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Utilizing the FIV model to understand dendritic cell dysfunction and the potential role of dendritic cell immunization in HIV infection

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Abstract

Dendritic cells (DC) are potent antigen presenting cells which initiate and coordinate the immune response making them central targets of and attractive candidates for manipulation in chronic lentiviral infections. Emerging evidence suggests that DC immune function is disrupted during both acute and chronic infection with human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV). Despite some early promising data, the use of DC for lentiviral immunotherapy has not fulfilled its expected potential and has been complicated by the large number of variables involved in DC harvesting, purifying, and antigen-loading. Pre-clinical studies aimed at identifying successful strategies for DC augmentation of current HIV treatment protocols are needed. Over the past two decades, the FIV model for HIV infection has increased the understanding of retroviral pathogenesis, and studies have begun using the FIV model to study DC dysfunction and DC-mediated immunotherapy. Careful consideration of the many variables involved in DC function and therapy should help develop protocols to explore the potential of DC vaccine-based therapies for lentiviral infection.

Keywords

dendritic cell; FIV; vaccination

Dendritic cells (DC) are considered to be the most potent antigen presenting cells, linking the innate and cell-mediated immune responses to pathogens. DC have been divided into two broad subsets, myeloid (mDC) and plasmacytoid (pDC), based on phenotype, function, and tissue localization (Banchereau et al., 2000; Moser et al., 2000). Although details of these subsets are debated and vary based on species, pDC are specialized early type 1 interferon-secreting cells that initiate antiviral adaptive immune responses. mDC differentiate from immature bone marrow(BM)-derived precursors and function as peripheral sentinels transmitting antigen-

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derived signals to draining lymph nodes (LN). mDC secrete high levels of interleukin-12 (IL-12) and are key players in amplifying adaptive immune responses. mDC are the subset most studied in feline immunodeficiency virus (FIV) infection, and they form the focus of this review (Bienzle et al., 2003; Sprague et al., 2005; Freer et al., 2005; Lehman et al., 2009). FIV infection produces a disease strikingly similar to AIDS with clinical phases analogous to those of HIV and is an important model to study retroviral pathogenesis (Elder et al., 1995). With recent advances in feline DC research, the FIV model system has contributed to the understanding of lentiviral-mediated DC dysfunction and demonstrates ongoing utility as an animal model in determining the potential for DC-based immunotherapy in HIV infection.

Characterization of feline DC

Interest in feline DC has expanded significantly since 1994 when feline Langerhans cells (LC), a subtype of BM-derived mDC, were first described (Tsagarakis et al., 1994). Kramer et al. (1996) confirmed that the ultrastructural features of feline LC, including the presence of Birbeck granules, were similar to that seen in humans. Soon afterwards a monoclonal antibody recognizing feline CD1a was developed that reacted with feline DC and showed tissue expression and biochemical characteristics similar to those described in other species (Woo et al., 1997). Further immunophenotypic evaluation demonstrated that, like human LC, feline LC expressed CD4, CD18, CD19, major histocompatibility complex (MHC) class II, and CD1a antigens (Saint-André Marchal et al., 1997).

In 2003, Bienzle et al. demonstrated that culture of feline blood and BM mononuclear cells with IL-4 and granulocyte-macrophage colony stimulating factor (GM-CSF) generated cells analogous to immature mDC in other species (Bienzle et al., 2003). The cultured DC expressed CD1a, CD1b, CD11b, CD14, and high levels of MHC class I and II as compared to macrophages or freshly-isolated blood monocytes. Consistent with phenotypic variability among species, CD14 retention has also been documented in cultured canine, bovine and porcine mDC (Hope et al., 2000; Carrasco et al., 2001; Bonnefont-Rebeix et al., 2006) but not in human and murine systems. Cultured feline DC also displayed cytoplasmic processes typical of DC, phagocytic, pinocytic, and receptor-binding antigen uptake abilities, and induced allogeneic lymphocyte proliferation. Sprague et al. (2003) similarly showed that, compared with macrophages, feline monocyte-derived DC have higher MHC class II and lower CD11b expression, increased basal IL-12 mRNA expression, and the ability to stimulate an allogeneic mixed leukocyte reaction (MLR). Dunham et al. (2004) further demonstrated that feline BM cells exposed to recombinant feline GM-CSF (rfGM-CSF) alone developed into cells with morphology typical of DC, and similar culture methods have since been used in combination with CD11c and MHC class II expression to purify DC for characterization and functional assays (Lehman et al., 2009). Feline DC have also been generated from tissue stromal cell cultures from newborn cats without addition of cytokines or growth factors (Avery et al., 2007). Feline monocyte-derived DC have been shown to mature in response to lipopolysaccharide (LPS) by up-regulating MHC class II and the co-stimulatory molecule CD80 and inducing increased allogeneic cell proliferation (Freer et al., 2005). The characterization of feline DC has enabled further study in mechanistic and immunotherapeutic research.

