

# Cross-talk between virus and host innate immunity in pediatric HIV-1 infection and disease progression

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## SUMMARY

Variability in the susceptibility to HIV-1 infection and disease progression depends on both virus and host determinants. Some exposed individuals remain HIV-1-uninfected and HIV-1-infected subjects develop disease at varying intervals with a small percentage remaining long-term non-progressors. As innate immunity is the earliest response to microbial entry and injury, host factors that impact innate immunity may play a role in viral infectivity and pathogenesis. In the pediatric population the interactions between the virus and the host may be of particular relevance due to the still developing adaptive immune system. Data indicate that genetic variants of defensins and Toll-Like Receptors (TLRs), key elements of innate immunity, play a role in mother-to-child transmission (MTCT) of HIV-1, and in the outcome of pediatric HIV-1 disease. Although the mechanisms by which these genetic variants influence HIV-1 interactions with the host are still largely unknown, defensins and TLRs, along with their link with regulatory T cells (Tregs), may play a critical role in the onset and persistence of immune activation, a hallmark of HIV-1 disease.

**KEY WORDS:** Immune activation, TLR9,  $\beta$ -defensins, Tregs.

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## INTRODUCTION

Mother-to-child transmission (MTCT) is the main source of pediatric HIV-1 infection. MTCT primarily occurs around the time of delivery, but breastfeeding is an additional route of viral transmission and accounts for about one-third of pediatric infections in resource-poor countries. The introduction of combined highly active anti-retroviral therapy (ART) has dramatically modified the natural course of HIV-infection and progression. ART-based prophylaxis regimens have reduced the MTCT from 15%-30% to below 2% in

most high-income countries (European Collaborative Study, 2005), but have been difficult to implement in economically constrained settings. Presently, about 2.5 million children are living with HIV-1 infection and 400,000 become newly infected each year.

Transmission of HIV-1 is a multifactorial event. The recent finding that ART prevents transmission of HIV-1 in discordant couples provides additional evidence that the level of viremia is one of the most important risk factors (Cohen *et al.*, 2011). However, the fact that some highly exposed individuals can remain seronegative despite repeated exposure to HIV-1 indicates that resistance to the virus does exist (Tomescu *et al.*, 2011). Even in the MTCT of HIV-1, maternal viral load is an important but not exclusive factor (European Collaborative Study, 1999). HIV-1-infected children exhibit a high degree of variability in their rate of disease progression and, in the absence of ART, children progress more rapidly than HIV-1-infected adults (De Rossi *et al.*, 1996).

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Infection as well as disease progression depend on virus-host interactions. Innate immunity is the earliest response to microbial entry and injury and may impact MTCT and disease outcome. The pediatric model affords several advantages for studying virus-host interactions, including the knowledge of the virus source and the time of exposure. Moreover, the effect of innate immunity may be of particular relevance because infants are exposed to HIV-1 and acquire infection when the adaptive immune system is still under development. The discovery of natural correlates of protection against transmission and disease progression could be important for the development of new strategies for the prevention and cure of HIV-1 infection. Here, we briefly review the role of key determinants of innate immunity, i.e. defensins and Toll-Like Receptors (TLRs), in the natural history of pediatric HIV-1 infection and we discuss the link between TLR and T regulatory cells (Tregs) in the onset and persistence of chronic immune activation, a hallmark of disease outcome in HIV-1-infected children.

