8-CD4 equilibrium-HIV risk model (**Figure 7D**). In response to comparable levels of antigenic stimulation associated with risk factors, otherwise healthy, young HIV– adults starting with elite CD8+ status may begin to manifest dichotomous phenotypes: AIIS susceptibility (diathesis to develop incipient or full-scale AIIS) vs. high-grade AIIS resistance associating with preservation of elite CD8+ status (**Figure 7D**). Chances of HIV seroconversion are lower

in those with elite CD8+ status at the time of HIV exposure (**Figure 7A-C**). The contributions of AIIS susceptibility vs. resistance to individual variation in risk of HIV acquisition may be substantial (**Figure 7D**). Approximately 65% of FSWs had incipient or full-scale AIIS at baseline; 87% of FSWs with these equilibrium states at baseline acquired HIV compared to only 13% of FSWs with elite CD8+ status (**Figure 7D**).

Taken together, our data support a possible cause (AIIS susceptibility/resistance)-effect (HIV susceptibility/resistance) relationship after controlling for level of risk factor-associated antigenic stimulation and HIV exposure. The association of incipient/full-scale AIIS vs. elite CD8+ status with higher vs. lower rates of HIV seroconversion provided the basis to propose that, in individuals with a high risk of exposure to HIV, HIV seropositivity and seronegativity are indicators of persons with AIIS susceptibility vs. high-grade AIIS resistance (**Figure 7D**). Post-infection, HIV viremia triggers full-scale AIIS, masking pre-infection AIIS status (**Figure 6G,7D**). However, regardless of HIV serostatus, AIIS is a potentially reversible trait (e.g., **Figure 6E,F** in HIV– FSWs and **Figure 3D,E** in HIV+ persons). Hence, cessation of antigenic stimulation attributable to HIV viremia or risk factors soon after HIV acquisition may allow for reconstitution of AIIS-free status (**Figure 7D**). Overall, in this model, incipient/full-scale AIIS elevates risk of acquiring HIV infection and contributes to post-infection outcomes.

Disease framework and indicator function of CMV serostatus

We posited that the framework of variable AIIS susceptibility-variable HIV risk model (**Figure 7D**) may extend to diseases/conditions other than HIV. In this model: (i) an

attribute of the host, i.e., lower immunologic integrity linked to susceptibility to develop incipient or full-scale AIIS, may antedate and contribute to development of diseases/conditions, (ii) after disease is established, antigenic stimulation or inflammation associated with disease may trigger the AIIS program, further skewing equilibrium toward full-scale AIIS, and (iii) with suppression of antigenic stimulation that antedated/triggered the disease and/or is consequence of disease, reconstitution of AIIS-free, or elite CD8+ status is feasible.

CMV seropositivity has been associated with mortality and a myriad of diseases (20-32, 101, 102). HIV and CMV infection share risk factors (103). These epidemiologic observations raised the possibility that akin to HIV serostatus, CMV serostatus has indicator functions of immunologic integrity linked to AIIS susceptibility/resistance, wherein, CMV seropositivity and seronegativity are indicators for AIIS susceptibility and resistance, respectively. With indicator function, some of the reported associations of CMV seropositivity may relate to a subset of CMV+ persons with heightened AIIS susceptibility vs. CMV infection *per se*. Hence, akin to the HIV model (**Figure 7D**), a tripartite model may be applied to CMV infection: (i) at time of exposure to CMV, chances of CMV seroconversion are greater in the presence of full-scale AIIS vs. AIIS-free status, predicting that CMV seropositivity is disproportionately overrepresented and underrepresented in persons with full-scale AIIS and elite CD8+ status, respectively, (ii) post CMV seroconversion, antigenic stimulation associated with CMV viremia (akin to HIV viremia; **Figure 3C**) further skews CD8-CD4 equilibrium toward full-scale AIIS, and (iii) skewing can be transient and reversed with mitigation of antigenic stimulation attributable

to CMV viremia [spontaneously/self-limiting or with therapy, akin to HIV viremia (**Figure 3D,E**)] or other causes. Hence, persistent incipient/full-scale AIIS in a CMV+ person may point to a host with a heightened susceptibility to activate the AIIS program in response to CMV viremia or other sources of antigenic stimulation vs. an attribute of CMV seropositivity *per se*. Contrarily, AIIS-free CMV+ persons may represent those who, subsequent to mitigation of antigenic stimulation, have re-attained AIIS-free status or individuals acquiring CMV with an AIIS-free status. We lacked a CMV seroconverting cohort to definitively establish this model; however, certain aspects of this model are supported by the following lines of evidence.

First, all renal transplant recipients with AIIS were CMV+ (**Figure 5C**) and, in the HIV– UCSD cohort, the hierarchy of the CMV seropositivity mirrored the HIV acquisition risk hierarchy: full-scale AIIS > incipient AIIS > elite CD8+ status (P<0.001 between ranks; compare **Figure 7E** vs. **7A**). Thus, CMV seropositivity was overrepresented vs. underrepresented in persons with AIIS vs. elite CD8+ status.

Second, associations attributed to CMV seropositivity may relate to AIIS susceptibility. Because CMV seropositivity increases with age, higher CD8+ levels in older persons have been attributed to CMV seropositivity (21, 22, 24, 104). However, rates of AIIS and CMV seropositivity increase with age (**Figure 5A**), and within each age stratum, CMV seropositivity rates were lower in those preserving elite CD8+ status (**Figure S14**).

Higher CD8+ T-cell levels in CMV+ persons is viewed as a hallmark characteristic of CMV seropositivity (21, 22, 104, 105). However, CD8+ levels are higher with AIIS (**Figure 2A,7A**). Hence, in CMV+ persons, the higher CD8+ levels linked to AIIS may be viewed as an attribute of the virus vs. the host, i.e., AIIS susceptibility. Consistent with the traditional viewpoint that CD8+ expansion is linked to CMV seropositivity, ranking of the HIV– UCSD cohort participants or HIV+ cohorts according to CD8+ levels in CD8-CD4 equilibrium states showed that groups with higher CD8+ levels also have higher rates of CMV seropositivity (**Figure 7E**; **Figure S11**). While overall median CD8+ levels were higher in CMV+ persons (**Figure 7A**), CMV serostatus explained a very small proportion of the variability of CD8+ levels in the overall HIV– UCSD cohort (r^2=0.08; *P*<0.001; n=644) or early HIV infection cohort (r^2=0.01; *P*<0.001; n=3791). Additionally, CMV seropositivity was observed across a range of median CD8+ levels linked to the CD8-CD4 equilibrium states and median CD8+ levels within the co-incident CD8-CD4 equilibrium states were not disproportionately higher in CMV+ persons (**Figure 7E**; **Figure S11**).

Furthermore, results presented (**Figure 3D-E**, **6E-F**) underscore that, regardless of CMV or HIV serostatus, incipient/full-scale AIIS may be transient. Additionally, while CMV seropositivity rates were highest in the CD8-CD4 equilibrium state with the highest CD8+ levels (IHG-III), such states can also be transient. FSWs are reported to have high rates of CMV seropositivity (up to 90%) (103). Our prospective evaluation of FSWs revealed that mitigation of antigenic stimulation linked to behavioral risk factors associated with reconstitution of AIIS-free status (**Figure 7F**). Contrary to the conventional cause (CMV

seropositivity)-effect (higher CD8+ levels) relationship, in the subset of HIV– FSWs with the highest CD8+ levels at baseline (IHG-III), levels were lower 6 years later (median: 1270 and 804 cells/mm^3). Thus, AIIS can be transient and mitigation of antigenic stimulation in FSWs associated with either preservation of IHG-I or a switch from worse grades (IHG-II, III, IV) to reconstitution of IHG-I, the most prevalent grade in younger adults (**Figure 7E** vs. **2B**).

Hence, dependent on when cross-sectional sampling is performed relative to level of antigenic stimulation, comparisons of persons according to CMV serostatus may result in misattributing features of the host (AIIS susceptibility) to the virus. To support this viewpoint, we evaluated the indicator functions of CMV serostatus and AIIS prevalence in the HIV– UCSD cohort with known recreational drug use status as a proxy for increased antigenic stimulation (106-108). Irrespective of drug use status, AIIS rates were lower in individuals with an indicator for AIIS resistance (CMV seronegativity) vs. an indicator for AIIS susceptibility (CMV seropositivity) (P<0.001; **Figure 7G**; **Figure S14**). Among persons with a positive urine test for recreational drugs, AIIS prevalence was higher in persons with an indicator for AIIS susceptibility (CMV seropositivity) vs. resistance (CMV seronegativity) (**Figure 7G**). Additionally, among persons with a proxy for AIIS susceptibility (CMV seropositivity), AIIS rates were higher in those with a positive urine test for recreational drugs. Taken together, these findings suggest that expression of AIIS and associated higher CD8+ levels in CMV+ persons is an indicator of a subset of persons with a heightened susceptibility to develop AIIS in response to ongoing antigenic stimulation vs. an invariant attribute of CMV seropositivity. This inference supports the

possibility that the reported associations of CMV seropositivity with higher CD8+ levels and diseases/mortality may relate to the subset of CMV+ with heightened AIIS susceptibility (**Figure 7E,G**).

HIV–, CMV–, and female sex: indicators of AIIS resistance

Taken together, the results presented thus far pointed to three proxies for or indicators of high-grade AIIS resistance: HIV seronegativity, CMV seronegativity, and female sex. Longitudinal birth cohorts were unavailable to test our posit that AIIS resistance vs. susceptibility contribute to a continua: differential disease risks, outcomes and treatment responses. Alternatively, we evaluated AIIS resistance proxies or indicators in an HIV model system of infection, disease progression, and treatment-associated immunologic reconstitution by testing five precepts (**Figure 8A**).

Precept 1 predicts that two AIIS resistance proxies (CMV seronegativity and female sex) are underrepresented in HIV– populations with AIIS (**Figure 8A**), a risk factor for HIV seroconversion (**Figure 7A-C**). Consistent with this precept, in the HIV– UCSD cohort, CMV+ males had the highest prevalence of AIIS (**Figure 8B**; **Figure S15**). Congruently, CMV seronegativity was underrepresented in HIV+ persons (~7%; **Figure S11**). These findings support the model (**Figure 7D**) that AIIS resistance/susceptibility rather than risk factors for HIV *per se* may influence HIV infection risk.

Precepts 2 and 3. Precept 2 predicts that HIV-induced AIIS rapidly masks pre-infection AIIS status and precept 3 predicts that AIIS resistance proxies associate with better

outcomes (**Figure 8A**). To test these precepts, we determined IHG status (i) before and after HIV seroconversion in the same FSWs (**Figure 6G**; **Figure S16**), (ii) within weeks of infection in participants of the primary HIV infection cohort (PIC) from UCSD (PIC-UCSD; **Figure S17**), and (iii) in the early HIV infection (EIC) cohort according to sex, CMV serostatus, and HIV viral load (**Figure 8C,D; Figure S18**). Better outcomes were defined as (i) preservation of IHGs overrepresented in HIV– persons (IHG-I or IHG-II; AIIS-free status) and (ii) lower HIV viral load.

