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HIV and the Macrophage: From Cell Reservoirs to Drug Delivery to Viral Eradication

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Abstract

Macrophages serve as host cells, inflammatory disease drivers and drug runners for human immunodeficiency virus infection and treatments. Low-level viral persistence continues in these cells in the absence of macrophage death. However, the cellular microenvironment changes as a consequence of viral infection with aberrant production of pro-inflammatory factors and promotion of oxidative stress. These herald viral spread from macrophages to neighboring CD4$^+$ T cells and end organ damage. Virus replicates in tissue reservoir sites that include the nervous, pulmonary, cardiovascular, gut, and renal organs. However, each of these events are held in check by antiretroviral therapy. A hidden and often overlooked resource of the macrophage rests in its high cytoplasmic nuclear ratios that allow the cell to sense its environment and rid it of the cellular waste products and microbial pathogens it encounters. These phagocytic and intracellular killing sensing mechanisms can also be used in service as macrophages serve as cellular carriage depots for antiretroviral nanoparticles and are able to deliver medicines to infectious disease sites with improved therapeutic outcomes. These undiscovered cellular functions can lead to reductions in persistent infection and may potentially facilitate the eradication of residual virus to eliminate disease.

Keywords Mononuclear phagocytes · Monocyte-derived macrophages · Human immunodeficiency virus · Viral persistence · Long acting slow effective release antiretroviral therapy · Cell ontogeny · Viral reservoirs

Introduction

Mononuclear phagocytes (MP; monocytes, macrophages and dendritic cells) serve as surveyors and protectors of the tissue microenvironment, secretors of inflammatory, growth and regulatory factors. Each and all enable intracellular killing, cell mobility, tissue and cellular repair and antigen presentation to maintain tissue homeostasis and affect T cell immunity for microbial and cancer surveillance (van Furth et al. 1972; Pistoia 1991; Mindell 2012). All serve in organ and body maintenance (Reviewed in Martinez and Gordon 2014). However, the same MPs that protect the host can also, under pathological and infectious circumstances incite the very disease states that they were designed to thwart (Bocchino et al. 2001; Giralt et al. 2009). This is certainly true for a host of viral, bacterial and parasitic infections where MPs serve as reservoirs of infectious agents, sites for microbial persistence, and inducers of end organ disease (Koziel et al. 1999; Langford et al. 2002; Buckner et al. 2011). Over the past decade yet another role for the MP has been uncovered. That role rests in utilizing the cell's own "high cytoplasmic content" to carry disease fighting drugs encased into stable nanoparticles (Edagwa et al. 2017). A discussion of the multifaceted MP in all its roles in health and disease has become the subject of this review with an eye towards how natural functions may be harnessed or even enhanced for the benefit of the host (Li et al. 2016). How the immune system affects its environment and vice versa characterize its multifaceted roles. When opportunities come available they can affect a functional change for the innate immune system (Moorjani et al. 1996; Kadiu and Gendelman 2011b). The MP's story is how a cell, when given opportunities, can protect and defend, elicit disease or be harnessed for therapeutic drug delivery (Edagwa et al. 2017).

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The Monocyte, Macrophage and Dendritic Cell

Hematopoietic Origins

From their inception during embryonic development, MP exemplify the principal that ontogeny recapitulates function. Human hematopoiesis occurs at a series of sites including the yolk sac by 17 days post-conception, fetal liver and spleen, and finally within bones (Reviewed in Baron et al. 2012). Each of these locations supplies waves of primordial myeloid cell migration to the periphery, giving rise to the first tissue resident macrophages (McGrath et al. 2015). The pervasiveness of these amoeboid cells throughout the body led the early founder of cellular immunity, Ilya Metchnikoff, to postulate their utility in engulfing debris. Metchnikoff characterized macrophages' phagocytic function as not only instrumental to tissue remodeling of tadpoles, but in taking up and killing foreign materials in starfish larvae as well (Reviewed in Gordon 2016). Hematopoiesis thus establishes populations of long-lived and self-replenishing resident phagocytes (aka histiocytes) in the brain (microglia), liver (Kupffer cells), and lungs (alveolar macrophages). In contrast, the skin and intestine are continuously replaced by circulating macrophage precursors known as monocytes (Baron et al. 2012). Monocytes appear in early circulation several days after the first macrophages (Schulz et al. 2012). Early work by Ralph van Furth and Zanvil Cohn founded the paradigm wherein bone marrow promonocytes differentiate into monocytes that enter the bloodstream and become macrophages as they extravasate into tissue (van Furth and Cohn 1968). Dendritic cells (DCs) represent the third of the mononuclear phagocyte trinity and act as immune sentinels capable of triggering adaptive immunity. DC may emerge from monocytes directly, a shared predecessor, or common lymphoid progenitors (Merad et al. 2013). MP contribute to a variety of processes during embryogenesis that reflect their ultimate functions (van Furth and Cohn 1968). Examples include erythrocyte formation (Rhodes et al. 2008), reshaping limbs and central nervous system (CNS) (Hopkinson-Woolley et al. 1994; Squarzoni et al. 2014), production of angiogenic growth factors (DeFalco et al. 2014), and fetal immunity (Zaccheo et al. 1989; Li et al. 2014). MP in post-natal organisms similarly overlap in their roles as protectors, cytokine secretors, and linkages to adaptive immunity (Polin et al. 2016).

Innate Surveyors

MP vigilantly patrol the body for signs of damage or pathogenic invasion. Endocytosis ensues when MP take in foreign particles that may be trafficked throughout the cell via endosomes or degraded in the lysosome (Merad et al. 2013). A variety of membrane receptors aid in this process. Innate leukocytes have evolved numerous membrane-bound pattern recognition receptors (PRR) that directly bind motifs common amongst foreign pathogens (pathogen associated molecular patterns, PAMPs) or damaged cells in distress (DAMPs) (Davis et al. 2011). Pre-coating of pathogens with complement or antigen-specific antibodies enhances receptor-mediated endocytosis by MP. Monocytes migrate along chemical gradients in the blood stream towards tissue sites of inflammation. Monocyte chemotactic proteins 1 and 3 (MCP1 and 3 or CCL2 and CCL7, respectively) discharged from other monocytes/macrophages, N-formylmethionyl-leucyl-phenylalanine residues, and inflammatory arachidonic acid metabolites each recruit monocytes out of circulation (Obrist et al. 1983; Locati et al. 1994). Two monocytic subsets predominate (Reviewed in Boyette et al. 2017). Classical monocytes (CD14++CD16-) travel throughout the cardiovascular system in search of PAMPs. They express high levels of CD14, a co-receptor that aids in the recognition of lipopolysaccharide from gram-negative bacteria. Non-classical monocytes (CD14lowCD16+), in contrast, patrol local endothelium for nearby inflammation and recognize antibody-opsonized pathogen via CD16. Though naturally not abundantly endocytic (Steinman and Cohn 2007), monocyte-derived DCs still exhibit complement- and C-type lectin opsonized phagocytosis. Much of DCs' capacity for uptake of extracellular materials is relegated to subsequent antigen presentation to T lymphocytes. Macrophages endocytose as part of normal physiological processes such as removing reticulocytes and aging red blood cells from the blood stream (splenic red pulp macrophages), bone remodeling (osteoclasts), and in pruning neuronal synapses (microglia). They phagocytose free floating pathogens by recognizing common motifs via C type lectin-, scavenger-, and N-formylmethionyl membrane receptors. Specialized opsonin receptors for complement (Mac-1) or antibody (FcR) further facilitate ingestion of microorganisms by macrophages. Finally, macrophages surveil for early signs of bacterial sepsis. Hepcidin, an acute phase reactant, prompts iron sequestration in macrophages to halt bacterial growth (Reviewed in Michels et al. 2015). MP accordingly employ endocytosis to respond to physiological demands and guard from immunological insult.

