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HIV PATHOGENESIS AND TREATMENT (A LANDAY, SECTION EDITOR)

Understanding and Controlling Chronic Immune Activation in the HIV-Infected Patients Suppressed on Combination Antiretroviral Therapy

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Abstract Combination antiretroviral therapy (cART) has resulted in tremendous gains in survival among HIV-infected patients, but as a group those who achieve undetectable viral loads on cART experience a greater degree of immune activation and inflammation than the general population. HIV-infected patients continue to experience premature immune senescence with earlier and more frequent non-AIDS events compared to HIV-uninfected individuals. Chronic immune activation during suppressive cART derives from a variety of sources mediated by cytokines, chemokines, coagulation, microbial translocation, immune regulators and $T_{effector}$ cell activation abnormalities, among others. Current investigational strategies to control immune activation target potential causes of persistently heightened immune activation during cART such as microbial translocation, co-infections, and comorbidities or mediators along a common final pathway. Although several interventions have shown promise in vitro or in preliminary clinical trials, no intervention has sufficient evidence for routine use, making control of immune activation during cART an unmet need.

Keywords Immune activation · HIV · Microbial translocation · Combination antiretroviral therapy (cART) · Co-infections · HIV pathogenesis and treatment · Chronic immune activation

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Introduction

One of the greatest achievements of medical science is the discovery of AIDS, the retrovirus that causes it, the natural history of HIV disease, identification of surrogate markers for HIV disease progression, the development and approval of over 26 unique antiretroviral agents (ARVs), a comprehensive understanding of how to use these medications to provide long-term viral suppression, immune recovery, prolonged survival and a means to prevent the sexual transmission of HIV. Within 30 years, these advances have converted HIV/AIDS from a lethal disease to a manageable chronic disease with lifelong combination antiretroviral therapy (cART). However, although cART results in near complete suppression of HIV replication and a significant recovery of the peripheral CD4+ T cell compartment in the majority of recipients, HIV-infected individuals as a group continue to have higher levels of immune activation [1•] and are more likely to develop non-AIDS morbidities and die of non-AIDS events at an earlier age than the HIV-uninfected population [2•, 3]. Possible causes of the increased immune activation and increased all-cause mortality despite cART include: incomplete restoration of the immune system, damage to other organs prior to starting cART; low-level residual HIV replication; co-infections, co-morbidities; premature or abnormal immunosenescence, poorly understood side effects of ARVs, or some combination of these (Fig. 1).

Over the last several years, numerous publications have correlated increased all-cause mortality and/or non-AIDS events such as cardiovascular disease, myocardial infarction and non-AIDS related cancers with biomarkers of inflammation, humoral and cellular immune activation, and/or coagulation. A discussion of the strength of these correlations is beyond the scope of this paper. In this manuscript, we review investigational strategies that either directly address potential causes of persistent immune activation during virally suppressive cART or aim to block downstream events along a

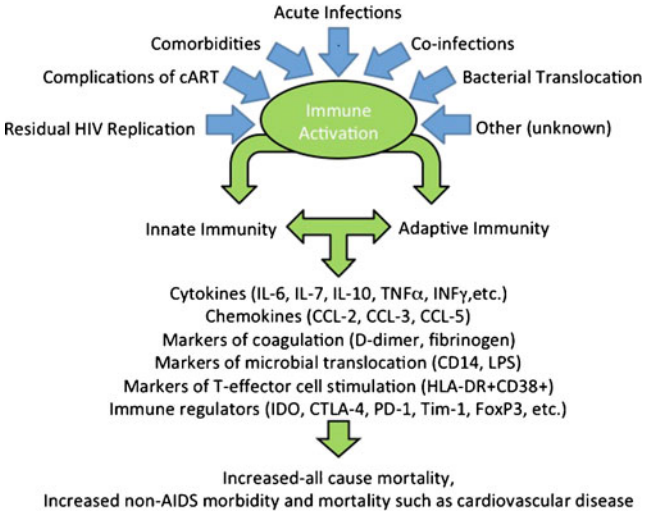

Fig. 1 Potential contributors to immune activation in HIV-infected individuals with undetectable plasma HIV RNA on cART and biomarkers of pathways that lead to increased morbidity and mortality in the same population

common final pathway of immune activation or inflammation that leads to increased morbidity and mortality. As shown in Fig. 1, these downstream events include perturbations of cytokines, chemokines, coagulation, immune regulators and $T_{effector}$ cell activation. We conclude with our opinions on the limitations of the available data and ongoing studies and identify some of the data that we feel would be important in advancing this field.