Supporting precept 2, in FSWs with available pre/post infection data, HIV-induced AIIS obscured pre-seroconversion AIIS status; however, some FSWs preserved AIIS-free status pre and post seroconversion (**Figure 6G**). After HIV infection, some FSWs with pre-seroconversion IHG-I preserved this status, whereas others switched to IHG-II (**Figure S16**). A switch from pre-seroconversion IHG-I to post-seroconversion IHG-II status reflects an adaptive response to preserve CD8-CD4 equilibrium similar to that observed in older persons (**Figure 2B**) and renal transplant recipients (**Figure 5C**), since it involves suppression of CD8+ levels during significant CD4+ lymphocytopenia. In PIC-UCSD participants enrolled within two weeks of their estimated date of HIV infection, nearly 43% preserved AIIS-free status; of these, most manifested the IHG grade representative of an adaptive response, i.e., IHG-II (**Figure S17**). However, in persons enrolled more than 2 weeks after their estimated date of infection, only 14% preserved AIIS-free status; of these, nearly 3 times as many preserved IHG-II than IHG-I (**Figure S17**). Evaluation of 4,883 HIV+ persons from the EIC showed that, while HIV infection associated with high rates of AIIS, overall or in the seroconverting portion of the cohort,

IHG-II at baseline was 1.5 or 1.8 times, respectively, more common than presentation with IHG-I (**Figure 8C**, far left; **Figure S17**). Thus, during the very early stages of high-grade antigenic stimulation (HIV viremia), a subset of HIV+ persons mount an adaptive response to preserve CD8-CD4 equilibrium that involves suppression of CD8+ T-cell expansion, despite relative CD4+ lymphocytopenia (IHG-II).

In the EIC, AIIS resistance proxies (female sex and CMV seronegativity) were associated with increased preservation of AIIS-free status (IHG-I and especially IHG-II), with higher rates of preservation in those classified as spontaneous virologic controllers, a rare subset who can spontaneously control HIV replication without ART (**Figure 8C**, right). Furthermore, the proportion of individuals with an HIV viral load <1000 copies/mL at baseline was significantly greater in those with IHG-I or IHG-II status at baseline, females, and CMV– persons (**Figure 8D**; **Figure S18**). Thus, CMV seronegativity rates were higher in persons with spontaneous virologic control status or viral loads <1000 copies/mL (**Figure 8C**; **Figure S18**). These findings support the third precept, as AIIS resistance proxies associated with better outcomes post HIV seroconversion: greater preservation of AIIS-free status (especially of IHG-II) and lower baseline HIV viral load (**Figure 8C,D**).

Precept 4 predicts that the likelihood of reconstituting AIIS-free status during ART is greater in persons with AIIS resistance proxies (**Figure 8A**). Congruently, both the rate (**Figure 8E**) and extent (**Figure 8F**) to which AIIS-free status was restored during ART was faster and greater in females and CMV**–** persons, and not attributable to differences in the interval between the estimated date of seroconversion and initiation of ART (**Figure**

S19). In HIV– persons, IHG-II was the most common grade among CMV– persons (**Figure 7E**) and, paralleling these findings, in HIV+ persons, nearly 86% of CMV– persons reconstituted AIIS-free status during long-term ART, and most had IHG-II status (**Figure 8F**, left). Thus, supporting precept 4, the CD8+ suppressor status (signified by IHG-II), a feature of AIIS resistance, is masked by HIV-induced AIIS in therapy-naïve patients and it is unmasked after HIV viremia is suppressed (**Figure 8F**, left).

In contrast, in HIV+ persons with a proxy for AIIS susceptibility (CMV seropositivity), the IHG profile reconstituted after ART was nearly indistinguishable from that of HIV– FSWs who subsequently seroconverted (**Figure 8F**, right). Hence, compared with the two proxies for AIIS resistance, a proxy for AIIS susceptibility associated with residual/persistent AIIS in a subset, despite early initiation of ART. These differences in reconstitution of AIIS-free status may relate to heightened sensitivity of AIIS-susceptible (proxied by CMV+) vs. AIIS-resistant (proxied by CMV–) persons to the effects of ongoing antigenic stimulation that may or may not be related to HIV (e.g., risk behaviors).

These observations reinforce that HIV+/CMV+ persons with residual AIIS after antiretroviral therapy may reflect a subset with heightened AIIS susceptibility (e.g., **Figure 7E,8F**). The traditional approach of using CD4+ counts or the CD4:CD8 ratio to monitor immune status pre and post ART would not have yielded this inference (**Figure 8G**). Instead, it would have demonstrated a robust increase in CD4+ counts and ratio values post-ART and that CMV+ vs. CMV– persons had higher CD8+ and lower ratio values post-ART.

Precept 5 pertains to the idea that AIIS resistance as reflected by preservation of elite CD8+ status is not stochastic and instead has a genetic basis. We evaluated whether HIV– persons with AIIS were less likely to carry genotypes containing a single-nucleotide polymorphism (SNP; rs2524054) in the major histocompatibility locus that, in genome-wide association studies of HIV– individuals, correlated with higher CD4:CD8 ratios and lower CD8+ levels (109).

In the overall HIV– UCSD cohort and its European-American component, homozygosity and heterozygosity for this major histocompatibility SNP were overrepresented in persons with elite CD8+ status with higher (IHG-I) or lower (IHG-II) CD4+ counts, whereas homozygosity for the SNP was absent in persons with AIIS (**Figure 8H,** left; **Figure S20**). Thus, this SNP is overrepresented or underrepresented in the same group of individuals distinguished by the lowest or highest CMV seropositivity rates, respectively (**Figure 8H**, right).

We termed this SNP as the AIIS-restricting SNP. Homozygosity for the AIIS-restricting SNP was associated with a 39% reduced likelihood of having CMV seropositivity; however, in the small sample size in which both genotype and CMV serostatus were available (n=635), this association did not achieve statistical significance at $P<0.05$ (OR=0.61; 95% CI: 0.31-1.19; P=0.146). Because of the linkage disequilibrium pattern between the AIIS-restricting SNP and an SNP in the *MICA* gene that correlates with lower HIV viral load (110), the associations of the AIIS-restricting SNP in HIV+ persons were

obscured (**Figure S20**). However, the association of the AIIS-restricting SNP in HIV– persons suggests that elite CD8+ status is genetically influenced (precept 5; **Figure 8A**) and preservation of this status (e.g., **Figure 7D,E**) is less likely to be due to the absence of CMV seropositivity.

Discussion

We have characterized two immune-related programs -- AIIS and ADIS -- that calibrate immunologic integrity; while they have unique causes, characteristics, and consequences, many features of AIIS have been likely misattributed to ADIS. Uniquely, AIIS and ADIS have distinct sexually dimorphic features: at any age, males are more susceptible to develop AIIS, whereas in older persons, ADIS is more common in females. We demonstrate that, depending on extrinsic (e.g., microbial burden) and intrinsic (genetics, sex) factors, immunologic integrity attributable to the AIIS program resides along a continuum quantifiable using CD8-CD4 equilibrium metrics. To uncover possible cause (lower immunologic integrity attributable to AIIS)-effect (disease risk) relationships, we performed a large-scale disease association analysis, and additionally focused on experimental model systems of HIV, CMV, and a cancer that has high incidence and recurrence rates in solid organ transplant recipients. The sum of our results suggest that identification of AIIS and ADIS provides a cogent framework for (i) mapping individual- and population-level trajectories of immune health as well as disentangling cause-effect relationships pertinent to understanding sex-specific differences in disease risks and lifespan in a nonconfounded manner, and (ii) reframing our understanding of the determinants that have shaped the HIV epidemic; our data suggest that a key factor for

HIV acquisition is a person's susceptibility to develop incipient/full-scale AIIS in response to heightened antigenic stimulation associated with risk factors for HIV infection. That is, likelihood of HIV acquisition is greater in persons with lower immunologic integrity linked to incipient/full-scale AIIS and therefore, HIV seropositivity is an indicator of persons with AIIS susceptibility.

Our findings indicate that lower immunologic integrity attributable to AIIS vs. ADIS is unrestricted vs. restricted to chronological age. While ADIS is linked to the aging process, AIIS is a previously unrecognized immunologic program linked to antigenic exposures, independent of age. Our data suggest that AIIS is the expression of an evolutionarily conserved immunologic program activated in response to varied antigenic stimuli correlating with disequilibrium between levels of peripheral blood CD8+ and CD4+ T-cells. AIIS reflects increased accumulation of host antigenic burden and is potentially reversible with a cause-specific decrease or cessation of antigenic stimulation. While AIIS may occur at any age conditional on host and environmental factors, an increase in antigenic exposures is an inevitable aspect of aging; hence, the rates of AIIS increase with age. Thus, in persons experiencing moderate- or high-grade antigenic stimulation, AIIS can occur at any age; however, AIIS status at older ages (without a condition associated with moderate/high grade antigenic stimulation) is attributable to cumulative host antigenic burden of low-grade, repetitive antigenic challenges accrued over a lifetime. After midlife, a decline in immunologic integrity may relate to ADIS and/or AIIS. Consequently, the lower immunologic integrity and immune traits linked to incipient/full-scale AIIS in older

persons has been misattributed to processes related to age and assigned monikers such as inflammaging, immune risk phenotype, and immunosenescence of age.

People vary in their proclivity to develop AIIS, regardless of the grade of antigenic stimulation. Our findings suggest that susceptibility to developing incipient or full-scale AIIS is a proximate risk factor for some age-associated inflammatory diseases (including features attributed to inflammaging), shorter lifespan, immunosuppressive conditions (some cancers), inferior vaccine responses, and susceptibility to and outcomes of chronic viral infections, such as HIV. While our studies focused on antigenic stimulation related to infectious diseases, antigenic stimulation via distinct gut microbiota may also mediate differential activation of the AIIS program. Since gut microbiomes are established by differences in environment and diet in early life (e.g., breast-fed vs. bottle fed (111)) and thereafter (e.g., high vs. low carbohydrate diets (112)), we suggest that AIIS vs. ADIS may serve as potentially proximate and modifiable vs. distal and nonmodifiable risk factors for human diseases.

We distinguished immunologic traits that track AIIS, ADIS, or both. Immunologic traits of AIIS (e.g., expansion of NK T-cells and senescent and terminally differentiated CD8+ T-cells) appear to share conserved characteristics across humans and non-human primates, regardless of HIV or SIV serostatus. Some of these traits are hallmarks of conditions/diseases associated with increased antigenic stimulation and lower immunologic integrity (e.g., chronic viral infections, some cancers, older age). In contrast, ADIS is likely an irreversible process attributable to physiologic involution of the immune

system that correlates with an aged CD8-CD4 equilibrium and a distinct set of immunologic traits, e.g., loss of naïve CD8+ T-cells, B cells and plasmacytoid DC.

While it is customary to compare immune traits by HIV serostatus, we found significant overlap in the CD8-CD4 equilibrium states of HIV– and HIV+ individuals. Thus, evaluating immunologic traits using a strategy suggested in Figure 4A may mitigate confounding and identify immune traits attributable to AIIS vs ADIS. Since the immunologic traits that distinguished AIIS from AIIS-free status were similar in HIV– humans and SIV+ sooty mangabeys, as well as HIV– and HIV+ persons (Okulicz et al, manuscript submitted), the identified differences in immune traits between HIV– and HIV+ persons may relate to study groups that differ with respect to the proportion of individuals with distinct equilibrium states. That is, immunologic traits differ by CD8-CD4 equilibrium states rather than HIV serostatus *per se.*

AIIS rates were higher in epidemiologic contexts with greater infectious disease burdens. Thus, evolutionary preservation of a trait associated with lower immunologic integrity suggests that immune processes leading to AIIS are part of an essential host defense strategy, potentially mitigating autoimmunity and reducing the effect of infections on host fitness in settings of higher infectious disease burden. The concept of disease tolerance as a defense strategy was first advanced by Medzhitov et al. (113).