Secretion and Homeostatic Regulation

MP control the balance between inflammation and repair by paracrine secretion of cytokines. Monocyte / macrophages promote their own replication by releasing granulocyte-monocyte colony stimulating factor (GM-CSF) (Pistoia 1991). This growth factor prevents apoptosis, stimulates antifungal responses, and induces the differentiation of monocyte lineages (Chao et al. 1998; Hercus et al. 2012; Subramanian Vignesh et al. 2013). Dendritic cell subsets equilibrate between aggressive and defensive immune states.

Plasmacytoid DCs produce abundant amounts of antiviral interferon-α when they sense viral nucleic acids in their cytoplasm (Santana-de Anda et al. 2013). Conventional DCs drive the maturation of anti-bacterial/fungal T_{H17} through interleukins 1, 6, and 23 (Espinosa and Rivera 2012). Contrastingly, tolerogenic Langerhans cells (skin DCs) bolster peripheral T cell anergy and poor lymph node migration by constitutively releasing IL-10 (Kissenpfennig et al. 2005; Satpathy et al. 2012). Macrophages, too, tip the inflammatory scale in either direction with cytokines (Reviewed in Martinez and Gordon 2014). Classically activated macrophages (M1) recruit additional leukocytes (TNFα and IL-1) or differentiate naïve CD4+ T cells to T_{H1} effectors (IL-12) in an effort to rid the host of intracellular bacteria or viruses. Classically activated macrophages predominate as sinus histiocytes (lymph node) and LysoMacs (Peyer's Patches in gut associated lymphoid tissues). Alternative activation of macrophages (M2) by interleukins 4, 13, or 10 instigates adaptive immunity against extracellular pathogens or propagates tissue repair. M2 uniformly stifle differentiation of lymphocytes into T_{H1} effectors by extruding IL-10. M2s thematically convert arginine to cell-proliferative polyamines (M2a) or proline building blocks (M2c). This latter subtype signals the end of inflammatory responses with IL-10 and fosters rebuilding by synthesizing collagen, tumor growth factor beta (TGFβ), and matrix metalloproteinases. Thus, MP immunomodulate themselves and their surrounding stroma with growth factors, cytokines, and enzymes.

Innate Killers

MP make use of hydrolytic enzymes and reactive metabolites to destroy pathogens. Phagocytosed bacteria progress through a series of progressively acidified endosomes prior to fusing with the lysosome (Flannagan et al. 2009). In the phagolysosome, hydrogen ions (pH 4.5) denature invader proteins themselves and secondarily activate hydrolytic proenzymes cathepsin, alpha-glucosidase, and phospholipases (Mindell 2012). The phagolysosomal membrane also houses phagocyte oxidases (Phox) and inducible nitric-oxide synthases (iNOS) that generate reactive-oxygen and -nitrogen species (Slauch 2011). Impairment of monocyte/macrophage NADPH oxidase exhibited increased infectivity by opportunistic parasites, *Candidal* yeasts, and *Staphylococcal* bacteria (Murray and Cohn 1980). M1 characteristically upregulate synthesis of these intermediates to combat intracellular pathogens. Antibody-dependent phagocytosis (ADPh) couples debris clearance with adaptive immunity's ability to target unique antigens. Activating membrane receptors present on macrophages / nonclassical monocytes that potentiate respiratory burst may tightly bind monomeric antibodies (FcγRI) or loosely attach immune complexes (FcγIII, FcγRIIa). In contrast, ligands of FcγRIIb abrogate phagocytosis in these cells

(Gordan et al. 2015). Antibody-dependent cell-mediated cytotoxicity (ADCC) serves as another bridge between innate and adaptive immunity. ADCC effectors puncture plasma membranes with perforin and thereafter inject pro-apoptotic granzymes. Though macrophages are not the chief mediators of ADCC under physiological conditions, they retain their capacity to lyse malignant cells in the presence of antibody therapeutics (Beers et al. 2008; Hubert et al. 2011). To this end, monocyte / macrophages are vital in directly ridding hosts of hostile elements.

Antigen Presenting Cells (APC)

DC and monocyte / macrophages orchestrate adaptive immunity by activating T lymphocyte responses. T cells rely on two signals to mature from CD4$^+$ naïve to T helper (T_H) lymphocytes. First, antigen-specific T cell receptors (TCR) complementarily bind to digested peptides displayed in major histocompatibility complex II (MHC-II) on the surfaces of APC. CD4 co-receptor secures TCR / MHC-II attachment. B7–1 / B7–2 (CD80/86) immunoglobulins on APC must then serve as ligands for CD28 on T cells for activation to occur (Kindt et al. 2007). A landmark finding that paved the way for the 2011 Nobel Prize awarded to Ralph Steinman days after his death was made possible through his collaborations with Zanvil Cohn. The work established the presence of a large MP population with abundant pseudopodia in multiple secondary lymphoid organs (van Furth et al. 1972). DC, as they became known, stimulate T lymphocytes over 100 times more effectively than other APC subclasses (Steinman and Witmer 1978). This is owed to their constitutive expression of MHC-II and costimulatory molecules (Kindt et al. 2007). Classical DCs (cDCs) capture antigen in tissue and migrate to draining lymph nodes where they stimulate T cells (Delamarre et al. 2005; Desch et al. 2014). Antigen cross presentation describes the loading of extracellular materials onto MHC class I molecules to prime CD8$^+$ cytotoxic T lymphocytes. Monocytes, unlike at least one population of DCs, require toll-like-receptor (TLR) stimulation for cross-presentation to occur (Segura et al. 2012; Larson et al. 2016). Monocyte / macrophages also differ from DC in that surface MHC-II and B7–1 / B7–2 co-stimulators appear primarily only after phagocytosis (Kindt et al. 2007). LY6C$^+$ monocytes migrate to murine secondary lymphoid organs, however debate persists whether they merely deliver antigen to lymph nodes for display by other APCs or activate T cells themselves (Reviewed in Jakubzick et al. 2017). Macrophages more closely parallel cDC in their capacity for antigen presentation. They successfully migrate to lymph nodes where they may induce T_{H1}, T_{H2}, or CD8 cytotoxic T lymphocyte responses (Desmedt et al. 1998; Moser 2001; Pozzi et al. 2005). MP thus stand as essential intermediaries for launching T lymphocyte defenses against specific pathogens.

