HARNESSING INNATE IMMUNE RESPONSES TO ENHANCE ANTI-HIV-1 THERAPIES

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Abstract—Innate immune responses represent the first line of defense against invading microbes including HIV-1. Macrophages and other myeloid cell populations mediate responses to viruses, bacteria and fungi in a rapid, non-specific manner following binding of moieties from these pathogens to innate immune receptors. Macrophages express several types of innate immune receptors called “pattern recognition receptors” that bind distinct pathogen-associated molecular patterns. Chief among the innate immune receptors in macrophages are a family of receptors called the Toll-like receptors that reside on the plasma membrane as well as within endosomal vesicles. The binding of ligand to Toll-like receptors and other types of innate immune receptors triggers a down-stream signaling cascade designed to rapidly induce expression of inflammatory mediators and innate immune molecules to thwart infection. Several nucleic acid based approaches have been developed to block or eliminate HIV-1 infection of macrophages and other myeloid cells, including the use of short-interfering RNA to block viral and cellular protein expression, and more recently, genomic engineering with CRISPR/Cas to cleave viral or cellular genes important in HIV-1 infection and replication. However, because these constructs are comprised of nucleic acids, they function as powerful ligands to activate select innate immune receptors, triggering anti-viral responses that effectively inhibit HIV-1 infection. This review will summarize the potential dual-benefits of nucleic acid-based therapies to block HIV-1 infection of macrophages.

Keywords—Innate Immunity, Macrophages, HIV-1, inflammation, Toll-like receptors

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INTRODUCTION

1. *Innate immunity to HIV-1*

1.1 *Innate immune responses to HIV-1*

HIV-1 is generally transmitted across mucosal surfaces of the genital or gastrointestinal tracts (Royce, Sena et al. 1997, Sagar 2014). These mucosal tissues are uniquely designed to prevent pathogenic invasion by the presence of a mucous barrier to trap pathogens (Carias, McCoombe et al. 2013), a confluent epithelial cell lining that can "sample" pathogens using receptors present on its apical surface (Nasu and Narahara 2010), and the presence of innate immune cells located in the submucosal region of these sites (Shen, Richter et al. 2014). Other factors such as an acidic pH and the presence of soluble molecules that bind to and inactivate pathogens can assist in suppressing the transmission of HIV-1 from mucosal sites to the periphery. For example, dendritic cells respond to HIV infection by the induction of interferon-regulatory factor transcription family (IRF) gene transcription (Yoh, Schneider et al. 2015). One of the IRF family members, IRF3, has several functional domains and is found in an inactive form in the cytoplasm. Upon phosphorylation, IRF3 forms a complex with a protein termed CREBBP that binds the cytoplasmic cyclic AMP-response element binding protein, and then translocates to the nucleus to induce production of the interferon-alpha (IFN-α) and interferon-beta (IFN-β) along with the interferon regulatory factor 7 (IRF7) that further increases type I interferon production. Interferons are potent anti-viral molecules that directly suppress pathogen replication and induce an inflammatory state to prevent subsequent infection (Buitendijk, Eszterhas et al. 2014).

1.2 *Innate immune receptors*

Innate immune receptors that detect pathogens represent the first line of defense against infection. These receptors are located on the cell membrane, within endosomal vesicles, and in the cytoplasm of various cells including monocytes, macrophages, dendritic cells, as well as on neutrophils and certain subsets of lymphocytes. These receptors include more than a dozen different Toll-like receptors (TLR) (Giraldo, Hernandez et al. 2015), cytosolic DNA sensing receptors (Swaminathan, Sui et al. 2014), phagocytic receptors (i.e. mannose receptors, scavenger receptors) that internalize pathogens (Olivetta, Tirelli et al. 2014), and chemotactic receptors such as f-Met-Leu-Phe that bind N-formulated peptides produced by bacteria and induce the trafficking of neutrophils to sites of infection (Westhorpe, Zhou et al. 2009). These receptors recognize repeating structures on pathogens termed pathogen associated molecular patterns (PAMPs). The binding of a pathogen to its particular receptor triggers an intracellular signaling cascade that ultimately induces gene transcription and production of innate immune molecules. Several of the TLR reside within endosomes and recognize microbial nucleic acids during endocytosis of pathogens at the time of initial infection. Viruses whose genome is composed of single-stranded RNA such as HIV-1 are recognized by TLR7 and TLR8. Once these TLR are activated, an intracellular signaling pathway is induced that is mediated via the cytoplasmic adaptor protein MyD88. MyD88 activation ultimately induces interferons as well as other inflammatory cytokines and chemokines. TLR3 recognizes double-stranded RNA molecules and in contrast to the other TLR that signal through MyD88, activation of TLR3 results in the phosphorylation and activation of IRF3.