A polymorphism (AIIS-restricting SNP) in the major histocompatibility locus was underrepresented in those with AIIS, suggesting that AIIS susceptibility may have a

genetic basis. However, irrespective of this genetic basis, females appear to resist AIIS throughout life. Women manifest a survival advantage and greater immunocompetence, reflected by resistance to infections, better vaccine responses and resistance to some age-associated diseases (e.g., some cancers) (5-7, 12-19). The female survival advantage is also seen in other species (5). Our data suggest that females are more successful in resisting AIIS – even in the context of high-grade antigenic stimulation – thus, preserving features of greater immunologic integrity, such as lower HIV viral loads and more rapid restoration of AIIS-free status after initiation of ART in HIV+ persons. Resistance to AIIS may underpin observations by others that women have lower HIV viral loads (114) and reservoir sizes (115). AIIS resistance may allow women to enter childbearing age with greater immunologic integrity, promoting reproduction and fetal health as well as lifespan. The tradeoff is that females are more susceptible to autoimmune diseases at a younger age (116, 117). Congruent with the idea that resistance to developing AIIS, i.e., preservation of greater immunologic integrity, is a correlate of autoimmunity, as the AIIS-restricting SNP has been associated with autoimmune diseases (109). Thus, we advance the idea that AIIS resistance vs. susceptibility may represent proximate risk factors for diseases that reside on the autoimmune vs. inflammatory disease spectrum as well as contribute to individual variation in lifespan.

We lacked a prospective longitudinal cohort to characterize the CD8-CD4 equilibrium/disequilibrium trajectory in individuals across the lifespan. Through comparisons of individuals binned in age strata from early adulthood to >90 years of age,

we characterized progressive shifts in CD8-CD4 equilibrium during aging. Our data suggest that ADIS reflects an 'aged' CD8-CD4 equilibrium status which is more common in females in later life. While we could not determine the disease correlates of ADIS, some age-associated diseases are more common in older females (e.g., Alzheimer disease) (7). Thus, our findings provide a potential framework to understand how immunologic integrity conditional on AIIS and ADIS contributes to sex-based differences in disease susceptibility.

We extend the idea first advanced by Levy and Ziegler (100) and Sonnabend et al (94) that immunosuppression may both precede and contribute to HIV infection. Our epidemiologic survey indicates a high rate of incipient/full-scale AIIS in HIV-seronegative persons with behavioral and non-behavioral (e.g., schistosomiasis) risk factors for HIV infection. The elevated rates of incipient/full-scale AIIS in persons with high-risk behaviors may relate to antigenic stimuli attributable to STIs and alloimmunization to sperm, semen, and/or blood antigens (94-100). In FSWs, the contribution of AIIS susceptibility to HIV acquisition was high, with nearly 87% of all seroconversions occurring in those with incipient or full-scale AIIS at baseline. Consistent with our findings, CD8+ and CD4+ T-cell values reflective of incipient AIIS predicted HIV seroconversion in hemophiliacs after controlling for the quantity of non-recombinant clotting products received (84). In a prophylaxis trial in men who have sex with men, those who subsequently seroconverted had CD8+ and CD4+ T-cell values consistent with incipient AIIS at baseline (118). In contrast, preservation of elite CD8+ status despite ongoing risk factor-associated increased antigenic stimulation (high-grade AIIS resistance) may be a correlate of

individuals designated previously as “highly exposed, HIV-seronegative individuals” (119).

Collectively, our findings suggest that a trifecta of factors favor HIV acquisition: (i) an AIIS-susceptible host; (ii) causes of antigenic stimulation (behavioral or nonbehavioral related) of enough severity and/or chronicity to induce incipient or full-scale AIIS which is more likely in the AIIS-susceptible host; and (iii) exposure to HIV in the presence of incipient or full-scale AIIS. Hence, HIV may be largely restricted to the subset of AIIS-susceptible persons experiencing moderate- to high-grade sources of antigenic stimulation. We therefore suggest that (i) susceptibility to develop incipient or full-scale AIIS in response to antigenic stimulation rather than the risk factors *per se* undergirds increased HIV acquisition risk, and (ii) in persons at high risk for exposure to HIV, seropositivity vs. seronegativity for HIV is an indicator of persons who, respectively, have a proclivity for developing vs. resisting incipient/full-scale AIIS. We suggest similar parallels for CMV seroconversion.

Additionally, our results support the contribution of the AIIS program along a continua: (i) seroconversion with HIV or CMV is more likely to occur in the presence of incipient or full-scale AIIS (AIIS susceptibility); (ii) following infection, viremia further precipitates immune skewing toward an AIIS-biased disequilibrium; and (iii) with cessation of virus-associated antigenic stimulation, skewness dissipates. In this continua, the indicator/proxy functions of viral serostatus are masked in settings of higher antigenic stimulation and revealed after stimulation is suppressed. While pre-existing immunosuppression linked to incipient

or full-scale AIIS may predispose individuals to HIV acquisition, full-scale AIIS induced by HIV-associated antigenic stimulation further compounds the immunosuppression post-infection. Thus, post-HIV infection, persons may have AIIS attributable to two causes of antigenic stimulation: risk factor-induced AIIS and HIV viremia-induced AIIS. However, only the latter cause of AIIS is responsive to ART.

The indicator function of CMV seropositivity of lower immunological integrity may explain its association with varied adverse outcomes (1, 11, 20-32, 101, 102); i.e., these associations reflect those of a subset of CMV+ persons with a proclivity to develop incipient or full-scale AIIS in response to varied antigenic stimuli rather than effects of CMV *per se*. Consistent with the idea that CMV seronegativity is an indicator of AIIS resistance, individuals with familial longevity in the Leiden Longevity Study have immunologic features consistent with AIIS resistance, including CMV seronegativity (120, 121). The indicator function of CMV serostatus has therapeutic implications. In immunocompromised HIV– persons (e.g., bone marrow transplantation), CMV seropositivity is correlated with adverse outcomes (23, 122). However, treatment of CMV infection may be insufficient, as the proximate defect may reside in the host, i.e., an increased susceptibility to develop AIIS in response to either CMV viremia or other triggers of the AIIS program.

Our findings have three practical applications for HIV prevention and treatment-associated immunologic reconstitution. First, although pre-exposure prophylaxis with antiretroviral medications may protect against HIV infection (123), in the absence of

barrier protection, antigenic stimulation associated with high-risk behavioral activity may induce AIIS and its associated sequelae. Second, our results point to a risk continuum linked to the AIIS program, as AIIS susceptibility predisposes to not only HIV acquisition but also poorer outcomes both before and during ART. Hence, persistent AIIS in HIV+ persons, despite early and durable suppression of viremia, may reflect effects of pre-existing or ongoing risk factor-associated antigenic stimulation vs. effects of HIV infection per se. Third, curative strategies may be less effective in persons who fail to reconstitute AIIS-free status after ART, and vaccines to prevent HIV infection may be less effective in those with incipient or full-scale AIIS.

Together, we describe an antigen-activated, sexually dimorphic, genetically influenced, immunosuppression-mediating program that shapes the trajectory of immunologic health and disease risks from early life. AIIS resistance vs. susceptibility may contribute to contrasting disease risks (autoimmune vs. inflammatory diseases), and some of the associations of HIV and CMV seropositivity with adverse outcomes may stem from hosts with heightened susceptibility to develop AIIS in response to antigenic stimulation, related or unrelated to HIV or CMV viremia. Results of an accompanying study using a large-scale transcriptomics approach, support these viewpoints (Manoharan et al.). Those studies also identified key genes, pathways, and proximate transcriptional regulators that appear to drive the AIIS immunologic program (Manoharan et al.), providing potential therapeutic targets to modulate AIIS status. Although further work is needed, our results (i) underscore the need to distinguish between AIIS and ADIS to avoid confounding interpretation of studies aiming to investigate disease mechanisms and lifespan; (ii)

provide a roadmap for personalized medicine, proposing CD8-CD4 equilibrium metrics as a facile method to monitor immunologic status, regardless of HIV serostatus or age; (iii) highlight that cross-sectional comparisons may be prone to ecological fallacy (124) and reversal of cause-effect relationships, i.e., misattributing characteristics of the host to the disease/condition; and (iv) advance the concept that selection of immunocompromised hosts (AIIS susceptibility) for infection by HIV may reflect an evolutionary strategy to promote the life cycle of HIV and sustain the HIV epidemic.

Methods

All studies were approved by the institutional review boards at the University of Texas Health Science Center at San Antonio and institutions participating in this study. Detailed methods, including immunophenotyping and statistical tests, are provided in Supplementary methods. Characteristics of the study participants are listed in Supplementary methods, **Tables S3** (SardiNIA), **S4** (HIV+ cohorts), **S7-S9** (FSWs and others), and **Figure S21**.

AUTHOR CONTRIBUTIONS

SKA conceived the idea; designed, supervised, and coordinated the study; interpreted the data; and wrote the manuscript. SKA and JFO obtained funding for the study. JFO, RAC, NH and WH provided conceptual contributions. JFO, LRM, JK, TBB, FAP, SL, DMS, MJB, PNH, KJW, CK, SJL, FC, TMF, and BKA provided access to, contributed to, and/or supervised data collection of human cohorts. GS directed accrual of cohort data from sooty mangabey monkeys. H-Y.Z and Y-T.Z provided access to data from the cohort of Chinese macaques. JL, MF, and MTH directed and contributed to data collection from CC mice. VO and EF performed immunophenotyping in the SardiNIA cohort. WH assisted in assembly of the datasets. MS and MJB performed statistical analysis for the SardiNIA and renal transplant recipient cohorts, respectively. NH and WH performed statistical analysis for all other datasets. AB and WH drafted the figures. FJ, AMS, JAM, AC, and LP provided experimental support. Online supplementary materials were prepared by WH, NH, AB, JAM, MSM and SKA. JFO, LRM, MS, NH, MJB, VO, EF, FJ, MSM, AMS, NG, KJW, EAW, EJW, R.R-B, NZ, DDR, KRF, BKA, RAC, GCL, and WH contributed to data interpretation and provided editorial suggestions. The order of the co–second authors was determined by their relative contribution to the study.

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DISCLAIMER

The authors have no conflicts of interest to disclose. The views expressed are those of the authors and do not necessarily reflect the official views of the Uniformed Services University of the Health Sciences; the National Institutes of Health; the Department of Health and Human Services; Walter Reed National Military Medical Center; Brooke Army Medical Center; Madigan Army Medical Center; Naval Medical Center San Diego; Naval Medical Center Portsmouth; the Department of Defense; the Departments of the Army, Navy, or Air Force; or the Henry M. Jackson Foundation for the Advancement of Military Medicine. Mention of trade names, commercial products, or organizations does not imply endorsement by the U.S. government. The investigators have adhered to the policies for protection of human subjects as prescribed in 45 CFR 46. The U.S. government is authorized to reproduce and distribute reprints for governmental purposes notwithstanding any copyright notation thereon.

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Figure legends

Figure 1. Concepts and metrics. (**A**) Concepts related to age-independent immunosenescence (AIIS) and age-dependent immunosenescence (ADIS). Ag, antigenic. (**B**) Successful vs. unsuccessful immune allostasis. (**C**) Hypothesis: indicator function of cytomegalovirus (CMV) and/or human immunodeficiency virus (HIV) serostatus may correct prevailing views of cause and effect relationships. **(D)** Key study cohorts. CC-RIX, Collaborative Cross recombinant inbred intercrossed; UCSD, University of California at San Diego; SardiNIA, cohort focused on age-related traits in Sardinia in association with the National Institute on Aging (NIA). (**E**) Concepts and immune metrics: CD8-CD4 equilibrium/disequilibrium states in response to antigenic (Ag) stimulation, immune allostasis, and immune health grades (IHGs). (**F**) Cutoffs of the CD4:CD8 T-cell ratio and CD4+ T-cell count used to define IHGs.