DC function during FIV infection

Although the decline of CD4⁺ T cell number and function during lentivirus infection has received much attention, accumulating data indicates that DC play a prominent role in HIV-induced immunodeficiency (Kawamura et al., 2003; Fantuzzi et al., 2004; Smed-Sorensen et al., 2004; Granelli-Piperno et al., 2006; Almeida et al., 2007; Fernandez et al., 2008; Huang et al., 2008; Hogue et al., 2008; Sachdeva et al., 2008; Velilla et al., 2008; Muratori et al.,

2009) (Table 1). DC are involved in T cell activation through the required presentation of antigen by MHC and direct interaction with CD40 ligand (CD40L) on CD4⁺ T cells. Underlining the importance of T cell activation, the breadth and magnitude of HIV-1-specific CD4⁺ and CD8⁺ T cell responses are associated with control of viremia and disease progression in HIV-1-infected individuals (Rosenberg et al., 1997; Betts et al., 2006; Dyer et al., 2008).

Study of feline DC during FIV infection has revealed many of the same interactions and functional defects documented in SIV and HIV infections and holds promise to explore areas yet unstudied in HIV infection. Acute FIV infection results in decreased numbers of p55⁺/S100⁺ thymic DC, similar to reports in acute SIV infection (Muller et al., 1993; Obert and Hoover, 2000). FIV crosses mucous membranes within hours of exposure and rapidly traffics via DC and T cells to LN (Obert and Hoover, 2002), a pathway similar to that thought to occur in the initial phase of HIV infection (Spira et al., 1996). Rogers et al. (2002) found FIV viral antigen associated with small numbers of S100⁺ cells and rarely with parafollicular and follicular interdigitating DC in lymphoid and intestinal tissues three weeks after intravenous viral inoculation. These results collectively suggest that FIV co-localizes with a small number of DC early after infection, however details of specific trafficking patterns during FIV infection await further investigation.

The role of DC in propagation and dissemination of lentiviruses is a topic of active study. Low level, productive infection of DC can be established *in vitro*, yet studies suggest that productive infection is not required for transmission to lymphocytes (Geijtenbeek et al., 2003). Whether productive infection is a required component of long-term *in vivo* persistence is unresolved (Burleigh et al., 2006; Granelli-Piperno et al., 2006). DC exposed to HIV and SIV form what has been described as an “infectious synapse” with T lymphocytes facilitating direct transfer of virus during normal DC: T cell interactions (Garcia et al., 2005; Frank et al., 2008). Controversy exists over whether viral transfer requires internalization of intact HIV virions into vesicles or whether virus remains associated with lipid rafts on the cellular membrane (Cavrios et al., 2008). Cultured, monocyte-derived feline DC incubated with FIV were shown to contain virus in membrane-bound vesicles within two hours of exposure (Sprague et al., 2008). Delayed addition of activated feline CD4⁺ T cells to allow degradation of uninternalized virus resulted in increased viral replication, implying transmission of virus from within the DC. Using BM-derived DC, van der Meer et al. (2007) were unable to show direct viral infection of DC *in vitro* but demonstrated enhanced replication in DC:thymocyte and DC:PBMC co-cultures that required direct cellular contact. Using feline monocyte-derived DC infected *in vitro* by spinoculation, Freer et al. (2007) detected intracellular viral antigen in immature and LPS-matured DC and detected free virus over time in supernatants from these cultures. Reggeti et al. (2008) similarly showed that although viral replication was lower in DC than in lymphocytes exposed to FIV *in vitro*, viral ultrastructure and DNA detection confirmed that feline DC expressed specific receptors and were infected productively with FIV. Our laboratory has documented increased viral replication when BM-derived DC from chronically FIV-infected cats are co-cultured with T lymphocytes (unpublished data). The accumulated data suggest that both monocyte- and BM-derived DC can be infected by strains of FIV and that exposed or infected DC can readily transmit FIV to resting and activated lymphocytes. However, *in vivo* FIV infection of DC is not yet fully understood.

