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CHAPTER

8

Innate Immunity in Simian Immunodeficiency Virus Infection

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OVERVIEW

Since the discovery of Toll-like receptors (TLR) in the late 1990s, an explosion of research describing the interaction of HIV with the innate immune system has emerged in the literature. Most of this work has fallen into two broad categories: (1) elucidating the cellular machinery and signaling pathways responsible for recognition of HIV-derived pathogen-associated molecular patterns (PAMPs) and (2) examining the fate of innate cells and their contribution to the control of virus in HIV-infected individuals. More recently, the concept has emerged that unmitigated stimulation of the innate immune system by HIV PAMPs may be a significant contributor to the systemic immune activation associated with HIV disease progression. Finally, a number of studies have attempted to exploit the potency of TLR ligands by using them as adjuvants to enhance adaptive immunity in a number of preclinical vaccine candidates or as potential microbicides.

The simian immunodeficiency virus (SIV)/macaque model has been invaluable in addressing several of these questions. The primary focus of this chapter is on summarizing current concepts of the interplay of innate immunity and HIV infection that have been shaped by work

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done in the SIV/macaque model. The chapter will be divided into five parts:

1. Non-human primate (NHP) models of SIV infection
2. Innate immune sensors of HIV and SIV
3. SIV infection and cellular innate immunity
4. Physical and mucosal immune barriers in SIV infection
5. Innate immunity as a driver of immunopathogenesis in HIV/SIV infection
6. In vivo administration of innate ligands to NHPs.

Several topics peripheral to innate immunity will not be covered in detail within this chapter, because they are beyond its scope or are directly addressed in an adjoining chapter, and we will not directly describe the considerable research on adaptive immune responses in SIV infection or major histocompatibility (MHC) alleles associated with viral control, or discuss in detail HIV/SIV restriction factors.

NHP MODELS OF SIV INFECTION

Characteristics of the SIV/Macaque Model

Experimental inoculation of Asian macaque species (*Macaca mulatta*—rhesus macaque (RM), *Macaca fasicularis*—cynomolgus monkey, and *Macaca nemestrina*—pig-tailed macaques) with SIVmac or SIVsmm has resulted in the most relevant NHP models of in vivo HIV-1 infection [1]. The course of disease progression after SIV infection in macaques has proven to have a high degree of variability, and depends on the SIV isolates and molecular clones. Disease severity can range from very low or none, as in the case of cloned SIVmac142, to rapid and pronounced, as observed for SIVmac239 or SIVmac251 infections, which causes death in 25% of animals by 3 months postinfection [2,3].

The clinical disease associated with SIVmac infection of Asian macaques is strikingly similar to that observed in HIV-1-infected humans and can be classified into three stages: acute, post-acute asymptomatic and AIDS [2]. During the acute phase, occurring in the first 2–3 weeks after inoculation, a massive viremia develops, accompanied by large decreases in the frequency and absolute numbers of $CD4^+$ T lymphocytes, both in the peripheral circulation and in the gastrointestinal (GI) tissues [4]. Other clinical signs during the acute phase, such as fever, lymphadenopathy, rash, and malaise, are characteristic of simian AIDS [3]. SIV infection generally enters a clinically asymptomatic phase, during which the viremia is controlled and maintained at a stable set point and $CD4^+$ T lymphocyte levels undergo a slow but continual decline [2]. During the later AIDS stage, there is severe depletion of $CD4^+$ T cells and concomitant occurrence of opportunistic

infections. Protozoal infections such as *Pneumocystis jiroveci* are usually the first to develop; however, viral (typically cytomegalovirus, adenovirus, and simian virus 40) and bacterial infections (*Mycobacterium avium*) are also prevalent [3]. Immunopathologies include hyperplasia within lymphoid follicles early in infection, switching to lymphoid depletion in advanced disease [3,5]. Thymic atrophy and inflammation of the lungs are also common [6–8]. Most pathogenic SIVmac viruses, such as SIVmac239, cause death from AIDS-like symptoms at approximately 1 year postinfection [9]. The similarities between human and simian AIDS suggest that the macaque model is highly relevant. The high efficiency of infection after challenge also makes it a useful model for vaccine efficacy studies.

Nonpathogenic/Natural Hosts of SIV Infection

Unlike SIV infection of Asian macaque species, infection of most African NHPs with their corresponding SIV does not induce an overtly appreciable disease. The notable exception is chimpanzees, in which pathology consistent with AIDS has been reported in experimental infection with HIV-1 [10] and in SIVcpz infections in the wild [11]. The best characterized natural host species are sooty mangabeys (SMs) and African Green monkeys (AGMs) [12]. SIV infection of natural hosts shares phenotypic similarities with pathogenic infection: a high level of plasma viremia (~10^5 copies/ml), tropism for $CD4^+$ T cells, and rapid but transient depletion of mucosal $CD4^+$ T cells during acute infection. Despite these similarities, in addition to remaining AIDS-free, SMs and AGMs lack many of the classical clinical features of pathogenic HIV/SIV: They maintain stable numbers of peripheral blood $CD4^+$ T cells and do not display bystander lymphocyte activation. The mechanisms by which SMs and AGMs remain AIDS-free have been extensively studied in recent years, and several hypotheses have emerged, as reviewed in Ref. [12]. One key difference appears to be tropism in key subsets of $CD4^+$ T cells: The $CD4^+$ T cells from SMs have a reduced expression of the SIV co-receptor CCR5 on central memory cells (Tcm) and $CD4^+$ Tcm cells harbor lower levels of SIV in vivo relative to their effector counterparts (Tem) [13]. A strong, inverse correlation between disease progression and preservation of the $CD4^+$ Tcm pool has been demonstrated in SIV-infected macaques; the ability of SMs to shunt the bulk of SIV replication away from this subset likely is linked to their ability to maintain stable bulk $CD4^+$ T-cell numbers. The precise immunological mechanisms by which natural host species remain AIDS-free are under intense investigation; however, they remain a crucial model system to dissect the immunological aspects of SIV infection and differentiate aspects of normal host response to a viral infection from a dysregulated, pathogenic immune activation driven by SIV. As we discuss in detail subsequently, in recent years, several studies have directly compared

the innate immune response to SIV among natural pathogenic hosts, and a clear picture of the differences among species has begun to emerge.

INNATE IMMUNE SENSORS OF HIV AND SIV

A number of innate receptors have been demonstrated to mediate recognition of HIV. The best characterized are the TLRs, which recognize viral-derived ssRNA and viral DNA. Most of the current knowledge of innate recognition has come from in vitro studies of HIV-1; however, in a few examples, these studies have been extended to primate studies, typically with the following aims: (1) to empirically test innate stimuli for its adjuvant activity with candidate anti-HIV/SIV vaccines, or (2) to understand the contribution of innate stimuli to pathogenic immune activation. A thorough discussion of the innate immune receptors shown to recognize HIV can be found in Iwasaki [14].