Figure 1

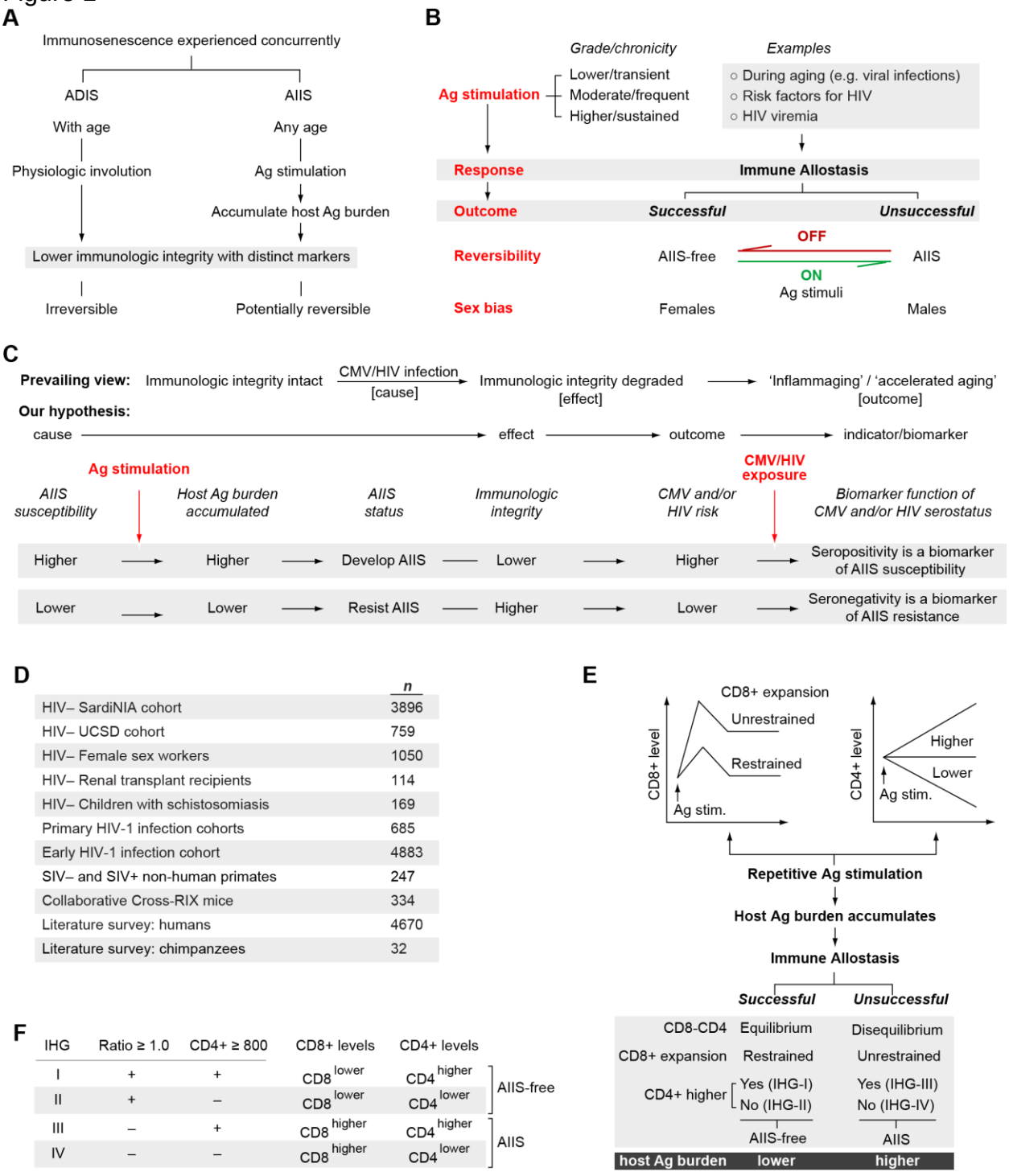

A
Immunosenescence experienced concurrently
ADIS
AIIS
With age
Any age
Physiologic involution
Ag stimulation
Accumulate host Ag burden
Lower immunologic integrity with distinct markers
Irreversible
Potentially reversible
B
Grade/chronicity
Examples
Ag stimulation
Lower/transient
Moderate/frequent
Higher/sustained
During aging (e.g. viral infections)
Risk factors for HIV
HIV viremia
Response
Immune Allostasis
Outcome
Successful
Unsuccessful
Reversibility
AIIS-free
OFF
ON
Ag stimuli
AIIS
Sex bias
Females
Males
C
Prevailing view:
Immunologic integrity intact
CMV/HIV infection
[cause]
Immunologic integrity degraded
[effect]
'Inflammaging' / 'accelerated aging'
[outcome]
Our hypothesis:
cause
effect
outcome
indicator/biomarker
Ag stimulation
CMV/HIV exposure
AIIS susceptibility
Host Ag burden accumulated
AIIS status
Immunologic integrity
CMV and/or HIV risk
Biomarker function of CMV and/or HIV serostatus
Higher
Higher
Develop AIIS
Lower
Higher
Seropositivity is a biomarker of AIIS susceptibility
Lower
Lower
Resist AIIS
Higher
Lower
Seronegativity is a biomarker of AIIS resistance
D
n
HIV– SardiNIA cohort 3896
HIV– UCSD cohort 759
HIV– Female sex workers 1050
HIV– Renal transplant recipients 114
HIV– Children with schistosomiasis 169
Primary HIV-1 infection cohorts 685
Early HIV-1 infection cohort 4883
SIV– and SIV+ non-human primates 247
Collaborative Cross-RIX mice 334
Literature survey: humans 4670
Literature survey: chimpanzees 32
E
CD8+ expansion
Unrestrained
Restrained
CD8+ level
Ag stim.
CD4+ level
Higher
Lower
Ag stim.
Repetitive Ag stimulation
Host Ag burden accumulates
Immune Allostasis
Successful
Unsuccessful
CD8-CD4 Equilibrium Disequilibrium
CD8+ expansion Restrained Unrestrained
CD4+ higher Yes (IHG-I) Yes (IHG-III)
No (IHG-II) No (IHG-IV)
AIIS-free AIIS
host Ag burden lower higher
F
IHG Ratio ≥ 1.0 CD4+ ≥ 800 CD8+ levels CD4+ levels
I + + CD8 lower CD4 higher AIIS-free
II + – CD8 lower CD4 lower
III – + CD8 higher CD4 higher AIIS
IV – – CD8 higher CD4 lower

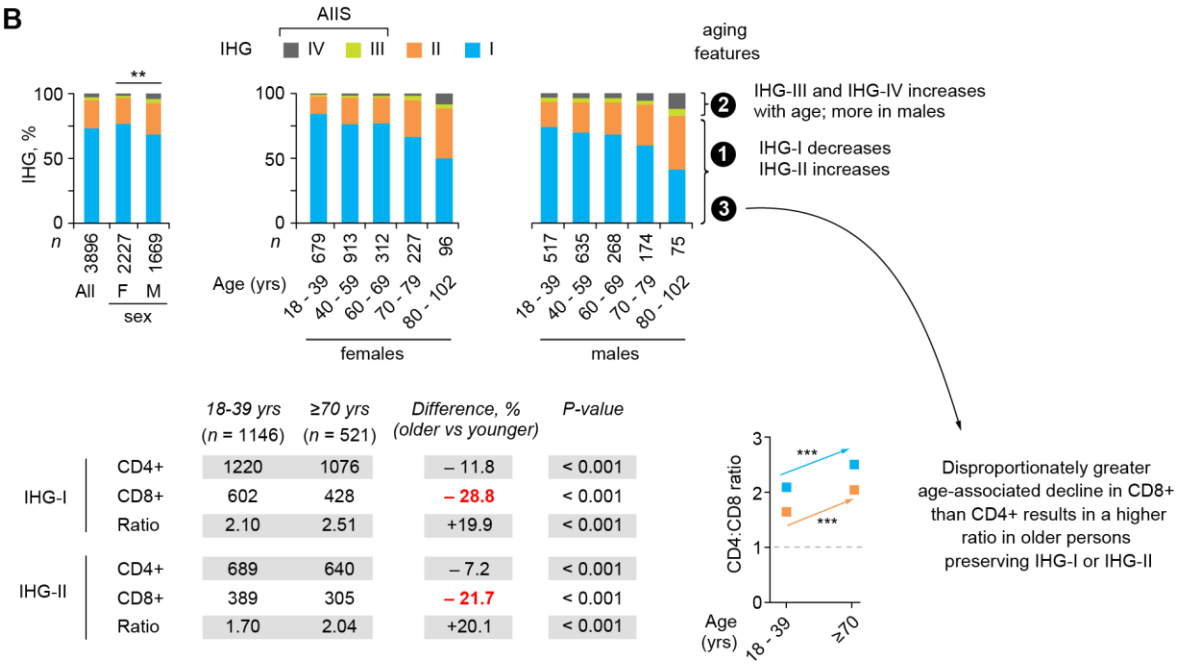
Figure 2. CD8-CD4 equilibrium states change with age in HIV− persons. All findings from the community-based HIV− SardiNIA cohort. (**A**) Median (interquartile range) CD8+ and CD4+ T-cell counts (cells/mm^3) by immune health grades (IHGs). (**B**) Three features of aging. Top, IHGs, overall and by age strata and sex. Bottom left values, median CD4+ and CD8+ counts (cells/mm^3) and CD4:CD8 T-cell ratio as well as percent differences in median levels for older (≥70 years) vs younger (age 18-39 years) persons with IHG-I or IHG-II. Bottom right plot depict median values of CD4:CD8 T-cell ratio by age strata. (**C**) Odds of having IHG-I vs. IHG-II by age strata (feature 1) or having IHG-III or IHG-IV vs. IHG-I or IHG-II (feature 2), relative to reference group (18-39 years). Depicted are odds ratios (ORs) with 95% confidence intervals adjusted for sex. (**D**) Plot of probability of AIIS vs age strata (used as a continuous variable) by sex with 95% confidence band. *P, by* logistic regression. (**E**) Median CD4:CD8 ratios in age strata (overall and by sex) illustrating potential of confounding by conflation of IHGs. ***P*<0.01, ****P*<0.001.

Figure 2

A

IHG	*n* (%)	CD8+	CD4+	Ratio	CD8+ expansion	CD8+	CD4+	CD8-CD4	AIIS Status
I	2852 (73.2)	525 (392-689)	1154 (980-1393)	2.26 (1.75-2.97)	Restrained	Lower	Highest	Equilibrium	AIIS-free
II	840 (21.6)	354 (267-447)	675 (592-746)	1.90 (1.44-2.38)	Suppressed	Lowest	Lower		
III	95 (2.4)	1195 (1033-1463)	988 (870-1197)	0.87 (0.76-0.93)	Unrestrained	Highest	Higher	Disequilibrium	AIIS
IV	109 (2.8)	781 (646-932)	616 (517-712)	0.82 (0.66-0.93)	Unrestrained	Higher	Lowest		

B



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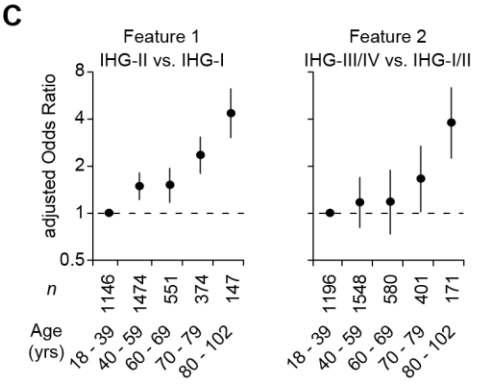

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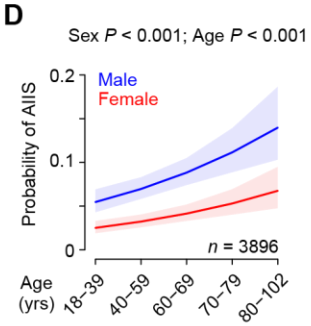