### Defensins and susceptibility to HIV-1

Defensins, important effectors of innate immunity, are small cationic peptides mainly produced by leukocytes and epithelial cells. According to size and binding patterns, defensins are subgrouped into  $\alpha$ ,  $\beta$  and  $\theta$  (Klotman *et al.*, 2006).  $\beta$ -defensins, in particular, have been studied in HIV-1 infection.  $\beta$ -defensin 1 is constitutively expressed by epithelial cells, while expression of  $\beta$ -defensins 2 and 3 can be induced by pro-inflammatory cytokines, such as tumor-necrosis-factor (TNF) and interleukin (IL)-1 $\beta$ . However,  $\beta$ -defensins have been detected in monocytes, macrophages and dendritic cells, and thus they are not exclusively associated to epithelial cells. Moreover,  $\beta$ -defensins and  $\alpha$ -defensins have been found in breast milk (Armogida *et al.*, 2004; Jia *et al.*, 2001). The antiviral activity of  $\beta$ -defensins involves several mechanisms, including direct interaction with viral envelopes and target cells. They may inactivate viruses by disrupting viral envelopes or by interacting with viral glycoproteins, such as gp120 of HIV-1.  $\beta$ -defensin 2 and 3 inhibit HIV-1 spreading and replication through viral inactivation and down-regulation of CXCR4, the coreceptors of HIV-1 X4 strains (Quinones-Mateu *et al.*, 2003; Seidel *et al.*, 2010).  $\beta$ -defensin

2 may also inhibit HIV-1 at the intracellular level (Sun *et al.*, 2005). The antiviral activity of  $\beta$ -defensins may also be related to their ability to attract cells of the immune system to target tissues (Yang *et al.*, 2002).  $\beta$ -defensin 1 is chemoattractive for immature dendritic cells and memory T-cells through the CCR6 chemokine receptor activation (Andresen *et al.*, 2011).

Exposed uninfected subjects (ESN) have been shown to have higher levels of  $\alpha$ -defensins than healthy controls (Trabattoni *et al.*, 2004). Moreover, oral and vaginal mucosa of ESN exhibited a greater expression of the  $\beta$ -defensins than HIV-1-infected subjects (Zapata *et al.*, 2008). Recent studies indicated that single nucleotide polymorphisms (SNPs) in the 5'- untranslated region of  $\beta$ -defensin 1 gene may influence susceptibility/resistance to infection by pathogen agents; these SNPs do not induce aminoacid changes, but may affect gene expression (Jurevic *et al.*, 2003; Tesse *et al.*, 2008). Three of these SNPs, the rs1800972 C>G, rs1799946 G>A, and rs11362 G>A, have recently been shown to be involved in the risk of MTCT and perinatal HIV-1 infection (Table 1). The rs1800972 CC genotype was found to be associated with increased risk of perinatal HIV-1 infection in Italian children (Braida *et al.*, 2004; Segat *et al.*, 2006). It is of note that genetic variants vary in frequency and function in different geographic and ethnic populations. In Brazilian cohorts (a mixture of African, Caucasian and American populations) the rs1800972 SNP did not seem to influence the risk of MTCT, whereas the rs1799946 GG and the rs11362 AA genotypes were associated with an increased risk of MTCT (Milanese *et al.*, 2006). However, these associations were not confirmed in other Brazilian cohorts (Segat *et al.*, 2009). A study in a large cohort of European white infants born to HIV-1-seropositive mothers who had not undergone ART therapy/prophylaxis during pregnancy demonstrated that the rs1799946 GG genotype and rs1800972; rs1799946 G;G haplotype had a protective role against HIV-1 infection. The protective role of the G;G haplotype was confirmed by evidence that this haplotype in mothers was associated with low levels of HIV-1 plasma viremia at the time of delivery and a low risk of MTCT (Ricci *et al.*, 2009) (Table 1). Of interest, in a Mozambican cohort, women with the rs1799946 GG genotype had significantly lower

TABLE 1 - Influence of genetic variants of  $\beta$ -defensin-1 and TLR9 genes on HIV-1 infection and disease progression.