HIV MP Interactions

Human Immunodeficiency Virus (HIV)

HIV distinguishes itself as a unique lentivirus within the family *Retroviridae*. Retroviruses carry positive-sense single stranded RNA in roughly 100 nm envelopes. They reverse-transcribe genomic RNA using viral enzymes into DNA, prior to returning to the central dogma in which DNA transcribes RNA that in turn translates protein. Common features of the lentiviruses include their utilization of monocyte / macrophage lineages as cellular hosts, extended incubation periods prior to terminal disease, viral persistence in face of vigorous immunity, and ability to cause progressive health decline in the absence of treatment (Reviewed in Desport 2010). HIV's approximately 10 kb genome encodes three canonical structures shared among all lentiviruses. *Gag* gives rise to core structural elements: matrix, capsid, and nucleocapsid. Enzymes necessary for survival that include reverse transcriptase, RNase-H, and integrase result from HIV's *pol* sequence. Finally, the *env* gene produces surface and transmembrane proteins utilized in recognition and entry of virus into target cells. Six accessory proteins are transcribed from an open reading frame in the HIV genome. Vpx transports pre-integrated viral DNA into the nuclei of non-dividing cells. Tat (trans-activator of transcription) enhances transcriptional elongation by recognizing stem loops known as the Tat activation region (TAR). Rev, the only accessory protein common to all lentiviruses, carries full-length and singly spliced HIV transcripts from the nucleus to cytoplasm. Vif increases viral infectivity and assembly. Vpu, found only in HIV-1, and Nef decrease CD4 expression leading to enhanced release of virus.

HIV Life Cycle

HIV gene products facilitate eight major steps in the viral lifecycle of HIV (Reviewed in Desport 2010). The process begins with (1) attachment of envelope glycoproteins that bind cellular primary CD4 receptors and chemokine co-receptors CCR5 or CXCR4. Next, (2) entry ensues as HIV transmembrane protein fuses with plasma membrane, followed by pore formation and deposition of viral core into the host cell. Uncoated viral products potentiate (3) reverse transcription and (4) integration of proviral DNA into the host's chromosomes. The number of proviral DNA copies present in each HIV-1-infected cell varies by tissue type (Arainga et al. 2016) and viral strain ranging from 0 to 2 in PBMC (Shiramizu et al. 2004) to 3–4 in splenocytes (Jung et al. 2002). Gene expression and post-transcriptional regulation represent the fifth (5) stage of productive infection by HIV. Full-length or singly spliced RNA transcripts encode *gag* and *pol*, or *env* proteins, respectively. Proteases cleave Gag and Env polyproteins, which are each subsequently fatty acylated or N-glycosylated. Small multiply spliced RNAs comprise a minority of HIV transcripts that translate into the aforementioned accessory proteins. Complexes of envelope glycoproteins and gag-polyproteins classically thought to form at the plasma membrane or late endosomes signal (6) assembly of proviral elements. This also triggers the virus to regulate cellular endocytic trafficking of proteins and cytoskeletal rearrangement, which culminate in (7) budding from the host-cell. Finally, (8) maturation progresses as gag polyprotein is cleaved during the penultimate step and thereafter, spawning new progeny virions.

Four phases characterize the disease course of HIV in the absence of antiretroviral treatment (Le 2017). During the first month post-infection, known as the window period, HIV RNA accumulates as CD4^{+} T lymphocyte counts begin to decline. Acute infection, marked by flu-like symptoms, presents in the following month. During this phase, viral RNA peaks while CD4^{+} counts reach a relative nadir, permitting for viral dissemination and seeding of lymphoid organs. HIV next enters clinical latency as cellular and humoral immune responses fight back against viremia. Symptoms subside from the third month to approximately five years after infection in concert with HIV RNA decline ($<10^4$ copies / mL) and CD4^{+} T lymphocyte rebound. HIV-1 is commonly latent, as marked by the absence of viral replication, however it differs from classical latency exemplified by herpes simplex virus that silence viral transcription by use of viral gene products (Desport 2010). Resting memory CD4^{+} T cells (Williams and Greene 2007) and monocytes (Gendelman et al. 1990) both harbor integrated HIV without demonstrating viral replication. Viral reactivation occurs once monocytes differentiate into macrophages upon reaching tissues (Meltzer et al. 1990). Final crisis results 8–11 years post-infection with HIV-1, but is vastly delayed in the case of HIV-2 (Marlink et al. 1994). Patients become progressively immunocompromised as viral replication depresses CD4^{+} counts below 400/mm^3. Acquired Immunodeficiency Syndrome (AIDS) is diagnosed when exceedingly rare infections manifest that fewer than 200 CD4^{+} T lymphocytes are left circulating per milliliter blood.

Cell Tropism

HIV readily infects MP due to the presence of necessary receptors on their plasma membranes. HIV envelope glycoprotein 120 (gp120) initiates attachment to host cell CD4, prompting a conformational shift that exposes sites on gp120 for secondary connections to chemokine receptors, CCR5 or CXCR4. Subsequent binding of cell membranes to viral gp41 allows HIV fusion and entry. Viral strains (R5) that selectively recognize CCR5 can replicate in macrophages (M-Tropic) or CD4^{+} T lymphocytes (Alkhatib et al. 1996; Ancuta et al. 2006), while others (X4) that principally utilize the CXCR4 co-receptor produce higher number of viral progeny

in CD4$^+$ T cells and T-cell lines (Reviewed in Iordanskiy et al. 2013). Coupling of HIV to CCR5 increases the likelihood for replicative potential in monocyte / macrophages, though this feature is neither necessary nor sufficient for macrophage tropism (M-tropism). Some primary R5-HIV isolates fail to infect macrophages and X4-viruses may be capable of entering macrophage lineage cells (Gorry et al. 2001). Monocytes display basal amounts of all three HIV receptors in their resting states but growth factors alter this expression upon differentiation to macrophages or DC. Monocyte colony stimulating factor (M-CSF) induces macrophages to upregulate CCR5 by 10-fold whereas GM-CSF less potently increases CCR5 but markedly decreases CXCR4 among dendritic cells (Lee et al. 1999). M-tropic strains predominate in both early (Reviewed in Desport 2010) and late (Li et al. 1999) disease states. This correlates with times of high transmissibility and terminal CD4$^+$ T lymphocyte depletion, respectively. In contrast, X4-viruses appear most frequently in patients transitioning between early and intermediate phases (Reviewed in Desport 2010). CCR5-mediated entry of HIV into macrophages indicates the underlying potential for disease state to progress.