Strategies Addressing Potential Causes of Persistently Heightened Immune Activation During cART

Control of Microbial Translocation

The gut associated lymphoid tissue (GALT) is home to nearly 95 % of the body's CD4+ T-cells. During acute HIV infection, this tissue compartment is essentially lost and is not restored when HIV replication is controlled by host immune responses or cART. The loss of gut mucosal CD4+ T cells particularly affects those with the Th17 phenotype [4, 5], and is associated with altered gut microbiota [6], intestinal mucosal inflammation with increased permeability, altered tight junction protein [7], and leakage of microbial products such as bacterial lipopolysaccharide (LPS) and 16 s DNA into the systemic circulation [8]. cART improves but does not restore gut immunity and function [9], and it does not usually return systemic microbial product levels to those seen in HIV-uninfected individuals [8, 10••, 11]. Others have reviewed the pathogenesis of microbial translocation and potential signaling cascades through which translocated microbial products may induce inflammation and increase immune activation [12•]. Systemic activation induced by translocated microbial products may adversely affect immune reconstitution and other outcomes [13]. Accordingly, interventions to control microbial translocation during cART are being explored in studies that target patients with poor CD4+ T cell response during cART since they are most likely to have high levels of activation [14••]. Current investigational agents have the potential to interrupt microbial translocation through a variety of mechanisms, e.g., by reducing the abnormal gut microflora (rifaximin), restoring gut microbial balance (pre- or pro-biotics), binding LPS in the gut (sevelamer), direct restoration of the endothelial barrier (Vit A/retinoic acid) or the use of T-cell homeostatic mechanisms to restore T-cells in the GALT.

Rifaximin

Rifaximin is a semisynthetic derivative of rifampin. Less than 1 % of the oral dose is absorbed and the remainder is largely excreted unchanged in stool [15]. Rifaximin has broad-spectrum *in vitro* activity against Gram-positive and Gram-negative aerobic and anaerobic enteric bacteria [16]. Studies in patients with inflammatory bowel disease suggest that rifaximin exerts counter-regulatory activities at the interface between enteric bacteria and intestinal epithelial cells that may contribute to maintenance of intestinal immune homeostasis [17]. Local effects of rifaximin can produce significant changes in systemic immune parameters. For example, intestinal decontamination with rifaximin in patients with alcoholic liver cirrhosis reduced circulating LPS by approximately 50 %, decreased levels of pro-inflammatory cytokines, and improved liver function [18, 19].

Administration of rifaximin and sulfasalazine to acutely SIV-infected pigtail macaques lowered microbial translocation (sCD14), immune activation ($CD4^+$ and $CD8^+$ T cells expressing HLA-DR and Ki-67), inflammation (interleukin-6 and RANTES) and mucosal CD4 + T cell depletion [20]. ACTG A5286 study (clinicalTrials.gov identifier NCT01466595) is the most advanced clinical trial of rifaximin in HIV-infected patients. This is a randomized open-label two-arm study evaluating the impact of 4 weeks of rifaximin on levels of translocated microbial products and markers of immune activation in 70 HIV-infected subjects with suppressed plasma HIV RNA. This study is fully enrolled and results are expected in the first half of 2013.

Sevelamer

This is a non-absorbable polymer used to treat hyperphosphatemia in renal dialysis patients. It has been shown to lower circulating levels of endotoxin and C-reactive protein (CRP) in renal dialysis patients [21, 22]. The impact of sevelamer in HIV-infected individuals is being evaluated in ACTG A5296, a single arm trial in which 40 chronically

HIV infected patients not on cART will be administered 8 weeks of sevelamer followed by additional 8 weeks of follow-up [NCT01543958]. The primary objective of A5296 is to evaluate effect of sevelamer on plasma endotoxin and soluble CD14 (sCD14) levels. A5296 is fully enrolled and results are expected in 2013.

Probiotics and Prebiotics

HIV alterations to the gut microbiota include reduced concentrations of immunologically beneficial bacteria such as lactobacillus and bifidobacteria and increased concentration of non-beneficial organisms like *Pseudomonas aeruginosa* and *Candida albicans* [6]. Since gut microbiome composition affects the GALT and immune activation [23] restoration of gut microbial balance through nutritional supplementation with probiotics or prebiotics is a potential intervention to modulate gut immunity. Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit on the host while prebiotics are non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria [24]. The potential effect of probiotic bacteria on microbial host defense, growth, and immune function in HIV was reviewed recently [25]. In summary, emerging data mainly from HIV-infected children suggest probiotics may modulate gut immune function, exert protective effects against inflammation and chronic immune activation, preserve CD4 + T cell count, and improve weight gain. Prebiotics have shown promise as well. Among ARV naïve HIV-infected patients, 12 weeks of an oligosaccharide prebiotics mixture (15 or 30 grams of short chain galactooligosaccharides/long chain fructooligosaccharides/pectin hydrolysate-derived acidic oligosaccharides daily) increased bifidobacteria and reduced pathogenic bacteria [26•]. A reduction in immune activation and improvement in NK cell activity was observed, but the mechanisms are unclear.

