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Review

Evolution and Diversity of Immune Responses during Acute HIV Infection

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SUMMARY

Understanding the earliest immune responses following HIV infection is critical to inform future vaccines and therapeutics. Here, we review recent prospective human studies in at-risk populations that have provided insight into immune responses during acute infection, including additional relevant data from non-human primate (NHP) studies. We discuss the timing, nature, and function of the diverse immune responses induced, the onset of immune dysfunction, and the effects of early anti-retroviral therapy administration. Treatment at onset of viremia mitigates peripheral T and B cell dysfunction, limits seroconversion, and enhances cellular antiviral immunity despite persistence of infection in lymphoid tissues. We highlight pertinent areas for future investigation, and how application of high-throughput technologies, alongside targeted NHP studies, may elucidate immune response features to target in novel preventions and cures.

INTRODUCTION

More than four decades after the first cases of acquired immunodeficiency syndrome (AIDS), we still lack effective human immunodeficiency virus (HIV) vaccines and curative treatments. The vast majority of those infected require daily anti-HIV therapy to stave off disease, with long-term adherence and uninterrupted access to treatment still ongoing global challenges.

Much of what is known about HIV infection comes from studying human samples from chronic infection or utilizing non-human primate (NHP) models with natural or human-engineered AIDS virus variants—simian immunodeficiency virus (SIV) and simian-human (SHIV), respectively (Estes et al., 2018; Garcia-Tellez et al., 2016). NHP models have demonstrated that features of initial pathology in the days immediately after infection can predict overall disease outcome and that tissue damage begins prior to the onset of plasma viremia (Evans and Silvestri, 2013; Policicchio et al., 2016). A more detailed understanding of host-pathogen interactions in humans during the entirety of the acute infection window, however, had been limited by the difficulty in screening and sampling at-risk populations during the earliest days after exposure, before peak viremia is achieved (McMichael et al., 2010). Characterizing acute HIV infection (AHI) in humans, especially relative to pre-infection, to identify responses linked to disease course is critical to inform future vaccines and therapeutics. More broadly, HIV could serve as a model for acute human viral infections in general (Hargreaves et al., 2020; Robb and Ananworanich, 2016), given its historical role in establishing many modern concepts in immunology (Abbott et al., 2018; Colomer-Lluch et al., 2018; Hughes and Andersson, 2015; Youngblood et al., 2012) and the native heterogeneity in disease course, ranging from progression to natural viral control (Walker and Yu, 2013).

In recent years, a combination of new technologies and the ability to perform longitudinal studies of uninfected persons in areas of high incidence have provided new insights regarding immune and viral dynamics from the onset of plasma viremia. One example is the Females Rising through Education, Support, and Health (FRESH) study in South Africa, in which uninfected 18–23-year-old women at high risk of infection are monitored twice weekly as part of an HIV-prevention and poverty-alleviation project (Dong et al., 2018). Others include the RV217 and RV254 studies in Thailand (Ananworanich et al., 2017; Robb et al., 2016), which rely on screening of blood donations for persons who are HIV RNA and/or antigen positive and antibody negative. These peripheral blood mononuclear cell (PBMC) and plasma samples have provided some of the first insights into human immune responses immediately after detectible infection. Moreover, application of transcriptomic and proteomic technologies, including single-cell RNA sequencing (scRNA-seq) and single-cell mass cytometry (CyTOF) (Coindre et al., 2018; Kazer et al., 2020; Sannier et al., 2020), to these and other rare samples has begun to generate more comprehensive, longitudinal data from the AHI time frame than ever before. Early treatment arms in these studies, where participants initiate antiretroviral therapy (ART) immediately after their first positive plasma viremia test,

have also begun to contextualize the effects of limiting acute antigen exposure on cellular and molecular responses, pinpointing dysfunctional immune activity and highlighting the impact of modulating host-pathogen interactions during peak plasma viremia.

Here, we review emerging data on the evolution of AHI in humans from the time of onset of plasma viremia, with inclusion of relevant data from NHP models. By including studies spanning both hyperacute (prior to peak viremia; i.e., approximately less than one month after transmission) and late-acute infection (approximately one to six months post-transmission), we discuss the timing, identity, and function of the diverse immune responses induced. We also consider the effects of early ART administration on these responses and highlight pertinent areas for future investigation, including cure research, as well as novel technological approaches that can be used to maximize the amount of information extracted from these precious primary human samples.

RAPID SYSTEMIC DISSEMINATION OF INFECTION AND INDUCTION OF PRO-INFLAMMATORY RESPONSES UPON INFECTION

The very earliest mucosal events in HIV infection have largely been inferred from tissue studies of SIV infection in NHPs. Intravaginal and intrarectal SIV infection lead to local viral replication within hours (Haase, 2011; Ribeiro Dos Santos et al., 2011) and systemic spreading by one to three days in the majority of animals (Barouch et al., 2016; Deleage et al., 2019; Rabezanahary et al., 2020). This is accompanied by a robust systemic pro-inflammatory response with induction of innate immune activity including components of the inflammasome, which is thought to contribute to suppression of host cell antiviral-restriction factors favoring viral replication (Barouch et al., 2016; Lu et al., 2014). The molecular and cellular events that enable and facilitate viral transmission through mucosal barriers have been reviewed recently elsewhere (Gonzalez et al., 2019).

After initial infection and local tissue inflammation, both pro-inflammatory and antiviral cytokines and chemokines are robustly upregulated systemically before onset of plasma viremia and during viral expansion (Figure 1) (Katsikis et al., 2011; Keating et al., 2016; McMichael et al., 2010; Muema et al., 2020; Stacey et al., 2009). Onset of plasma viremia in humans is characterized by increased levels of interferon (IFN)-α, CXCL10 (formerly termed IP-10), interleukin (IL)-8, IL-10, IL-12, and CCL2 in the periphery (Muema et al., 2020; Stacey et al., 2009). Interspecies studies of pathogenic SIV infection (rhesus macaque; leads to AIDS) in comparison with studies of non-pathogenic SIV infection (African green monkey; persistent viremia without immunodeficiency) highlight that the timing of the pro-inflammatory and antiviral responses in tissues and the periphery associate with disease progression (Bosinger and Utay, 2015; Bosinger et al., 2012). In non-pathogenic SIV infection, inflammatory responses quell within days post-infection, and upregulated IFN-stimulated genes (ISGs) return to baseline within 30 days post-infection, in stark contrast to pathogenic infection, in which these responses are prolonged for weeks and years, respectively (Bosinger and Utay, 2015), highlighting the need to better understand early events in AHI and how they correspond to long-term outcome. Relatedly, longitudinal multicellular scRNA-seq of FRESH study participants confirmed a ubiquitous ISG response to HIV in the hyperacute window (Kazer et al., 2020). In NHPs, higher levels of cytokines like IFN-α, IFN-γ, IL-18, IL-1β, and others associated with pathogenic infection suggest that the extent of inflammatory and IFN-related cytokine production during acute infection could contribute to long-term immunodeficiency (Keating et al., 2016) (see Box 1 for further discussion of pro-inflammatory and antiviral responses and insights from NHP models). A similar study in humans demonstrated significant correlations between peak levels of IFN-α and soluble IL-2R during hyperacute infection and peak viral load (Muema et al., 2020). Higher levels of peripheral CXCL10, which modulates immune responses by activation and recruitment of leukocytes, have also been shown to correlate with disease progression in humans (Jiao et al., 2012; Liovat et al., 2012). Thus, the timing and magnitude of pro-inflammatory and antiviral responses within tissues and the periphery could tip the balance of future disease progression or control (Figure 1); similarly, in certain respects, NHP models can recapitulate critical features of human infection.