The beta-chemokine receptor, CCR5, has long been recognized as important to HIV-1 infection. Mutations in CCR5 may confer HIV resistance in human hosts by halting entry of M-tropic HIV-1 clades (Dean et al. 1996; Cheng-Mayer et al. 1997) and delay AIDS progression in heterozygotes (Samson et al. 1996; Zimmerman et al. 1997). A G protein-coupled receptor, CCR5 serves as a docking site for CC motif chemokine ligands MIP-1α (CCL3), MIP-1β (CCL4), and RANTES (Struyf et al. 2001). High concentrations of these proteins correlated with low levels of viremia in patients, and were essential to natural killer (NK) cell cytotoxicity of infected CD4$^+$ T lymphocytes (Kottilil et al. 2003). Furthermore, HIV-1 patients with low or undetectable plasma levels of virus, known as long-term non-potentiators (LTNPs), were found to maintain elevated blood concentrations of MIP-1α, MIP-1β, and RANTES (Walker et al. 2015). Thus, physiological blockade of CCR5 presents one adaptive means of interrupting HIV infection in macrophages.

HIV Persistence

One advantage offered by macrophages to HIV is their ability to survive for months to years. HIV ensures increased cellular longevity by making cells resistant to TNFα-induced apoptosis, increasing anti-apoptic proteins Bcl-2 and Bcl-XL, and decreasing pro-apoptotic Bax and Bad (Guillemard et al. 2004). Viral envelope glycoprotein itself induces M-CSF secretion, which upregulates anti-apoptotic genes *bfl-1* and *mcl-1* in macrophages (Swingler et al. 2007). HIV-1 also exerts biphasic control of Bcl-2 in monocytes to silence any early replication in preference for viral latency (Aillet et al. 1998).

Finally, HIV can promote cell survival in MP. This occurs in microglia directly via Tat stimulation of PI-3-K/AKT signaling (Reviewed in Le Douce et al. 2010). HIV indirectly forces cell cycle arrest by activating cyclin-dependent kinase inhibitor, p21 (Allouch et al. 2014). MP therefore provide HIV with prime resources with which to establish persistent infection.

HIV manipulates normal sorting of endocytic vesicles to promote its own life-cycle (Fig. 1a). During HIV assembly, viral Gag polyprotein accumulates in a subset of late endosomes known as multivesicular bodies (Gousset et al. 2008). HIV also redirects late endosomes to carry viral metabolites to its assembly sites (Chertova et al. 2006; Gerber et al. 2015). Endosomes further provide viral progeny with material for envelope encasement, as evidenced by the abundance of late-endosomal markers HIV virion envelopes (Pelchen-Matthews et al. 2003). Additionally, HIV utilizes late endosome machinery in macrophages to aid in budding or direct spread amongst MP. High levels of host annexin II and ESCRT I in late endosome extracts collected from HIV-1-infected macrophages suggest that these membrane bending proteins play a role in viral budding (Chertova et al. 2006). Moreover, retrograde transport of HIV from late endosomes back to the endoplasmic reticulum and golgi complex enables these organelles to serve as staging locales for intercellular infection via bridging conduits (Kadiu and Gendelman 2011a). HIV reorganizes myosin II to construct bridging conduits between macrophages that perpetuate viral spread without exposure to host immune defenses (Kadiu and Gendelman 2011b). Thus, HIV capitalizes on late endosomes to advance viral assembly, budding, and direct spread to adjacent MP.

HIV and MP Function

Phagolysosomal degradation of antigen is also impaired at multiple stages in HIV-1-infected macrophages. Firstly, HIV-1 reduce antibody-dependent phagocytosis by post-transcriptionally downregulating FcγR on monocyte-derived macrophages (MDM) (Leeansyah et al. 2007). HIV additionally weakens downstream Syk-phosphorylation and cytoskeletal rearrangements needed for ADPh (Reviewed in Kedzierska et al. 2003). HIV disrupts phagocytosis of C3b-opsonized materials by elevating MP cyclic AMP (Azzam et al. 2006). Secondly, viral Nef protein interferes with AP-1 mediated endosome formation at macrophages' plasma membrane (Mazzolini et al. 2010). HIV pass directly from early endosomes to more hospitable recycling endosomes and multivesicular bodies, representing a tertiary means of lysosomal escape (Kadiu and Gendelman 2011b). Fourthly, HIV impedes phagolysosomal fusion immediately upon gp120-CD4 attachment and post-entry with Vpr (Moorjani et al. 1996; Dumas et al. 2015). Each of these mechanisms diminish the chance that lysosomal protons, reactive oxygen / nitrogen
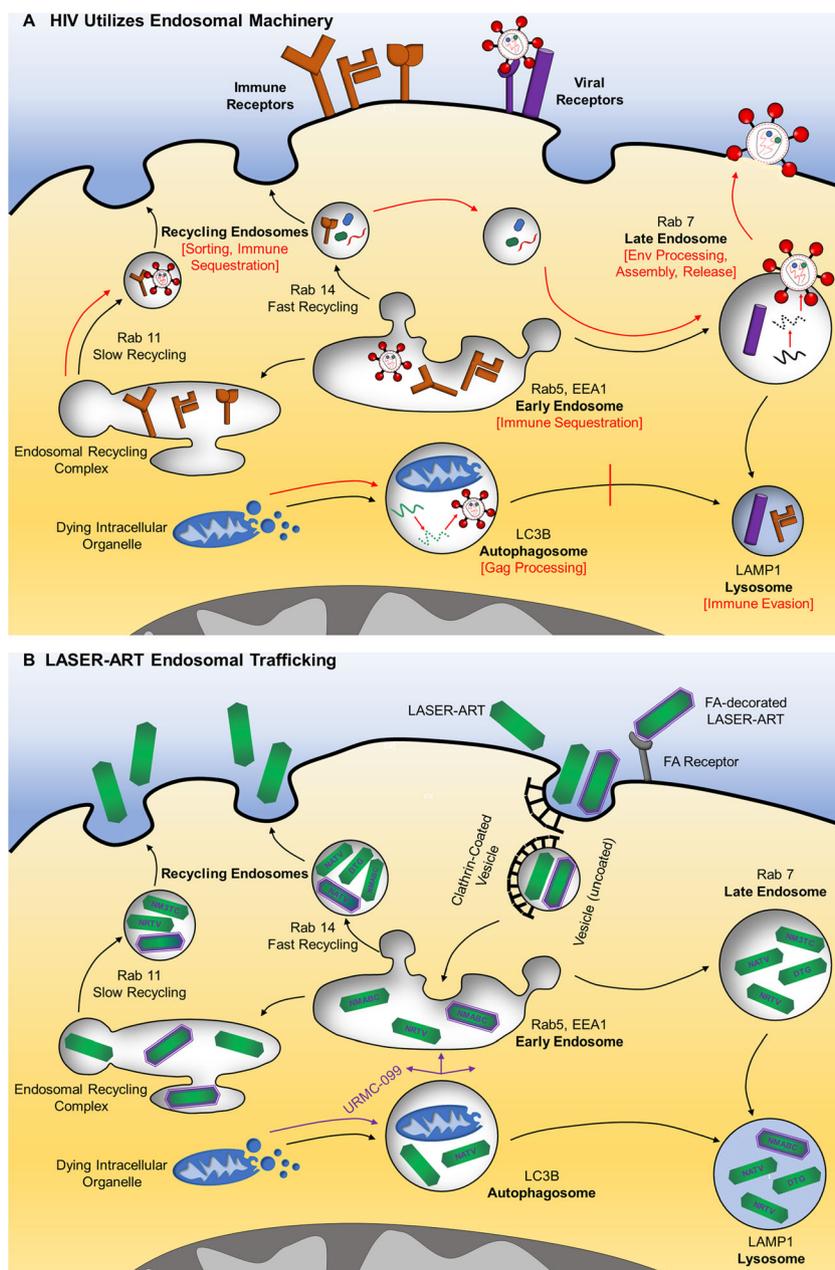
Fig. 1 Utilization of endocytic machinery in **a** HIV Infection: Endocytosis and autophagy direct extracellular materials or dying organelles to the lysosome, respectively (black arrows). HIV binds CD4 and CCR5 or CXCR4 to enter host cells. Provirus resides in early, recycling, and late endosomes in addition to within autophagosomes. HIV manipulates subcellular organelle trafficking to its advantage (red arrows). Early endosomes sequester MHC-I and immune checkpoint inhibitor, CTLA-4. Recycling endosomes aid in sorting proviral proteins prior to assembly, as well as in sequestration of CTLA-4 and co-stimulatory molecules, CD80/86. HIV promotes autophagosome formation, where Gag polyprotein processing occurs, and inhibits its own degradation in the lysosome. Late endosomes assist in envelope protein processing, proviral assembly in MP, and viral release. HIV evades adaptive immunity by degrading CD4 and MHC-I in the lysosome. **b** LASER-ART delivery is shown. Nanoformulated nanocrystals accumulate in high concentrations within recycling and late endosomes and to lesser levels in early endosomes or lysosomes. URMC-099, a mixed lineage kinase-3 inhibitor, enhances shuttling of antiretroviral particles from the autophagosome to each of these compartments, where they inhibit key HIV processes due to their proximity to virus. Recycling endosomes also provide for sustained release of free-drug from nanocrystals present in MP. N- nanoformulated; M- myristoylated; FA- folic acid; 3TC- lamivudine; ABC- abacavir; ATV- atazanavir; DTG- dolutegravir encased in europium doped cobalt-ferrite; RTV- ritonavir