Optimizing Gut CD4+ T Cell Population

Gut mucosal recovery after the initial catastrophic loss of CD4+ T cells, particularly Th17 cells, is limited. Accordingly, interventions are needed to reverse or minimize ongoing damage to gut immunity. Interleukin (IL)7 attracted interest in part because it is needed for normal lymphoid development, and it has shown promise as a potential agent to improve CD4+ T cells in HIV-infected patients. Specifically, recombinant human IL-7 (rhIL-7) administered weekly to cART suppressed HIV-infected participants resulted in brisk and sustained (through 52 weeks) increase in output of total, naïve, central memory CD4+ T-cells [27••]. Preliminary data from the follow up study confirmed the previous CD4+ T cell findings and demonstrated increases in the number and proportion of gut-associated CD4+ T cells, particularly those of the central memory phenotype [28]. Of note, there was no change in Th17 cells and microbial translocation (sCD14) but D-dimer levels decreased.

IL 21, a cytokine needed for Th17 cell generation, is also being investigated as a potential intervention, although it has yet to be evaluated in HIV-infected patients. In chronically SIV infected rhesus macaques, the extent of depletion of CD4+ IL-21 + T cells in blood and rectal mucosa was shown to correlate with magnitude of Th17 cell loss, and treatment with IL21 increased levels of Th17 cells [29•]. Based on the experience with IL-2, additional work is necessary to show that IL-7 or IL21 induced CD4+ T cells are functionally normal.

Maximal Suppression of HIV Replication

cART is generally considered successful if it leads to sustained suppression of viremia at levels below 50 copies/mL. However in a representative study, laboratory-based real-time PCR detected residual viremia of 1 to 49 copies/mL (median of 2.6 copies/mL) in 63 % of patients who had “undetectable” (< 50 copies/mL) HIV RNA on clinical assays [30]. The source of this residual viremia is not completely understood though viral release from latently infected long-lived cells or cellular reservoirs in privileged sites is often implicated. Recent data suggest that the mechanism of action of ARVs may also contribute, e.g., entry inhibitors have been shown to increase extracellular concentrations of HIV in vitro, possibly by preventing viral products produced by cryptic replication from entering CD4+ cells [31]. There is controversy as to whether low-level (cryptic) viral replication is ongoing during successful cART and whether it contributes to residual viremia and/or immune activation.

A number of cART intensification studies have attempted to dissect the relationships between cryptic replication (if occurring), residual viremia and immune activation. The general approach in these studies is to add a selected ARV agent to intensify the regimen (hence presumably block cryptic replication) in patients who already have plasma HIV RNA<50 copies/mL on cART. Impact of the cART intensification is subsequently estimated by quantifying plasma/cellular markers of viral replication and immune activation. Table 1 shows that raltegravir, an integrase inhibitor, is the most commonly used ARV agent in cART intensification studies and that results of these studies have been contradictory. While a few studies have suggested the possibility of an association between residual viremia and immune activation [32–34], other studies found no effect of cART intensification on immune activation [35–37]. In general, these latter studies also did not detect evidence of cryptic replication or any effect of cART intensification on residual viremia, in agreement with other studies [38–41]. In

Table 1 Selected antiretroviral intensification studies with immune activation endpoints

ARV drug	Design (Time[a])	N	Key outcome measures	Results	Reference
Raltegravir (RAL)	RCT (12 weeks)	50	2-LTR circles, total cellular HIV-1 DNA, T cell activation	At week 12, there was no change from baseline in 2-LTR circles, total HIV-1 DNA, ratio of 2-LTR circles to total HIV-1 DNA, or CD4 and CD8 T cell co-expression of CD38 and HLA-DR.	35
	RCT (12 weeks)	18	HIV-1 RNA by SCA and immune activation in CSF and blood	At week 12, raltegravir intensification did not significantly change CSF or 36 plasma HIV-1 RNA. Blood CD8 and CD4 CCR5 expression and CSF co-expression of CD38 and HLA-DR increased with intensification. CSF and blood neopterin and other activation markers were not significantly affected	36
	RCT (48 weeks)	69	HIV-1 DNA (integrated and unintegrated), T cell activation	By 24 weeks, RAL intensification transiently increased 2 LTR circles in 29 % of subjects. CD8 cell activation markers reduced significantly in this subgroup. At week 48, RAL intensification was associated with lower activation of CD8 but not CD4 cells. No change in total and integrated HIV-1 DNA, or HIV-1 RNA <5 copies/mL.	32, 33
	RCT (24 weeks)	75	Plasma HIV-1 RNA, CD4 cell count, T cell activation, microbial translocation	24 week intensification with RAL or hyperimmune bovine colostrum had no significant effect on plasma HIV-1 RNA, CD4 cell count, LPS, 16 s ribosomal DNA, sCD14, or T cell CD38 HLA-DR co-expression.	37
	Single arm (14 weeks)	[b]7	Plasma HIV-1 RNA, cell associated HIV (RNA and DNA) in the blood and gut (duodenum, colon, rectum), T cell activation.	5 of 7 pts had a mean decrease in unspliced HIV RNA of 2,520 copies/106 CD4 cells in the ileum but no consistent decrease in the plasma, PBMC, duodenum, colon, or rectum. There was a trend towards decreased T cell activation in all sites, which was greatest for CD8+ cells in the ileum and PBMC; and a trend towards increased CD4+ T cells in the ileum	34
Maraviroc (MVC)	RCT (24 weeks)	34	CD4 count, T cell activation, apoptosis	MVC intensification had no effect on CD4 cell count but markers of immune activation and apoptosis declined	90
	[c]RCT cross over design (8 weeks)	10	Serum and CSF, SCA, IgG index, CD4 count	No significant changes in CSF and plasma HIV RNA levels, CSF and plasma neopterin, CSF and serum b2-microglobulin, IgG index, albumin ratio, or CD4 count	95
	RCT 24 weeks	45	T cell activation in serum, and rectal biopsies	MVC increased activated CD8 cells in plasma and activated CD4 and CD8 cells in rectum. No effect on peripheral CD4 count	94