Alongside rapid viral spreading and inflammation after transmission, HIV integrates into the genome of $CD4^+$ T cells that then transition into resting memory cells, creating a persistent viral reservoir (Davey et al., 1999). If ART is interrupted in HIV-infected individuals, infection can resume from latent viruses hidden in these resting memory cells (Chun et al., 1997; Finzi et al., 1997; Wong et al., 1997). The viral reservoir is established within days after infection, as ART initiated at Fiebig Stage I still permits viral rebound after treatment interruption (Colby et al., 2018). Indeed, in a pathogenic SIV infection model, the viral reservoir is seeded before detectible plasma viremia; the reservoir is established in the gastrointestinal (GI) tract no later than three days post-infection (Cantero-Pérez et al., 2019; Whitney et al., 2014, 2018) and in splenic and mesenteric lymph nodes (LNs) as early as four days post-infection, where it is preferentially harbored in T follicular helper (Tfh) and T effector memory cells (Rabezanahary et al., 2020). Sequencing of viral RNA/DNA from the central nervous system of acutely infected individuals demonstrated distinct mutations from transmitter/founder viruses, providing further evidence for early establishment of viral sanctuaries, tissue sites that facilitate persistent viral replication despite successful ART treatment (Tovanabutra et al., 2019). NHP studies showing exclusion of $CD8^+$ T cells from germinal centers (GCs) in LNs and secondary lymphoid organs implicate these structures as important viral reservoirs and potential viral sanctuaries that likely contribute to ongoing chronic inflammation (Fukazawa et al., 2015).

INNATE IMMUNE CELLS RESPOND DURING HYPERACUTE INFECTION AND ORCHESTRATE THE INITIAL ANTIVIRAL RESPONSE

Innate immune cells are the first line of defense against previously unencountered foreign pathogens and subsequently instruct adaptive immunity (Altfeld and Gale, 2015; Iwasaki and Medzhitov, 2015). Nevertheless, the roles of these cells throughout AHI are not completely understood. Here, we describe what is known about various innate immune cell subsets in hyperacute infection and highlight what remains to be elucidated.

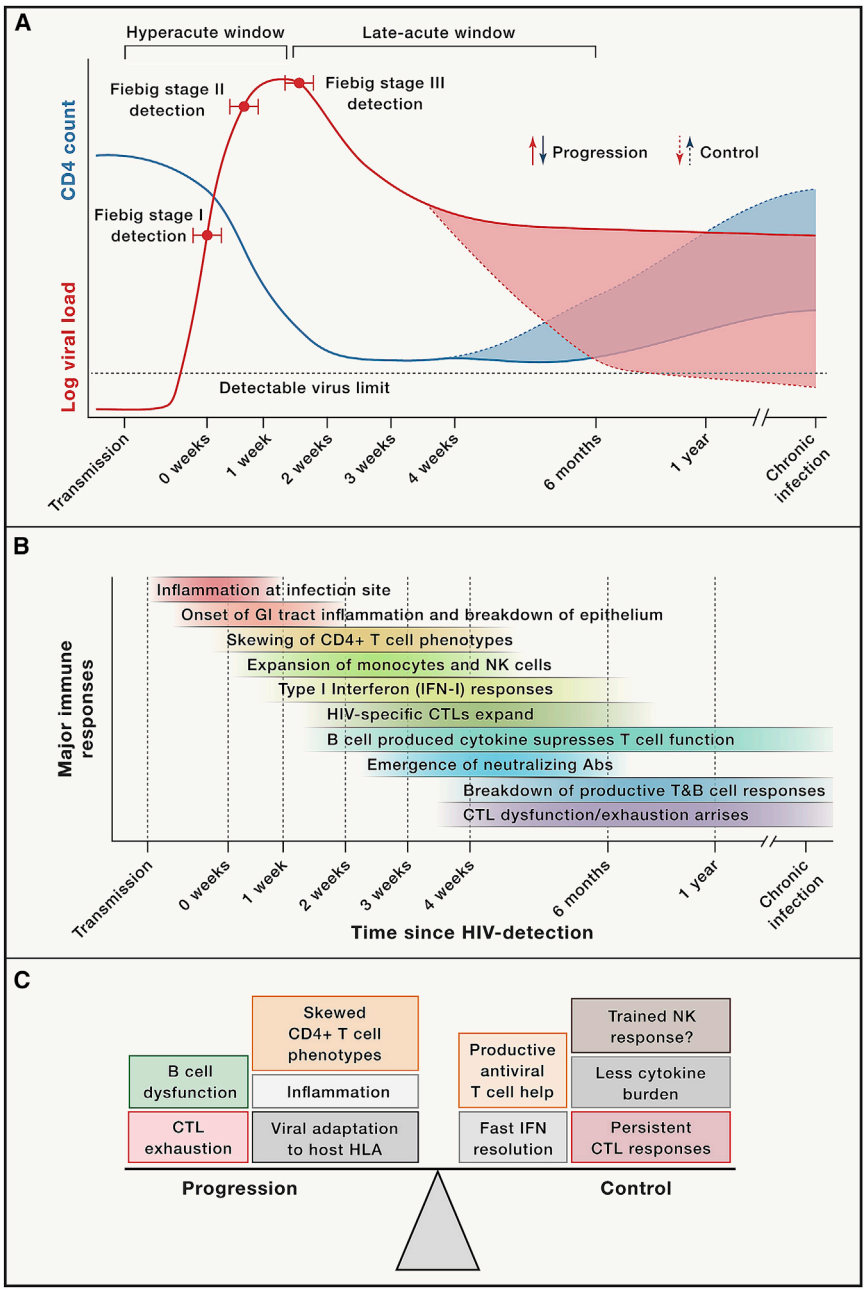

Figure 1. Acute HIV Infection Dynamics

(A) Representative dynamics of plasma viral load and peripheral CD4$^+$ T cell counts during acute HIV infection. Individuals that develop control of HIV without ART can maintain low chronic viral setpoint and reestablish pre-infection CD4$^+$ T cell levels (elite or viremic controllers). Individuals and animals can also maintain high chronic viral setpoint and recover CD4$^+$ T cell counts (long-term non-progressors [LTNP] and natural hosts, respectively).

(B) Representative immune responses and their timing along the course of acute HIV infection. Without structured cohorts to reliably sample before Fiebig Stage I, the earliest responses are inferred from SIV/SHIV models and limited human studies.

(C) Cellular and molecular factors in AHI that contribute to disease progression or control. The progression-control spectrum has been linked to the magnitude, timing, and function of several distinct immune responses. Box colors represent different cell types or cytokine responses.

Early work in NHP models pinpointed plasmacytoid dendritic cells (pDCs) as instigators of systemic antiviral type-I IFN production after infection. After sensing viral RNA/DNA by TLR7 and TLR8, pDCs produce IFN-α and IFN-β in LNs and the GI tract (O'Brien et al., 2013). A treatment interruption study in ART-adherent individuals with undetectable viremia who began ART therapy at onset of plasma viremia demonstrated that pDCs upregulate migration and activation markers before detectible viremia but exhibit a transient decline (compared to prior to treatment) in their ability to produce IFN-α *in vitro* (Mitchell et al., 2020). Multicellular analysis in the FRESH study also showed that pDCs do not express type-I IFN genes or interferon regulatory factor 7 (*IRF7*) prior to peak viremia, but they do upregulate ISGs and signaling factor genes, supporting a hypothesis that pDC function in the periphery could be to recruit other immune cells rather than produce IFN-α/β (Kazer et al., 2020). Nevertheless, multiple other peripheral immune subsets upregulate *IRF7* during hyperacute infection, suggesting that although pDCs likely play an important role in directing IFN signaling in LNs, both direct viral sensing and IFN feedback from other cell types that contribute to the overall peripheral response. Novel, low-input genomic technologies (Corces et al., 2017; Skene and Henikoff, 2017; Stubbington et al., 2017) will provide new opportunities to study pDCs from tissue biopsies, fine need aspirates, and small amounts of blood to better understand their compartment-specific roles (see Box 2 for a brief description of these technologies and their utility in studying HIV).