Gene	SNP	Genotype	Model	Haplotype	Effect (study population)	References
<b><math>\beta</math>-defensin 1</b>						
	rs1800972	CC	Codominant		High risk of MTCT (Italian children)	Braida <i>et al.</i> , 2004
	rs1800972	CG	Codominant		Slow disease progression (white children)	Freguja <i>et al.</i> , 2012
	rs1799946	GG	Codominant		High risk of MTCT (Brazilian children)*	Milanesi <i>et al.</i> , 2006
	rs1799946	GG	Recessive		Low risk of MTCT (white population)	Ricci <i>et al.</i> , 2009
	rs11362	AA	Codominant		High risk of MTCT (Brazilian children)*	Milanesi <i>et al.</i> , 2006
	rs1800972;rs1799946			G;G	Low risk of MTCT (white population)	Ricci <i>et al.</i> , 2009
	rs1800972;rs1799946			G;G	Low viral load at delivery (white population)	Ricci <i>et al.</i> , 2009
	rs1800972;rs1799946			G;G	Slow disease progression (white children)	Freguja <i>et al.</i> , 2012
<b>TLR9</b>						
	rs352139	GG	Codominant		Slow disease progression (Swiss HIV cohort)	Bochud <i>et al.</i> , 2007
	rs352139	AA	Recessive		Slow disease progression (white children)	Freguja <i>et al.</i> , 2012
	rs352140	AA	Codominant		Slow disease progression (Swiss HIV cohort)	Bochud <i>et al.</i> , 2007
	rs352140	AA	Recessive		High viral load, low CD4 cell count, rapid disease progression (Spanish adults)	Soriano-Sarabia <i>et al.</i> , 2008
	rs352140	GG	Dominant		Low viral load, slow disease progression (Seattle primary infection cohort)	Pine <i>et al.</i> , 2009
	rs352140	AG	Underdominant**		Rapid disease progression (white children)	Freguja <i>et al.</i> , 2012
	rs352139;rs352140			G;A	Low risk of MTCT (white population)	Ricci <i>et al.</i> , 2010
	rs352139;rs352140			G;G	Rapid disease progression (white children)	Freguja <i>et al.</i> , 2012
	rs5743836;rs352140			C;G	Rapid disease progression (Seattle primary infection cohort)	Pine <i>et al.</i> , 2009

\*These associations were not defined in other Brazilian cohorts (Segat *et al.*, 2009). \*\*The underdominant model may explain the unusual association between a heterozygous status and disadvantageous condition (Altrock *et al.*, 2010).

levels of HIV-1 RNA in breast milk than women carrying the rs1799946 GA or AA genotypes (Baroncelli *et al.*, 2008). It has been suggested that breast milk has higher levels of  $\beta$ -defensin 1 than other compartments; higher levels of hormones during lactation may upregulate  $\beta$ -defensin 1 expression (Jia *et al.*, 2001; Lama *et al.*, 2007). By modulating gene expression, polymorphisms of  $\beta$ -defensin 1 may curtail HIV-1 levels and exert protective effect against HIV-1 transmission during breastfeeding.

Few data are available on the role of defensins in HIV-1 disease outcome. Specific variants of  $\beta$ -defensin 1 gene may protect against disease progression by increasing the protein expression at mucosa level. A recent study on the natural history of pediatric HIV-1 infection suggested that the rs1800972; rs1799946 G;G haplotype of  $\beta$ -defensin 1 also had a protective effect against disease progression (Freguja *et al.*, 2012) (Table 1).

#### **TLRs and susceptibility to HIV-1 infection**

TLRs are type 1 transmembrane proteins differentially expressed in immune cells. They recognize and bind conserved pathogen-associated molecular patterns (PAMPs) shared by large groups of microorganisms. Following interactions with specific ligands, the cytoplasmic portion of TLRs (similar to that of IL-1 receptor family, therefore called the Toll/IL-1 receptor (TIR) domain) through adaptor proteins trigger activation of signalling pathways that ultimately induce dendritic cell maturation and cytokine production, including type 1 interferons IFN- $\alpha$  and IFN- $\beta$  (Akira *et al.*, 2006). While TLR2 and TLR4 recognize viral components at the cell surface, TLR3, TLR7, TLR8 and TLR9 are expressed in endosomal compartments (Uematsu *et al.*, 2006). Notably, TLR7 and TLR8 recognize ssRNA viruses, including HIV-1. TLR9 recognizes unmethylated-cytidine-phosphate-guanosine (CpG) DNA motifs in bacteria and viruses; activated TLR9 alerts the immune system, triggering the activation of pro-inflammatory reactions that induce dendritic cell maturation and production of cytokines (Barton *et al.*, 2009). TLR4 is an essential receptor for lipopolysaccharide (LPS) (Shimazu *et al.*, 1999). LPS is an indicator of microbial translocation that sustains chronic immune activation in HIV-1-infected subjects (Brenchley *et al.*, 2006).