[a] Time= duration of intensification

RCT = randomized controlled trial

[b] 5 patients received RAL

[c] 4 weeks of MVC (n=7) or lopinavir (n=3) plus 4 weeks of enfuvirtide

the study by Chun et al., residual viremia was found to correlate with proviral DNA (reservoir) size but neither of these parameters correlated with soluble or cellular markers of activation or inflammation [30]. The lack of a clear correlation between residual viremia and immunologic activation in patients suppressed on cART contrasts the situation in untreated HIV infection where expression of the activation marker CD38 on CD8+ T cells correlates positively with HIV RNA level and may be a stronger predictor of survival than HIV RNA or CD4 count [42].

Taken together, cART intensification studies to date have not provided proof that cryptic replication exists during cART. There is also no proof that cART intensification can eliminate residual viremia or normalize immune activation even if cryptic replication is occurring. Notably, only a few studies have evaluated tissue compartments or employed the most sensitive markers of low-level replication. As such,

additional studies are needed before cryptic replication as a contributor to chronic immune activation can be definitively excluded. Determining whether intensification with agents that block translation of HIV DNA into RNA, packaging and/or release of progeny viral particles will impact cryptic replication and/or residual viremia will have to wait until such agents are available for study.

Co-Infections

The HIV-infected population is at increased risk for co-infections that can independently activate host immune system. Because some co-infections compound HIV-induced immune activation and HIV disease progression, it is plausible that treatment or chronic suppression of such co-infections will favorably influence the course of HIV disease in patients not on cART and reduce chronic immune activation in individuals with undetectable HIV on cART. Indeed, a systematic review of 18 studies in HIV-infected patients who were not receiving cART found that treatment of co-infections (tuberculosis, syphilis, malaria, intestinal helminths, schistosomiasis, filariasis and HSV) resulted in a mean HIV RNA decrease of 0·04 $\log_{10}$ copies/mL to 3·47 $\log_{10}$ [43•]. In another study, HSV 2 seropositive pregnant women with untreated HIV and median HIV RNA of 4 $\log_{10}$ copies/mL were administered valacyclovir 500 mg twice daily from 34 weeks of gestation to 1 year postpartum [44]. HSV suppression was associated with a 0.6 $\log_{10}$ copies/mL reduction in HIV RNA 6 months postpartum and 0.3 $\log_{10}$ copies/mL reduction at 12 months postpartum, but there was no significant impact on CD38+/HLADR + CD4+ and CD8+ T cells. The study did not report possible effects on the soluble markers of immune activation or inflammation. Thus, treatment/suppression of co-infections may indirectly lower HIV RNA in patients with untreated HIV, but changes in immune activation may not be detected.

Fewer studies have examined co-infections in HIV-infected patients suppressed on cART to determine the contribution of co-infections to chronic immune activation. The probe studies to date have targeted HSV or CMV. Like other herpesviruses, HSV and CMV establish are more prevalent in HIV-infected patients than in the general population, and maintain latency through mechanisms that are not well understood, and a. However, there are effective and safe antiviral agents for treatment and chronic suppression of HSV and CMV.

HSV

Approximately 60 % of HIV-infected adults are HSV 2-infected, which is three times the prevalence in the general US population [45]. In the Multicenter AIDS Cohort Study (MACS), HSV-2 co-infection was associated with increased risk for subclinical coronary atherosclerosis in HIV-infected men (RR 4.12; 95 % CI=1.58-10.85) after adjusting for age, race/ethnicity, cardiovascular risk factors, and HIV infection related factors [46]. Infection with a greater number of herpesviruses was also identified as a risk factor. The effect of HSV 2 treatment on markers of immune activation and inflammation was not reported. Of note, the reported association between HSV 2 co-infection and increased atherosclerosis risk in HIV patients awaits confirmation and potential mechanisms are yet to be elucidated. One hypothesis is that HSV 2-induced immune activation even in the absence of clinically apparent HSV–2 reactivation [47] increases the risk of cardiovascular disease [48]. An ongoing randomized controlled clinical trial (VALacyclovir for Inflammation Attenuation Trial Pilot, VALIANT) is addressing whether valacyclovir can attenuate markers of inflammation and immune activation in HSV 2 co-infected HIV-infected patients suppressed on cART [NCT01176409].