E

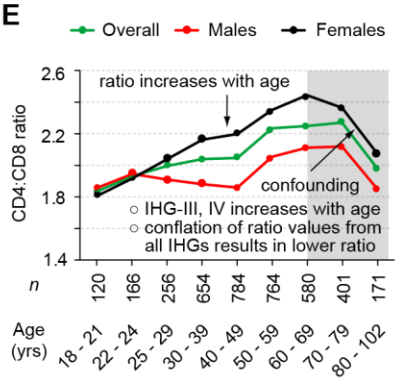


Figure 3. Sexually dimorphic CD8-CD4 equilibrium states. (**A**) CD8:CD4 equilibrium states by age. Immune health grade (IHG)-I and IHG-II with ratio values ≥2.5 signifies age-dependent immunosenescence (ADIS) in older (≥40 years). AIIS, age-independent immunosenescence. (**B**) CD8-CD4 equilibrium states in HIV− University of California, San Diego (UCSD) cohort. Plots are Kernel density estimates of the CD4:CD8 T-cell ratio. Abscissa shows IHG status categorized into ADIS, elite CD8+ status, incipient or full-scale AIIS, using criteria shown in Figure 3A. *P*, differences in distribution. (**C, D**) CD4-CD8 equilibrium states (as derived in panel B) in HIV+ participants from the (C) primary HIV infection cohort (PIC) and (D) early HIV infection cohort (EIC). C, Equilibrium states in individuals enrolled within the indicated time intervals from the estimated date of infection (EDI). D, Equilibrium states at initiation of antiretroviral therapy (ART) (pre-ART) and 2-year windows after initiating virally suppressive ART in participants who received ART for at least 8 years. (**E**) Equilibrium states (as derived in panel B) before (pre-ART) and during the indicated times after ART initiation in participants from the EIC and PIC compared with individuals from the HIV− UCSD cohort stratified by CMV serostatus and age. Age, median age. (**F**) Left two plots: odds of having incipient AIIS (in those without AIIS) and of preserving elite CD8+ status (in those who do not have the indicator for ADIS) by sex, adjusted for age. Middle plot: odds of having full-scale AIIS by age and sex. Right-most plot: odds of having the indicator of ADIS (in those without AIIS) by age and sex. Depicted are odds ratios (ORs) with 95% confidence intervals in the HIV− SardiNIA cohort. ns, non-significant. ***P*<0.01, ****P*<0.001.

Figure 3

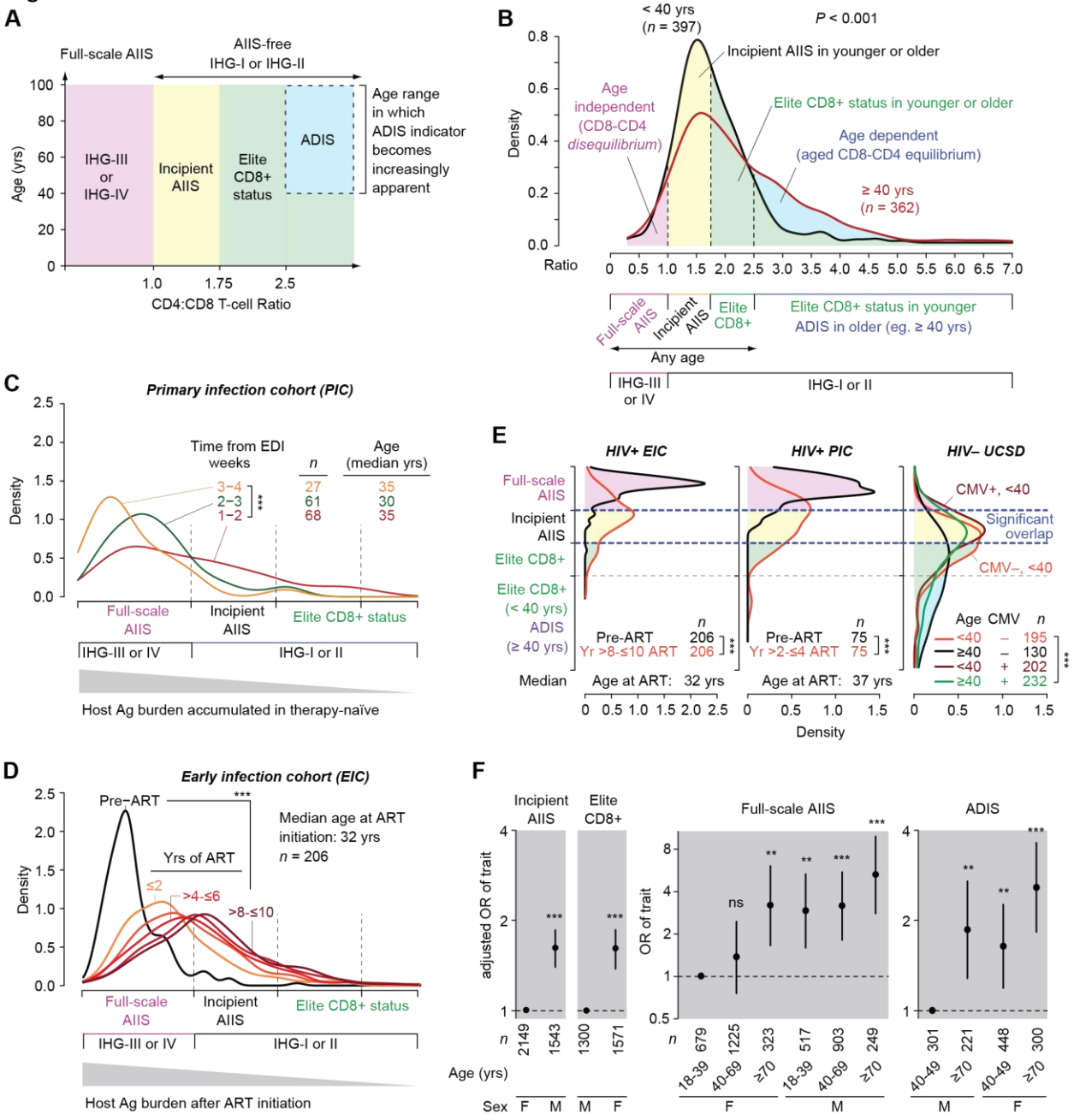

A
Full-scale AIIS
AIIS-free
IHG-I or IHG-II
Age (yrs)
IHG-III or IHG-IV
Incipient AIIS
Elite CD8+ status
ADIS
Age range in which ADIS indicator becomes increasingly apparent
CD4:CD8 T-cell Ratio
B
< 40 yrs (n = 397)
P < 0.001
Incipient AIIS in younger or older
Elite CD8+ status in younger or older
Age independent (CD8-CD4 disequilibrium)
Age dependent (aged CD8-CD4 equilibrium)
≥ 40 yrs (n = 362)
Density
Ratio
Full-scale AIIS
Incipient AIIS
Elite CD8+
Elite CD8+ status in younger
ADIS in older (eg. ≥ 40 yrs)
Any age
IHG-III or IV
IHG-I or II
C
Primary infection cohort (PIC)
Time from EDI weeks
n
Age (median yrs)
3–4
2–3
1–2
27
61
68
35
30
35
Full-scale AIIS
Incipient AIIS
Elite CD8+ status
IHG-III or IV
IHG-I or II
Host Ag burden accumulated in therapy-naïve
D
Early infection cohort (EIC)
Pre–ART
Yrs of ART
≤2
>4-≤6
>8-≤10
Median age at ART initiation: 32 yrs
n = 206
Host Ag burden after ART initiation
E
HIV+ EIC
HIV+ PIC
HIV– UCSD
Full-scale AIIS
Incipient AIIS
Elite CD8+
Elite CD8+ (< 40 yrs) ADIS (≥ 40 yrs)
CMV+, <40
Significant overlap
CMV–, <40
Pre-ART
Yr >8-≤10 ART
206
206
Pre-ART
Yr >2-≤4 ART
75
75
Age CMV n
<40 – 195
≥40 – 130
<40 + 202
≥40 + 232
Median
Age at ART: 32 yrs
Age at ART: 37 yrs
Density
F
Incipient AIIS
Elite CD8+
adjusted OR of trait
n
2149
1543
1300
1571
Full-scale AIIS
ADIS
OR of trait
ns
679
1225
323
517
903
249
301
221
448
300
18-39
40-69
≥70
40-49
Age (yrs)
Sex
F
M

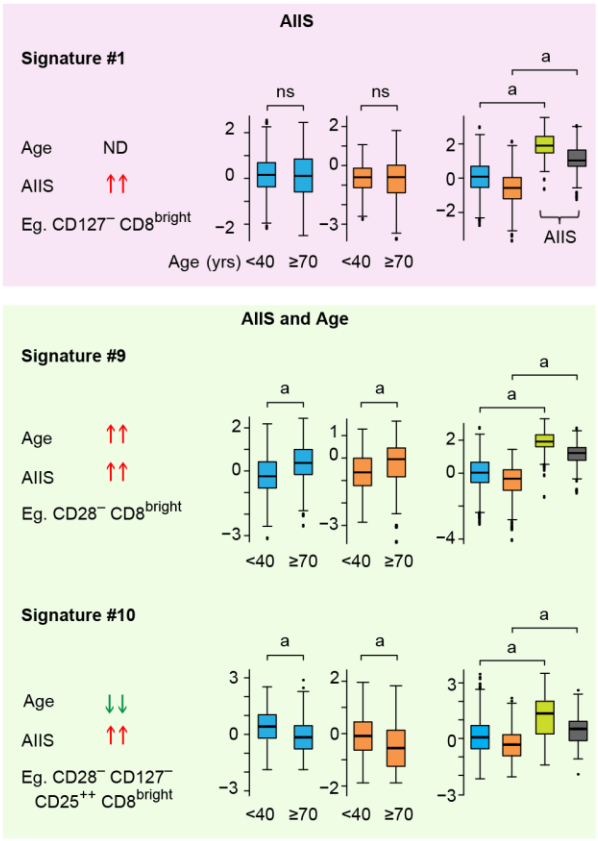
Figure 4. Immunologic traits of age-independent immunosenescence (AIIS) vs age and evolutionary conservation of AIIS. (**A**) Top, 75 immune traits subgrouped into 19 signatures that associate with either AIIS, age (proxy for ADIS), AIIS and age, or neither (neutral) in the HIV− SardiNIA cohort. Bottom, levels of a representative example (Eg.) of a trait in the indicated signature. Trait levels were normalized using inverse normal transformations with normalized values ranging from −3 to 3, and the covariate-adjusted residuals are shown. Arrow (significant difference at *P* <1.67E-4) or ND (no difference at *P* <1.67E-4) refer to the directionality of trait levels according to AIIS status (by immune health grade [IHG]-I vs. III and IHG-II vs. IV, after adjusting for age and sex) and age (<40 vs. ≥70 yrs within IHG-I or IHG-II after adjusting for sex). Two arrows indicate both comparisons for AIIS (IHG-I vs. III and IHG-II vs. IV) or age (<40 vs. ≥70 yrs within IHG-I and IHG-II) are significant at *P* <1.67E-4 and one arrow indicates only one of the comparisons for AIIS or age is significant at *P* <1.67E-4. Bottom right, median number of individuals evaluated by IHG status and by age within IHG-I or IHG-II. *a*, *P*<1.67E-4 is the significance threshold after adjusting for multiple comparisons (75 traits x 4 comparisons). ns, non-significant. (**B**) IHGs and their associations in seronegative simian immunodeficiency virus (SIV−) and seropositive (SIV+) sooty mangabeys. From left to right: distribution of IHGs in sooty mangabeys by sex and SIV serostatus; SIV viral load by IHG; levels of indicated CD8+ T-cell immune traits by IHG status in SIV+ monkeys, and distribution of AIIS (IHG-III or IHG-IV) and AIIS-free (IHG-I or IHG-II) status within the indicated age strata and SIV serostatus groups. ***P*<0.01, ****P*<0.001.