species, and hydrolases will lethally maim HIV. Lysosomes are additionally central to autophagy and antigen presentation in MP (Fig. 1a). Autophagy describes the ability of cells to extend their lifespans by directing worn cytoplasmic organelles from autophagosomes to lysosomes. HIV-1 requires autophagy in macrophages but not CD4+ T lymphocytes for

active replication (Espert et al. 2009). Gag polyprotein processing transpires in autophagosomes, however HIV Nef halts lysosomal fusion to protect proviral constituents (Kyei et al. 2009). Nef further sequesters MHC-I (Dirk et al. 2016), CTLA-4 (El-Far et al. 2013) immune checkpoint inhibitors, and CD80/86 (Chaudhry et al. 2008) costimulatory molecules in early / recycling endosomes. Lastly, HIV escapes adaptive immunity by augmenting MHC-II. Viral Nef protein accelerates endocytic removal of mature antigen presenting MHC-II as well as CD4 to lysosomes (Cenac et al. 1975; Chaudhry et al. 2009; Amorim et al. 2014). HIV also reduces lysosomal processing of antigen and MHC-II peptide loading in monocytes (Polyak et al. 1997). In this regard, HIV takes full advantage of a key organelle in MPs- the lysosome.

Secretory Responses

Secretion of inflammatory mediators by MP in response to HIV advances viral spread and facilitates immune exhaustion. Without antiretroviral therapy (ART), macrophages produce IL-12 and TNFα which skew differentiation of naïve CD4$^+$ T cells to T_{H1} effectors. These lymphocytes, in turn, prompt macrophages to (1) produce reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), and phagolysosomal enzymes, (2) upregulate antigen presenting MHC-II and T cell costimulatory molecule B7, and (3) secrete additional cytokines that promote antiviral immunity. Macrophages from ART-treated patients produce immunosuppressive IL-10 while only expressing IL-12 and TNFα in response to appropriate stimuli such as LPS (Bocchino et al. 2001). Monocytes from off-therapy HIV-1 patients exhibit amplified signaling along the JAK-STAT1-IFNγ axis, suggesting further enactment T_{H1} immune responses (Alhetheel et al. 2008). Peroxisome proliferator-activated receptor (PPAR)-gamma antagonism in macrophages during HIV-1-infection likewise tipped homeostatic balance in favor of inflammation and lipodystrophy (Giralt et al. 2009). Administration of rosiglitazone, a PPAR-gamma agonist, halted brain inflammation and HIV-1 replication in murine HIV-encephalitis subjects (Potula et al. 2008). Chemokines and growth factors contribute to viremia by recruiting CD4$^+$ T lymphocytes and making cells more permissive for infection. CXCR3 expressed on the surface of CD4$^+$ cells enables uninfected T cells to chemotax towards CXCL10 and CXCL11 released by infected macrophages in lymph nodes (Foley et al. 2005) and the central nervous system (Poluektova et al. 2001). HIV infection of MDMs induces HIV co-receptor expression, viral shedding, and differentiation of new macrophage hosts by M-CSF (Kutza et al. 2002). In summary, monocytic-lineage release of T_{H1} proinflammatory cytokines, lymphocyte recruiting chemokines, and growth factors assist in prolonged viral spread throughout the body.

HIV Disease Pathogenesis

Viral Reservoirs and Disseminators

Control of viral transcription in MP stations these cell types to distribute HIV to numerous organ systems. Monocytes in vivo serve as latent reservoirs devoid of active viral replication (Gendelman et al. 1990; Aillet et al. 1998). Monocytes suppress Cyclin T1, a component of positive transcription elongation factor, via micro RNA silencing (Sung and Rice 2009). Infected monocytes may also therefore use this mechanism to quell Tat-driven expression of HIV genes. Microglia subdue HIV-1 transcription at the epigenetic level. CTIP2, a transcription factor abundant in microglia, establishes heterochromatic environments in the vicinities of HIV promoters (Marban et al. 2007). In contrast, HIV readily exits latency upon transitioning to macrophage states. Macrophages harbor abundant amounts of circular episomal viral DNA that is stable for up to two months (Marban et al. 2007). Persistent transcription of this unintegrated DNA has further been demonstrated in macrophages (Kelly et al. 2008). These represent but a few of the molecular means that situate MP as viral reservoirs.