CMV

CMV seroprevalence varies across geographic and demographic groups [49]. Approximately 90 % of HIV-infected individuals in the US are co-infected with CMV. In HIV-infected individuals, CMV co-infection has been associated with higher immune activation [50] and T cell immune senescence [51]. Symptomatic CMV disease (hepatitis, colitis, retinitis, and pneumonia) in HIV-infected patients is virtually restricted to untreated patients with advanced immune suppression. In the Women's Interagency HIV Study higher CMV IgG antibody levels were associated with increased prevalence of carotid artery lesions among HIV-infected women who had achieved HIV suppression on cART, but not among viremic or untreated HIV-infected women [52]. CMV-specific T cell responses have been shown to be higher in HIV-infected patients on cART than in HIV untreated patients or HIV-negative patients [53] and higher CMV-specific CD8 IFN-γ production has been associated with more atherosclerosis [54].

To evaluate whether asymptomatic CMV might contribute to immune activation in HIV-infected individuals suppressed on cART, 30 CMV seropositive individuals with CD4+ T cell count <350 cells/mm^3 who had been on cART for at least 6 months and had a≥10 % activated CD8+ T-cells (CD38+ HLA-DR+) were administered valganciclovir 900 mg daily or placebo for 8 weeks in a randomized trial [55••]. About two-thirds of the participants had HIV RNA<75 copies/mL and the median CD4+ T cell count was 190 cells/mm^3. Valganciclovir treated patients had approximately 4 % reduction in percentage of activated CD8+ T cell (from about 20 % to 16 % CD38+HLA-DR+) while no change was observed in placebo recipients. The impact of valganciclovir remained evident in analysis restricted to those with HIV RNA<75 copies/mL. There was also a reduction in CD8+ T cell activation, though

not statistically significant, in those with negative HSV 2 serology or continued acyclovir prophylaxis. Critically, CMV DNA was detected in the saliva in 30 % and in the seminal plasma in 60 % of patients prior to valganciclovir but was undetectable up to 12 weeks after treatment compared to no change in the placebo group. This study suggests that CMV and/or other herpesvirus replication may be a significant cause of immune activation (up to 25 % of the CD8+ T cell activation) during cART, at least in patients with low CD4+ T cell counts. It has yet to be determined whether effects observed during short-term suppression of CMV are sustained long-term and whether they translate into a clinical benefit.

EBV

EBV is an oncogenic herpesvirus implicated in immune activation and chronic inflammation. EBV co-infection is found in 80–100 % of HIV-infected patients with Hodgkin lymphoma. EBV DNA load in treated and untreated HIV infection correlates with HIV viremia and with level of LPS, pro-inflammatory cytokines (IL-6, IL-10 and TNF-α), and B cell activation [56•]. Although acyclovir, ganciclovir and forscanet have in vitro activity against EBV, these agents are active only during lytic EBV infection and are not expected to be effective in latency. Thus, they cannot address the potential impact of chronic EBV infection in treated HIV-infected patients. Clinical studies demonstrating a link between markers of immune activation and clinical outcome have not evaluated EBV as a potential contributor or confounder between the association of soluble markers of inflammation and clinical outcome. However, even in the absence of the immune activation data the connection of EBV to non-Hodgkin lymphoma is clear.

Chronic Viral Hepatitis

Approximately 1.3 % of the U.S. population has chronic HCV [57]. HCV is more common in HIV-infected persons than the general population and has been linked to immune activation. In a cohort (N=59) of natural HIV suppressors (defined as HIV RNA <400 copies/mL while not receiving cART), those who were HIV/HCV-co-infected had lower mean CD4+ T cell counts and elevated levels of immune activation (CD38+HLA-DR+CD8+ T-cells). The increased levels of immune activation were not associated with sex, HLA B57 status, or injection drug use [58], suggesting an independent effect of HCV on immune parameters in this population. Markers of soluble markers of immune activation and inflammation were not reported. Two studies assessing immune activation before and after treatment for HCV infection with the newer peg-INF-g sparing regimens in HIV-infected individuals suppressed on cART are underway. Although a significant proportion of HIV-infected individuals are co-infected with HBV, the ability to use tenofovir/emtricitabine as the nucleos(t)ide backbone of the regimen allows clinicians to treat HIV and HBV concurrently. An effective HBV vaccine is also available. Therefore, the contribution of HBV to chronic immune activation may be limited and waning.