Toll-like Receptors

Toll genes and their corresponding proteins, termed TLRs, were originally described for their antifungal properties in the *Drosophila* species [15]. Eleven mammalian homologs of TLRs that recognize different components of microbes, called PAMPs, have been identified to date [16,17]. Different leukocytes express specific profiles of TLRs; in humans, the plasmacytoid dendritic cells (pDCs) express only TLR7 and TLR9 [18], both of which, along with TLR3 and -8, belong to a TLR subfamily that detects PAMPS intracellularly [19–21]. Toll-like receptor-7 binds single-stranded viral RNA, whereas TLR9 binds hypo-methylated cytosine-phosphate-guanine (CpG) dinucleotides that are enriched in bacterial DNA but are less prevalent in eukaryotic DNA [20,22]. Signaling occurs when the ligands are endocytosed and transported to lysosomal compartments, where they bind their cognate receptors, TLR7 or TLR9 [23,24]. The ligand–receptor complex recruits the global TLR adaptor molecule MyD88, which initiates a signaling cascade of transcription factors including, nuclear factor-κB, activator protein-1, c-Jun N-terminal kinases, and extracellular signal-regulated kinases [19,24,25].

The best-studied TLR recognizing HIV and SIV is TLR7, which is expressed endosomally and recognizes ssRNA [26]. It is expressed at high levels in pDCs, and signaling via TLR7 induces high expression of interferon-alpha (IFN-α) [27]. Whereas HIV and SIV induce high levels of IFN-α by pDCs, these cells are only productively infected at low levels [28]. However, nonproductive internalization of HIV by pDCs is sufficient to induce IFN-α secretion [29,30], as noted with aldothiothreitol-inactivated SIV, which is capable of inducing high levels of IFN-α induction in RMs

and SMs [31]. HIV-infected $CD4^+$ T cells are more potent inducers of IFN production in pDCs than purified virion [32], and autophagy is required for efficient IFN production, which suggests that infected $CD4^+$ T cells, rather than HIV itself, may be the primary stimuli in vivo. Upon stimulation, TLR7 interacts with the MYD88 adaptor molecule and activates phosphorylation of IRF7, which translocates to the nucleus and *trans*-activates type I IFN transcription [33]. During viral infection, IRF7 associates with TLR7 in early endosomes and initiates primarily IFN production. However feedback mechanisms induce maturation of the endosomes, consequently the IFN produced leads to an up-regulation of surface co-stimulatory molecules. In contrast to other viruses and TLR7 ligands, activation of TLR7 by HIV causes the TLR7/IRF7 signaling complexes to remain sequestered in early endosomes, with prolonged production of IFN-α [34].

Emerging Innate Receptors in the Recognition of HIV

Toll-like receptors that recognize HIV are expressed within endosomes, and require virion fusion and delivery to the endosome to become activated. However, until recently, it was unclear how cytosolic recognition of HIV occurs. HIV-1 virions harbor two positive-sense viral RNA genomes. The RIG-I family of helicases has been demonstrated to recognize viral RNA in the cytoplasm, and recently it has been reported that HIV genomic RNA activates RIG-I, the prototypical member of this family [35,36]. Activation of RIG-I in virally infected cells may be masked by HIV's ability to target the IRF3 transcription factor for degradation [35,37,38] and suppress IFN-beta expression. The lattice of HIV capsid also interacts with the restriction factor TRIM5 and enhances innate signaling [39]. Finally, an endogenous receptor for HIV DNA intermediates that arise during reverse transcription of the HIV genome has been hypothesized [14]. Activation of this receptor is masked by the cellular expression of TREX1, a host exonuclease that digests ssDNA [40] and prevents HIV DNA from accumulating to high enough levels to stimulate the as-yet undescribed sensor [41]. With the exception of TRIM5, as described subsequently, the role of these factors in infection at the organism level in either humans or NHPs has not yet been described.

Restriction Factors in SIV Vaccine Studies

Restriction factors are intracellular proteins that act directly to block viral infection at the intracellular level. Although they have primarily been studied for their activity against HIV and SIV in vitro, recent genetic data demonstrated that they can affect viral load and transmission in SIV infection. The most well-studied restriction factors against HIV are TRIM5a [42], APOBEC3G [43,44], Tetherin/BST-2 [45,46], and SAMHD1 [47,48],

each of which has been able to demonstrate highly effective blockade against HIV infection in vitro. These restriction factors have been hypothesized to be important in safeguarding against transmission of lentiviruses between species [49]. TRIM5α is the best studied of the lentiviral restriction factors. Human TRIM5 has low activity against HIV; however, TRIM5 in RMs demonstrates much higher activity in vitro against SIV [42]. Furthermore, TRIM5 in macaques is highly polymorphic, and macaques inheriting restrictive alleles of TRIM5 have lower viral load [50,51] and are more resistant to low-dose challenge by the intrarectal [52] or penile [53] routes. Importantly, these studies have employed SIVsmm strains of SIV; other groups have shown that TRIM5 genotypes have no effect on viral replication or transmission of SIVmac strains [54]. Owing to their considerable influence on viral load and transmission, the prevalence of restrictive or permissive TRIM5 genotypes is an important consideration in SIV vaccine studies.

SIV INFECTION AND CELLULAR INNATE IMMUNITY

Dendritic Cells

The primary function of dendritic cells (DCs) is to act as potent or professional antigen-presenting cells that activate the adaptive immune response. Resident DCs at sites of infection express a multitude of innate pathogen recognition receptors (PRRs) described previously at the plasma membrane, cytoplasm, and endosome, with which they recognize PAMP-containing structures in foreign microorganisms such as viruses. Upon recognition, DCs become activated and traffic to secondary lymphoid organs, where they present foreign antigen via MHC class I and II surface molecules to $CD4^+$ and $CD8^+$ T cells. In this manner, DCs represent the interface between innate and adaptive immunity. Because of their primary importance in modulating T-cell responses, modulation of DC response by vaccination and adjuvants has been a subject of interest in the development of HIV vaccines; we will describe methodologies targeting the innate machinery in the subsequent section. In addition, over the past decade, it has been demonstrated that HIV and SIV are also capable of hijacking sentinel DCs and using them to disseminate from primary sites of infection to secondary lymphoid organs, which will be discussed later in more detail.

Dendritic Cell Subsets

Unlike lymphocytes, DCs are classified by both their cell-surface markers and anatomic location. In blood, the best-studied human myeloid DC subsets are typically defined as $CD11c^+$ and CD1c/$BDCA1^+$ Lin-cells,

sometimes referred to as conventional or classical DCs (cDCs). Phenotypically, these cells have been demonstrated to be similar in RMs and SMs [55]. However, in RMs, several lineage markers are present on cDCs that are absent on their human counterparts, including CD16, CD56, and CD11b [56,57]. More recently, novel blood DC subsets have been described in humans: $CD141^+$/$BDCA3^+$ DCs, capable of efficient cross-presentation and representing the functional analog of mouse CD8α DCs [58]. However, similar subsets of these cells have not been reported as yet in NHPs. A third subset of nonmyeloid blood DC, the pDC (discussed subsequently), differs significantly from myeloid DCs in that its primary function appears to be the secretion of high amounts of IFN-α, rather than antigen presentation. Within the skin and mucosa, Langerhans cells (expressing langerin) are prevalent in the epidermal layers as two dermal DC subsets: $CD1a^+$ or $CD14^+$. More recently, gut-resident $CD103^+$ DCs have been described in RMs and humans [59] that promote tolerance by induction of T-regulatory cell response and maintain homeostasis of the gut epithelial barrier (described in detail later). Of interest, this subset is also notable for being depleted from the mucosa during SIV infection [60].