Studies of peripheral cytokines upregulated in AHI (e.g., CXCL9, CXCL10, IL-10, etc.; Jiao et al., 2012; Katsikis et al., 2011; Stacey et al., 2009) implicate monocytes and macrophages as key players in orchestrating cellular trafficking and antiviral factor production. Comparison of complete blood counts pre-infection and at peak viremia in the FRESH study revealed expansion of monocytes after onset of plasma viremia (Muema et al., 2020). Transcriptional analysis showed that monocytes upregulate genes encoding many known pathogen recognition receptors (PRRs) and HIV-restriction factors during hyperacute infection (e.g., RIG-I, APOBEC3 family, LGALS3BP, etc.) (Kazer et al., 2020). Monocytes were also shown to upregulate CCL2 and CXCL10 within the first four to six days of Zika virus infection, implicating their importance in sensing and responding to viral pathogens more broadly (Michlmayr et al., 2020). Moreover, monocytes also persistently

Box 1. Inflammation and Type-I IFN Signaling in Progressive and Non-progressive SIV Infection Models

Early events in the GI tract could be particularly revealing regarding the extent of local and peripheral cytokine response due to it being the site of the majority of the lymphoid tissue in the body (Brenchley et al., 2006; Redd et al., 2009). Acute infection leads to dramatic depletion of both activated and resting $CD4^+$ T cells in the gut, already evident within four days of experimental NHP infection (Li et al., 2005; Mattapallil et al., 2005). This massive cell death during hyperacute infection manifests chronic inflammation and permanent damage to the gut epithelial barrier, promoting microbial translocation and IL-17 production in the tissues (Klase et al., 2015; Klatt et al., 2010). SIV infection of African green monkeys (non-progressive infection), compared to Rhesus Macaques (progressive infection) revealed induction of inflammation-induced genes *TNF* and *IL10* in PBMCs and LN lymphocytes at five and 10 days post-infection only in non-progressive infection, suggesting that early inflammation could reduce antiviral signaling and lead to further $CD4^+$ T cell depletion. Indeed, characterization of the GI tract during hyperacute SIV infection in African green monkeys demonstrated reduced apoptosis of both enterocytes and lymphocytes despite transient increases in pro-inflammatory cytokines and chemokines in the plasma and increased T-cell activation in the blood (Raehtz et al., 2020). RNA-seq analysis confirmed limited transient antiviral and T-cell activation programming during the first two weeks of infection, but no genes associated with microbial translocation, which was corroborated by lipopolysaccharide immunohistochemistry. Thus, one path to reduce overall disease burden could be protection of the gut epithelium to resist infection and inflammatory signaling, potentially by modulating resident innate lymphoid cells (Shah et al., 2017), macrophages (Swan et al., 2017), or the epithelial cells themselves (Ordovas-Montanes et al., 2020).

Persistent type-I IFN signaling in pathogenic SIV and HIV infection can lead to broad dysfunction in both innate and adaptive immunity during chronic infection (Bosinger and Utay, 2015; Cheng et al., 2017; Soper et al., 2018; Wang et al., 2017; Zhen et al., 2017). Understanding how levels and timing of the initial inflammation at the site of infection, GI tract, and in the periphery impacts when and how antiviral type-I IFN responses form and ultimately transition from productive to dysfunctional (Wang et al., 2017) is crucial to mitigate chronic inflammation and deter their persistence. Given the roles of tissue resident macrophages (Grainger et al., 2017), DCs (Sun et al., 2020), ILCs (Branzk et al., 2018), and epithelial cells (Okumura and Takeda, 2017) in maintaining tissue homeostasis and propagating both inflammatory and type-I IFN signaling in the GI tract, further work is needed to understand how their depletion or perturbation in AHI impacts outcome.

upregulate HLA-DR throughout hyperacute and late-acute infection (Chen et al., 2017; Liu et al., 2019), potentially contributing to prolonged antigenic stimulation of T cells and subsequent T cell exhaustion. Human beta defensin 1 was also demonstrated to be upregulated in circulating monocytes three months post-infection (Corleis et al., 2017), suggesting that monocytes could encounter and recognize components of HIV directly in addition to responding to type-I IFN, and/or durable changes to myeloid progenitors could occur. Whether tissue resident macrophages play similar roles to peripheral monocytes as signal transducers in hyperacute infection, or potentially adopt more niche-specific roles (Guilliams et al., 2020; Lavin et al., 2015), needs to be further explored in NHP models and what limited tissue samples from humans are available.

Innate-like cytotoxic lymphocytes also expand and activate during hyperacute infection. Peripheral $CD56^{dim}CD16^+$ natural killer (NK) cells expand (Alter et al., 2005), activate (Naranbhai et al., 2013), and express cytolytic gene modules starting within one week post-onset of plasma viremia (Kazer et al., 2020). Early proliferation of NK cells was also associated with subsequent viremia control in two FRESH study participants. Their role in tissues during AHI, however, is unclear. In a pathogenic vaginal SIV challenge model, NK cells were recruited to the female genital tract (FGT), albeit in small numbers, during the first week of infection but lacked CD107a and Ki67 expression (Shang et al., 2014), indicating that they did not adhere to their traditional cytotoxic roles. Nevertheless, data in HIV models suggest that NK cells could be protective against infection. Treatment of healthy human NK cells with IL-15 superagonist *in vitro* before injection into humanized mice challenged with HIV inhibited infection and could directly suppress viremia in the spleen (Seay et al., 2015). Moreover, the demonstration of adaptive-like NK cells in SIV or SHIV infection restricted to DCs cross-presenting Gag and Env peptides hints that these cells could play a more important role in acute infection than currently known and could conceivably be primed to respond during acute HIV infection (Reeves et al., 2015). Indeed, Wang et al. confirmed this finding in humans, showing the expansion of $CD94^+$ memory NK cells by TCF7 in HIV infection (Wang et al., 2020). With improved NK cell manipulation in NHP models, it will be essential to further test the potential for NK-cell-mediated protection during acute infection, especially before cytotoxic T lymphocyte (CTL) responses fully mature. Moreover, the realization of both adaptive and innate training in NK cells (Adams et al., 2016) makes them a potential vaccine target alongside T cells.

Both mucosal-associated invariant T (MAIT) and $\gamma\delta$T cells change in frequency during hyperacute infection and potentially play cytolytic and regulatory roles after onset of plasma viremia. Although initially shown to be depleted in late-acute infection (Cosgrove et al., 2013), a recent study demonstrated that MAIT cells actually expand and upregulate genes associated with IFN-γ production and innate mediated cytotoxic immunity during hyperacute viremia (Lal et al., 2020). Conversely, $\gamma\delta$T cells, which are known to target conserved regions of bacteria present at mucosal surfaces, skew in proportion away from the traditionally cytotoxic subset (Vδ2) toward the tissue resident effector memory subset (Vδ1 in the periphery during AHI; Bhatnagar et al., 2017; Juno and Eriksson, 2019). In their review on the role of $\gamma\delta$T cells throughout acute and chronic HIV infection, Juno and Eriksson indicate that the expansion of the Vδ1 subset is likely in response

Box 2. "Omic" Technologies and Their Utility in Studying Acute HIV Infection

With the advent of next-generation sequencing and steady improvements in low-input and single-cell profiling technologies, high-content measurements of gene expression, protein expression, open chromatin, and other cellular features are becoming increasingly accessible and affordable substantial insights into disease biology in heterogeneous tissue environments (Hartmann and Bendall, 2020; Shema et al., 2019; Stubbington et al., 2017). Critically, many of these assays can be used to study rare cell types from low-input samples like tissue biopsies, fine-needle aspirates, and small amounts of blood. Below, we briefly describe some of these new technologies, their use thus far in HIV infection, and some of what we stand to learn through future applications. For a more extensive discussion of single-cell technologies that can be applied to study tissues in human disease, please see Rozenblatt-Rosen et al. (2020) and Slyper et al. (2020).