Monocytes and their descendants are also instrumental in transmitting virus to and from anatomic sites. HIV penetrates gut mucosa early in disease. Infection of lamina propria macrophages leads to the loss of tight junctions in intestinal epithelia (Kotler 2005). R5-tropic virus has the highest transmission efficiency in female genital mucosal tissue, another early site of pathogen introduction, causing HIV-positivity in macrophages by 96 h post-infection (Gupta et al. 2002). The "Trojan Horse" paradigm posits that HIV-1-infected monocyte / macrophages traffic the virus into the central nervous system (CNS) across the blood-brain-barrier, which normally excludes most pathogens and host proteins. Macrophages, however, undergo diapedeses between choroid plexus epithelial- and brain-endothelial tight junctions, introducing virus to microglia (Reviewed in Meltzer et al. 1990). Distinct populations of HIV-ferrying monocytes have been classified immunophenotypically as CD14$^+$CD16$^+$CD11b$^+$Mac387$^+$ and capable of migration in response to a CCL2 concentration gradient (Buckner et al. 2011). Lectins on plasma membranes anchor cells together for adhesion. HIV-1 has been shown to induce myeloid cell sialic acid-binding immunoglobulin-type lectin-1 (siglec-1) expression, a receptor that binds viral membrane gangliosides, and enhances transinfection of other cells (Pino et al. 2015). Langerhans (epithelial dendritic) cells transfer R5-tropic HIV to surrounding T lymphocytes in a CCR5 dependent manner, indicating that virus spreads locally upon occupational exposures that break skin (Kawamura et al. 2008). Monocyte lineages are thus critical in exposing gut, CNS, genital, and dermal leukocytes to HIV infection.

MP and HIV Neuropathogenesis

Macrophages, in the context of HIV-1, mount free radical stress and toxic responses that damage uninfected neurons. Normal microbicidal activity in phagocytes includes superoxide (O_2^-) and peroxide (HOOH) formation. Peroxide, in turn, degenerates to hydroxyl free radicals (HO·). Activated macrophages induce nitric oxide synthase that eventually leads to nitric oxide radicals ($NO_2·$) from peroxynitrite ($ONOO^-$) intermediates. Each of these radicals damage pathogen DNA. HIV dysregulates mitochondrial superoxide dismutase leading to sustained intracellular superoxide anions and peroxynitrite (Aquaro et al. 2007). HIV-1 further insults monocyte / macrophage natural responses to free radicals by limiting the production of heme oxygenase 1 (HO-1), prompting accumulation of heme (Cross et al. 2011) and other neurotoxins in the CNS. Glutamate, an excitatory neurotransmitter, damages neurons when high levels amass as has been shown to occur during neuroinflammation due to HIV-1-infected macrophages (Gill et al. 2015). Adoptive transfer of HIV-1-infected human MDM into basal ganglia of immunodeficient mice has provided a murine model by which to study HIV-encephalitis (HIVE) and neurocognitive dementia (HAND). Anti-inflammatory agents, platelet activating factors and TNFα inhibitors were applied to this system and successfully protected against neuronal injury (Persidsky and Gendelman 2002). To this end, altered biochemical processes in HIV-1-infected macrophages negatively affect neuronal survival.

Macrophages are central to many of the processes that precipitate dementia in adults with HIV. HAND severity has been known since the mid-1990s to correlate with the number of macrophages and microglia in the CNS and not HIV gp41 immunoreactivity, AIDS duration, or the use of antiretrovirals (Glass et al. 1995). Rapidity of dementia progression was also associated with macrophage activation (Bouwman et al. 1998). Peripherally circulating monocytes can cross the blood-brain barrier (BBB) and infect CNS microglia in addition to brain perivascular macrophages. Infected cells release inflammatory cytokines (IL-12, TNFα), chemokines (CXCL10, CXCL11), amino acids (glutamate), reactive oxygen / nitrogen species, and eicosinoids, all of which contribute to neuronal death (Reviewed in Ramesh et al. 2013). Moreover, aberrant conductance through voltage-gated potassium channels on microglia has been associated with some of the early symptoms of HAND (Reviewed in Keblesh et al. 2008). Monocyte / macrophages from seropositive patients with HAND also secrete less defense proteins, such as lysozyme, leaving the CNS open to concomitant infection that can advance cognitive decline (Sun et al. 2004). Some of these opportunistic infections will be noted below.

HIV can disrupt healthy brain parenchyma leading to behavioral symptoms that characterize HIV-associated encephalitis. The importance of HIV-1-infected MDM to abnormal behavior has been validated using the murine HIVE model (Persidsky and Gendelman 2002). Post-mortem brain tissue from HIVE cases shows adherence of platelet-monocyte complexes (PMCs) in CNS postcapillary venules (Singh et al. 2014). It is hypothesized that PMCs precipitate neuroinflammation by secreting chemokines that further recruit activated monocytes to the CNS. Second, PMCs likely also damage the blood-brain-barrier during trans-endothelial migration. Viral gene product-, cytokine-, phospholipid-, and eicosanoid secretions by HIV-1-infected microglia are a third implication of MP in HIVE pathology (Reviewed in Gelbard and Epstein 1995). CNS-permeable ART reduced monocyte chemotactic protein 1 (MCP-1) and viral RNA contained in HIV-patient cerebrospinal fluid (De Luca et al. 2002). The same was seen in rhesus macaques treated with minocycline, an antibiotic with anti-inflammatory and neuroprotective properties (Zink et al. 2005). In aggregate, these findings support early damage caused by activated monocytes as an etiology for HIVE, which can be pharmacologically combatted.

HIV infection of the MP potentiates opportunistic infection of the CNS as well as peripheral neuropathy. Several forms of damage to brain white matter have been linked to HIV infection of monocyte / macrophages. Severe demyelinating leukoencephalopathy transpires in ART-refractory patients as HIV-carrying monocytes damage brain capillaries, demyelinate axons, and inflame astrocytes (Langford et al. 2002). High MP chemokine levels in cerebrospinal fluid (CSF) samples also point to impaired immune clearance of AIDS-defining infections. Progressive multifocal leukoencephalopathy, brought on by JC polyomavirus reactivation in immunocompromised patients ($CD4^+ < 200/mm^3$), often presents with elevated cerebrospinal fluid MCP-1 concentrations (Marzocchetti et al. 2005). Undiagnosed AIDS individuals with symptomatic *Cryptococcal* meningitis ($CD4^+ < 100/mm^3$) similarly test high for macrophage secreted IL-13 and MIP-1α in CSF. This finding correlates with poor outcome of early intervention (Scriven et al. 2015). Newly recruited monocytes to the CNS become alternatively activated M2 under this cytokine milieu, thereby suppressing appropriate effector responses. Peripheral neuropathy is the most common neurological symptom in HIV-1 patients (Reviewed in McArthur et al. 2005), manifesting as weakness, numbness, and pain often in the hands in feet. HIV-1-infected macrophages in the endoneurial sheath generate cytokine-mediated autoimmunity against peripheral nerves (Dalakas and Cupler 1996). Other causes of neuropathy in HIV patients relate to the degeneration of long axons, loss of unmyelinated fibers, macrophage infiltration into peripheral nerves and dorsal root ganglia, and terminal effects of cytomegalovirus and herpes zoster coinfections (Pardo et al. 2001). To this end, MP shuttle HIV into the nervous system where they

damage neurons as well as generate permissive environments for opportunistic pathogens.