Highjacking of DCs for Viral Entry and Dissemination

Early studies in vaginal transmission models of SIV demonstrated that SIV enters the mucosa and nonproductively infects resident DCs [61] and LCs [62]. Within 24 h of infection, however, the HIV-infected DCs are detectable in the draining LNs [61]. HIV-1 binds to a C-type lectin receptor, DC-SIGN (CD209), on the surface of mucosal DCs, and potently enhances *trans*-infection of $CD4^+$ T cells. Similar activities have been demonstrated for DC-SIGN + DCs in RMs, chimpanzees, and AGMs in vitro [63–65].

Plasmacytoid DCs

pDCs are a unique subset of DCs that are present at low frequencies in peripheral blood. Unlike most DCs, the primary function of pDCs does not seem to be antigen presentation, but production of massive levels of the antiviral cytokine IFN-α [66]. pDCs express high levels of the interleukin (IL)-3Rα chain, CD123 [67–70], as well as BDCA-2 and -4 [71]. Although the function of BDCA-2 is still being defined, BDCA-4 is identical to neuropilin-1, a neuronal receptor for axon guidance factors and a receptor on endothelial and tumor cells for vascular endothelial growth factor (VEGF-A) [71–73]. Isolated pDCs are characteristically smooth round cells, whereas mDCs have prominent dendrites [74]. These morphological differences suggest that pDCs in blood are generally more immature, a finding supported by low cell surface expression of the T-cell co-stimulatory molecules CD40 and CD80/CD86 [75]. pDCs were first recognized

in human lymph nodes as cells with plasma cell morphology but devoid of B cell and plasma cell markers [76]. They were originally referred to as plasmacytoid T cells because of their close association with T cells in lymph nodes [77], but the nomenclature was later changed to plasmacytoid monocytes when they were found to share some common markers with myelomonocytic cells [74]. Several research groups recognized concurrently that stimulating peripheral blood mononuclear cell (PBMC) cultures with viruses or virus-infected cells resulted in high concentrations of IFN-α accumulation in the supernatants, but only a small number of cells appeared to be responsible for the bulk of IFN-α production [78–80]. These cells, dubbed natural interferon-producing cells (nIPCs), lacked T, B, NK, and monocytic markers, but expressed MHC-II molecules, and were clearly distinct from classical peripheral blood DCs [78,80,81]. After nearly a decade of independent study, both Siegal et al. [82] and Cella et al. [83] determined that plasmacytoid monocytes and nIPCs in peripheral blood and secondary lymphoid tissues were the same cell type, whereupon the designation pDCs was adopted.

In response to a wide range of viruses and bacteria, pDCs produce up to 10 pg of IFN-α/activated cell, which is 10-fold greater than the amount of IFN-α produced by activated monocytes [84]. Although IFN-α1 is predominant, human pDCs secrete most of the 13 IFN-α subtypes, as well as moderate levels of IFN-β [85,86]. Interferon regulatory factors (IRFs) control expression of both IFN-α and -β, with IRF-5 and -7 controlling IFN-α secretion in response to virus infections. pDCs express most of the IRF genes, and in particular constitutively express high levels of IRF-5 and IRF-7, which can be detected by the presence at both the mRNA and protein levels [86]. The high level of constitutive IRF-7 expression in pDCs is thought to contribute to rapid IFN-α synthesis in response to virus infections [87]. pDCs are adept at recognizing and becoming activated in response to a variety of viruses including hepatitis C virus (HCV), human T-lymphotropic virus (HTLV), and herpes simplex virus 2 (HSV-2). pDCs have been directly linked to resolution of disease in respiratory syncytial virus, Dengue fever virus, and HSV-2 [88–90], but are also depleted in a number of diseases including HTLV, HCV, severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), and HIV [91–97].

pDCs have been well studied during HIV infection. Human pDCs stimulated in vitro with infectious or inactivated HIV, or even with recombinant gp120, secrete IFN-α, and up-regulate CD40, CD83, and CD86 [98–102]. Compared with uninfected controls, circulating pDCs in HIV-infected patients express higher levels of CD86 on the cell surface [103]. Although both TLR7- and TLR9-dependent mechanisms of HIV-mediated pDC activation in vitro have been described, activation in vivo primarily depends on TLR7 pathways and contributes to the overall immune activation observed in advanced HIV disease [104–106].

However, in chronically infected HIV patients, the frequencies and absolute numbers of circulating pDCs are reduced, as is pDC-dependent IFN-α production ex vivo; these reductions generally correlate with increasing viral loads and decreasing numbers of $CD4^+$ T cells [103,107–110]. Downregulated CCR7 expression on pDCs in HIV patients is also related to viral load [111]. After the administration of highly active antiretroviral activity, circulating $CD4^+$ T-cell numbers usually increase and plasma viremia declines; however, pDC numbers are only partially restored and IFN-α secretion remains defective [112,113]. Furthermore, pDCs isolated from HIV patients were impaired in their ability to stimulate allogeneic T cells, and not only harbored proviral DNA, but also transferred virus to $CD4^+$ T cells in co-culture [100,114,115]. HIV-1 isolates that used both R5 and X4 infected human pDCs productively in vitro, although R5-tropic viruses tended to replicate more efficiently [116–118]. In whole PBMC cultures infected with R5-using strains, pDCs had higher proviral loads than $CD4^+$ T cells, which suggests that pDCs are preferentially infected [119].

Similar to what has been observed in HIV, pDC depletion has been demonstrated during acute and chronic SIV infection of RMs, pig-tailed, and cynomolgus macaques [56,120,121], but not nonpathogenic host species of SIV such as SMs and AGMs [122–124]. Interestingly, studies of acute pathogenic SIV infection in macaques have demonstrated a transient increase of pDCs in peripheral blood after rapid egress from the bone marrow, followed by depletion of circulating pDCs and accumulation of apoptotic pDCs in lymph nodes [57,120]. Furthermore, recent evidence from multiple laboratories has shown that pDCs are not necessarily depleted during pathogenic SIV infection, but rather, accumulate in large numbers in the GI tract [124–126]. Of note, this phenomenon appears to be absent in nonpathogenic SIV infections.

Natural Killer Cells

Natural killer (NK) cells are generally thought of as the primary effector cells of the innate immune system. Although NK cells are prototypically thought of as cytotoxic cells, in which they are among the first cells to intersect pathogens and eliminate neoplastic cells, they also have more recently been shown to have significant homeostatic and regulatory functions [127–132]. In humans, two subsets of NK cells predominate [129]. The dominant subset in peripheral blood is $CD16^+CD56^{dim}$, which primarily mediates cytotoxic activity and secretes relatively little IFN-γ. In contrast, a distinct subset of $CD16^{-/low}CD56^{hi}$ NK cells displays little cytotoxic activity but secretes relatively large amounts of IFN-γ. The $CD56^{hi}$ subset also expresses CCR7 and CD62L and is the dominant population found in lymph nodes and in tonsillar tissues [133]. Mucosal NK cells have also been identified throughout the digestive tract, from the labial and

oropharyngeal mucosae to the large and small intestines [132,134–136]. Mucosal NK cells are typically $CD56^+$ and can be found both within the lamina propria and localized to intraepithelial tissues. Multiple mucosal NK cell subpopulations described in both humans and macaque models reveal significant diversity, but generally express low levels of cytotoxic enzymes such as granzyme and perforin and robust secretion of IFN-γ and tumor necrosis factor-α (TNF-α) [132,137–139]. NK cells have also been found throughout the female reproductive tract and have a major role in placental implantation and modulation of pregnancy [127–132].