- *Single-cell RNA-sequencing (scRNA-seq)*: High-throughput scRNA-seq methods, using droplets (Macosko et al., 2015; Zheng et al., 2017), picowells (Gierahn et al., 2017; Han et al., 2018), or split-pool approaches (Cao et al., 2017; Rosenberg et al., 2018) enable simultaneous whole-transcriptome measurements of thousands of single cells. We have applied scRNA-seq to blood samples in AHI to discern multicellular responses and identify subsets of innate immune cells associated with viral control (Kazer et al., 2020). In chronic infection settings, heterogeneity in T cell phenotype has also been explored (Liu et al., 2020; Sannier et al., 2020). Use in LN and gut tissues could help discern response features associated with CTL exclusion from GCs or polyfunctionality and clonality (Azizi et al., 2018; Tu et al., 2019) of $CD4^+$ T cells, as well as tissue-level adaptations to the temporary or permanent loss of direct viral targets or virally/antiretrovirally driven inflammation.
- *Single-cell mass cytometry (CyTOF):* Multiplexed measurement of protein expression from single cells using mass cytometry (Atkuri et al., 2015) has expanded the range of traditional single-cell measurements like flow cytometry to over 50 simultaneous protein and RNA markers. Applied to measure NK cell diversity (Strauss-Albee et al., 2015) and $CD4^+$ T cell infection susceptibility (Manganaro et al., 2018), its potential to understand changes in cellular protein expression during AHI is just emerging. Combined with imaging (Baharlou et al., 2019), it will be possible, for example, to discern cell-cell interactions in tissues with ongoing HIV infection and validate the roles of DCs in orchestrating pro-inflammatory and antiviral signaling.
- *Assay for transposase-accessible chromatin by sequencing (ATAC-seq):* Technological advances allowing for low-input (Corces et al., 2017) and single-cell (Buenrostro et al., 2015; Cusanovich et al., 2015) measurements of chromatin accessibility have begun to link phenotype and gene expression to epigenetic state across immune cells in disease settings (see review by Shema et al., [2019]). Thus far, ATAC-seq has been used to understand changes to chromatin accessibility in central memory and effector memory $CD4^+$ T cells (Einkauf et al., 2019) and DCs (Johnson et al., 2018) from HIV-infected individuals. Further application to both innate and adaptive immune subsets before and throughout AHI, in the presence and absence of prophylactic interventions, will help determine how innate immune cells could acquire protective responses (e.g. trained immunity or memory; Netea et al., 2020; Wang et al., 2020) and ascertain when and where T cells begin to acquire features of exhaustion, among other insights.
- *Cleavage under targets (CUT) for chromatin profiling:* CUT&RUN for low-input samples (Skene and Henikoff, 2017) and now CUT&Tag for single-cell samples (Kaya-Okur et al., 2019) enable efficient, detailed profiling of diverse chromatin components like histone modifications and transcription factor binding sites. CUT&RUN demonstrated the role of TCF7 in generating memory NK cells during HIV infection (Wang et al., 2020), but its application to other cell types and specifically during AHI has yet to be accomplished. In addition to highlighting epigenetic marks associated with activation or silencing, this approach could identify and validate transcription factors contributing to cellular dysfunction and nominate putative targets for restricting cytokine signaling.

to increased exposure to antigens, suggesting that these cells could be responding to microbial translocation resulting from breakdown of mucosal barriers like the gut epithelium. Whether changes in MAIT and $\gamma\delta$T cells directly affect disease trajectory in AHI through antiviral or other means is unknown.

Helper innate lymphoid cells (ILCs) are a recently described class of innate immune cells in mucosal tissues that play similar roles to T cells but are not engaged through an adaptive immune receptor (i.e., T cell receptor), instead reacting to tissue perturbations independent of antigen stimulation (Eberl et al., 2015). Studies in HIV/SIV infection (Shah et al., 2017) and other disease settings (Ardain et al., 2019; Ebbo et al., 2017; Stehle et al., 2018) have implicated ILC2s, ILC3s, and ILC progenitors (ILCPs) in mounting productive (and sometimes deleterious; Pantazi and Powell, 2019) immune responses upon pathogenic or inflammatory assault. Thus far, studies of these cells in AHI has been limited. Circulating ILC2s and ILC3s are irreversibly depleted during hyperacute HIV infection; transcriptionally, these cells exhibited gene programming associated with apoptosis and cell death, suggesting that they undergo cell death rather than migrating into tissues (Kløverpris et al., 2016), although the impact of their loss on disease progression is unclear. Confirming the depletion of ILCs during AHI, Wang and colleagues also tested their depletion *in vitro*, demonstrating that γ-chain cytokines (e.g., IL-2, IL-4, IL-15), which are known to be elevated during HIV infection, restricted Lin^-CD127^+ ILC numbers through JAK3 signaling (Wang et al., 2020). This suggests that these cells are lost as bystanders in the pro-inflammatory and antiviral cytokine milieu at the onset of plasma viremia. Although not yet measured in humans, SIV studies have shown depletion of ILC3s in the infant NHP gut (Hueber et al., 2020), as well as depletion and phenotypic skewing of $NKp44^+$ ILCs during acute infection in adult gut (Li et al., 2014), and suggested protective qualities of ILC3s in adenovirus-SIV vaccination and

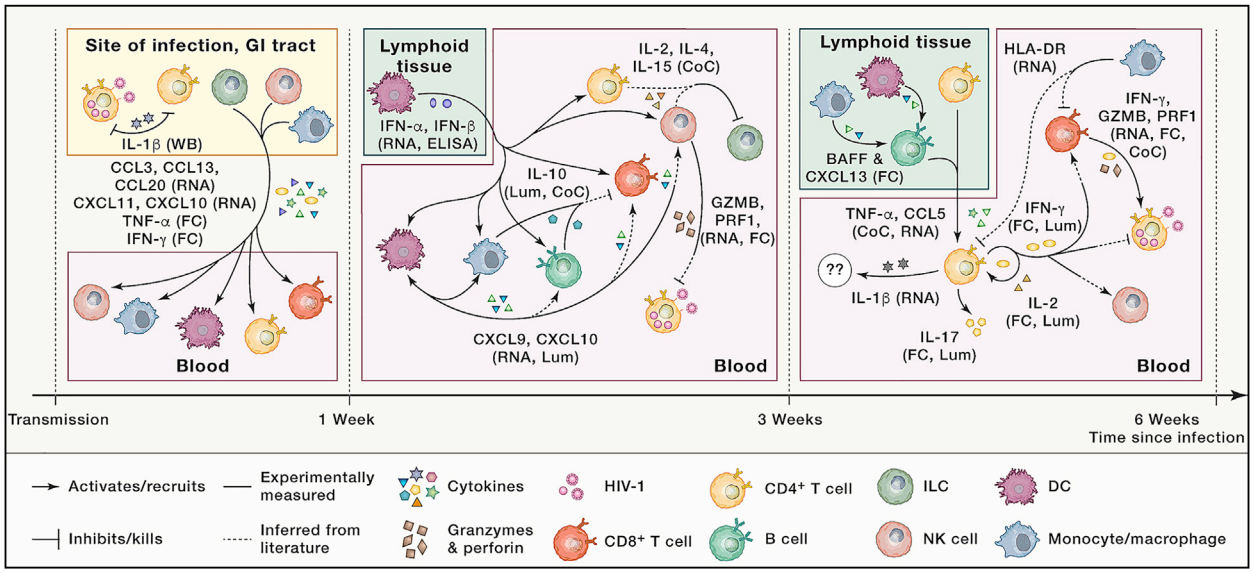

Figure 2. Known and Inferred Immune Cell Signaling during Untreated Acute HIV Infection

Understanding the interplay between distinct immune subsets in acute HIV infection is essential for disentangling the effects of key cytokines (e.g., type I and II IFNs, IL-10, IL-17, etc.) on each cell type. Moreover, realizing the compartment-specific responses and their effects will allow for more targeted treatment. Experimentally measured interactions are depicted with solid arrows. Interactions for which only the signaler or receiver has been measured are shown by arrows with hybrid solid and dashed lines, which are inferred from literature on chronic infection or other models. The supporting assay for each interaction is listed with each cytokine or factor. GZMB, granzyme B; PRF1, perforin; WB, western blot; RNA, RNA-sequencing; FC, flow cytometry; ELISA, enzyme-linked immunosorbent assay; Lum, luminex multiplex assay; CoC, *in vitro* coculture.

mother-to-child transmission (Hueber et al., 2020; Rahman et al., 2019). Groups 1 and 3 ILCs present in the GI tract during SIV infection potentially contribute to pro-inflammatory responses by producing higher levels of IFN-γ, tumor necrosis factor (TNF)-α, and IL-22 during the breakdown of the epithelium (Cogswell et al., 2020); however, when these pro-inflammatory ILCs arise is unknown. Further work demonstrating functional roles for helper ILC subsets in T-cell-rich tissues is necessary to determine their roles in hyperacute HIV infection and the potential of targeting these cells in vaccines and treatments.