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Because of the intrinsic role of TLR in response to pathogen infections, agonists to several TLR have been examined for their ability to inhibit or moderate HIV infection in animal and tissue culture models. Victoria et al., reported that the TLR2 agonists zymosan yeast and Pam3Cysk4 reduced HIV replication in macrophages if added prior to the addition of HIV-1. The induced cytokines responsible for the anti-HIV-1 effect were IL-10 and the β-chemokines CCL3, CCL4 and CCL5 (Victoria, Temerozo et al. 2013). Zhou, et al., used poly (I:C) and showed a similar suppression of HIV-1 infection in macrophages (Zhou, Wang et al. 2010). An interesting finding was reported by Franchin et al., who showed that bacterial-derived lipopolysaccharide (LPS), classically a ligand for TLR4, inhibited macrophage infection by HIV-1 by altering the recycling of CCR5, leading to the reduced expression of this important HIV co-receptor (Franchin, Zybarth et al. 2000).

Our own work (Buitendijk, Eszterhas et al. 2013, Buitendijk, Eszterhas et al. 2014) showed that agonists to TLR3, 7, 8 and 9 inhibited HIV-1 infection in both lymphocytes and macrophages by several different mechanisms. Gardiquimod, a TLR7 agonist, functions both as a reverse transcriptase inhibitor and a potent inducer of IFN-α. Agonists for TLR8 and 9, although purported to function through MyD88, were also capable of suppressing HIV-1 infection in the presence of a MyD88 inhibitor, suggesting the activation of a second intracellular signaling pathway. Most likely some of these agonists function by activating RNAse L, an enzyme induced by interferon that destroys cellular and viral RNA (Chakrabarti, Jha et al. 2011), or by activating protein kinase RNA-activated (PKR), an enzyme induced by IFN and activated by viral RNA (Munir and Berg 2013). These molecules likely function as alternative anti-viral sensors in the cytosol of several types of immune cells, including cells that do not express TLR.

1.3 Innate immune molecules

Innate immune cells produce several soluble molecules that can directly bind to and inactivate pathogens. Production of these mediators are induced following binding of ligand to one of several innate immune receptors, or are constitutively produced and are found in the cytoplasm. The most potent of these molecules are the type I and type II interferons (IFN-γ, IFN-α, IFN-β), cytokines and chemokines, and other anti-viral molecules including TRIM5 (Sayah, Sokolskaja et al. 2004, Stremlau, Owens et al. 2004, Jia, Zhao et al. 2015), cytophilin A (Franke and Luban 1995, Sokolskaja and Luban 2006), apolipoprotein B mRNA -editing catalytic 3G (Apobec3G) (Sheehy, Gaddis et al. 2002, Harris, Bishop et al. 2003, Bishop, Verma et al. 2008), human β-defensins (Feng, Dubyak et al. 2006, Mehlotra, Zimmerman et al. 2013, Jia, Zhao et al. 2015), and SAM-domain and HD-domain containing protein 1 (SAMHD1) (Ayinde, Casartelli et al. 2012). A family of interferon-stimulated genes including myxovirus A (MxA), 2'- 5' oligoadenylate synthase (OAS) and PKR, have also been well characterized and have been shown to block viral infection of macrophages (Burugu, Daher et al. 2014, Engelmann, Dubos et al. 2015, Hancks, Hartley et al. 2015, Lohofener, Steinke et al. 2015). Production of these anti-viral molecules depends on cellular expression of the receptors that induce them. For example, myeloid derived cells primarily express TLR3 and TLR8, whereas plasmacytoid dendritic cells macrophages, B cells, and CD4+ and CD8+ T lymphocytes express

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TLR7 and TLR9 (Caramalho, Lopes-Carvalho et al. 2003, Akira, Uematsu et al. 2006, Kawai and Akira 2006, Douagi, Gujer et al. 2009, Song, Zhuang et al. 2009, Cros, Cagnard et al. 2010). Thus, interaction of a particular pathogen with various innate immune cells may yield different outcomes depending on receptor expression. In addition to cell-specific production of anti-HIV molecules, the degree to which these proteins contribute to the anti- HIV-1 effect is likely due to an additive or synergistic effect of the types and quantities of known innate immune factors induced.

Our previous findings demonstrated that the addition of sunitinib maleate, a tyrosine kinase inhibitor, reversed the anti-HIV-1 activity following TLR8 and TLR9 activation by specific agonists (Buitendijk, Eszterhas et al. 2014). As this specific tyrosine kinase inhibitor has been reported to inhibit the anti-viral proteins RNAse L and PKR (Jha, Polyakova et al. 2011), this suggests that these two proteins are potent inhibitors of HIV-1. Further, as these two molecules lie further downstream in the signaling pathway from the MyD88 adaptor protein, it is likely that their induction is independent of activation of MyD88. Of interest is that sunitinib maleate is being used to treat non-AIDS defining cancers that arise in HIV patients (Deeken, Pantanowitz et al. 2009), and although this compound may reduce cancer growth, it may increase HIV replication.