MP and the Lung

Pulmonary coinfections with HIV stem from altered immunity of lung resident macrophages. Alveolar macrophages from HIV-1-infected patients produce decreased TNFα in response to TLR2 and TLR4 stimulation, indicating that the virus diminishes innate immune responses to opportunistic infection (Nicol et al. 2008). Toll-like receptor 2 is critical for phagocyte recognition of gram-positive bacteria while TLR4 binds a broader variety of pathogenic motifs such as LPS on gram-negative bacteria, protozoal phospholipids, fungal mannan, and viral envelope proteins. Higher HIV-1 RNA was measured in bronchioalveolar lavage (BAL) fluid of patients coinfected with *Pneumocystis carinii*, *Mycobacterium avium complex* (MAC), *Nocardia sp.*, and *Aspergillus fumigatus* than in matched HIV-1 patients, further demonstrating HIV's ability to impair innate immunity (Koziel et al. 1999). A prime concern of clinicians caring for AIDS individuals is their susceptibility to progressive primary tuberculosis. Unlike in immunocompetent adults in whom *Mycobacterium tuberculosis* (Mtb) remains latently confined to the lung in the absence of reactivation, Mtb primary infection of AIDS patients hematogenously disseminates to reticuloendothelial organs, bone/vertebrae, and meninges. Alveolar macrophages collected from newly-treated ART patients displayed weakened ability for proteolytic cleavage of proteins, a necessary step in antigen presentation to T cells (Jambo et al. 2014). These macrophages not only failed to facilitate Mtb-specific T_{H1} effector responses, which are essential to clearance of tuberculosis, but were also defective in IL-10 attenuation of immune inflammation (Tomlinson et al. 2014). These observations together suggest that, in the context of HIV, alveolar macrophages promote chronic inflammation that hinders both innate and adaptive immune clearance of pathogens.

MP and the Gastrointestinal Tract

HIV upregulation of proinflammatory cytokines along the gastrointestinal (GI) system derails macrophage prevention of Kaposi's sarcoma and liver fibrosis. Kaposi's sarcoma, a neoplasm of vascular endothelial cells in immunocompromised patients (CD4$^+$ < 500/mm^3) who are co-infected with human herpesvirus 8 (HHV-8), presents as violaceous nodules on skin, lung parenchyma, and GI lining. HIV Tat protein promotes growth of macrophages, which are among the only HHV-8 infected cells until late disease (Reviewed in Gallo 1998). HHV-8 itself is not regarded as oncogenic. Rather, endothelial cells perpetuate sustained microinflammation by secreting IL-6, IL-10, and IL-13. These cytokines polarize macrophages to differentiate into tumor associated

macrophages (TAMs) that promote tumor formation instead of viral clearance (Bhaskaran et al. 2017). The prototypical ability of macrophages to aid in intracellular pathogen destruction is also lost in HIV-1-infected resident liver macrophages. HIV infection of Kupffer cells enhances expression of TLR4 and its co-receptor, CD14, leading to overproduction of IL-6 and TNFα. These cytokines were shown to bolster liver fibrosis (Mosoian et al. 2017). Moreover, fewer Kupffer cells in liver biopsies from HIV-hepatitis C coinfected patients correlated with progression to hepatic fibrosis (Balagopal et al. 2009). Thus, many of the GI pathologies seen in HIV-1 patients arise from macrophages' inability to effectively clear coinfecting microorganisms.

MP and ART

ART is the standard of care for HIV-1 patients in the Western world, however its curative potential remains far from realized. Dosing of HIV-1 patients with nucleoside reverse-transcriptase inhibitors (NRTIs) plus protease inhibitors, together comprising the backbone of ART, was discovered in the late 1990s as a means of suppressing over 99% of free HIV-1 in plasma. Pharmacodynamic studies detail viral clearance kinetics as a two-phase system. ART first clears HIV from short-lived productively infected cells after several days (Perelson et al. 1997). HIV-1-infected macrophages or other MP require upwards of 3 weeks to 3 years of consistent ART treatment to reduce viral stores (Cavert et al. 1997; Perelson et al. 1997; Murray et al. 2007). As much as one-quarter of patients who attain the treatment goal of <50 HIV copies/mL plasma within one year of initiating ART enjoy only modest increases in CD4$^+$ lymphocyte counts (Benveniste et al. 2005; Aiuti and Mezzaroma 2006; Marziali et al. 2006; Taiwo et al. 2010). Furthermore, ART achieves little if any reduction of HIV replication in anatomic reservoirs such as the lymph node, spleen, GALT, CNS, and genital tissues (Lorenzo-Redondo et al. 2016; Edagwa et al. 2017). Drug resistance to highly active antiretroviral therapy emerges when patients fall below 95% adherence to designated regimens (Lima et al. 2008). Poor access to ART not only contributes to ART-resistance, but perpetuates viral transmission in endemic regions as well. The World Health Organization reports that as of 2016, fewer than 34% of HIV-1-infected individuals in eastern Mediterranean, western and central African countries receive ART (World Health Organization 2016). Future therapeutic strategies must therefore address these issues along with the plethora of previously noted HIV-associated comorbidities.

Erythrocyte ART Delivery

Due to fact that macrophages readily phagocytose erythrocytes under physiological conditions, red blood cells (RBCs)

became early vectors for antiretroviral delivery beginning in the 1990s. A barrier to the success of conventional nucleoside reverse-transcriptase inhibitors is the low level of phosphorylation in target cells needed to activate them. Erythrocyte encapsulation of the pre-phosphorylated nucleotide reverse-transcriptase inhibitor ddCTP, prompted IgG-mediated phagocytosis by macrophages in retrovirally infected mice along with nearly complete inhibition of HIV replication and acute infection symptoms (Magnani et al. 1992). This method also demonstrated increased drug stability and prophylactic protection of macrophages from HIV infection when the pre-phosphorylated zidovudine dinucleotide was loaded into RBCs (Magnani et al. 1996). Chemotherapeutic fludarabine embedded in erythrocytes has also been utilized to selectively kill HIV-1-infected monocyte / macrophage populations in vitro (Magnani et al. 2003), and delay in viral rebound after therapy cessation in primates (Cervasi et al. 2006). These observations provided early justification for the employment of macrophages as drug targets.

Long Acting Slow Effective Release ART (LASER-ART)

MPs position at the crossroads of immune defense and HIV hosting primes them as ideal conduits for novel treatment schemes. Long-acting slow effective release (LASER) antiretroviral particles tip macrophage biology in favor of viral clearance. LASER-ART are generated by chemically converting existing antiretrovirals to more hydrophobic prodrugs, which are then encased in lipophilic excipients. The increased hydrophobicity of these prodrugs better potentiates drug-crystal formation, while the raised lipophilicity of drug-excipient nanoparticles eases their passage across cell membranes. Together, these improvements boost the apparent half-lives of antiretrovirals by ensuring slow release of active drug from macrophages. LASER-ART also seek to enhance patient adherence, target anatomic sanctuaries of HIV, and serve as candidates for pre-exposure prophylaxis in medically underserved areas (Edagwa et al. 2017).