The role of TLRs in HIV-1 infection is multifac-

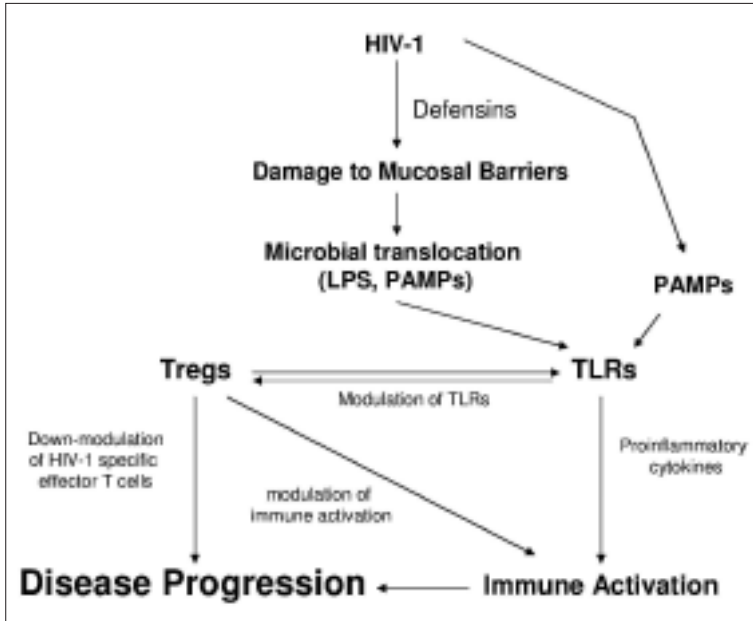
eted and a growing body of data supports a role of SNPs in several TLRs that modulate the risk of bacterial and viral infection. An association between a TLR4 SNP and a higher susceptibility to tuberculosis in HIV-1-infected patients in Tanzania has been reported (Ferwerda *et al.*, 2007). Moreover, two SNPs in *TRL4*, the rs4986790 A>G and the rs4986791 C>T, were linked to the level of HIV-1 plasmaviremia (Pine *et al.*, 2009). A functional TLR8 variant was associated with the clinical outcome of HIV-1 infection (Oh *et al.*, 2008). Two SNPs in *TLR9*, the rs352139 G>A and the rs352140 A>G were linked to viral load and disease progression in HIV-1-infected adults (Bochud *et al.*, 2007; Soriano-Sarabia *et al.*, 2008; Pine *et al.*, 2009). In particular, the rs352139 GG and rs352140 AA genotypes were found to be protective against disease progression in a Swiss cohort of HIV-1-infected adults (Bochud *et al.*, 2007). However, the rs352140 AA genotype was also found to be strongly associated with high viral load, low CD4 T cell count and rapid disease progression in a second study (Soriano-Sarabia *et al.*, 2008), while the protective effect of the rs352140 GG genotype was confirmed in a later study (Pine *et al.*, 2009). Recent studies in European white children demonstrated that the rs352139; rs352140 A;A and G;G haplotypes were associated with a higher risk of perinatal HIV-1 infection compared to the prevalent G;A haplotype (Ricci *et al.*, 2010). Notably, while the rs352139 AA genotype appeared to be associated with slow disease progression, the rs352140 AG genotype, and the rs352139; rs352140 G;G haplotype were strongly associated with rapid disease progression in HIV-1-infected children (Freguja *et al.*, 2012) (Table 1).