Because of the significant interest in NK cell biology in the context of tumor biology and infectious diseases, including HIV/SIV, accurate definitions of NK cells in animal models, including NHPs, are of critical importance. Recent studies have accurately identified macaque NK cells in the peripheral blood as being $CD3^-CD8\alpha\alpha^+NKG2A^+$ cells, and then further divided them into three distinct subpopulations: a major NK cell population that is $CD16^+CD56^{-/dim}$ and two minor populations, $CD16^{-/dim}CD56^{hi}$ and $CD16^-CD56^-$. A subsequent publication by another group validated this definition of macaque NK cells and demonstrated that it applies to SMs [140]. The CD56/CD16 functional profiles found in human NK cells seem to be analogous in NHPs, but interestingly, whereas $CD16^+$ NK cells predominate in peripheral blood, most NK cells in tissues are $CD56^+$ or DN.

NK cells have long been shown to inhibit HIV replication in in vitro cultures with β-chemokine-dependent inhibition and NKG2D ligand-mediated killing as a dominant mechanism [141]. Interestingly, acute HIV disease is associated with expansion of the $CD56^{dim}CD16^+$ NK cell subsets in humans, which suggests a virus-driven increase in cytolytic activity [142], and long-term nonprogressors have increased NK cell cytotoxicity compared with viremic individuals [143]. With the advent of more precise molecular profiling, it has recently been shown that KIR3DS1 and its putative ligand HLA-B Bw4-80I are also associated with slower disease progression, and the interaction of the two molecules leads to inhibition of HIV-1 replication in vitro [144].

A number of studies suggest that NK cells can help mediate control of SIV infection. NK cells have been shown to readily lyse SIV-infected T cells or cells pulsed with SIV [145]. Acute infection with SIVmac251 induces activation of macaque NK cells, reflected by the up-regulation of CD69 expression and increased lysis of susceptible K562 cells [146]. Longitudinal studies following SIV infection demonstrate that early NK cell responses can lead to long-term virus control in RMs, as well as in the natural host species, SMs and AGMs [140,147]. Mathematical models suggest that NK cells can modulate SIV replication and disease outcome in SMs, but not in RMs [148]. During SIV infection, NK cells in the mucosa up-regulate cytotoxic functions, and an overall increase in mucosal homing of NK cells is

evident [138,139,149]. In pig-tailed macaques infected with SIV, an inverse association between the magnitude of the NK cell response and the probability of development of neuro-AIDS was observed [145]. Macaque studies have thus far yielded conflicting results on the role of Killer cell immunoglobulin-like receptors (KIRs) in controlling SIV infection, with some studies suggesting positive control in the presence of cognate ligands [150,151]; others have suggested that KIRs are related to loss of control of SIV replication [152]. Although imperfect, macaque models have allowed for partial depletion of NK cells in vivo. Depletion of NK cells using a JAK3 inhibitor resulted in transient increases in viral loads, which suggests that there may be some role for NK cells in regulating viral control [153]. However, anti-CD16 depletion [154,155] showed no effect on early infection, but these studies may have been limited by inadequate depletion in tissues and a lack of effect on non-CD16 NK cell subpopulations.

Although NK cells generally exert their functions, either cytolytic or regulatory, directly on target cells, they can also cooperate with the adaptive immune system. Antibody-dependent cell-mediated cytotoxicity (ADCC) responses develop within the first few weeks postinfection [156] and are thought typically to be exerted by NK cells, but can also involve neutrophils. ADCC activity has been associated with delayed progression to AIDS in SIV-infected macaques [157], involved in the efficacy of live attenuated SIV vaccines [158], and with reducing viral loads in various nonreplicating vaccine modalities [159–161], including the RV144 HIV vaccine trial [162,163]. Anti-HIV ADCC antibodies have also been observed in highly exposed seronegative individuals [164], and are associated with the control of virus replication in HIV elite controllers and slow progressors [165,166].

Th17 Cells in HIV/SIV Infection

Another prominent $CD4^+$ subset in the mucosa that is profoundly affected by HIV and SIV infection are Th17 cells [167,168]. The Th17 subset represents an independent lineage from other Th cell populations in both function (high production of IL17A, IL17F IL22, IL21, and IL26) and differentiation (induced by the RORγτ transcription factor). Although they are rare in the periphery, Th17 cells are highly enriched in the lamina propria (LP) [168,169]. Th17 cells function to coordinate innate immunity against bacterial and fungal properties by regulating granulopoiesis, recruitment of neutrophils, and induction of antimicrobial peptides [170]. Th17 lymphocytes are also integral to maintenance of an intact epithelial barrier via secretion of $IL22^{170}$. Interleukin-22 acts on epithelial and other nonhematopoietic cells to promote differentiation and proliferation, and triggers production of β-defensins, S100 family proteins, RegIIIγ, and other antimicrobial compounds [167,170,171]. During HIV and pathogenic SIV

infection [172,173], Th17 are depleted from the mucosa but are maintained in natural hosts such as SMs. HIV-infected long-term non-progressors also maintain higher Th17 levels than patients with progressive disease. The loss of Th17 cells and other IL17-producing cell types such as innate lymphoid cells (ILCs) [60], and $CD8^+$ IL17 cells [174], also occurs during pathogenic infection. In 2006, a seminal work by Brenchley and Douek and colleagues showed that during pathogenic HIV and SIV infections, a significant amount of LPS and other microbial byproducts could be detected in the plasma [175]. This process, which they termed microbial translocation (MT) (described in greater detail subsequently), was argued to be a natural sequelae of the massive $CD4^+$ T-cell depletion and disruption of lymphoid architecture observed in the mucosa, and could be a significant source of the systemic immune activation observed in HIV and SIV infection. Later, Raffatellu et al. [172] demonstrated that the loss of Th17 cells was associated with translocation of *Salmonella* in SIV-infected macaques, providing strong evidence linking Th17 loss with breakdown of the epithelial barrier, a role that seems logical considering their role in IL22 production [172]. Providing another link between the loss of Th17 cells and SIV pathogenesis was the demonstration that whereas Th17 are depleted from SIV-infected macaques, they are maintained in infected, nonpathogenic species such as SMs [173].

The mechanism by which Th17 cells are depleted is an area of active investigation. Although it has been reported that Th17 can be efficiently infected in vitro [176], they are not preferentially infected in vivo [173]. Furthermore, that other cells capable of secreting IL17 that do not bear CD4 are also depleted from the mucosa during SIV infection suggests a mechanism independent of direct infection of IL17-producing cells. One likely mechanism may be the loss of IL21-producing cells, because the depletion of $IL21^+CD4^+$ T cells correlates with the loss of Th17 cells [177], and exogenously given IL21 attenuates the loss of Th17 cells during SIV infection [178]. An alternative, overlapping mechanism, may be due to the dysregulated metabolism by activated DCs and APCs expressing the indoleamine 2,3-dioxygenase (IDO1) enzyme responsible for the catabolism of tryptophan. IDO1 activity and its catabolites inversely correlate with Th17 levels in HIV-infected individuals [179].