Figure 4

A

	AIIS				Age (proxy for ADIS)				AIIS and Age				Neutral						
Signature #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
AIIS	↑↑	↓↓	↑↑	↑↑	ND	ND	↑	↑	↑↑	↑↑	↓↓	↓↓	ND	↑	↓	↑	↓	ND	ND
Age	ND	ND	↑	↓	↑↑	↓↓	↑↑	↓↓	↑↑	↓↓	↑↑	↓↓	ND	↑	↓	ND	ND	↑	↓
# of traits	5	1	4	3	5	15	1	1	2	5	1	2	12	1	1	2	2	10	2

ND - No difference; ↑↑ or ↓↓ - both comparisons for AIIS or age are significant; ↑ or ↓ - only one of the comparisons for AIIS or age is significant



IHG		*n*, median (range)
AIIS-free	I	2665 (2321-2852)
	II	790 (711-840)
AIIS	III	85 (76-95)
	IV	103 (96-109)

IHG	Age (yrs)	*n*, median (range)
I	<40	910 (801-956)
	≥70	309 (266-334)
II	<40	181 (165-190)
	≥70	180 (158-187)

a, $P < 1.67E\text{-}4$

B

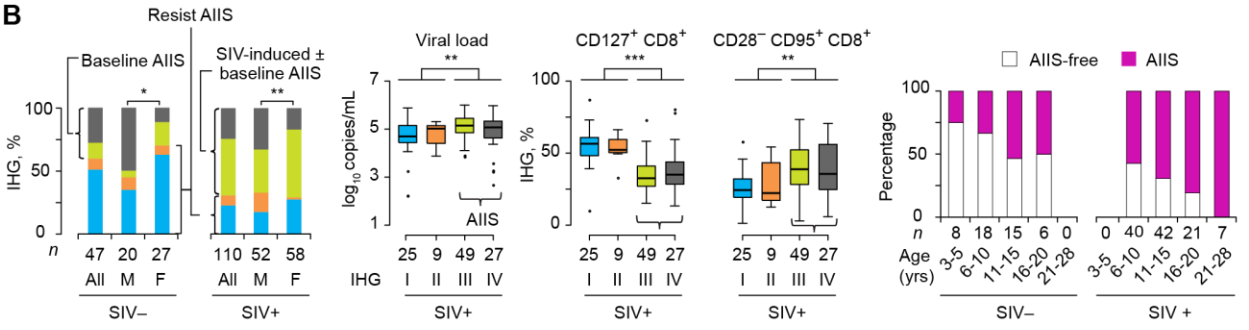


Figure 5. Inducers and associations of age-independent immunosenescence (**AIIS).** (**A**) Inducers and associations of AIIS determined through a combination of a literature survey and our primary datasets. IHG, immune health grade; Flu, influenza; CMV, cytomegalovirus; FSW, female sex workers; &, interquartile range. (**B**) IHG distribution and the indicated immunologic trait in Kenyan HIV− children with *Schistosoma haematobium* infection by urine egg counts. (**C**) IHG distribution and its associations in HIV− renal transplant recipients. Upper left, IHG distribution by sex; upper right, hazard of second instance of cutaneous squamous cell cancer (CSCC) by IHG status; middle left; distribution of levels of CD57+CD8+ T-cells stratified as higher vs. lower by median values; middle right, CMV serostatus by IHG; bottom, model of associations with AIIS resistance (preservation of AIIS-free status) in renal transplant recipients. Ag, antigenic (e.g., alloimmune responses). **(D)** IHG by Ebola virus infection outcomes in Collaborative Cross recombinant inbred intercrossed (CC-RIX) mice. The infection outcomes of CC-RIX strains were adapted from Ref. (34). The IHG distributions are for the strains of uninfected mice which correspond to the strains of mice that were infected with Ebola virus. **(E)** Hazard ratio of progressing to AIDS (1993 criteria (125)) by IHG grade at presentation in early HIV infection cohort adjusted for age, sex, and ethnicity. (**F**) Model: AIIS induced by various risk factors for HIV precedes and contributes to HIV seroconversion; seroconversion risk varies by AIIS susceptibility, and higher AIIS rates in people infected with schistosomiasis may account for co-spatial distribution of helminthic infections and HIV in Africa (adapted from Ref. (83)). Heatmap, relative prevalence of schistosomiasis (left) and HIV (right). *P<0.05, **P<0.01, ***P<0.001.

Figure 5

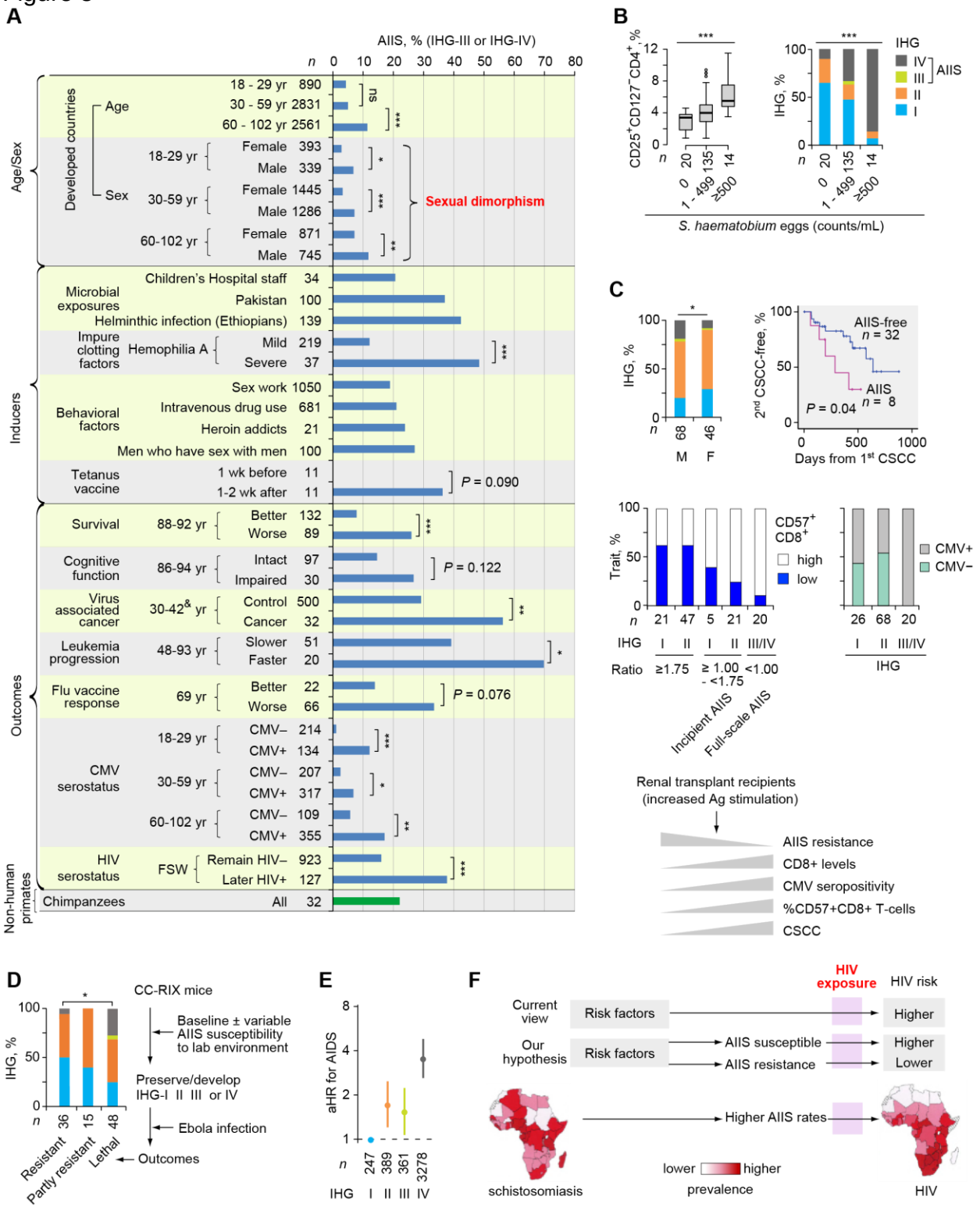

A
AIIS, % (IHG-III or IHG-IV)
n 0 10 20 30 40 50 60 70 80
Age/Sex
Developed countries
Age
18 - 29 yr 890
30 - 59 yr 2831
60 - 102 yr 2561
ns
***
Sex
18-29 yr
Female 393
Male 339
*
30-59 yr
Female 1445
Male 1286
***
60-102 yr
Female 871
Male 745
**
Sexual dimorphism
Inducers
Microbial exposures
Children's Hospital staff 34
Pakistan 100
Helminthic infection (Ethiopians) 139
Impure clotting factors
Hemophilia A
Mild 219
Severe 37
***
Behavioral factors
Sex work 1050
Intravenous drug use 681
Heroin addicts 21
Men who have sex with men 100
Tetanus vaccine
1 wk before 11
1-2 wk after 11
P = 0.090
Outcomes
Survival
88-92 yr
Better 132
Worse 89
***
Cognitive function
86-94 yr
Intact 97
Impaired 30
P = 0.122
Virus associated cancer
30-42& yr
Control 500
Cancer 32
**
Leukemia progression
48-93 yr
Slower 51
Faster 20
*
Flu vaccine response
69 yr
Better 22
Worse 66
P = 0.076
CMV serostatus
18-29 yr
CMV– 214
CMV+ 134
***
30-59 yr
CMV– 207
CMV+ 317
*
60-102 yr
CMV– 109
CMV+ 355
**
HIV serostatus
FSW
Remain HIV– 923
Later HIV+ 127
***
Non-human primates
Chimpanzees
All 32
B
CD25+CD127−CD4+, %
IHG, %
IHG
IV
III
II
I
AIIS
n 20 135 14
0 1 - 499 ≥500
S. haematobium eggs (counts/mL)
C
IHG, %
n 68 46
M F
2nd CSCC-free, %
AIIS-free n = 32
AIIS n = 8
P = 0.04
Days from 1st CSCC
Trait, %
CD57+ CD8+
high
low
n 21 47 5 21 20
IHG I II I II III/IV
Ratio ≥1.75 ≥ 1.00 - <1.75 <1.00
Incipient AIIS
Full-scale AIIS
CMV+
CMV−
26 68 20
I II III/IV
IHG
Renal transplant recipients
(increased Ag stimulation)
AIIS resistance
CD8+ levels
CMV seropositivity
%CD57+CD8+ T-cells
CSCC
D
IHG, %
n 36 15 48
Resistant
Partly resistant
Lethal
CC-RIX mice
Baseline ± variable AIIS susceptibility to lab environment
Preserve/develop IHG-I II III or IV
Ebola infection
Outcomes
E
aHR for AIDS
n 247 389 361 3278
IHG I II III IV
F
HIV exposure
HIV risk
Current view
Risk factors
Higher
Our hypothesis
Risk factors
AIIS susceptible
AIIS resistance
Higher
Lower
Higher AIIS rates
lower
higher
prevalence
schistosomiasis
HIV

Figure 6. Associations of behavior and biologic risk factors with outcomes (CD8-CD4 equilibrium states and HIV seroconversion) in HIV− female sex workers (FSWs). (**A**) Disribution of immune health grade (IHG) at baseline (top) and subsequent HIV seroconversion rates (bottom) in FSWs who were HIV− at baseline stratified according to the indicated behavioral and biological risk factors. Behavioral risk factors were duration of sex work, condom use (1, never; 2, <50%; 3, ≥50%; and 4, always), clients/week, ∆ (clients – condoms) (the difference between the number of clients/wk and condoms used/wk), and a behavioral activity score (BAS) that accounts for the latter 3 behavioral characteristics. Biological risk factors were direct and indirect indicators of sexually transmitted infections (STIs). Scores were derived for the direct (syphilis [rapid plasma reagin test] and gonorrhea) and indirect (vaginal discharge, abdominal pain, genital ulcer, dysuria, and vulvar itch) indicators of STIs (Supplementary Methods). (**B**) Kernel density estimates of baseline CD4:CD8 T-cell ratio and CD8-CD4 equilibrium states (akin to Figure 3B) for HIV− FSWs by baseline BAS and total STI scores or both. Baseline median age is reported in the far right. (**C**) Kernel density estimates of baseline CD4:CD8 ratio and equilibrium states at baseline for FSWs who subsequently seroconverted vs. those who remained HIV−. (**D**) Kaplan-Meier plots depicting hazard of HIV seroconversion in FSWs who, in year 6 of HIV− follow-up manifested a CD8-CD4 equilibrium status that switched from better at baseline to worse (better→worse) or preservation of full-scale AIIS (worse→worse) vs. rest. (**E**) Characteristics of 27 HIV− FSWs with CD8-CD4 equilibrium and BAS data available within every two-year window for 10 years. Left to right: IHG distribution within time intervals from cohort entry; BAS and CD8+ and CD4+ levels within the indicated time intervals. (**F**) Kernel density estimates of the CD4:CD8 ratio and CD8-CD4 equilibrium states for the subset of HIV− FSWs with availble laboratory data at baseline and in year 6 of HIV-seronegative follow-up. Insets show BAS (box and whisker plots) at baseline and in year 6 of follow-up for whom BAS data was available. (**G**) IHG distribution before and after HIV infection in FSWs. ns, not significant (>0.1). **P*<0.05, ***P*<0.01, ****P*<0.001.