Metabolic Syndrome/Obesity

Metabolic syndrome is a cluster of risk factors for cardiovascular disease and diabetes, defined as presence of three or more of the following five criteria: increased waist circumference (country and race specific); fasting glucose >100 mg/dl (insulin resistance); triglycerides >150 mg/dl; HDL-Cholesterol <40 mg/dl in men or <50 mg/dl in women; and hypertension >130/85 mmHg [59]. Metabolic syndrome is common in HIV-infected persons with a prevalence of 20 % among ARV naïve patients who enrolled in ACTG studies. After initiating cART, the incidence of metabolic syndrome was 8.5 per 100 patient years. Risk factors included CD4 + T cell counts <50 cells/mm^3, BMI >25 kg/m^2, age >30 years, HIV RNA >400 copies/mL and protease inhibitor use [60]. Some ARVs, especially protease inhibitors have been associated with components of the metabolic syndrome. Given associations between metabolic syndrome and pro-inflammatory changes in adipose tissue (increased levels of INFγ, IL-6 and TNF) [61], there is a need to understand the impact that treating or preventing metabolic syndrome (diet and exercise, fibrates, niacin, statins, omega-3 fatty acids) would have on immune activation/inflammation during HIV infection. Aspirin has an anti-inflammatory effect as well as an anti-platelet effect. While the benefit of aspirin in secondary prevention of cardiovascular disease in the general population is well established, its role in primary prevention appears limited to subgroups with very high risk such as diabetics whose 10-year risk of cardiovascular events exceed 10 %. The major risk with chronic aspirin use is bleeding. Although there is benefit in understanding the effect aspirin has on chronic immune activation in the HIV population, the experience from non-HIV infected patients indicate that safety concerns must be considered when designing such studies.

Blocking Mediators Along the Common Final Pathway

Statins

Statins are primarily prescribed for cardiovascular risk reduction because they inhibit the 3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme and reduce low density lipoprotein (LDL) cholesterol levels. In addition, statins interrupt several steps in the inflammatory cascade

underlying atherogenesis and cardiovascular disease such as endothelial dysfunction, inflammatory cell infiltration, vascular remodeling and plaque rupture [62]. Potential pathways through which statins exert these effects include inhibition or down regulation of adhesion factors, thrombosis, monocyte/T-cell recruitment, and inflammatory cytokines/chemokines. Statins block IFN-γ induced upregulation of the major histocompatibility complex (MHC) class II molecules in a variety of cells [63, 64]. These immuno-inflammatory properties are independent of the effects on serum lipids [65–67] and have been implicated in the improvement of brachial artery flow-mediated dilation (FMD) and reduction in cardiovascular risk observed in individuals on statins [62].

Some studies suggested that statins can reduce HIV replication in vitro [68, 69], but this has not been demonstrated in vivo. Interest has therefore shifted to exploring whether immuno-inflammatory properties of statins can be exploited to control chronic inflammation/immune activation in HIV-infected patients receiving ART.

Studies evaluating the impact of statins on biomarkers of inflammation during HIV infection have generated conflicting results. In a retrospective study of HIV-infected patients on protease inhibitor based cART, rosuvastatin, atorvastatin and pravastatin resulted in comparable decline in CRP (approximately −20 %) and TNF ∝ (approximately −30 %) while no significant change was detected in IL-6 levels [65]. Another retrospective study involving 58 of 83 patients enrolled in the VIHstatine study (90 % of whom had HIV RNA<400 copies/mL) concluded that pravastatin or rosuvastatin use was associated with lower CRP levels [66]. In contrast pravastatin and fenofibrate had no significant impact on inflammatory markers among HIV-infected patients in ACTG A5087 (N=74), including the subset with HIV RNA<50 copies/mL [70]. These discrepant findings on CRP changes with statins may be due to differences in the study populations, underscoring the need for additional research.

Studies evaluating effect of statins on T cell activation have been generally found a benefit. Atorvastatin reduced immune activation (CD38+CD8+ T cells) in a case–control study of HIV-infected patients on successful cART [71]. Baseline levels of mean CD38 percentage (Q25, Q75) were similar between cases and controls: 49 % (29;70) vs. 51 % – (32; 70). At week 48, there was a significant difference between cases and controls: 37 % (23; 46) vs. 42 % (25; 56) P=0.039. Similarly, in HIV-infected patients not receiving cART, administration of high dose atorvastatin (80 mg daily) resulted in reductions in circulating proportions of CD4+ HLA-DR+(−2.5 %), CD8+ HLA-DR+(−5 %), and CD8+ HLA-DR+ CD38+ T cells (−3 %) [67]. There appear to be differences in the immune modulatory potency of different statins. In healthy volunteers, 14 days of atorvastatin 20 mg daily downregulated HLA DR and CD 38 expression on T cells but this effect was not seen with simvastatin dosed at 20 mg or 40 mg daily [72]. Effects on soluble markers of immune activation and inflammation were not reported.

Preliminary studies suggest that statin-induced biomarker changes may translate to clinical benefit. For example, 8 weeks of atorvastatin improved FMD among patients with HIV RNA<50 copies/mL during protease inhibitor based cART [73]. Also, among HIV-infected patients who had achieved HIV-1 RNA<400 copies/mL, statin use (1538) was associated with significant reduction in mortality during a median 570 days of observation. Only 7 of 85 deaths recorded in the study occurred in patients on statins; by multivariate Cox regression, statin use was associated with a relative hazard (RH) of 0.33 (95 % CI: 0.14, 0.76, p=0.009) [74]. Most deaths were due to malignancy, non-AIDS-defining infection and liver failure with no difference detected in death from cardiovascular disease between statin users and none users, but the number of evaluated patients was small. Another observational study reported an association between statin use and lower incidence of non-Hodgkin lymphoma [75].