Innate Lymphoid Cells

Recently, a subpopulation of mucosae-restricted cells that bear features similar to both NK cells, Th17 and Th22 cells, has been identified in mice, humans, and RMs [139,180–186]. These cells have been termed ILCs and are partially identifiable by the high expression of NKp44; they are predominately found in mucosa-associated lymphoid tissues, including all components of the GI tract, including gut-draining lymph nodes, tonsils,

and lungs. ILCs express high levels of the Th17 cell transcription factor RORγt and share a phylogenetic lineage with lymphoid tissue-inducing cells (LTis) [187], but are generally non-cytotoxic and secrete large quantities of IL17, IL22, IL26, and CCL6. Unfortunately, due to limited access to mucosal tissues in humans, ILCs have been problematic to study and their functional niche in primates is unclear. This caveat has been partially alleviated by the more recent description of this cell type in nonhuman primates by multiple groups [139,188,189]. Interestingly, during SIV infection ILCs are massively depleted in number and have a significantly altered functional repertoire. Furthermore, loss of ILCs negatively correlates with breaches in the gut epithelia during chronic SIV infection, suggesting they may be necessary for the maintenance of gut homeostasis [60]. The link is likely to be functionally related given the prominent roles IL-17 and IL-22 play in the regulation of gut integrity. Although the mechanism of ILC suppression during SIV infection is not entirely clear, it appears to be partially linked to increases in soluble inflammatory mediators in the GI tract and an accumulation of indoleamine 2,3-dioxygenase 1 (IDO1) catabolites [139].

PHYSICAL AND MUCOSAL IMMUNE BARRIERS IN SIV INFECTION

Innate Physical Nonimmune Barriers in HIV/SIV Infection

In general, the rate of HIV/SIV transmission in the absence of vaccines is relatively low, partially because of low virus infectivity and sufficient innate immune responses. However, although most transmission events occur at vaginal or rectal mucosal surfaces, the role that physical barriers have in these tissues in blocking transmission has been traditionally ignored. Empirical evidence suggests that physical barriers may limit many transmission events because intravenous infection (where such barriers are obviously absent) has significantly higher rates of transmission compared with mucosal HIV infection [190]. Furthermore, intravenous (IV) infections characteristically have greater numbers of viral variants, with as many as 16 founder viruses that have been observed in samples from HIV-1–infected intravenous drug users. By comparison, vaginal transmission is typically characterized by one to three founder populations and rectal transmission, both experimentally and in cohorts of men who have sex with men (MSM), has a greater number of founder virus populations than vaginal transmission, but fewer than intravenous transmission [191–194]. Finally, although founder virus populations have yet to be enumerated in cases of oral HIV transmission, this route of infection is generally thought to be inefficient. These data detailing the relative

success rates of virus transmission by various routes offer clues about the substantial role that physical barriers have in preventing HIV infection.

During mucosal transmission of HIV/SIV, infection likely involves one of four mechanisms: (1) direct infection of epithelial cells; (2) transcytosis through epithelial cells; (3) transmigration of infected cells; or (4) direct virus entry through breaches in the epithelial layer. All of these methods feature a role for the mucosal epithelia. Interestingly, HIV has evolved to disrupt mucosal epithelia even in the absence of infection [195], which further suggests that these physical barriers put selection pressures on the virus. Thus, we can ask which characteristics of the various epithelia may be involved in delaying spread of virus or blocking infection entirely. Most HIV transmissions occur across the vaginal epithelia, but transmission rates are higher across the rectal mucosa. The mean thickness of human epithelia in rectum is 24.6 mm, compared with vaginal epithelia, with a mean thickness of 215.5 mm, a 10-fold difference [196]; and the rectal mucosa is far more vulnerable to epithelial breaches during intercourse. Furthermore, most of the vaginal epithelia are stratified squamous arranged in layers compared with single-layer columnar epithelium in the rectal mucosa. However, the thick vaginal epithelial layer thins during the luteal phase of the menstrual cycle as a result of increased progesterone levels, and elevated infection rates are observed during this period. In fact, experimentally induced thinning of the vaginal epithelia in macaque models using Depo-Provera treatment is commonly used to increase infection efficacy [197].

Intestinal epithelia are generally thought of as simply providing a physical barrier; however, they have an active role in mucosal immunity by production of a broad array of mucins, inflammatory cytokines, and chemokines [198], including β-chemokines capable of blocking HIV/SIV infection directly. Increased elasticity of vaginal mucus and a low pH in the follicular phase are also thought to prevent transmission, and during the luteal phase, mucus thinning and increase in the pH correlate with increased transmission rates [197]. Cervicovaginal mucus inhibits HIV diffusion and penetration, and provides another physical barrier to infection [199].

Finally, oral HIV/SIV infection occurs at low rates during mother-to-child transmission [200], but it is debatable whether sexual oral transmission occurs at a detectable frequency. Regardless, oral transmission is inefficient compared with other routes of transmission. This may be partly because of particularly strong physical barriers in the oral mucosa, including the thickest epithelial layer among mucosal surfaces, with a mean thickness of 263 mm [196]. The oral mucosa is also bathed in saliva, which contains multiple salivary proteases and amylases that are known to inhibit virus infectivity, as well as mucins that can physically trap virus particles [201].

Mucosal Immunity

The epithelial lining that includes luminal mucus layers provides a considerable physical and biochemical barrier to microbes. The chemical barriers include secretory immunoglobulin A (IgA), defensins, RegIII proteins, and other soluble factors [202]. Underlying and within the epithelial lining is a complex mucosal immune system composed of its own specialized structures and diverse immunological subsets [203]. The mucosal immune system faces a unique challenge in the constant barrage of foreign antigens represented by the microbiota present in the intestinal lumen. The mucosal compartment is a vibrantly dynamic system in which each of the three major components (the epithelial barrier, the mucosal immune structure including the cells that form the structure, and commensal microbiota) all actively contribute to the development and homeostasis of the system as a whole. Perturbations to one part of the system, as occurs during HIV/SIV infection, can cause significant disruption of the others [171].

Structurally, the mucosal immune system has several features analogous to the classical immune system. Activation of adaptive responses is initiated in inductive sites such as mesenteric LNs, Peyer patches (PP), and isolated lymphoid follicles (ILFs) [203]. A cardinal feature of the PP and ILF that distinguishes them from nonmucosal LNs is the encapsulation by specialized follicle-associated epithelium that contains pinocytotic M cells, which are responsible for sampling the antigenic content of the intestinal lumen. After presentation to T and B cells, they migrate to mucosal effector sites such as the LP and the basal layer underlying the gut epithelium, and within the intra-epithelium lymphocyte compartment (IEL).