Figure 6

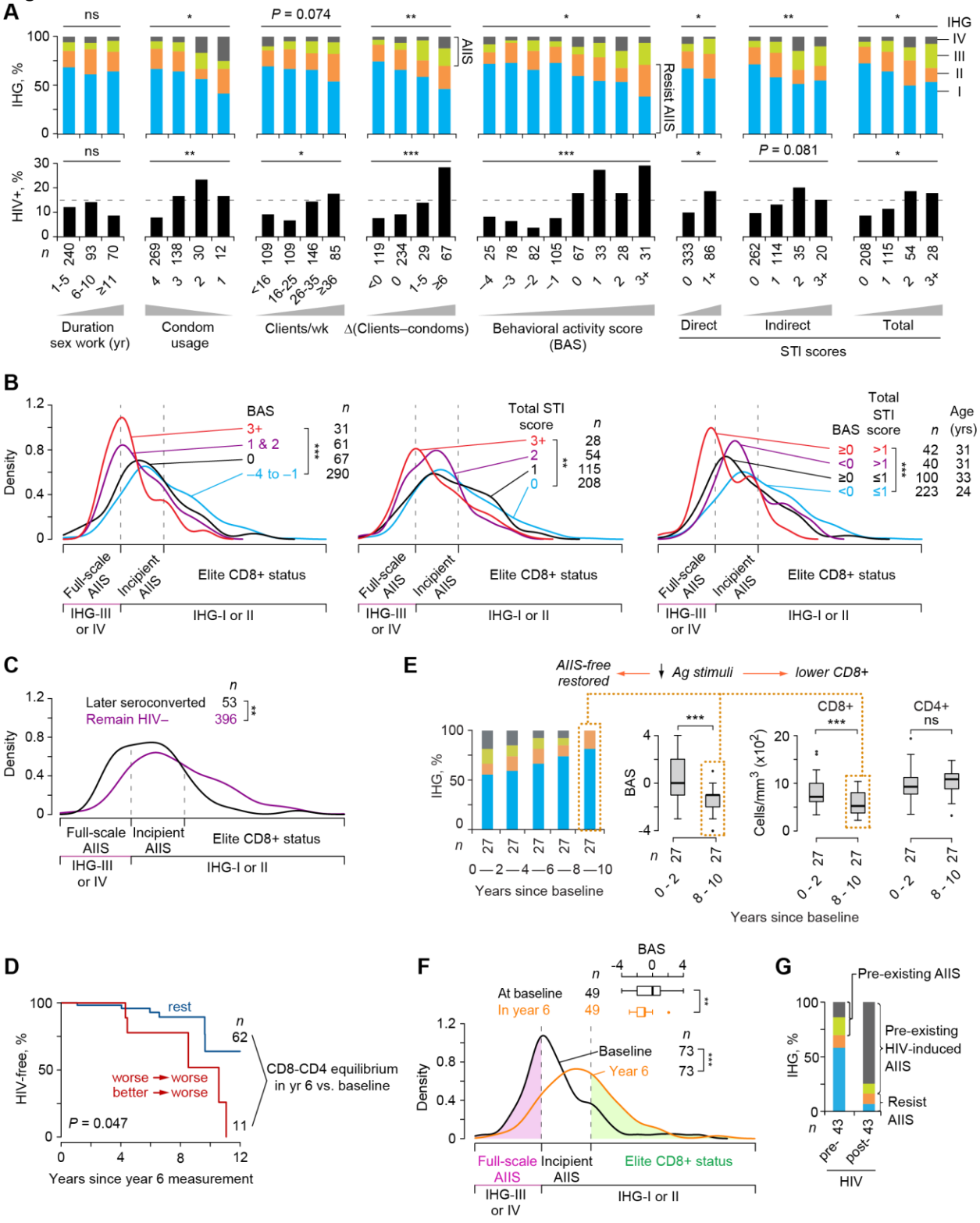

A
ns
*
P = 0.074
**
*
*
**
*
IHG
IV
III
II
I
AIIS
Resist AIIS
IHG, %
HIV+, %
ns
**
*
***
***
*
P = 0.081
*
n
240 93 70
269 138 30 12
109 109 146 85
119 234 29 67
25 78 82 105 67 33 28 31
333 86
262 114 35 20
208 115 54 28
1-5 6-10 ≥11
4 3 2 1
<16 16-25 26-35 ≥36
<0 0 1-5 ≥6
−4 −3 −2 −1 0 1 2 3+
0 1+
0 1 2 3+
0 1 2 3+
Duration sex work (yr)
Condom usage
Clients/wk
Δ(Clients–condoms)
Behavioral activity score (BAS)
Direct
Indirect
Total
STI scores
B
Density
BAS
n
3+ 31
1 & 2 61
0 67
−4 to −1 290
***
Total STI score
n
3+ 28
2 54
1 115
0 208
**
Total STI BAS score
n
Age (yrs)
≥0 >1 42 31
<0 >1 40 31
≥0 ≤1 100 33
<0 ≤1 223 24
***
Full-scale AIIS
Incipient AIIS
Elite CD8+ status
IHG-III or IV
IHG-I or II
C
Density
n
Later seroconverted 53
Remain HIV− 396
**
Full-scale AIIS
Incipient AIIS
Elite CD8+ status
IHG-III or IV
IHG-I or II
D
HIV-free, %
rest
worse → worse
better → worse
P = 0.047
n
62
11
CD8-CD4 equilibrium in yr 6 vs. baseline
Years since year 6 measurement
E
AIIS-free restored
Ag stimuli
lower CD8+
IHG, %
BAS
CD8+
CD4+
***
***
ns
Cells/mm3 (x10²)
n
27 27 27 27 27
0 2 4 6 8 10
Years since baseline
0-2 8-10
Years since baseline
F
BAS
n
At baseline 49
In year 6 49
**
Density
Baseline 73
Year 6 73
***
Full-scale AIIS
Incipient AIIS
Elite CD8+ status
IHG-III or IV
IHG-I or II
G
Pre-existing AIIS
IHG, %
Pre-existing HIV-induced AIIS
Resist AIIS
n
43 43
pre- post-
HIV

Figure 7. Indicator/biomarker function of HIV and cytomegalovirus (CMV) serostatus. (**A**) Top and middle, Median CD8+ and CD4+ T-cell counts (cells/mm^3) and CD4:CD8 T-cell ratio and HIV seroconversion rates in female sex workers (FSWs) within the indicated CD8-CD4 equilibrium states. Bottom, odds ratios (ORs) with 95% confidence intervals (unadjusted) for HIV seroconversion indexed to IHG-I with elite CD8+ status. IHG, immune health grade. (**B**) Odds of future HIV seroconversion by behavioral activity score (BAS), total sexually transmitted infection (STI) score, and CD8-CD4 equilibrium states stratified as full-scale AIIS, incipient AIIS, or elite CD8+ status classified as in panel A. Depicted are odds ratios (ORs) with 95% confidence intervals in univariate (left) and multivariate (right) analysis. (**C**) Odds by multivariate model of HIV seroconversion in persons categorized according to three parameters: BAS, total STI scores, and CD8-CD4 equilibrium states classified as in panel A. (**D**) Summary and indicator/biomarker function of HIV serostatus inferred by the associations shown in panels A-C; post-HIV seroconversion events are based on Figure 3C-E. Ag, antigenic. (**E**) Co-distribution of CD8-CD4 equilibrium states and CMV serostatus in participants of the HIV− University of California San Diego (UCSD) cohort. Top, median CD8+ levels (cells/mm^3) by CMV serostatus in the overall cohort and in persons with the indicated CD8-CD4 equilibrium states. (**F**) IHG status at baseline and in the sixth year of follow-up in 73 HIV− FSWs shown in Figure 6F. Median CD8+ levels are depicted on the top. Half-moon arrows point to the change from baseline in year 6 of follow-up in the overall subset of HIV− FSWs (n=73) and by their IHG status at baseline. (**G**) IHG status in HIV− UCSD cohort by CMV serostatus and results of a urine drug screen for recreational drug use (e.g., methamphetamine). *P<0.05, **P<0.01, ***P<0.001. ns, non-significant.

