Pharmacologic inhibition of IDO1 blunts features of SIV-related chronic inflammation

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Introduction

The immunomodulatory and neurotoxic activities of kynurenines derived from tryptophan catabolism by indoleamine-2,3-dioxygenase (IDO1) play a role in sustaining a cycle of inflammation and immune dysfunction during HIV infection. Such a cycle may lead to increased risks of non-AIDS diseases, such as liver disease and neurocognitive impairment, despite effective cART. We hypothesized that inhibition of IDO1 may break this cycle, reversing immune dysfunction, decreasing systemic inflammation, and ultimately decreasing the incidence of non-AIDS diseases. We tested this hypothesis in a pilot study of virologically suppressed, SIV-infected rhesus macaques treated with an optimized regimen of an IDO1 inhibitor (IDOi) currently under clinical investigation (INCB024360).

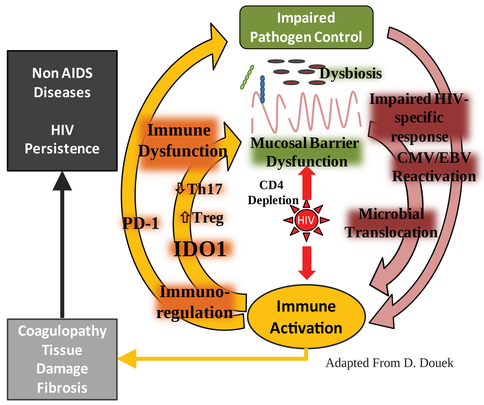
Methods

Twelve rhesus macaques were infected with SIVmac251, treated with cART, and divided into two groups after virologic suppression to receive either placebo or IDOi for 8 weeks while continuing ART. Blood, lymphatic tissue, and mucosal tissues were evaluated to determine the pharmacologic, immunologic, metabolic, microbiological, and transcriptomic features of IDOi during SIV infection.

Results

SIV infection increased plasma levels of kynurenine even after ART and kynurenine was transiently suppressed after each IDOi dose. Pharmacologic inhibition of IDO1 led to increased CD4/CD8 ratio in blood and significantly decreased markers of T cell and monocyte activation in blood and lymphoid tissues. In gut mucosa, we observed a trend to increased frequency of CD4+ T cells producing IL-17, IL-22, and/or IL-21. Interestingly, we found increased plasma levels of microbial metabolites of tryptophan, such as indoleacetate, which are AHR agonists that promote intestinal barrier function through induction of IL-22 and have neuroprotective properties.

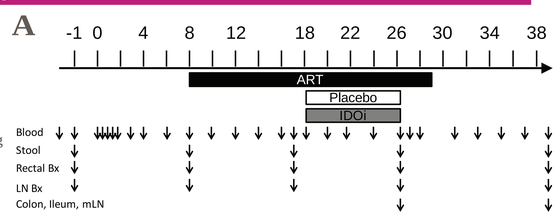
Vicious Cycle of Immune Dysfunction During HIV Infection



Adapted From D. Douek

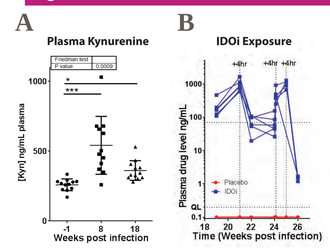
HIV infection sets in motion a vicious cycle of immune dysfunction and inflammation that drives non-AIDS diseases . This cycle is initiated by acute inflammation and CD4+ T cell depletion at mucosal surfaces which impairs mucosal barrier function. The microbial products translocated as a result of this dysfunction are immunostimulatory, driving immune activation. When this occurs chronically and systemically, immunoregulatory mechanisms, such as induction of IDO1 activity, impair immune function, leading to further dysfunction at mucosal surfaces, intestinal dysbiosis, and outgrowth of dormant viruses such a EBV or CMV. These events are proinflammatory, perpetuating a feed-forward loop. Chronic inflammation in tissues leads to tissue damage, organ dysfunction, and non-AIDS diseases such as cardiovascular disease, liver fibrosis, and neurocognitive impairment.