In summary, both the myeloid and lymphocyte innate immune compartments respond robustly after onset of plasma viremia. Although monocytes and pDCs clearly contribute to cell traffic signaling and amplify the type-I IFN response (Figure 2), the immediate antiviral functions of these cells as well as innate lymphocytes are still unclear. Moreover, changes in myeloid subsets during other acute viral infections are increasingly being shown to be associated with disease outcomes (Arts et al., 2018; Heung and Hohl, 2019; Kwissa et al., 2014; Michlmayr et al., 2020). Vaccine studies demonstrating protection conferred by NK cells and ILCs (Gorini et al., 2020; Rahman et al., 2019) suggest that these cells likely play more direct antiviral roles in controlling infection spread after onset of plasma viremia than is currently understood. Critically, how the timing and the magnitude of the initial innate responses to HIV infection impact the development of productive T and B cell responses has yet to be adequately explored.

EARLY ADAPTIVE IMMUNE RESPONSES ARE DYSFUNCTIONAL

HIV-specific T and B cell responses arise in the days to weeks after peak plasma viremia and exert pressure on HIV forcing sequential immune escape mutations (Fischer et al., 2010). Both HIV-specific and non-HIV-specific lymphocyte responses, however, have been shown to exhibit early dysfunction, skewing in phenotype, and lack of reactivity starting in hyperacute infection (Figure 1). Here we discuss both the productive and dysfunctional adaptive immune responses starting in hyperacute infection and throughout late-acute infection and their potential impact on disease trajectory.

As the primary targets of HIV, $CD4^+$ T lymphocytes are the first subset to demonstrate significant phenotypic changes during hyperacute infection. Gut homing $CD4^+$ T cells expressing $\alpha4\beta7$ (Arthos et al., 2008) are depleted in the GI mucosa starting at onset of plasma viremia but are not depleted in the periphery until after peak viral load is achieved (Sivro et al., 2018). Another AHI cohort corroborated the targeted depletion of these cells, specifically T helper (Th) 17 and Th1 subsets (Lu et al., 2016). Globally, peripheral $CD4^+$ T cells were shown to upregulate genes associated with differentiation and downstream of pro-inflammatory cytokines IL-1β and TNF-α starting after peak viremia (Kazer et al., 2020). The phenotypes and roles of HIV-specific $CD4^+$ T cells during AHI, however, are more diverse.

Despite the targeted loss of Th17 cells, HIV-specific $CD4^+$ T cells produce IL-17, IFN-γ, and IL-2 during AHI (Yue et al., 2008) and in response to Gag p24 antigen *ex vivo* (Chevalier et al., 2016), indicating that at least a portion of Th cells are functional. However, IL-17 is not classically antiviral (Iwasaki and Medzhitov, 2015), and its production by HIV-specific $CD4^+$ T cells could indicate skewing away from, or depletion of, the antiviral Th1 phenotype. The extent of cytokine production by HIV-specific $CD4^+$ T cells was shown to be positively associated with the magnitude of peak viral load (Chevalier et al., 2016),

although whether these cytokines effectively inhibit viral replication is unclear. In addition to higher frequencies of Gag-specific T cell responses in the late-acute window (Schieffer et al., 2014), the frequency of cytolytic HIV-specific $CD4^+$ T cells producing granzyme A during the hyperacute window also negatively correlated with viral load set point (Soghoian et al., 2012). These findings suggest that those $CD4^+$ T cells that do exhibit Th1 activity could respond through diverse antiviral mechanisms variably over time. Notably, the extent of activation marker expression by memory $CD4^+$ T cells in the periphery during late-acute infection correlated with higher $CD4^+$ T cell counts two years post-infection (Maenetje et al., 2010; Xia et al., 2018), indicating the importance of T cell help during AHI. Others have shown that this association could be subset specific; Pušnik and colleagues demonstrated that frequencies of stem-cell-like memory ($CCR7^+CD27^+CD95^+CD45RA^+$) $CD4^+$ T cells, potentially induced by Fas upregulation, were associated with increased HIV replication and rapid disease progression (Pušnik et al., 2019). Re-polarization of Th17-like $CD4^+$ T cells or redirection of pro-inflammatory signals during AHI may help prevent further T cell loss, misdirected T cell help, and dysfunction as well as promote proliferation of naive T cells (Figure 1C).

T-B cell interactions are also inhibited during AHI but not until late-acute infection. Investigation of Tfh cells, known to expand in chronic infection (Lindqvist et al., 2012; Roider et al., 2018), at two months revealed a shift in helper phenotype toward Tfh1 that negatively correlated with chronic viral set point and was predictive of p24-specific plasma IgG titers at one year after infection (Baiyegunhi et al., 2018). *In vitro* cocultures using primary cells from the RV254 study, however, demonstrated that the quality of Tfh-B cell reactions begin to deteriorate in late-acute infection, with poorer survival of resting memory B cells ($CD21^+CD27^+$) isolated from Fiebig Stage III in comparison with Stages I and II (i.e., earlier in infection trajectory; Figure 1A) (Muir et al., 2016). Moreover, these B cells showed skewing of cytokine production, with reduced IL-10 production and higher levels of CCL5 (RANTES) and TNF-α, highlighting B cells as a potential source of pro-inflammatory cytokines during both hyperacute and late-acute infection. Production of IL-10 by B cells during AHI (Liu et al., 2014) could have deleterious effects on early CTL responses, which are inhibited by IL-10 (Brockman et al., 2009). Secreted factors by other cells, especially macrophages and DCs, likely also influence T-B cell interactions and must be investigated further.

Relatedly, B cell activating factor (BAFF), which has been shown to be produced by monocytes and pDCs during late-acute HIV infection (Borhis et al., 2016), has been posited to dysregulate Tfh-B cell responses in GCs (Borhis et al., 2017). Indeed, an anergic subset of $CD21^-CD27^+IgD^+$ B cells (termed marginal zone B cells) was found to emerge in AHI and expand during chronic infection (Liechti et al., 2019). Treatment of SIV-infected macaques throughout hyperacute infection with a BAFF antagonist, however, inhibited the proliferation of GC B cells and delayed the upregulation of IFN-α and CXCL10 (Borhis et al., 2020), suggesting that levels of BAFF could need to be finely tuned to promote productive antibody responses. More generally, the extent of cytokine/chemokine production and signaling, rather than its presence or absence, likely tips the balance between disease and control (Figure 1).

Peripheral B cells are activated starting in hyperacute infection and persist throughout AHI. Longitudinal sampling of acutely infected individuals in the FRESH study revealed a relative depletion of resting memory B cells from the blood during hyperacute infection alongside gradual increases in tissue-like and activated memory B cells and plasmablasts in the late-acute stage (also confirmed in other cohorts; Liechti et al., 2019; Muir et al., 2016), consistent with increased levels of BAFF and CXCL13 in the plasma (Mabuka et al., 2017). Interestingly, levels of CXCL13 at several time points during AHI were predictive of the development of cross-neutralizing antibodies at one year (Mabuka et al., 2017), although the direct relationship or mechanism is unknown (NB the development of broadly neutralizing antibodies is complex and outside the scope of this review; for additional details see Dashti et al., 2019; Sadanand et al., 2016; Wang and Zhang, 2020). The majority of antibodies targeting critical HIV proteins Gag, Pol, and Env produced throughout AHI are non-neutralizing (Kardava et al., 2014; Tomaras et al., 2008). Knox and colleagues, however, showed that T-bet^+ B cells specifically contribute to effective HIV Env (i.e., binding protein) memory response (Knox et al., 2017), suggesting that targeted transcription factor induction could direct productive B cells. B cell mediated cytokine signaling, especially in the hyperacute infection window, must be explored further to ascertain their relative contribution to blocking successful T cell responses.