Figure 7

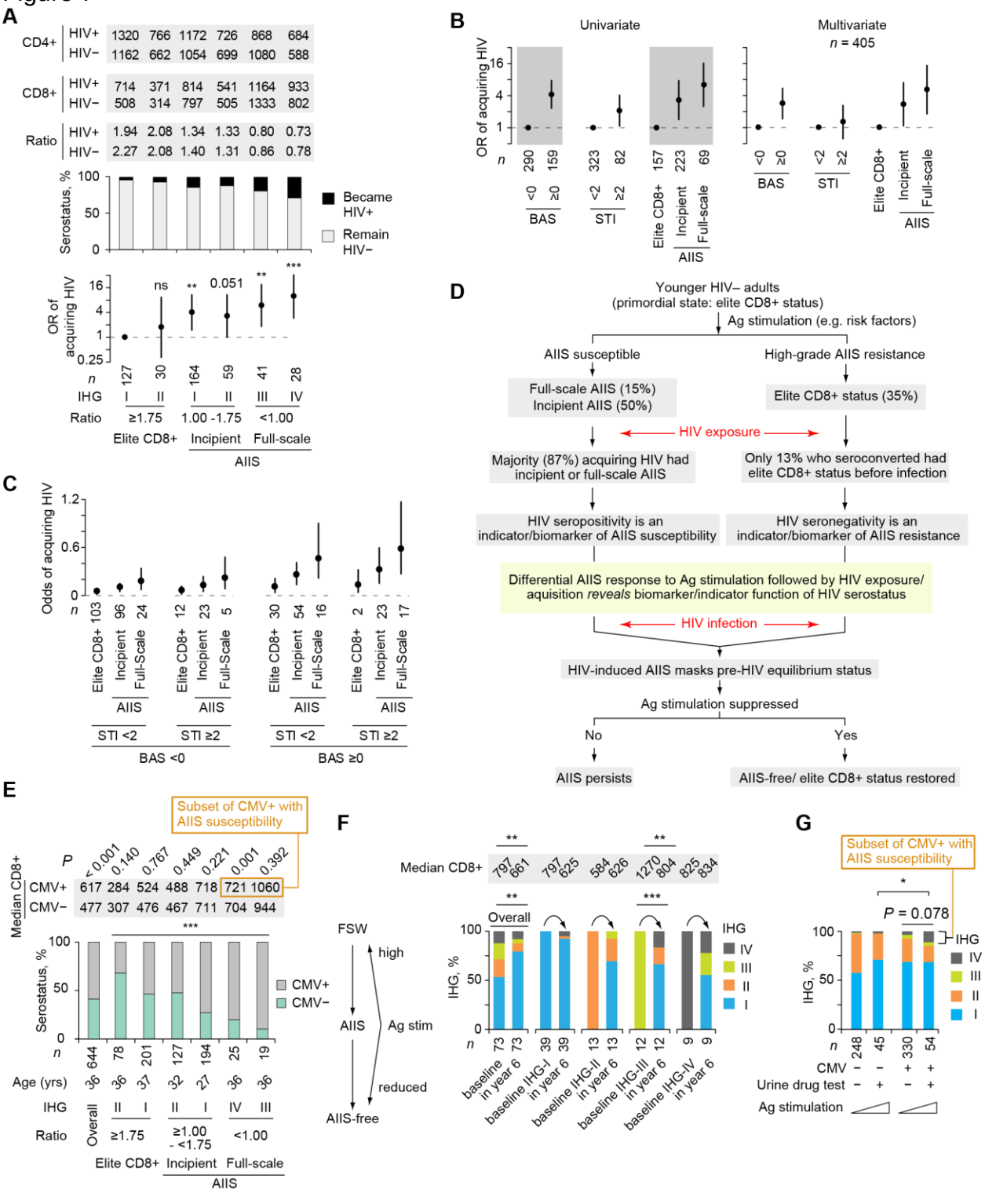
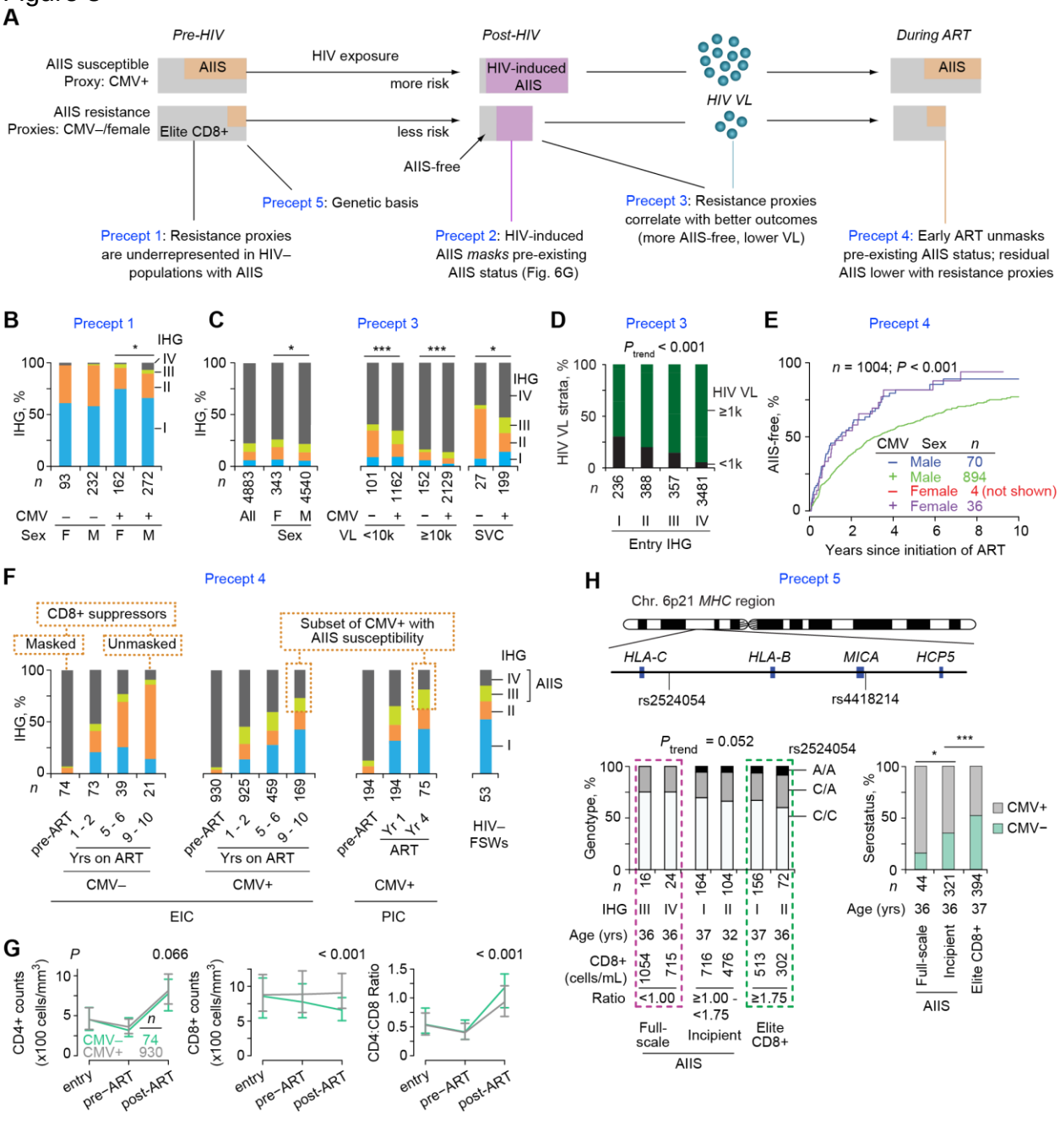

A
CD4+ HIV+ 1320 766 1172 726 868 684
HIV− 1162 662 1054 699 1080 588
CD8+ HIV+ 714 371 814 541 1164 933
HIV− 508 314 797 505 1333 802
Ratio HIV+ 1.94 2.08 1.34 1.33 0.80 0.73
HIV− 2.27 2.08 1.40 1.31 0.86 0.78
Serostatus, %
Became HIV+
Remain HIV−
OR of acquiring HIV
ns
0.051
n 127 30 164 59 41 28
IHG I II I II III IV
Ratio ≥1.75 1.00 -1.75 <1.00
Elite CD8+ Incipient Full-scale
AIIS
B
Univariate
Multivariate n = 405
OR of acquiring HIV
n 290 159 323 82 157 223 69
<0 ≥0 BAS
<2 ≥2 STI
Elite CD8+ Incipient Full-scale AIIS
C
Odds of acquiring HIV
n 103 96 24 12 23 5 30 54 16 2 23 17
Elite CD8+ Incipient Full-Scale
AIIS
STI <2 STI ≥2 STI <2 STI ≥2
BAS <0 BAS ≥0
D
Younger HIV− adults
(primordial state: elite CD8+ status)
Ag stimulation (e.g. risk factors)
AIIS susceptible
High-grade AIIS resistance
Full-scale AIIS (15%)
Incipient AIIS (50%)
Elite CD8+ status (35%)
HIV exposure
Majority (87%) acquiring HIV had incipient or full-scale AIIS
Only 13% who seroconverted had elite CD8+ status before infection
HIV seropositivity is an indicator/biomarker of AIIS susceptibility
HIV seronegativity is an indicator/biomarker of AIIS resistance
Differential AIIS response to Ag stimulation followed by HIV exposure/ aquisition reveals biomarker/indicator function of HIV serostatus
HIV infection
HIV-induced AIIS masks pre-HIV equilibrium status
Ag stimulation suppressed
No
Yes
AIIS persists
AIIS-free/ elite CD8+ status restored
E
Subset of CMV+ with AIIS susceptibility
P <0.001 0.140 0.767 0.449 0.221 0.001 0.392
Median CD8+
CMV+ 617 284 524 488 718 721 1060
CMV− 477 307 476 467 711 704 944
Serostatus, %
CMV+
CMV−
n 644 78 201 127 194 25 19
Age (yrs) 36 36 37 32 27 36 36
IHG Overall II I II I IV III
Ratio ≥1.75 ≥1.00 - <1.75 <1.00
Elite CD8+ Incipient Full-scale
AIIS
F
FSW
high
AIIS
Ag stim
reduced
AIIS-free
Median CD8+ 797 661 797 625 584 626 1270 804 825 834
Overall
IHG, %
IHG IV III II I
n 73 73 39 39 13 13 12 12 9 9
baseline in year 6
baseline IHG-I in year 6
baseline IHG-II in year 6
baseline IHG-III in year 6
baseline IHG-IV in year 6
G
Subset of CMV+ with AIIS susceptibility
P = 0.078
IHG, %
IHG IV III II I
n 248 45 330 54
CMV − − + +
Urine drug test − + − +
Ag stimulation

Figure 8. Durability of indicators of resistance to age-independent immunosenescence (**AIIS).** (**A**) Five precepts tested along the risk continuum of HIV acquisition, immune depletion, and immunologic reconstitution during virally suppressive antiretroviral therapy (ART). VL, HIV viral load. (**B**) Distribution of immune health grades (IHGs) by sex and cytomegalovirus (CMV) serostatus in HIV− University of California, San Diego (UCSD) participants. (**C**) Distribution of entry (baseline) IHGs in therapy-naïve HIV+ participants from the early HIV infection cohort (EIC) by sex, entry HIV VL (copies/ml; k, x 1000), CMV serostatus, and spontaneous virologic controller (SVC) status. (**D**) Distribution of VL (< vs. ≥1000 copies/ml) strata by IHG status in EIC participants. (**E**) Rate of achieving AIIS-free status by sex and CMV serostatus following initiation of ART in EIC participants. (**F**) IHG distribution at entry, at pre-ART, and during the indicated years after initiating ART in the EIC and primary HIV infection cohort (PIC). Baseline IHGs in HIV− FSWs who subsequently seroconverted is depicted in the far right as a reference group. (**G**) Line plots connecting median (IQR) of CD4+ and CD8+ T-cell counts and CD4:CD8 ratio at three timepoints in participants from the EIC who received ART. Post-ART values reflect the best value achieved during ART and corresponds to the same timepoints at which the IHGs were derived in panel F. (**H**) Top, map of major histocompatibility locus and two single-nucleotide polymorphisms (SNPs) studied. Bottom left, distribution of the rs2524054 SNP in all HIV− UCSD participants categorized as having full-scale or incipient AIIS vs. elite CD8+ status. Similar data for European subset of the cohort are shown in **Supplementary Figure S20**. Analysis was restricted to persons with available CMV serostatus. Bottom right, CMV seropositivity rates in the same cohort by full-scale or incipient AIIS vs. elite CD8+ status. *P<0.05, ***P<0.001.

Figure 8


A
Pre-HIV
Post-HIV
During ART
AIIS susceptible
Proxy: CMV+
AIIS
HIV exposure
more risk
HIV-induced AIIS
HIV VL
AIIS
AIIS resistance
Proxies: CMV–/female
Elite CD8+
less risk
AIIS-free
Precept 5: Genetic basis
Precept 1: Resistance proxies are underrepresented in HIV– populations with AIIS
Precept 2: HIV-induced AIIS masks pre-existing AIIS status (Fig. 6G)
Precept 3: Resistance proxies correlate with better outcomes (more AIIS-free, lower VL)
Precept 4: Early ART unmasks pre-existing AIIS status; residual AIIS lower with resistance proxies
B
Precept 1
IHG
IHG, %
CMV
Sex
C
Precept 3
All
Sex
VL
SVC
D
Precept 3
HIV VL strata, %
HIV VL
Entry IHG
E
Precept 4
n = 1004; P < 0.001
AIIS-free, %
Years since initiation of ART
F
Precept 4
CD8+ suppressors
Masked
Unmasked
Subset of CMV+ with AIIS susceptibility
Yrs on ART
ART
HIV– FSWs
EIC
PIC
G
CD4+ counts (x100 cells/mm3)
CD8+ counts (x100 cells/mm3)
CD4:CD8 Ratio
entry
pre-ART
post-ART
H
Precept 5
Chr. 6p21 MHC region
HLA-C
HLA-B
MICA
HCP5
rs2524054
rs4418214
Genotype, %
Serostatus, %
Age (yrs)
CD8+ (cells/mL) Ratio
Full-scale
Incipient
Elite CD8+
AIIS