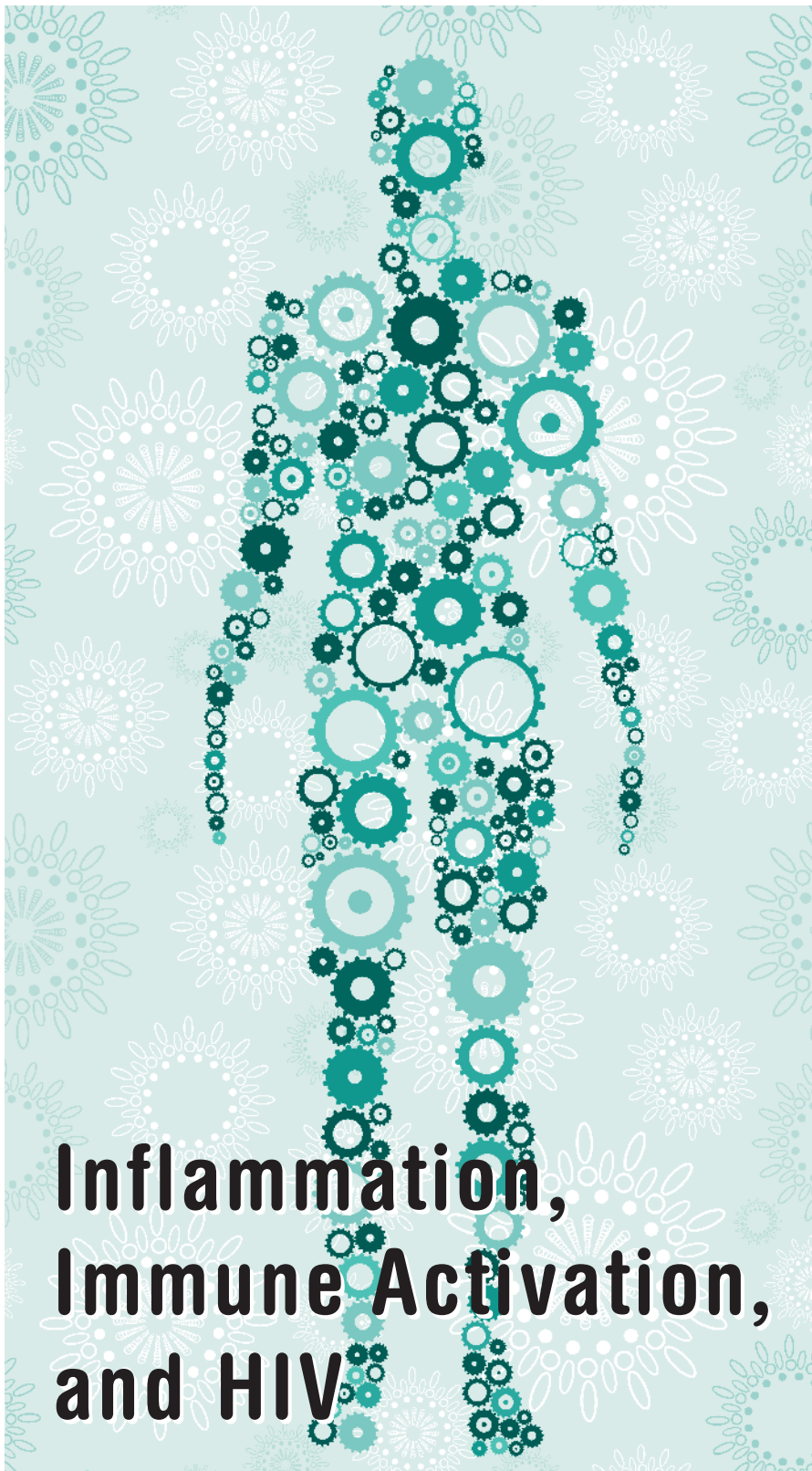Liz
Highleyman

Inflammation, Immune Activation, and HIV

Inflammation is a broad term for what happens in the body when the immune system is activated to counter a threat. A healthy immune response is key to good health, but ongoing immune activation and inflammation due to a persistent threat such as chronic HIV infection can lead to many different problems throughout the body.

Among people with HIV, combination antiretroviral therapy (ART) has dramatically reduced the risk of AIDS-defining opportunistic illnesses (OIs) and mortality directly related to immune suppression. But as HIV positive people survive longer, they are at increased risk for a variety of non-AIDS conditions even when their CD4 cell counts are high.

At the annual Conference on Retroviruses and Opportunistic Infections (CROI) in February, some attendees dubbed 2010 "the year of inflammation." A growing body of evidence implicates chronic inflammation and immune activation in the development of non-AIDS conditions, and some experts blame inflammation for what looks like accelerated aging in people with HIV.

"Untreated HIV infection causes inflammation and, despite ART, it does not normalize," Steven Deeks, professor of medicine at the University of California-San Francisco (UCSF), explained at a post-CROI workshop

> ## Immunosenescence:
>
> Age-related decline in immune function.

sponsored by Project Inform. "This leads to all sorts of 'badness.' Some 20 presentations showed this same phenomenon linking HIV, inflammatory biomarkers, age-related symptoms, and immunosenescence."

WHAT IS INFLAMMATION?

Inflammation refers to the complex cascade of events that happen when the immune system recognizes a threat and goes into action, including migration and activation of various types of white blood cells (leukocytes) and release of chemical messengers known as cytokines.