HIV-specific $CD8^+$ T cells also activate after peak plasma viremia and are associated with enhanced control of viremia. Both the FRESH and RV254 studies have shown that HIV-specific $CD8^+$ T cells expand in the periphery and upregulate cytotoxic effector molecules perforin and granzyme B within the first month of infection (Demers et al., 2016; Ndhlovu et al., 2015; Takata et al., 2017). The extent of this response has also been shown to associate with slower disease progression (Streeck et al., 2014), supporting the theory of protective HLA alleles against HIV (Figure 1) (Carlson et al., 2015; Goulder and Walker, 2012). The magnitude of the activated ($HLA\text{-}DR^+CD38^+$) $CD8^+$ CTL response also negatively correlated with viral load setpoint (Ndhlovu et al., 2015). Interestingly, activated HIV-specific $CD8^+$ T cells were found to be present in cerebral spinal fluid (CSF) during AHI (Kessing et al., 2017). The ability of these cells to traffic to sites of viral persistence is likely a key factor; relatedly, SIV-specific CTLs are restricted from entry into germinal GCs in LN in both acute and chronic infection (Fukazawa et al., 2015; Li et al., 2019), indicating tissue-/microenvironment-specific control of their migration. The exclusion of CTLs from LN starting early in infection (though unclear in humans; Petrovas et al., 2017) could enhance early seeding of the reservoir in these tissues and must be further explored in HIV.

The first HIV-specific CTL responses appear to have a detectable antiviral effect, but this is not durable in most persons. CTLs sampled from five participants during late-acute infection and cocultured with primary $CD4^+$ T cells infected with various transmitter/founder strains inhibited viral replication *in vitro* (Freel et al., 2012). However, when cocultured with target cells infected with HIV containing known early escape mutations, these cells relatively underperformed, suggesting that any given HIV-specific response could be limited in its usefulness over time. Indeed, recent work suggests that although CTLs can kill

infected cells and induce escape mutations in HIV during late-acute infection, these cells lack cross-reactivity, are dysfunctional, and become exhausted and/or do not develop into long-term effector memory cells (Du et al., 2016; Ndhlovu et al., 2015; Takata et al., 2017). HIV-specific cells expressing CD38 but reduced levels of CD8 and CD27 within the hyperacute window were shown to have reduced efficacy in inhibiting HIV replication and also made up to 40% of all HIV-specific cells (Eller et al., 2016). The expansion of these cells was associated with $CD4^+$ T cell depletion, suggesting that a loss of $CD4^+$ T cell help could lead to a dysfunctional CTL phenotype. Stimulating $HLA\text{-}DR^+CD38^+$ CTLs collected at various Fiebig Stages (I–III) *ex vivo* through the T cell receptor (TCR) revealed reduced IFN-γ, TNF-α, and IL-2 production from those sampled at Fiebig Stage III, indicating dysfunction begins within 1-month post-detection (Takata et al., 2017).

Impaired function of CTLs with constant antigen exposure, termed T cell exhaustion, has been widely explored in chronic viral infections and cancer (McLane et al., 2019). Studies in acute SIV infection suggest that SIV-specific CTLs could be primed for exhaustion due to persistent high expression of PD-1 (Petrovas et al., 2007). In addition to reduced T cell help, several putative mechanisms of CTL dysfunction during AHI have been proposed, including the following: (1) dysregulation of T-bet expression leading to lower levels of perforin (Demers et al., 2016), (2) apoptosis after activation without transition to effector memory (Ndhlovu et al., 2015), and (3) altered metabolism (Takata et al., 2017; Trautmann et al., 2012). These various dysfunctional states likely represent concerted CTL programming induced by changes in T cell help in addition to other immune signaling events. Whether dysfunction arises first in HIV-specific T cells, hindering helper and antibody responses through uncontrolled viremia, or improper help and cytokine production induce dysfunction in HIV-specific T cells, is unclear. Moreover, exacerbated viral replication in the absence of productive HIV-specific $CD8^+$ T cell responses could further fuel functional changes to naive T and B cells. Understanding precisely when, where, and how CTLs begin to fail is critical to develop novel interventions to prevent dysfunction during AHI (Figure 1).

Collectively, the depletion and subsequent phenotype skewing of $CD4^+$ T cells during hyperacute infection likely imparts lasting dysfunction on other adaptive lymphocytes, leading to skewed cytokine production in B cells and a pro-apoptotic phenotype in HIV-specific $CD8^+$ T cells. Improper neutralization or killing of virally infected cells throughout AHI could lead to persistent antigen presentation, potentially by monocytes and DCs (Chen et al., 2017; Kazer et al., 2020; Liu et al., 2019), and thus contribute to early T cell exhaustion. Targeting differentiation and class-switch programming in $CD4^+$ T cells and B cells, respectively, could enhance helper and antibody responses during AHI and promote effective CTL killing and memory generation.

UNDERSTANDING MULTICELLULAR IMMUNE RESPONSES THROUGHOUT AHI

Although recent cell-type-centric studies of various lymphocyte and myeloid cells have begun to highlight specific cell states, circulating factors, and PRRs as critical "lynch pins" to target during AHI, their relationships with one another are only beginning to be understood. We propose how the measured responses in various cell types could form both productive and dysfunctional immunity during untreated AHI, as depicted in Figure 2. After inflammatory responses at mucosal tissue sites, peripheral myeloid cells expand and orchestrate a multicellular antiviral type-I IFN signal to induce cell trafficking. These cells also prime B-T cell interactions through BAFF and CXCL13 production, likely in GCs. B cells produce pro-inflammatory cytokines, which could skew $CD4^+$ T cell differentiation and helper function. Persistent B cell activation and uncontrolled viremia potentially inhibit neutralizing antibody production. Deficient T cell help also hinders effective and durable $CD8^+$ T cell responses in the periphery, which in turn could allow for persistent antigenic stimulation and subsequent lymphocyte exhaustion throughout the body.

It is critical that future work incorporate longitudinal multicellular studies across tissue compartments to discern who, when, and where the balance is tipped between control and progression of viremia. In hyperacute infection, NK and MAIT cells exhibit direct antiviral activity through cytolytic killing, whereas monocytes and DCs serve to recruit antiviral and inflammatory agents. Whether the loss of helper ILCs during the hyperacute window impacts disease trajectory must be further explored. Throughout the late-acute window, $CD8^+$ T cells, $CD4^+$ T cells, and B cells directly combat viremia through killing, IFN-γ secretion, and antibody production, respectively. IL-17-producing $CD4^+$ T cells and activated monocytes, however, could hinder antiviral responses in this time frame; these skewed responses could stem from pro-inflammatory signals produced in LNs and the GI tract. To understand the persistent role for various innate immune cell subsets in helping or impeding adaptive responses and/or directly fighting viremia, more granular characterization of these cells across tissue compartments will need to be performed, likely in NHP models given current standard of care (i.e., treatment at detection) and a dearth of samples. NHP models also provide opportunities to functionally test both already established cell-cell relationships, and their antiviral contributions, and those yet to be discovered.