Our laboratory has been studying BM-derived DC expanded *ex vivo* with rFGM-CSF from naïve and FIV-infected cats. Stimulation of DC from FIV-infected cats through pathogen sensing molecules called Toll-like receptors (TLRs) resulted in an altered cytokine response (Lehman et al., 2009). Compared to control animals, ligation of TLRs 4, 7, and 9 in DC from chronically FIV-infected cats resulted in a significant alteration in the ratio of IL-12 to IL-10 that was not due to virus-associated changes in TLR expression as determined by TLR mRNA

levels. Altered cytokine production was also seen with CD40 ligation of DC from FIV-infected cats as both IL-10 and IL-12 mRNA levels increased without, however, altering the IL-12/IL-10 ratio. Although the *in vivo* significance of these changes remains unknown, alterations in the ratio of immunoregulatory cytokines produced by BM-derived mDC in response to specific pathogen-derived signals is likely relevant to the increased risk of opportunistic infections seen in lentiviral infection. Increased production of IL-10 compared to IL-12 creates a cytokine balance predicted to hamper cell-mediated immunity. Further investigations into the mechanisms of altered DC cytokine production induced by FIV infection are in progress.

The ability to stimulate proliferation of resting allogeneic lymphocytes is considered a hallmark of normal DC function. We have recently determined that, unlike DC from naïve cats, BM-derived DC from cats chronically-infected with a clade C FIV virus are unable to stimulate T cell proliferation (Lehman et al., manuscript submitted). Several studies have shown that human monocyte-derived DC infected with HIV have reduced ability to stimulate allogeneic and mitogen-stimulated lymphocyte proliferation *in vitro* (Macatonia et al., 1989; Knight et al., 1993; Knight et al., 1997; Fantuzzi et al., 2004; Granelli-Piperno et al., 2004; Shan et al., 2007). In contrast to our studies, Freer et al. (2008) showed that *in vitro* infection of monocyte-derived DC with a clade A FIV-petaluma virus did not impair subsequent lymphocyte proliferation. Conflicting study results may be due to the viral strain used, DC source, or differences in duration of infection and warrants further investigation.

The potential for DC therapy in lentiviral infection

Although circulating HIV RNA may become undetectable with effective antiretroviral therapy leading to significantly decreased morbidity and prolonged lifespan, viral reservoirs that can be reactivated persist (Finzi et al., 1999; Blankson et al., 2002; Chun et al., 2008). Additionally, antiretroviral therapy is costly, can have serious side-effects, requires life-long therapy, decreases anti-HIV T-cell frequencies, and can select for treatment-resistant viral strains (Rinaldo et al., 2000; Metzner et al., 2007; Moyle, 2007; Bader et al., 2008; Adjé-Touré et al., 2008). Consequently, other immunotherapies that enhance functional virus-specific effector T-cell immunity without leading to toxicity or T cell exhaustion would be ideal adjunctive therapeutics for lentiviral infections.