The mucosal immune system is also enriched in several unique cellular subsets that are found only minimally in the periphery. Immunoglobulin A–producing B cells and plasma cells reside in the LP and produce polymeric IgA, which are transported to the intestinal lumen and contribute to the soluble immunity provided by the extra-epithelial mucus layer. Within the IEL, most T cells are $CD8^+$ as either CD8αβ heterodimers or CD8αα homodimers [169,203,204]. The IEL $CD8^+$ compartment is split into a TCRαβ subset that resembles conventional $CD8^+$ T cells, but is enriched for cells expressing TCRγδ chains [203]. The frequency of γδ T cells is about 4% of the T-cell population in the peripheral blood, but can comprise up to 40–50% of the mucosal T cells [171]. Gamma-delta T cells express TLR1 and TLR2 and produce chemokines that recruit other effector cells during bacterial infections and can make IL17 [205]. In humans and NHPs, these cells express the TCR Vδ1 or Vδ2, and the Vδ1 population expands, but their ability to produce IL17 is impaired [205,206].

In contrast to the IEL, most T cells in the lamina propria express CD4 [169]. Lamina propria–resident $CD4^+$ T cells are predominantly activated and express CCR5, which makes them efficient targets for

HIV/SIV infection [167,207]. Whereas the $CD4^+$ compartment is heterogeneous, there are three predominant populations: Th1, Th17, and $CD25^+$ Treg cells [169]. The fate of mucosal T cells during HIV and SIV infection has been well studied (reviewed in Ref. [208]) and they undergo a rapid and massive depletion [209–211] that is largely refractory to reconstitution during antiretroviral (ART) administration.

INNATE IMMUNITY AS A DRIVER OF IMMUNOPATHOGENESIS IN HIV/SIV INFECTION

Persistent Activation of Bacterial PRRs Induced by Microbial Translocation

As described earlier, disease progression in HIV infection has been demonstrated to be intimately linked to persistent activation of the immune system manifested as elevated rates of T-cell turnover [212], expression of activation markers on T and B lymphocytes [213], and elevated plasma levels of proinflammatory cytokines [214], among others. The cause of immune activation is likely multifactorial, and one of the widely accepted hypotheses is that the persistence of virus provides unabated stimuli for activation of both the innate and adaptive immune systems [122]. However, immune activation is not completely eradicated in HIV-infected patients who have effectively suppressed virus after the institution of ART [215]. The residual immune activation in virologic responders has been argued to result from low levels of viral replication, but it could also be caused by alternative mechanisms of immune activation that are independent of viral replication [216]. In the late 1990s and early 2000s, several groups demonstrated that HIV and SIV infection caused a rapid and near total depletion of $CD4^+$ T cells in the GI tract [209–211,217,218]. These findings led Brenchley and Douek to propose a model in which the initial insult to the mucosal lymphocyte compartment during primary infection established a permanent perturbation to the host immune system [208]. Shortly thereafter, the same group demonstrated that elevated levels of bacterial structural components such as lipopolysaccharide (LPS) could be detected in the plasma of HIV-infected patients and SIV-infected RMs in the absence of overt bacteremia, and correlated with lymphocyte markers of immune activation [175]. These findings provided a mechanism by which immunological damage inflicted during early infection could translate into chronic immune activation. These views led to coining of the term "microbial translocation," and the model postulates that depletion of gut $CD4^+$ T cells results in disruption of the epithelial barrier structures of the GI tract that allows for penetrance of microbial byproducts to enter the systemic circulation, where they provide continual stimuli for innate

pattern recognition receptors [167,219]. Evidence of elevated microbial components in plasma in HIV infection has since been replicated by several groups [220–223] Data from the NHP model provided direct visualization of LPS in breaches of the mucosal barrier in SIV-infected RMs [224], and the absence of MT in natural host species has helped link MT to pathogenesis [175].

Studies of MT in HIV infection have involved the detection and quantitation of plasma levels of LPS or sCD14 using appropriate assay systems; however, other models have demonstrated that translocation of several microbial byproducts capable of activating multiple PRRs also occurs, including peptidoglycan, lipoteichoic acid, flagellin, and CpG motifs in bacterial DNA. Recently, expansion of several viral species has been demonstrated to occur in the mucosa of SIV-infected macaques, but it is not yet known whether these viruses can be detected systemically [225]. The translocation of these products correlates with elevated levels of the proinflammatory cytokines IL6, IL8, and IFN-α. Moreover, IFN-α expression co-localizes with bacterial proteins in the GI tract of SIV-infected RMs. In this manner, microbial translocation may be an alternate mechanism of pathogenic stimulation of viral and bacterial innate pathways during HIV infection.

Plasmacytoid Dendritic Cell Activation and Chronic Production of Type I Interferon

Most chronic viral infections are efficiently controlled by the immune system and remain largely latent except for minor bursts of viral replication [226]. HIV and SIV differ from most chronic viral infections in that in the absence of ART therapy, HIV and SIV replicate at high levels (~10^5 RNA copies/ml plasma) in most subjects for life. The persistent presence of viral particles provides perpetual stimuli for both innate pattern recognition receptors and antigen-specific cells, and is considered one of the primary sources for the chronic immune activation observed in pathogenic HIV/SIV infection. Unabated antigen stimulation as a cause of immune activation and $CD4^+$ T-cell depletion have been extensively described [227]. In recent years, more attention has been directed toward the effects that chronic virus replication has on the innate system. In particular, the role of activated pDCs and subsequent IFN-α production as a factor in driving pathogenesis has garnered significant interest [228]. As described earlier, exposure of HIV particles to pDCs initiates high levels of IFN-α and inflammatory cytokine production via the TLR7 and TLR9 receptors and the activation of the transcription factor IRF7. Several lines of in vitro evidence have implicated pDCs and/or IFN-α production in HIV pathogenesis: (1) pDCs activated by viral particles induce apoptosis of $CD4^+$ T-cell through the up-regulation of TNF-related apoptosis inducing ligand (TRAIL/TNFSF10) [229,230], and

(2) HIV-derived RNA treatment of pDCs stimulates the production of IDO, which skews T-cell maturation toward Treg development, which typically suppresses antiviral immunity. An overview of models of the contribution of pDCs to chronic immune activation is summarized in Figure 8.1. In pathogenically infected rhesus, cynomolgus, or pig-tailed macaques, SIV infection initiates and sustains the chronic induction of interferon-stimulated gene (ISG) expression, which lasts indefinitely [231–235]. Similarly, persistent ISG expression is detected in HIV-infected humans [236,237]. In contrast, natural hosts of SIV that avoid AIDS, such as SMs and AGMs, demonstrate a transient ISG induction that disappears during chronic infection [232–235]. pDCs have been reported to be depleted from the blood of SIV-infected RMs [238]; subsequent studies demonstrated that they relocate to the mucosa in large numbers rapidly after infection [124,125,239] and co-localize with strong ISG expression.