Figure 1. Study Design



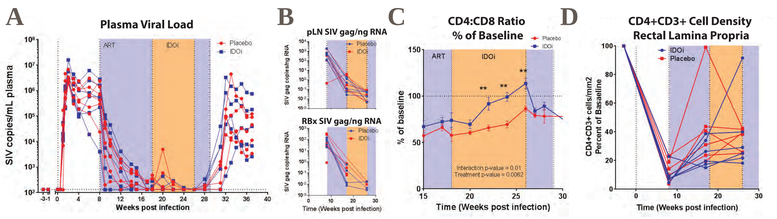
Twelve RM were infected with SIVmac251 IV. After 8 weeks antiretroviral therapy (ART) was initiated (detailed below). At 18 weeks post infection the animals were randomized to receive 75mg/kg INCB024360 or placebo twice daily, orally, under sedation. Samples were collected as described in (A).

Figure 2. Pharmacokinetics and Pharmacodynamics



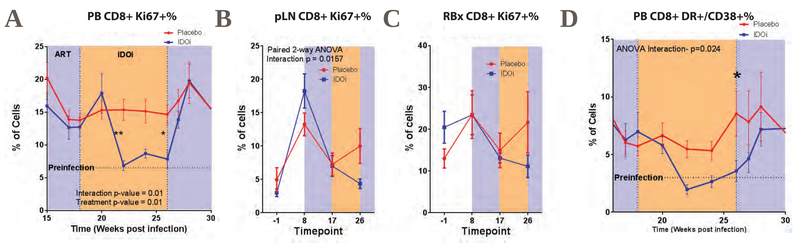
We confirmed that plasma Kyn was induced during acute infection and remained elevated despite ART (B). Plasma exposure of IDOi was found to be above therapeutic levels when measured at +4hrs relative to dose, but not at trough timepoints (C). In concordance with IDOi exposure we found that plasma Kyn was significantly suppressed in treated animals, but only when measured at +4hrs from dose and had returned to unsuppressed levels at trough timepoints.

Figure 3. SIV levels in blood and tissues are unaffected by IDOi, but CD4:CD8 ratio is increased in peripheral blood



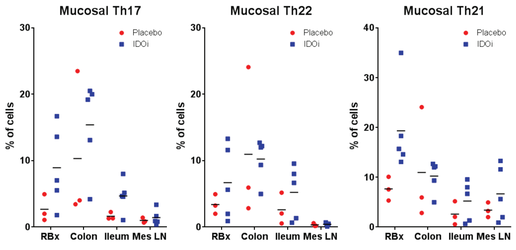
ART consisted of daily SQ administration of 20mg/kg TDF coformulated with 40mg/kg 3TC, 300mg brecanavir IM once weekly, and 50mg/kg cabotegravir IM every 3 weeks. SIV was efficiently suppressed in all animals over 8-10 weeks of ART, reaching the lower limit of detection in plasma (A). Tissue RNA was suppressed but remained detectable in lymph node and rectal mucosa (B). IDOi significantly increased CD4:CD8 ratio in blood (C). CD4+ T cell levels in rectal tissue were not affected by IDOi (D)