EARLY INITIATION OF ART DRASTICALLY MITIGATES SYSTEMIC AND CELLULAR IMMUNE RESPONSES DURING AHI IN THE PERIPHERY

In addition to developing insight into some of the earliest responses during untreated AHI, recent studies have provided invaluable opportunities to understand the impact of early treatment (at HIV detection, Fiebig Stage I–III) on both AHI trajectory and long-term disease progression (Ananworanich et al., 2017; Dong et al., 2018). Alongside results from NHP early treatment models and chronic infection studies, it is becoming clear that the timing of ART makes a significant difference in the dynamics and quality of antiviral immunity and disease trajectory in humans (Figure 3) (Crowell et al., 2016; Hellmuth et al., 2019; Ndhlovu et al., 2019; Schuetz et al., 2014). Critically, early treatment can be utilized as a control of sorts to compare against progressive infection and identify the timing and quality of viral spread, as well as observe the impact of dysfunctional and/or overreactive immune responses during AHI. Moreover, it can

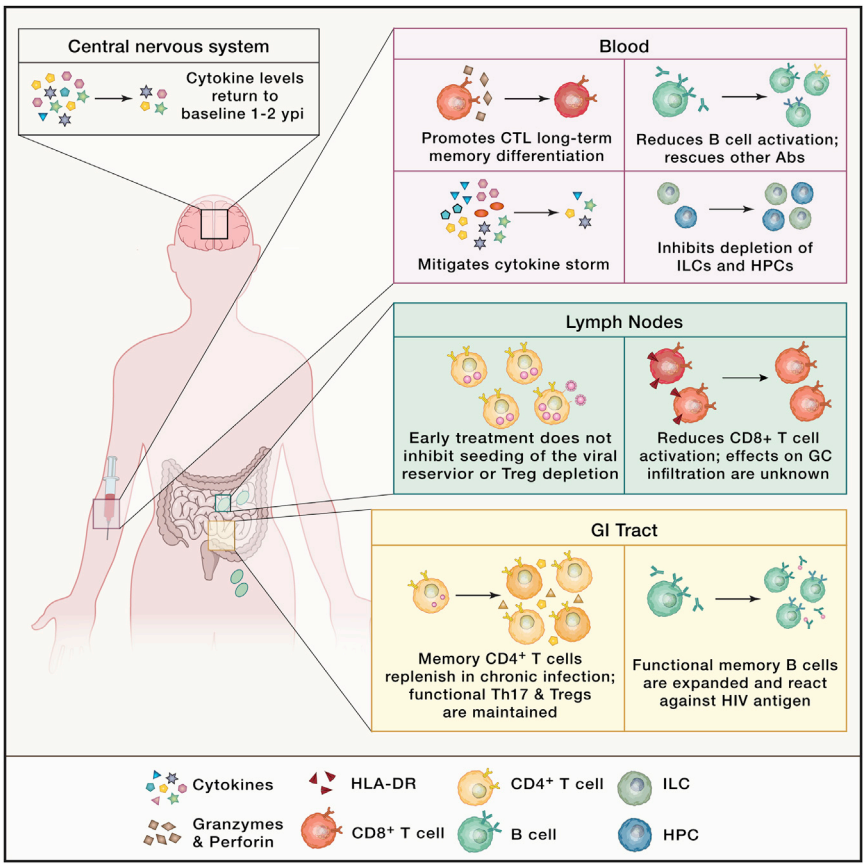

Figure 3. Effects of Early ART Administration during Acute HIV Infection on Peripheral and Tissue Immune Responses

We are just beginning to understand the effects of early ART on AHI with improved NHP models and cohorts treating at HIV detection throughout Fiebig Stages I–III. Overall, early ART mitigates peripheral cytokine production and adaptive immune cell dysfunction. Samples from early ART-treated individuals in prospective cohorts provide a unique opportunity to explore similarities and differences in cellular response and phenotype across tissue compartments. Whether we can use our understanding of these improved antiviral responses to inform long-term cure strategies or vaccines is still unknown. Abs, antibodies; HPC, hematopoietic precursor cell.

be used to examine how different tissues dynamically adapt (or maladapt) to the loss of direct viral targets, potentially elevating risk for conditions associated with chronic inflammation (Deeks et al., 2013; Somsouk et al., 2015), as well as the ability of early treatment to reestablish homeostatic function across tissues.

Profiling of the viral reservoir in early treated individuals (ETIs; at onset of plasma viremia) has shown that immediate ART administration restricts the detected copies of HIV DNA (per million cells) in PBMCs, gut tissue, and LNs (Ananworanich et al., 2012; Crowell et al., 2016; Leyre et al., 2020). In the long term, ETIs demonstrate lower levels of cell-associated HIV DNA 10 years after infection and faster decay of HIV-infected cells in the periphery when compared to individuals treated during chronic infection (Buzon et al., 2014). An in-depth longitudinal study of two ETIs sampling multiple tissue sites starting from Fiebig Stage I showed that even near complete depletion of detectible HIV RNA or DNA from LN, bone marrow, and CSF through two years after infection in one individual (but not the other) still resulted in viral rebound after ceasing ART with HIV sequences matching those measured during AHI (Henrich et al., 2017). Given the direct association of granzyme-B-producing HIV-specific CTLs and reduced viral reservoir in early infection (Yue et al., 2017), treatments that facilitate CTL trafficking to lymphoid GCs in conjunction with ART could be required for HIV eradication. Although early ART can mitigate the spread and magnitude of the viral reservoir, it cannot prevent seeding in humans. Thus, with current prophylactics, only pre-exposure prophylaxis, or post-exposure prophylaxis initiated within 72 h post-infection (Barouch and Deeks, 2014), appear able to inhibit the establishment of the viral reservoir.

Given that ETIs exhibit decreased seroreactivity and occasionally lack HIV-specific antigen and/or antibody in the periphery (Dong et al., 2018; Manak et al., 2019), the frequencies of other circulating factors like cytokines and chemokines could also be reduced or altered during AHI. Indeed, cytokine profiling in the FRESH study demonstrated that pro-inflammatory and type-I IFN cytokines in plasma stay closer to baseline levels in ETIs treated in Fiebig Stages I and II throughout the first month post-detection (Muema et al., 2020). ETIs in the FRESH and RV254/304 studies (Hellmuth et al., 2019; Sereti et al., 2017), however, show increased levels of CXCL13 and soluble CD14 ~36 weeks after infection and C-reactive protein, TNF, soluble IL-6R, soluble CD14, CXCL10, and CCL2 ~96 weeks after infection, in comparison with levels seen in uninfected individuals. These persistent elevated cytokine levels suggest that even early ART administration cannot mitigate systemic changes to circulating immune factors. Moreover, increased levels of inflammatory cytokines TNF-α and IL-6R could reflect ongoing viral replication or tissue damage throughout the body despite mitigating systemic viral spread. Interestingly, Hellmuth and colleagues demonstrated that in the CSF, however, cytokine levels return to uninfected levels by 96 weeks (Hellmuth et al., 2019), indicating that timing of treatment also affects compartmentalization of the immune response.

Peripherally, early ART administration mitigates the emergence of dysfunctional adaptive immune cells and prevents the loss of innate lymphoid cells. ART initiation before six months after infection reduced peripheral $CD8^+$ T cell counts closer to healthy levels two years post-infection in comparison to counts in ART naive individuals or those treated in chronic infection (Cao et al., 2016). Multiple studies demonstrated that HIV-specific CTLs in AHI from ETIs exhibit less expression of cytotoxic and activation markers and genes compared to untreated infection (Ndhlovu et al., 2019; Takata et al., 2017). Moreover, early

treatment prevents apoptosis of these cells and shifts their memory differentiation trajectory toward effector and central memory, resulting in longer-lasting and more functional (IFN-γ and proliferation) CTLs. This more "normal" function could be imparted by HIV-specific $CD4^+$ T helper cells, which demonstrate better proliferation capacity and IFN-γ production in ETIs, although the directionality of this relationship is unknown.

B cell responses are also markedly altered with early ART. B cell activation is mitigated, and resting memory cells are maintained near baseline levels (Mabuka et al., 2017; Moir et al., 2010; Muir et al., 2016), potentially due to reduced cytokine levels. Moreover, Moir et al. showed that the number of antibody-secreting cells after influenza immunization are increased in ETIs in comparison with the numbers in those who started ART during chronic infection, suggesting sustained improvement in B cell and GC function. Finally, ETIs maintain normal levels of ILCs and lymphocyte hematopoietic progenitor cells in the periphery, whereas these cells are depleted without treatment (Bordoni et al., 2018; Kløverpris et al., 2016). The contributions of the absence of virus/ongoing replication, reduced peripheral cytokine levels, or something yet to be described to the overall reconstitution of adaptive immune responses in ETIs is unclear. Understanding differences in innate immune subsets and antigen-presenting cells, and their frequencies and durations of function, is critical to further contextualize these differences in adaptive immune function and antiviral activity.