Several groups have investigated the potential use of DC as an immunotherapy for HIV. In the late 1990s a pilot clinical trial administering HIV antigen-pulsed allogeneic and autologous monocyte-derived DC to HIV-infected patients demonstrated increased HIV-specific lymphocyte proliferative responses in three out of six patients although no effect was seen on viral load (Kundu et al., 1998). In 2003, Lu et al. (2003) documented a fifty-fold reduction in circulating viral DNA and a thousand-fold reduction in peripheral viral RNA in SIVmac251-infected rhesus monkeys after administration of aldrithiol-2 (AT-2)-inactivated virus-pulsed autologous monocyte-derived DC. Viral load reductions were maintained over the 34-week study period. The same group had previously shown that DC loaded with AT-2-inactivated HIV expanded CD8+ T cell activity *in vitro*, and their work using this technique has shown the most positive results thus far (Lu et al. 2001). In 2004, Lu et al. (2004) evaluated a similar therapeutic DC vaccine for chronic HIV-1 infection. Eighteen patients who were HIV positive for 13-65 months and not on antiretroviral therapy were administered three treatments of autologous mDC pulsed with AT-2-treated autologous HIV-1. Eight of the eighteen study participants demonstrated viral load suppression greater than 90% during the initial 112 days of the study. The remaining participants had weaker or transient responses. In responding individuals, the reduction in viral DNA and RNA remained significant for 224 days and 365 days respectively. A transient increase in CD4+ T cell counts was noted that then returned to baseline for the remainder of the study year.

Despite the potential demonstrated in these initial reports, subsequent work utilizing DC therapy in HIV infection has been less successful. A phase one clinical trial using peptide-loaded DC vaccination followed by treatment interruption during chronic HIV-1 infection reported no peptide response in two of the four study participants, limited responses in the other two participants, and no suppression of viral rebound (Ide et al., 2006). Garcia et al. (2005) saw weak, transient cellular immune responses and suppressed viral rebound in four of twelve patients after treatment interruption using therapeutic immunization with monocyte-derived DC loaded with heat-inactivated autologous HIV-1 in patients with chronic HIV-1 infection. Further research to determine the conditions required for efficacious use of DC in lentiviral immunotherapy strategies is needed, and studies using the FIV model have begun to address these issues.

Several groups are currently using feline DC in preventative and therapeutic immunotherapy trials for FIV infection. Reggeti et al. (2008) found that immunization of cats with allogeneic BM-derived DC did not induce cross-protective immunity against FIV that had been propagated in the same cells *in vitro*. Freer et al. (2008) evaluated the potential of feline monocyte-derived DC loaded with AT-2-inactivated virus to provide protective immunity. DC were generated from peripheral blood mononuclear cells cultured in autologous plasma with GM-CSF and IL-4, loaded with AT-2-inactivated FIV, and matured with LPS. Three subcutaneous DC injections were given at four-week intervals two months prior to challenge. Although FIV-specific proliferative responses and detectable antibody responses were noted, the DC vaccines failed to prevent infection or alter viral levels.

Feline DC have also been used in attempts to modify existing FIV infection. Freer et al. (2008) used monocyte-derived DC as a potential immunotherapy in chronic FIV infection. Feline monocyte-derived DC loaded with AT-2-inactivated homologous FIV, and matured with LPS, were shown to have high MHC class II and B7.1 expression and to stimulate MLRs as is characteristic of mature, functional DC. Chronically FIV-infected cats received three DC vaccines at one-month intervals in sites topically pre-treated with imiquimod. A TLR 7 agonist, imiquimod has been shown to stimulate DC and induce responses in cats (Hemmi et al., 2002; Gill et al., 2008). Although FIV-specific lymphoproliferative responses were increased, viral loads and CD4+ T cell depletion were not altered in treated cats. Similar to human trials, evaluation of potential causes for the lack of efficacy of feline DC immunotherapy studies remains to be completed.

Current issues in DC immunotherapy for lentivirus infection

Understanding of DC biology has advanced significantly in recent years, although many questions remain that likely affect their use in immunotherapy protocols. Peripheral blood and BM from multiple species can be cultured with IL-4 and/or GM-CSF to generate large numbers of mDC for use in DC trials (Inaba et al., 1992; Romani et al., 1994; Dunham et al., 2004; Freer et al., 2005; Lehman et al., 2009). Although such techniques are crucial to the study of DC immunotherapies, the impact of culture conditions and cytokine exposure on DC function is not yet fully understood.