Figure 4. Systemic immune activation is decreased by IDOi



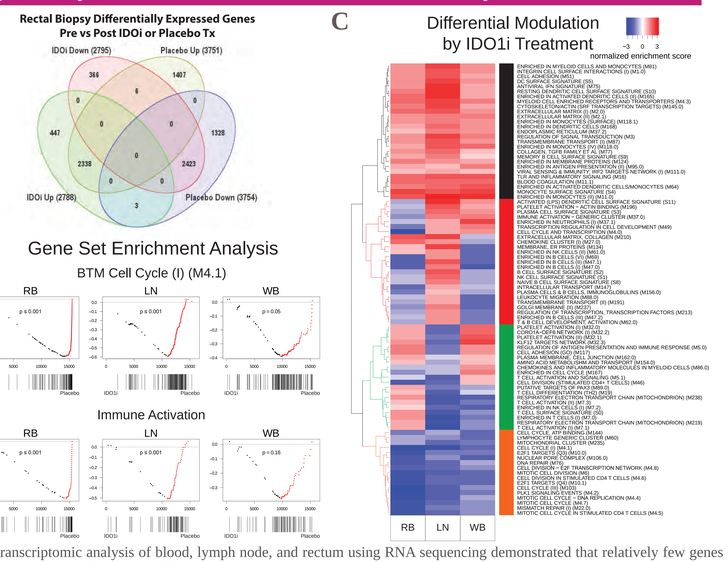
Markers of T cell activation were measured by flow cytometry in cells isolated from blood, lymph node, and intestinal mucosa. We found that IDOi significantly reduced T cell activation as marked by Ki67 (A, B) or CD38/HLA-DR (C) in CD8+ T cells found in peripheral blood (A, D), peripheral lymph node (B), or rectal mucosa (C). Corresponding response in decreased CD4+ T cell activation by both markers in multiple tissues was observed but is not shown.

Figure 5. Trend to increase in mucosal Th17, Th22, Th21 with IDOi



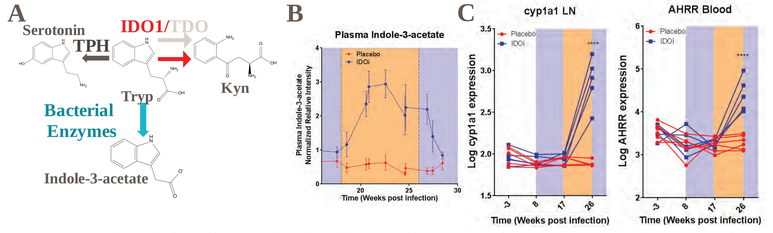
An important feature of HIV/SIV-associated immune dysfunction is the loss of IL-17, IL-22, and IL-21 producing CD4+ T cells in the intestinal mucosa, thought to be related to IDO activity. We found that cells capable of IL-17, IL22, and/or IL-21 production trended to increased frequency in IDOi treated animals. Cells producing these cytokines were also less likely to produce IFNg and more likely to produce IL-2 (not shown). This trend is indicative of qualitative reconstitution of intestinal immune function.

Figure 6. Systemic immune activation is decreased by IDOi



Global transcriptomic analysis of blood, lymph node, and rectum using RNA sequencing demonstrated that relatively few genes were statistically significantly modified by IDOi treatment (A, rectal biopsy). However, gene set enrichment analysis using the whole dataset revealed a global reduction in cell cycling and immune activation (B) in the IDOi treated animals, resulting in an expression pattern characteristic of non-pathogenic SIV infection. Distinct modules of genes were modulated (C), including cell cycle (orange), T cell activation (green), B cell activity (red), and monocyte activity (black).

Figure 7. IDOi leads to alternative Tryp metabolism



Tryptophan metabolism is balanced between the IDO/TDO-governed kynurenine pathway, the TPH-governed serotonin pathway, and conversion by an array of bacterial enzymes into metabolites such as indole-3-acetate (IAA) or indole-3-aldehyde (A). We found that while IDOi suppressed the kynurenine pathway, the bacterial pathway was enhanced, likely due to increased substrate, evidenced by increased plasma levels of IAA (B). IAA is an aryl hydrocarbon receptor agonist and AHR response genes such as Cyp1A1 or AHRR were strongly induced specifically in treated animals (C).

Conclusions

These results support the hypothesis that IDOi disrupts the vicious cycle of IDO1-mediated inflammation and immune dysfunction during SIV infection. This effect is associated with a shift in microbiota function characterized by increased production of anti-inflammatory postbiotic metabolites that may serve as novel biomarkers of disease and therapeutic responses. These data suggest further investigation of IDOi as a strategy to blunt or prevent non-AIDS diseases in suppressed HIV infection by targeting host and microbial metabolism in the setting of chronic inflammation.

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All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.