#### **Immune activation: a link between innate immunity and HIV-1 disease outcome**

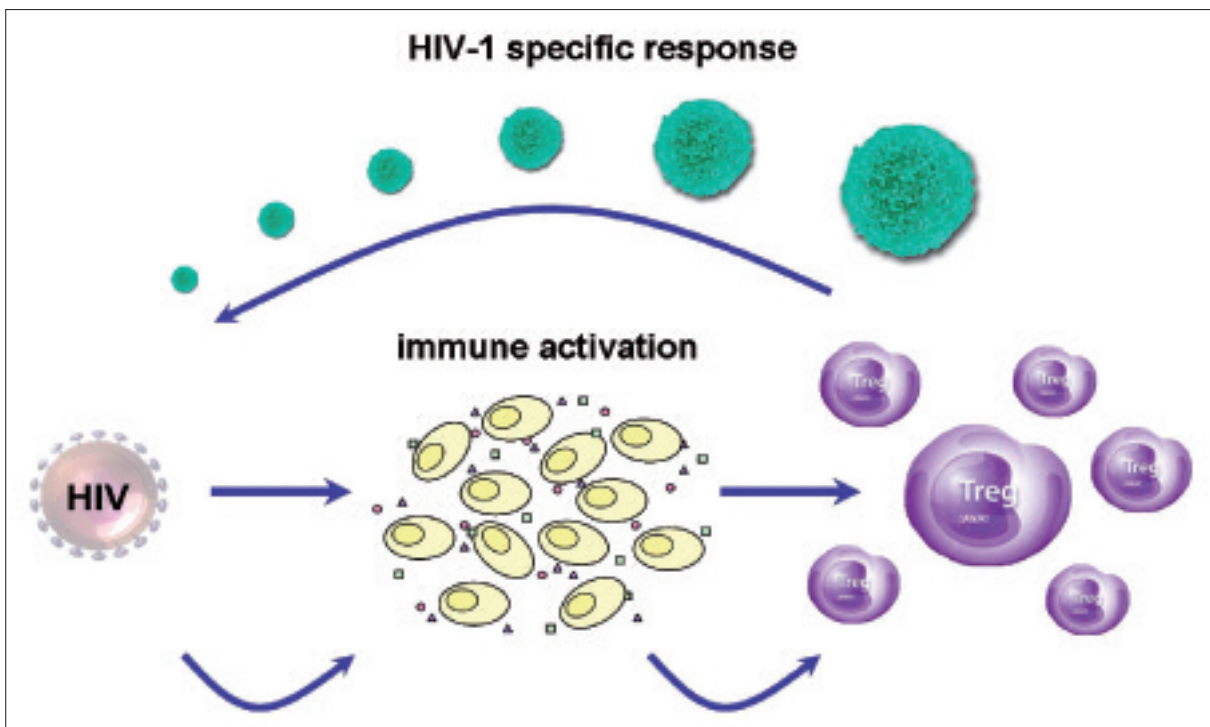
The mechanisms by which genetic variants of defensins and TLRs may influence disease outcome are still largely unknown. TLRs and defensins play important roles in controlling the overall responses to pathogens (Akira *et al.*, 2006; Menendez *et al.*, 2007). By modulating the expression and functional ability of their encoded proteins, genetic variants of these genes may modulate immune activation. Chronic immune activation and disease progression are deeply connected in HIV-1-infected subjects (Brenchley *et al.*, 2006).

Chronic immune activation is overwhelmingly detrimental; it results in the generation of activated T cells that are target for the virus, thus fur-

ther driving viral replication and CD4 cell depletion (Hunt *et al.*, 2003). In children, as well as in adults, immune activation is a hallmark of disease



**Figure 1** - Interactions between HIV-1 and key components of innate immunity during the course of HIV-1 infection. Defensins may counteract the damage to mucosal barriers exerted by HIV-1. HIV-1, as well as components of microbial translocation, such as LPS, and other PAMPs activate TLRs. T cells with regulatory activity (Tregs) potentially have a dual role: detrimental by suppressing the specific effector response to HIV-1 and beneficial by curtailing the hyperactivity of TLRs and the consequent immune activation. LPS: lipopolysaccharide; PAMPs: pathogen-associated molecular patterns.