Several randomized clinical trials are ongoing/planned to further evaluate the effects of statins on biomarkers of immune activation and inflammation in virally suppressed HIV-infected patients. The statins in all of these studies are atorvastatin, pravastatin or rosuvastatin partly because of concerns about potential drug-drug interactions between other statins and protease inhibitors, and the lack of effect of simvastatin in preliminary trials. Other studies are further evaluating preliminary associations between statin-induced biomarker changes and clinical benefits.

Chloroquine and Hydroxychloroquine

Chloroquine is an antimalarial drug while its analogue hydroxychloroquine is most often used to treat autoimmune disorders. These agents have similar biochemical properties and are expected to have similar immunomodulatory properties [76]. Chloroquine and hydroxychloroquine have weak anti-HIV activity in vitro, possibly by inhibiting posttranscriptional modification of gp120 [77, 78]. On the other hand, chloroquine increases endosomal pH, which promotes release of entrapped viral particles and potentially increases astrocyte permissiveness to HIV infection [79]. The endosomal pH increase also inhibits endosomal maturation and nucleic acid binding to toll like receptor (TLR)7 and TLR9 [80]. Because TLR mediates T cell activation by stimulating IFN-γ production in dendritic cells, chloroquine has been proposed as an adjuvant to cART in order to control immune activation [81]. Chloroquine and hydroxychloroquine may

also dampen T cell activation through inhibition of calcium signaling [82] and macrophage antigen catabolism [83]

Effects of chloroquine and hydroxychloroquine during HIV infection have been studied in untreated HIV patients as well as those on virally suppressive ART. In patients with untreated chronic HIV, chloroquine 250 mg or 500 mg was shown to lower LPS, CD8+ memory T cell activation and also Ki-67 expression in CD4+ and CD8+ memory T cells [84]. Also, 800 mg of hydroxychloroquine once daily (which is higher than the clinically approved dose) was reported to suppress HIV replication and reduce markers of immune activation such as IL-6 [85, 86]. The maximum approved dose of hydroxychloroquine for clinical use (400 mg) was evaluated in a recent clinical trial [87••] and the results were dramatically different from those obtained using 800 mg hydroxychloroquine. Compared to placebo, 400 mg hydroxychloroquine was associated with a 0.38 $\log_{10}$ increase in HIV RNA and a 62 cells/mm^3 decline in CD4+ T cell count over 48 weeks. CD4+ T cell decline to <350 cells/mm^3 (the level for ART initiation in the study), and influenza-like illness were more common among subjects in the hydroxychloroquine arm. There was no significant difference in immune activation as measured by CD38 and HLA DR expression on CD4+ or CD8+ T cells between the two groups. The different results with 400 mg and 800 mg of hydroxychloroquine may be related to dose-dependence of the effects on endosomal pH [76]. Overall, there is currently no role for chloroquine or hydroxychloroquine in HIV-infected patients who are not on cART while concerns have emerged about the safety of hydroxychloroquine in this patient subgroup considering the increase in HIV RNA and decrease in CD4+ T cell count observed with clinical dose of hydroxychloroquine.

Critically, the results from patients not on cART appear to differ from those of patients who have already achieved viral suppression. In a single-arm study [88], patients with immunologic non-response (CD4 <200 cells/mm^3 or CD4 count increase <5 % in the preceding 12 months despite HIV RNA <50 copies/mL on cART) were administered hydroxychloroquine 400 mg daily for 6 months. Hydroxychloroquine use was associated with broad immune benefits including reduction in levels of LPS, IFNα-secreting plasmacytoid dendritic cells, IL-6, TNF-α, and TLR expression/signaling while increasing Tregs and percentage of circulating CD4$^+$ T cells. An ongoing ACTG study (A5258) is evaluating the effect of chloroquine 250 mg daily on immune activation in different HIV populations: i) those who are ARV naïve or off cART for at least 6 months; and ii) those who have received at least 24 months of cART and have suppressed HIV RNA below detection limits with CD4+ T cell count < 350 cells/mm^3 [NCT00819390]. This study is expected to shed more light on the immunomodulatory effects of chloroquine and hydroxychloroquine in chronic HIV infection.

Maraviroc

Maraviroc (MVC) prevents HIV entry into target cells by blocking the chemokine receptor CCR5. In theory, CCR5 blockage may also prevent ß chemokines such as CCL3, CCL4 and CCL5 from interacting with CCR5 and triggering T cell activation and proliferation [89]. MVC inhibits the chemotactic activity of monocytes, macrophages and dendritic cells [90].