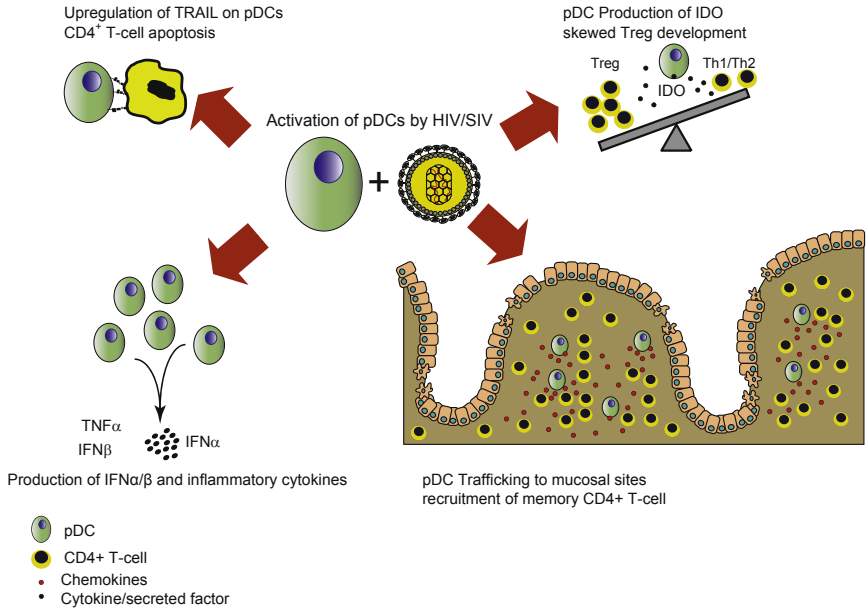

FIGURE 8.1 Current hypotheses of pDC-driven immune activation in HIV/SIV infection. Activation of pDCs by HIV/SIV leads to their activation and secretion of high levels of IFNα and -β and other inflammatory cytokines. Plasmacytoid dendritic cell activation by HIV or TLR7/8 ligands up-regulates surface expression of the TRAIL molecule, and TRAIL+pDCs are capable of driving apoptosis in CD4+ T-cell lines by TRAIL-dependent pathways. Activated pDCs produce high levels of IDO that influence CD4+ T-cell maturation toward a Treg phenotype and inhibit classical CD4+ T-cell responses. Finally, in early SIV infection, pDCs migrate to the vaginal and rectal mucosa, where they are associated with expression of ISGs and recruitment of CD4+ T cells by chemokines such as MIP3α. (See color plate at the back of the book.)

Plasmacytoid dendritic cells do not accumulate in the gut of SIV-infected SMs [124]. The absence of long-term ISG expression and pDC re-localization in species that resist SIV-disease suggests that aberrant activation of pDC contributes to pathogenesis in HIV/SIV-infected humans and macaques, respectively. The molecular mechanisms underlying the divergence between natural hosts and pathogenic species are currently unknown: A previous study reported that IFN-α production by SM pDCs was defective because of species-specific mutations in IRF7 [55]; however, those observations were later demonstrated to be largely the result of technical errors and sequencing artifact [232]. Further elucidation of differences in the innate signaling pathways between pathogenic and disease-resistant model species will likely provide valuable insight into the molecular basis of pathogenesis.

IN VIVO MANIPULATION OF THE INNATE IMMUNE SYSTEM IN NHPs

Over recent years, a number of studies have attempted to manipulate the innate system in NHPs, to affect SIV transmission rates during experimental vaccine studies or alter disease progression. The goals of these studies have largely fallen into two categories: (1) to understand the role of innate signaling as a cause of chronic pathogenic immune activation in SIV infection, and (2) to test the efficacy of TLR ligands in enhancing protection from SIV challenge.

In vivo Pathogenesis Studies

In what are now considered classic studies, immune activation has been demonstrated to be a stronger predictor of mortality and disease progression in HIV infection than CD4 counts and/or other markers [240]. These have been complemented by comparative studies of SIV infection in pathogenic or nonpathogenic infection [241]. Rhesus and pig-tailed macaques, which both ultimately develop disease during SIV infection, show widespread innate signaling and interferon responses well into chronic infection; in contrast, nonpathogenic species such as AGMs and SMs rapidly control this response to pre-infection levels.

Two primary pathways have been implicated as the most likely candidates for pathogenic innate signaling: (1) bacterial ligands and associated TLR receptors (TLR2 and TLR4), and (2) unabated stimulation of innate receptors for viral PAMPs (TLR3, TLR7, TLR9, and TLR9) provided by persistent replication of HIV/SIV. The rationale for bacterial signaling stems from a seminal study in which HIV-infected patients and SIV-infected macaques were demonstrated to have elevated plasma levels of

LPS owing to increased infiltration of micro-particles from gut-resident bacteria [175]. This phenomena, referred to as microbial translocation, is thought to be the natural sequelae of the massive depletion of mucosal $CD4^+$ T cells [218], Th17 cells [173], and $CD103^+$ mucosal dendritic cells [60], and subsequent disruption to mucosal structural integrity [224]. In a logical progression from the microbial translocation model, two studies administered LPS to nonpathogenic SIV+AGMs to determine the potential for inducing immune activation in a manner similar to that seen in macaques. A single bolus injection of LPS resulted in elevation of activation markers on $CD4^+$ T cells that lasted several days [242]. A follow-up study using multiple doses showed that exogenous LPS could also induce elevated levels of coagulation markers similar to those observed in pathogenic SIV and HIV infections [243].

Other facets of the innate system that have been considered a likely candidate for driving host immune activation are viral PRRs engaged by the persistent stimuli provided by HIV/SIV replication. The persistent interferon signaling that is observed in humans and pathogenic hosts, but not nonpathogenic hosts, provided some of the rationale for this hypothesis [232–235,244]. Prolonged administration of viral TLRs in mice results in lymphopenia and degradation of lymphoid architecture [245]. To test the role of innate signaling and resultant chronic interferon in pathogenesis, Vanderford and colleagues [246] administered recombinant IFN-α2 to SIV-infected SMs that were otherwise clinically benign. Although the IFN-α elicited transient increases in activated $CD8^+$ T cells, this activation was not sustained, and no $CD4^+$ T-cell depletion was observed in blood, lymph node, or mucosa. Recently, Kader and colleagues performed the reciprocal experiment, attempting to block TLR7 and TLR9 signaling using a synthetic antagonist in SIV-infected RMs [247]. No decrease was observed in $CD4^+$ or $CD8^+$ T-cell activation in animals receiving blockade compared with saline controls. However, blockade was also unable to inhibit the expression of interferon-stimulated genes in vivo, which made it difficult to interpret the link between ISGs and T-cell activation.

As yet, modulation of the innate system in vivo has been unable to unequivocally reverse SIV-related immune activation in macaques. Attempts to break the nonpathogenic phenotype maintained by natural hosts using in vivo manipulation of the innate system have resulted in relatively modest increases in activation but have not induced widespread dysregulation of the immune system observed in pathogenic hosts. This likely reflects the considerable technical difficulty in performing in vivo interventions in NHPs, and that the mechanisms of immunopathology (or nonpathogenesis) are multifactorial and difficult to target with a single modality. Nevertheless, these studies provide a technical framework for future studies.