DC viability and function can be compromised during the collection, generation, purification, preparation, and maturation processes involved in study and use of the cells. Unfortunately, assessing DC viability prior to injection does not necessarily correlate with DC ability to traffic or interact with T cells. Activated, antigen-loaded DC can be rapidly eliminated *in vivo*, and exposure to loaded, nonviable DC can induce tolerance in immature DC (Hermans et al., 2000; Liu et al., 2002). The life-span of injected DC and their potential to induce tolerance rather than strengthen the immune response represent obvious concerns with current DC vaccine protocols. Although significantly more costly and labor-intensive, some recent studies

suggest that autologous-based antigen may enhance T cell responses to HIV, and autologous virus was used in the Lu group's DC immunization studies (Altfeld et al., 2003; Lu et al., 2003; Lu et al., 2004; Streeck et al., 2008). Due to issues with tolerance and the high mutation rate and creation of quasi-species by lentiviruses, antigen-loaded DC, even using autologous virus, may not initiate an effective immune response. Alternate DC loading strategies are under investigation including use of cell antigens, exosomes, recombinant viral proteins, liposomes, plasmids, and microbial vectors.

DC maturation status is another critical factor in their potential use as immune-modulators. Immature DC are poor T cell stimulators, and the DC maturation process involves up-regulation of LN homing receptors such as CCR7 allowing for effective trafficking (Sallusto et al., 2000; Cools et al., 2007). Mixtures of highly immunogenic inflammatory cytokines such as TNF α , IL-6, IL-1 β in combination with prostaglandin E $_2$ (PGE $_2$) have been traditionally used to mature DC *ex vivo* (Mehlhop et al., 2002; Trepiakas et al., 2008). Prostaglandin is thought to be important for up-regulation of CCR7 allowing DC to traffic to LN to present antigen (Luft et al., 2002; Scandella et al., 2002), although PGE $_2$ is also known to induce DC indoleamine 2,3-dioxygenase (IDO) activity (Braun et al., 2005; Krause et al., 2007). IDO, an enzyme involved in tryptophan degradation, is immunosuppressive and induces T cell apoptosis and regulatory T cells (Katz et al., 2008). Interestingly, PGE $_2$ was not used in the two most successful lentivirus DC therapy studies (Lu et al., 2003; Lu et al., 2004). Recent reports suggest trimeric, recombinant CD40L, which replicates the direct interaction with CD4 + T cells, can potentially induce DC maturation and prime DC for maximal CD8+ T cell stimulation (Huang et al., 2008). Comparative data concerning the ideal DC maturation protocol for immunotherapy remains incomplete.

Conclusion

The inability to consistently replicate the early and expected success in DC immunotherapy highlights the need to further investigate the many variables involved in effective utilization of DC in HIV infection. Continued research into DC function and therapy will help define the best means of using such potent cells to help control lentiviral disease. FIV-associated DC studies have confirmed and expanded many of the observations of lentiviral-mediated DC dysfunction seen with HIV infection. Recent and ongoing work has demonstrated the safety and potential use of the FIV model to explore DC immunotherapy for lentiviral infection.

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Table 1
Proposed Roles of Myeloid DC During Lentiviral Infection

Dendritic Cell:	Role in Infection:
Initial encounter with virus at mucous membranes	Enhanced viral transmission
Trafficking of virus to lymph nodes	Increased infection of T cell targets
Formation of 'infectious synapse' with T cell	Increased infection of T cell targets
Sequestration of virus	Decreased immune response to virus
Productive infection	Source of chronic infection/viral reservoir
Impaired DC maturation	Decreased immune function
Altered TLR-induced cytokine production	Decreased immune function
Altered CD40L-induced cytokine production	Decreased immune function
Decreased stimulation of T cell proliferation	Decreased amplification of immune response
Induction of CD8+ T cell apoptosis	Decreased cell-mediated immunity