LASER-ART modifications improve drug uptake and release by monocyte-derived macrophages (Singh et al. 2016; Guo et al. 2017; Sillman et al. 2018). Existing HIV therapies may be optimized through nanoformulation for macrophage phagocytosis. Particle size, surfactant coating, surface charge, and shape predicts uptake by MDM and antiretroviral efficacy (Nowacek et al. 2011). One advantage of nanoformulated ART lies in the ability to encapsulate multiple drugs in a single particle. Co-delivering lopinavir plus ritonavir together with tenofovir amplified intracellular drug concentrations 50-fold and sustained plasma concentrations 3.5 times longer than free drug (Freeling et al. 2015). Furthermore, conversion of native antiretroviral therapies to hydrophobic prodrugs enables prolonged host enzyme (e.g. carboxyesterase) cleavage. Nanoparticles of myristoylated abacavir (MABC) and lamivudine (M3TC) owed much of their improved efficacy to greater uptake by- and depot formation within MDM (Singh et al. 2016; Guo et al. 2017). To this end, LASER-ART utilizes macrophage predilection for phagocytosis to create cellular stores of drug.

LASER-ART further inhibits HIV by colocalizing to macrophage subcellular compartments used for viral assembly (Fig. 1b). Ritonavir-nanoparticles move from early to recycling endosomes, where particles' antiretroviral activities are spared from lysosomal degradation (Kadiu et al. 2011). Atazanavir (ATV) and lamivudine nanoparticles traffic to recycling and late endosomes, where HIV claims sanctuary (Guo et al. 2014a; Guo et al. 2017). ART may also be pharmacologically modulated by induction of autophagy. URMC-099, a MLK-3 inhibitor and autophagy promoter, raised dolutegravir release from

Fig. 2 LASER-ART drugs target HIV reservoirs. **a** Key anatomic reservoirs of HIV include the liver, spleen, lymph nodes, lungs, central nervous system (CNS), gut-associated lymphoid tissue (GALT), and genitourinary tract (GU). **b** LASER-ART drugs accumulate at high concentrations within the liver, spleen, and lymph nodes. Moderate biodistribution of LASER-ART drugs has been observed in GALT. Low levels of drug are found in the CNS and GU
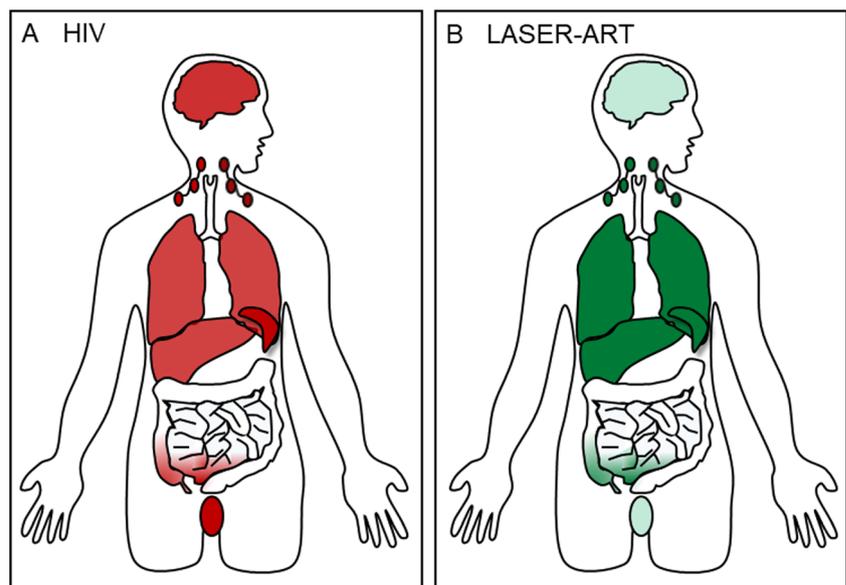
MDM 50-fold by extending cellular longevity (Gnanadhas et al. 2017). In this manner, URMC-099 exchanges an identical ploy used by HIV for survival in favor of extended drug release (Kyei et al. 2009). URMC-099 additionally boosted nanoATV levels in early, late, and recycling endosomes (Zhang et al. 2016). These tactics indicate that LASER-ART alone or with URMC-099 pinpoints HIV within infected cells.

A last benefit conferred by MP rests in their ability to shuttle LASER-ART to anatomic HIV reservoirs (Fig. 2). Indinavir nanoparticle-loaded bone marrow-derived macrophages successfully mitigated the number of HIV-1-infected cells in murine lymph nodes, spleen, liver, lungs, and CNS (Dou et al. 2006; Dou et al. 2009). Injection of cell-free nanoformulations into humanized SCID mice demonstrated antiviral effects, neuroprotection, and that drug-carrying macrophages indeed pass drug through brain microvascular endothelial cells (Dash et al. 2012; Kanmogne et al. 2012). Nanoformulation affords the opportunity to attach targeting-ligands to antiretrovirals. Folate decoration of ATV/r and M3TC facilitated greater MDM uptake and delivery to liver, spleen, and lymph nodes of HIV-1-infected humanized mice (Puligujja et al. 2013; Singh et al. 2016). A final technical achievement in LASER-ART design rests in the ability to house nanoformulations in theranostic particles. Drug encapsulation by materials like magnetite (Guo et al. 2014b), 111 indium oxine (Gorantla et al. 2006), europium doped cobalt-ferrite (Kevadiya et al. 2017) permit real-time biodistribution tracking by MRI, SPECT, or gamma-scintigraphy. This methodology potentiates for the eventual correlation between radiographic signal and projected pharmacokinetics / pharmacodynamics profiles without the need to harvest tissue. To conclude, macrophages bring LASER-ART treatments and diagnostic particles to HIV-1-infected areas that conventional therapies may inadequately reach.

Future Directions and Conclusions

HIV treatment has progressed significantly since the discovery of macrophages as viral hosts and drug targets. Though HIV promotes chronic-inflammatory programs in macrophages that drive CNS, pulmonary, cardiovascular, GI, and systemic pathologies, some of these aberrant processes serve as levers for therapeutic inhibition. Advances in bioengineering and medicinal chemistry, such as erythrocyte-loaded and nanoparticulated drug generation have allowed conventional antiretroviral therapies to become even more effective. Future developments in the field of macrophage-HIV eradication should apply CRISPR-Cas9 gene editing to splice out proviral sequences from host genomes. This approach has already met some success in vitro prophylactically in human embryonic stem cell-derived macrophages and primary CD4+ T cells (Liao et al. 2015), as well as therapeutically in infected-patient T lymphocytes (Kaminski et al. 2016). Adoptive transfer of antigen loaded antigen presenting cells capable of eliciting cellular and humoral immune responses (Cobb et al. 2011) is perhaps another exciting prospect on the horizon of HIV vaccinology. To this end, macrophages are paramount to the establishment of HIV infection and its eventual eradication.

Compliance with Ethical Standards

Conflict of Interest None.

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