Harnessing of the Innate System as Adjuvants in HIV Vaccines

Rationale of NHPs in SIV Challenge Studies

Many of the most effective vaccines are live attenuated strains of the pathogen. To date, live attenuated SIV strains have demonstrated by far the highest efficacy of experimental vaccines against SIV in their ability to protect from acquisition in challenge studies. Although safety issues preclude the use of live attenuated HIV as a human vaccine, the superior protection afforded by whole virus has fueled inquiry aimed at defining the underlying immunological mechanisms responsible. In contrast, subunit vaccines targeting individual viral particles or combinations of them are typically much poorer immunogens on their own. Improvement in vaccination efficacy has been shown to be enhanced through the use of adjuvants, which for the better part of the century have chiefly been accomplished with the use of formulations of aluminum salts or oil–water emulsions [248]. However, over the past decade, the study of vaccinology as a science has largely shifted from an empiric science to that of reductionist immunology and continues to evolve, now employing high-throughput "omics" approaches [249]. Whereas the concept has long been observed that nonself signals provided by live attenuated organisms provide a robust activation signal, the demonstration that individual TLR ligands could polarize T-cell responses toward Th1 or Th2 activity [250] or target specific DC subtypes [251,252] suggests that manipulation of the innate immune system could result in fine-tuning vaccine responses. Since the early 2000s, much progress has been made in understanding the effect of adjuvants composed of bacterial and viral TLR ligands and how they affect vaccine responses; the section that follows reviews data derived from TLR adjuvants used in NHP/SIV vaccine studies.

Non-human Primates and Adjuvant Development for HIV Vaccines

RMs have been the workhorse animal model for testing and evaluating candidate HIV vaccines, primarily owing to their ability to be infected by SIV and experience disease in a manner highly parallel to HIV infection of humans [1,253]. However, the use of NHPs in vaccine preclinical development is due to more than just their ability to be infected by SIV. Most adjuvants involve stimulation of TLRs or other PRRs in DCs, yet there are significant differences in the biology of DC populations between rodents and primates, as well the cellular distribution of TLRs [248]. Compared with mice, NHPs share a much higher degree of similarity with humans in both their DC populations, and are much more concordant in their cellular expression of PRRs and response to ligands [254]. In addition, the typical administration of vaccines in mice is via intraperitoneal

or intravenous routes, rather than subcutaneous or intramuscular routes as in humans and primates, which leads to adjuvants/vaccines that are being presented to differing subsets of DCs [248]. For these reasons, adjuvant immunogenicity data derived in RMs offer a more direct translation to human studies, and subsequently increased use of NHPs in studies of non-SIV vaccines in recent years.

Early work in mice demonstrated that injection of either the TLR7/8 agonist R848 or TLR9 ligand CpG oligodeoxynucleotides were capable of eliciting high levels of cytokine production and phenotypic maturation of conventional DCs, but that R848 was a poor adjuvant for raising antigen-specific $CD4^+$ Th1 and $CD8^+$ T-cell activity toward an HIV-Gag protein; however, these responses could be improved by direct conjugation of the Gag antigen to a TLR7/8 analogue [255,256]. A parallel study was performed in RMs and demonstrated that a TLR7/8 ligand-Gag protein conjugate significantly enhanced Gag-specific $CD4^+$ and $CD8^+$ T cells cytokine production (IL2, TNF-α, and IFN-γ) and included enhancement of antibody avidity [257]. Combination of TLR7/8 or TLR9 ligand-Gag conjugates with additional oil-based adjuvant, Montanide ISA 51, also led to enhanced cytokine release and a higher degree of polyfunctional cytokine responses in Gag-specific CD4 and CD8 compared with Gag + Montanide alone, and these enhancements were also observed after boost with adenoviral vectors expressing Gag. Direct comparison of TLR ligand adjuvants using the Gag-protein prime/Ad-Gag boost system demonstrated that Gag + TLR3 ligand followed by Gag + TLR7/8 and Gag + TLR9 ligands were most effective at inducing Gag-specific $CD4^+$ and $CD8^+$ T cells [258]. Although none of the ligands was able to augment resistance to SIV challenge, animals receiving TLR adjuvants had reduced viremia after infection [258]. Kwissa and colleagues observed a similar effect for TLR9 and TLR7/8 adjuvants for improving responses to a vaccine regimen composed of DNA prime and modified vaccinia virus Ankara boost [259].

The ability of innate adjuvants to enhance the immunogenicity of vaccines is becoming well established. However, basic experiments in mice demonstrate that the combinations of TLR ligands together in a single adjuvant formulation could have synergistic effects and improve immunogenicity even further [248]. This principle was driven home dramatically by studies of the yellow fever vaccine, one of the most efficacious vaccines developed, which was demonstrated to activate TLR2, TLR7, TLR8, and TLR9 [260]. Following up on these insights, Kasturi and colleagues developed a candidate composite TLR adjuvant of nanoparticles that approximate viruses in terms of size and carry both a TLR4 ligand (MPL) and TLR7 ligand (R837 in mice and R848 in monkeys) that enabled long-lived antibodies against influenza in both mice and RMs [261]. This adjuvant regimen is currently in preclinical studies to test its efficacy against SIV challenge.

Innate immune system activating ligands have demonstrated a robust ability to enhance the immunogenicity of vaccines, and their application will continue to be intensely studied. However, there have been instances in which innate stimuli have been demonstrated to be detrimental to host immune responses against SIV challenge, owing to their ability to recruit potential target cells to the site of infection. Intravaginal application of either CpG oligodeoxynucleotides (TLR9 ligand) or imiquimod (TLR7/8 ligand) induced a proinflammatory milieu and cellular infiltrate of $CD4^+$ T cells and other immune cells in the cervicovaginal mucosa. Subsequent high-dose challenge with SIV did not lead to protection; in fact, animals receiving TLR9 or TLR7 ligands developed higher set-point viremia compared with control animals [262]. Conversely, intravaginal treatment of monkeys with glycerol monolaurate, an antimicrobial that suppresses production of inflammatory cytokines and chemokines, suppressed production of CCL20/MIP3A and recruitment of $CD4^+$ T cells; and most remarkably, GML treatment led to protection from SIV transmission when exposed to an intravaginal high-dose challenge [263]. These studies demonstrate that although innate immune system activating ligands have a robust capacity to enhance the immunogenicity, immune functionality, and persistence of responses induced by a vaccine, they also have the potential to increase target cell recruitment and aid infection. Although the innate immune responses induced by adjuvants are expected to wane shortly after vaccination and not affect target cell recruitment, there is a paucity of data on the long-term effects of adjuvants.

CONCLUSIONS

The early 2000s saw an explosion in our understanding of the innate immune system at the cellular and molecular levels. The description of the innate system provided a theoretical framework for understanding how HIV and SIV cause chronic immune activation. In more recent years, several groups have translated murine studies using innate immune system activating ligands as adjuvants to empirically demonstrate that modulation of the innate immune system can enhance vaccine immunogenicity in primates and, in some cases, improved protective efficacy against SIV challenge. Future research efforts aimed at optimizing TLR combinations and defining the effect on target cells will be needed to maximize the protective potential of vaccines and move toward clinical trials. Comparative studies of SIV infection between species that develop AIDS and natural hosts have been informative in highlighting differences in the innate immune system that are important for pathogenesis. Finally, a handful of studies have begun to modulate the innate system in vivo in monkey models, and these will become increasingly important for the development of therapeutics capable of ameliorating disease.

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