There is no consensus on the immunomodulatory effects of MVC in virally suppressed HIV patients. In the single-arm ACTG A5256 study, intensification with MVC did not achieve the primary objective of increasing CD4+ T cells in virally suppressed poor immune responders, but activated T cell subsets (CD38+, CD38+/HLA-DR+, and Ki67+ CD4+ and CD8+ T-cells) and markers of apoptosis declined [91•]. These changes reversed partially with MVC discontinuation. Another single-arm study [92] and a randomized study [93] reported a decrease in % CD38+/HLA-DR+ CD4+ and CD8+ cells with MVC intensification. Contrasting these, MVC intensification paradoxically increased the percentage of CD38+ CD8+ T cells in one study [94], and intensification with MVC or lopinavir/ritonavir for 4 weeks followed by enfuvirtide for 4 weeks did not alter CSF or blood HIV RNA, neopterin, β2-microglobulin, or immunoglobulin G index in another study [95].

Other Anti-Inflammatory Drugs

Methotrexate is an anti-inflammatory agent used for the treatment of autoimmune disorders and also some malignancies. Although the mechanisms for its anti-inflammatory and immune modulatory effects are uncertain, postulated pathways include inhibiting antigen-stimulated T cell proliferation, induction of T cell apoptosis, reducing levels of inflammatory cytokines and, perhaps most importantly, increasing extracellular concentrations of adenosine [96, 97]. Low-dose methotrexate (10 mg weekly) is strongly associated with reduced cardiovascular events and mortality in rheumatoid arthritis patients [98, 99], presumably reflective of its beneficial anti-inflammatory effects. A recent meta-analysis of ten observational studies of methotrexate use and risk of cardiovascular disease (N=66,235) found a 21 % reduction in all cardiovascular events and an 18 % reduction in risk of MI [100]. A prospective randomized clinical trial of 7,000 patient study evaluating the effect of low dose methotrexate in the general population is planned to confirm the findings in the observational studies, but is not yet enrolling. Currently, HIV-infected individuals are excluded from the study, but may be included later once the safety of low dose methotrexate in the HIV-infected population has been established in the HIV population. (personal communication Paul Ridker). The ACTG is developing a placebo-

controlled randomized clinical trial A5314 [NCT00819390] to evaluate safety of low-dose methotrexate in HIV patients on ART. The study also aims to evaluate effects of low-dose methotrexate on brachial artery FMD, and inflammatory biomarkers of cardiovascular disease (CVD) risk (CRP, IL 6, d dimer and sCD14) among other endpoints.

Another study [NCT1090102] is exploring the effect of mesalamine (5 amino salicylic acid, an anti-inflammatory drug for inflammatory bowel disease) on immune activation and microbial translocation in patients who have received ART for at least 6 months and have HIV RNA<40 copies/mL and CD4+ T cell count < 350 cells/mm^3.

Conclusion

There is mounting evidence that residual chronic immune activation despite virally suppressive cART contributes to non-AIDS morbidity and all-cause mortality in HIV-infected patients. Accordingly, control of residual immune activation in this population is a priority. Several potential interventions have been identified based on their mechanisms of action, impact on other chronic inflammatory conditions and/or preliminary data, but no intervention can be recommended at this time, pending further evaluation.

Limitations of ongoing studies must be considered when translating research findings into clinical care. The first limitation is that most ongoing studies have a small sample size and are primarily designed to quantify biomarker changes following a specific intervention. Such studies are prone to inter- and intra-laboratory variability of results, underscoring the need to standardize procedures for sample collection, processing, freezing, shipping, thawing, assessing viability of cells (for cell based assays) and analysis across laboratories used by the various cohorts and networks. Also, there is a dearth of validated biomarker surrogates of clinical events, which implies that data from studies employing change in biomarker levels as the primary endpoint may not significantly inform treatment guidelines or future studies by themself. The utility of such studies may be in confirming that the biomarker is amenable to change in the HIV-infected population and identifying investigational interventions that warrant evaluation in large, randomized studies with clinical endpoints. However, the number of patients needed to confirm clinical endpoints, current economic considerations, and the lack of biomarkers unique to the HIV population means the potential interventions will need to be prioritized. Importantly, both biomarker and clinical endpoint studies may be confounded by variables like co-infections and co-morbidities that can independently provoke the immune system. Improved understanding of the contribution of these potential confounders and others like CD4 + T cell nadir is necessary to isolate the effect of the intervention being evaluated. There is also a need to illuminate currently unrecognized or poorly understood activation or inflammation pathways that may confuse the interpretation of research findings. Finally, long-term safety studies need to be conducted to monitor the safety and efficacy of new interventions. Whether these are required prior to approval of new agents or can be done as part of the post marketing surveillance is open for discussion. However, the experience with the COX-2 inhibitors needs to be kept in mind as we advance this field. Meanwhile, cART will remain the cornerstone treatment for HIV-infected individuals for the foreseeable future. Despite its inability to normalize immune activation, cART dramatically lowers activation from levels seen in untreated HIV infection, and it reliably prolongs survival. As currently recommended in the United States, cART should be offered to all HIV-infected patients as soon as feasible after diagnosis.

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- • Of importance
- •• Of major importance

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