GUT AND LYMPHOID TISSUES ARE STILL IMPACTED EVEN WITH EARLY ART INITIATION

ART administration studies in NHP models within hours to days after infection have established the near un-avoidable depletion of $CD4^+$ T cells in the GI tract during acute SIV infection. Although early ART does not prevent $CD4^+$ T cell loss, central memory $CD4^+$ T cells have been shown to repopulate to near pre-infection levels by six months post-infection in the GI tract (George et al., 2005; Verhoeven et al., 2008), similar to natural SIV hosts that experience non-pathogenic infection. Limited sampling in early treated humans has also demonstrated recovery of $CD4^+$ T cell numbers in the GI tract in this time frame (Allers et al., 2016; Ananworanich et al., 2012), although the full extent of clonal diversity and polyfunctionality of these cells has not yet been determined. Th17 cells, however, are mostly preserved if treatment is started during Fiebig Stages I and II (Kök et al., 2015; Schuetz et al., 2014). Moreover, their cytokine production polyfunctionality remains intact (IL-17 and IL-22 subsets) only when treated this early. Deleage et al. also showed that neutrophil infiltration in the GI tract is reduced in ETIs in comparison with those in untreated individuals 24 weeks after ART initiation (Deleage et al., 2016). Immunohistochemistry revealed reduced proliferating $Ki67^+$ cells when treated during Fiebig Stages I and II, but levels of TNF-α^+ cells were similar between ETIs and untreated individuals. On the cellular level, frequencies of resting memory B cells and Tfh were preserved and expanded, respectively, in the gut compared to frequencies in untreated individuals, suggesting improved GC function in secondary and tertiary lymphoid tissue sites in the GI tract (Planchais et al., 2018). Indeed, HIV Env gp140-reactive memory cells were shown to expand in ETIs. It is unclear, however, how maintaining Th17, Tfh, and resting memory B cells in the gut directly contributes to antiviral activity, impacts CTL function, or affects long-term disease outcome and to what degree early treatment can restore pre-treatment functionality and clonal diversity.

Progressive SIV models show establishment of viral reservoir and $CD4^+$ T cell death, likely by inflammation-mediated pyroptosis (Doitsh et al., 2014), in lymphoid tissues as early as four to six days post-infection (Lu et al., 2015; Rabezanahary et al., 2020). ART initiation five weeks post-SIV infection failed to mitigate Tfh and GC B cell proliferation and activation, likely facilitating ongoing viral infection during treatment (Hong et al., 2017). However, when ART was started at four days post-infection, mesenteric LNs contained lower frequencies of $HLA\text{-}DR^+$ and $CD39^+$ $CD8^+$ T cells, but Tregs were still depleted in relation with Th17 cells (Yero et al., 2019). This is in contrast to the blood, where frequencies of Tregs were restored and $CTLA\text{-}4^+PD\text{-}1^-$ memory $CD4^+$ T cells (known to contribute to viral persistence in SIV; McGary et al., 2017) were diminished in comparison with frequencies in untreated acute infection. Given the potential role of the LN in harboring ongoing HIV infection during ART (McManus et al., 2019), sampling this tissue during AHI could provide critical insight for developing novel treatments to eliminate HIV in both acute and chronic infection.

We summarize the known effects of early ART administration in acute HIV and SIV infection in Figure 3. It is critical to further distinguish molecular and cellular differences in the earliest stages of AHI, before complete suppression of viremia, between ETIs and untreated individuals. These earliest time points provide the best opportunity to discern how mitigating viremia, and potentially inflammation, affect the longevity and quality of both adaptive and innate immune responses, as well as tissue functionality. Moreover, linking these events to long-term disease progression and comorbidities (e.g., inflammation) could inform clinical or cellular metrics and indicate alternative, or adjunctive, treatments to daily ART for life (e.g., therapeutic vaccines [Stephenson, 2018] or monoclonal broadly neutralizing antibody treatments [Ananworanich et al., 2015; Dashti et al., 2019]).

CONCLUDING REMARKS

Studies of acute HIV and SIV infection have been instrumental in determining how the quality and quantity of the immune response affects long-term outcome and persistent disease. Moreover, these findings have highlighted how both adaptive and innate immune subsets develop dysfunctional phenotypes. However, the extent to which this dysfunction imparts subsequent disadvantages to other cell types, modulates overall tissue function, impacts antiviral activity, and affects the durability thereof without ongoing viral infection are unknown. Further perturbations of putative cell-cell signaling events in NHP models and *in vitro* coculture settings will help determine which cell types and signals should be targeted by vaccines and/or treatments. Moreover, as multiple measurements accumulate from the same individuals in prospective and early infection studies, systems biology approaches using machine-learning-based classifiers (like those successfully applied in systems serology

vaccine settings; Chung and Alter, 2017) could begin to causally link cellular and molecular features of the AHI response to disease outcome. More broadly, the extensive, valuable literature of AHI provides a guide and foil for investigating host responses to other acute viral infections, and demonstrates the need for longitudinal, early sampling to ascertain the phenotypes and dynamics of immune responses to target with treatments or therapeutics.

For HIV specifically, NHP models have been incredibly productive in depicting the roles of the antiviral and pro-inflammatory responses in the periphery, $CD4^+$ T cell privileged tissues, and at sites of transmission. As more data are generated from studies of AHI and the effects of early ART administration, repurposing of these models to test human-infection-informed hypotheses could yield fruitful results to guide future vaccine and therapeutic efforts. Critically, innate immune subsets—especially NK cells, helper ILCs, monocytes, and macrophages—and their roles during acute infection are poorly described in SIV models and must be further functionally characterized and subsequently perturbed to contextualize recent findings from hyperacute infection studies (Kazer et al., 2020; Kløverpris et al., 2016; Muema et al., 2020; Wang et al., 2020). Given the literature suggesting that HIV controllers have enhanced subsets of myeloid cells (Kazer et al., 2020; Martin-Gayo et al., 2015, 2018; Sáez-Cirión et al., 2011; Walker et al., 2015), changes to their phenotype during, or before, AHI could impart and/or reflect protective function in chronic infection. Indeed, a body of literature describing innate immune cell training in NK cells and macrophages in many infection and vaccine settings (Arts et al., 2018; Kaufmann et al., 2018; Netea et al., 2020) suggests that these cells could be potential targets alongside antibody- and T-cell-based vaccine approaches. With the advent of high-throughput clonotype sequencing, NHP models are also being used to study how T and B cell clones are shared across tissues and individuals and relate to disease progression (Price et al., 2009; Starke et al., 2020). While applied to chronic infection in humans (Costa et al., 2015; Meyer-Olson et al., 2010), this approach could further reveal the interplay of host-pathogen dynamics and link immune repertoire evolution to disease progression in future NHP and human acute infection studies.

With more advanced and sensitive tools to measure cellular state and phenotype from low-input human samples and multiomic approaches (reviewed in Chappell et al., 2018), the community is better equipped than ever to thoroughly map immune responses throughout the earliest stages of acute HIV infection. Moreover, by applying them to both tissue samples (biopsies or fine-needle aspirates) and matching peripheral blood samples, we will begin to understand viral and immune dynamics in peripheral blood in comparison with different tissues. Critically, further investigation across tissues is needed to understand how the earliest host-pathogen interactions in $CD4^+$ T-cell-rich tissues impact long-term disease outcome. Together, new genomic technologies, paired with more traditional approaches, have demonstrated their capacity to inform actionable hypotheses (Shalek and Benson, 2017; Shema et al., 2019) and will nominate a new set of vaccine and therapeutic strategies for not only HIV infection but also other viral, bacterial, and fungal infections moving forward.

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