**Figure 2** - In HIV-1-infected children, Tregs positively correlated with HIV-1 plasmaviremia and the number of activated CD8CD38+ cells. These findings suggest that HIV-1 induces immune activation with a selective expansion of Tregs. These cells suppress the specific response to HIV-1 but lack the ability to suppress immune activation. As a result there is an increase of HIV-1 burden and immune activation.

progression, and activated CD8CD38<sup>+</sup> cells increase in relation to HIV-1 RNA plasma viremia (Resino *et al.*, 2006; Anselmi *et al.*, 2007). In the earliest phases of disease, when the first line of defence fails to control the pathogens, the gut is one of the major sites of CD4 T cell depletion; this damage may impair the mucosal barrier allowing for microbial translocation (Douek *et al.*, 2009). Specific variants of  $\beta$ -defensin may protect against disease progression by increasing the expression of defensins at this mucosa level. Microbial translocation, with entry into the blood stream of bacterial components such as LPS, triggers TLR-mediated immune activation with a consequent production of pro-inflammatory cytokines (Chang *et al.*, 2009, Piconi *et al.* 2010) (Figure 1). Responsiveness to TLR7 and TLR8 stimulation, which have been shown to recognize HIV-1 ssRNA, did not decrease in chronic infection, and may be a contributing factor to ongoing T-cell immune activation in chronic viremic HIV-1 infection (Chang *et al.*, 2012). Of note, immune activation was shown to persist in children who did not respond well to ART; thus immune activation may be also associated with an impaired immune reconstitution (Anselmi *et al.*, 2007).

Chronic immune activation triggered by TLRs may be controlled by different mechanisms. Regulatory T cells (Tregs) play an important role in this setting, by producing immunosuppressive cytokines and regulating unwanted T cell activation (Belkaid, 2007). They are a small population of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T cells with suppressive activity; Tregs selectively express TLRs (TLR2, 4, 5, 7, 8) that can induce their proliferation once stimulated by TLR ligands, and in turn may suppress the TLR hyperactivity (Belkaid, 2007; Dai J *et al.*, 2009). This suppressive activity of Tregs may limit the magnitude of effector responses against a variety of pathogens, but may also suppress immune activation. Multiple studies have demonstrated that Tregs turnover is positively associated with immune hyperactivation (Xing *et al.*, 2010; Weiss *et al.*, 2010). Nevertheless, the role of Tregs in HIV-1 infection is still a matter of debate. Circulating HIV-1 leads to an expansion of this cell subset in an attempt to minimize immune activation. Consequently, Tregs suppressive function can limit HIV-1 specific immune responses by down-modulating the cytotoxic activity of effector T-lymphocytes. However, Tregs may

be also infected by HIV-1, hampering their function and making them unable to control immune activation (Kinter *et al.*, 2004; Antons *et al.*, 2008). In children it has been found that Tregs positively correlated with levels of HIV-1 plasma viremia and numbers of activated CD8CD38<sup>+</sup> cells, but inversely correlated with the number of CD4<sup>+</sup> T cells, thus suggesting their selective expansion along with increased viremia and CD4 T cell depletion (Freguja *et al.*, 2011). These findings indicate that viral load stimulates the immune activation resulting in an expansion of Tregs subset; these Tregs fail to control hyperactivation, but suppress the HIV-1 specific immune response allowing the virus to replicate and promoting disease progression (Freguja *et al.*, 2011) (Figure 2).

## CONCLUSIONS

A body of evidence has established that host genetic factors are important determinants of MTCT and HIV-1 infection outcome. Conversely from other host resistance factors, such as genetic variants of viral coreceptors and their ligands (reviewed in De Rossi, 2007), the effects of defensins and TLRs appear to occur regardless of viral tropism. However, it is clear that the function and impact of genetic polymorphisms differ according to race and ethnicity. Moreover, no single genetic variant is a crucial factor in HIV-1 pathogenesis, and the risk of transmission and progression depends on multiple interactions between virus and host. Because of the complexity of these interactions, many hypotheses have been proposed. Chronic immune activation is now accepted as an important hallmark of HIV-1 pathogenesis. Genetic variants of defensins and TLRs by modulating the expression of encoding proteins, may modulate immune activation and thus play a role in disease outcome. Potent ART may prevent transmission and change the natural history of HIV-1 infection by shutting down HIV-1 replication. However, ART prophylaxis does not prevent all MTCT, and HIV-1 infected children cannot be maintained on ART throughout their lives. The discovery of natural correlates of protection against transmission and disease progression could be important for the development of new strategies for the prevention and cure of HIV-1 infection.

## Competing interests

The authors declare that they have no competing interests.

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