1.4 *Nucleic acid-based anti-HIV therapeutics.*

The use of siRNA to silence gene expression has become an effective way to interrogate the role of cellular and viral proteins in HIV-1 infection and replication (Banerjee, Benjamin et al. 2014). For example, we previously established that siRNA specific for the cellular receptors CD4 and CCR5 effectively inhibited HIV-1 infection in a female reproductive tract tissue explant model (Eszterhas, Ilonzo et al. 2011). In this study, siRNA to silence either CD4 or CCR5 transcripts was encapsulated in cationic nanoparticles and added to female reproductive tract tissue explants from the endometrium, endocervix or ectocervix from pre-menopausal women (Eszterhas, Ilonzo et al. 2011). Two days later, the explants were exposed to HIV-1 and infection was measured on day 8 post-HIV-1 addition by measuring the presence of HIV-1 reverse transcripts. Compared to explants exposed to irrelevant siRNA, those exposed to siRNA specific for CD4, CCR5, or the combination of CD4 and CCR5 demonstrated significant suppression of HIV-1 DNA. This suppression correlated with an increase in IFN- , suggesting an induction of innate immune responses likely due to TLR7 activation following siRNA binding. Interestingly, corollary studies in a humanized mouse model demonstrated suppression of vaginal transmission of HIV-1 following intra-vaginal instillation of nanoparticles encapsulating either the combination of siRNAs to CD4 and CCR5, or an irrelevant siRNA, suggesting a more global induction of anti-viral responses regardless of the specificity of the siRNA (unpublished observations). In these studies, murine as well as human tumor necrosis factor alpha (TNF-α) levels were enhanced in the vaginal tract of treated mice compared to the control group. This suggests that localized inflammatory innate immune responses likely contributed to the suppression of vaginal transmission of HIV-1. Wheeler et al., demonstrated that siRNAs could be used intravaginally as an effective microbicide to block sexually transmitted pathogens including herpes simplex virus (HSV) as well as HIV (Wheeler 2014). Topically applied nucleic acids could

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function both to block transmission of viral sexually transmitted pathogens in a non-specific manner, as well as confer durable knockdown of gene expression in a specific manner (Wheeler 2014). As an extension of these studies, de Fougerolles et al., (de Fougerolles, Vornlocher et al. 2007) and Berhout et al., (Berkhout and ter Brake 2009) proposed the use of RNA-based therapeutics to control sexual transmission of human papilloma virus (HPV), HIV and HSV. These and other reports also found that siRNA delivery can trigger a type I interferon response (Hornung, Guenthner-Biller et al. 2005, Judge, Sood et al. 2005, Robbins, Judge et al. 2008). More recently, attention has focused on the use of genomic engineering using components of the CRISPR/Cas prokaryotic innate immune system to cleave critical cellular and viral genes in HIV infection (Khalili, Kaminski et al. 2015). This approach, unlike the use of the protein- based zinc finger nucleases (ZFNs) (Wayengera 2011) or transcription activator- like effector nucleases (TALENs) (Strong, Guerra et al. 2015), employs a single-stranded RNA molecule to bind to a specific target gene sequence in the human genome. Once bound, the complementary "guide" RNA directs the double-stranded cleavage of the target gene by the use of an associated enzyme termed Cas9 (Drake and Bates 2015). Direct transfection of target cells with plasmid DNA encoding both the guide RNA and the Cas9 gene has been demonstrated in vitro (Maggio, Holkers et al. 2014), but it is clear that alternative methods need to be developed to achieve in vivo targeting of the guide RNA and Cas9 gene editing system. Some approaches include transduction of cells with adenoviral vectors carrying the guide RNA and Cas9 sequences (Bi, Sun et al. 2014), or lentiviral vectors expressing the guide RNA/Cas9 transgene (Wang, Ye et al. 2014). Because the transgene delivered by lentiviral vectors is in the form of a single-stranded RNA molecule, it is possible, and probably likely, that this could induce anti-viral responses independent of a specific gene cleavage event. Thus, careful analysis of intracellular signaling pathways following delivery of transgenes using viral-based vectors need to be considered when evaluating efficacy.

1.5 Conclusion

The induction of innate immune protective mechanisms in myeloid cells can induce a potent anti-HIV effect and is likely a key player in the natural control of mucosal HIV -1 infection. This effect results from activation of innate immune receptors, including TLR, present in various cell types causing an anti-viral signaling cascade. The use of nucleic acid-based therapeutics can have the unintended effect of triggering innate immune mechanisms via binding to specific innate receptors. Thus, a more potent anti-HIV effect may be achieved by the combination of the activation of a non-specific intracellular pathway together with the specific suppression of viral or cellular gene expression. The development of nucleic acid based therapies such as siRNA or CRISPR/Cas gene editing approaches that exploit this potent additive anti-HIV response in innate immune cells including macrophages are highly desirable and may alter the current standard of HIV treatment from a maintenance based system to a potentially curative platform of care.

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