The word "inflammation" often brings to mind the immune system's immediate response to acute injury or infection. When bacteria enter the body through a cut, for example, these microorganisms, the toxins they produce, and other signals from injured cells and blood vessels alert macrophages and other leukocytes present in tissues. A cellular protein called nuclear factor kappa-B (NF-kB) is released and switches on genes involved in immune response. Newly activated macrophages then begin producing pro-inflammatory cytokines, including interleukin 1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-alpha).

Soon, neutrophils and other immune cells migrate to the site, where they ingest pathogens (a process called phagocytosis) or kill them by releasing toxic substances. Reactive forms of oxygen and nitrogen generated by these cells cause oxidative stress and damage to DNA (genetic material) in the body's cells. Basophils and mast cells release

Cytokines, Mediators, and Biomarkers

The table below lists key chemical messengers contributing to inflammation that are discussed in this article; the immune system uses hundreds of signaling chemicals and this list is by no means complete. Some of these chemicals build up in the bloodstream and can be measured in laboratory tests as biomarkers of inflammation, coagulation, or endothelial dysfunction. Biomarkers may not play a direct causal role in these processes, but their presence in the blood can provide clues about immune system activity.

NAME	FUNCTION
Adiponectin	anti-inflammatory adipokine hormone, signals increased inflammation
C-reactive protein (CRP)	acute phase protein, signals increased inflammation
D-dimer	byproduct of blood clot breakdown, signals increased coagulation
Fibrinogen	acute phase protein, mediator of blood clotting, signals coagulation
Intercellular adhesion molecule 1 (ICAM-1)	cell adhesion molecule, enables leukocytes to bind to endothelial lining, signals endothelial dysfunction
Interleukin 1 (IL-1)	pro-inflammatory cytokine, signals increased inflammation
Interleukin 4 (IL-4)	anti-inflammatory cytokine
Interleukin 6 (IL-6)	pro-inflammatory cytokine, signals increased inflammation
Interleukin 10 (IL-10)	anti-inflammatory cytokine
Leptin	pro-inflammatory adipokine hormone
Monocyte chemoattractant protein 1 (MCP-1)	inflammatory chemokine, promotes monocyte migration
Macrophage inflammatory protein 1 (MIP-1)	inflammatory chemokine, promotes neutrophil migration
Plasminogen	acute phase protein, mediator of blood clot breakdown, involved in wound healing
P-selectin	cell adhesion molecule, enables leukocytes to move along endothelial lining, signals endothelial dysfunction
Transforming growth factor-beta (TGF-beta)	anti-inflammatory cytokine
Tumor necrosis factor-alpha (TNF-alpha)	pro-inflammatory cytokine, promotes death of cancer cells, signals increased inflammation
Vascular adhesion molecule 1 (VCAM-1)	cell adhesion molecule, enable leukocytes to bind to endothelial lining, signals endothelial dysfunction

histamine, best known for its role in allergic reactions. This *innate branch* of the immune system is nonspecific—it responds to multiple threats.

Within cells, signals produced by these "first responders" stimulate release of locally acting hormones known as prostaglandins. They also

tivation mediated by the lymphocytes: T-cells, B-cells, and natural killer cells. Antigen-presenting cells such as macrophages capture pathogens and display pieces of them (antigens) on their surface. Lymphocytes interact with these cells and learn to recognize and directly target those particular pathogens. This

The inflammatory response, therefore, is the result of a complex interplay of many different types of immune cells that use hundreds of chemical messengers to communicate among themselves, forming cascades, chains of command, and feedback loops. (See sidebar on page 13 for a partial list of some important cytokines and mediators that play a role in inflammation.)

Differentiating Immune Cells

Lymphocytes and other immune cells are classified according to cell-surface molecules designated by a CD ("cluster of differentiation") number. Human leukocytes have more than 300 CD markers, and an individual cell may carry several of them. Helper T-cells with the CD4 marker, for example, coordinate immune responses, while killer T-cells carrying CD8 attack virus-infected and malignant cells.

CD molecules are not simply markers, however, but also have their own functions. Many act as cell surface receptors or ligands (molecules that bind to receptors), often triggering signaling cascades. HIV uses the CD4 receptor as a gateway to enter helper T-cells, macrophages, and dendritic cells.

CD markers also indicate the status of immune cells. The CD25, CD38, and CD69 markers show that a CD4 or CD8 T-cell is activated. The CD45 marker indicates whether a T-cell or B-cell is a naive (CD45RA) or memory (CD45RO) cell. T-cells expressing CD57 in the absence of CD28 are typically senescent, having exhausted their ability to proliferate.

CHRONIC INFLAMMATION AND IMMUNE ACTIVATION

Under normal circumstances, the immune response is self-limiting and turns itself off when no longer needed—for example, when a wound heals or a bout of infection resolves. But inflammation can become chronic if the trigger persists or if suppressive control mechanisms do not work properly.

Pro-inflammatory prostaglandins and acute-phase proteins are short-lived and their effects are temporary unless there is an ongoing signal to produce more. And just as some chemical messengers promote immune activation, opposing signals act to dampen responses. Anti-inflammatory cytokines include IL-4, IL-10, and transforming growth factor-beta (TGF-beta).

This fine-tuned system can go awry, however, when the immune system is faced with a threat it cannot overcome. This occurs, for example, during persistent infection. Other causes of chronic inflammation include autoimmune conditions (in which the immune system attacks the body's own tissues), obesity, chronic stress, and exposure to toxins such as tobacco smoke.

Numerous pathogens, including HIV, hepatitis B and C viruses (HBV and HCV), and herpes viruses, can remain in the body over the long term. Though the immune system may respond by producing antibodies and activating killer T-cells, this response is not always enough to clear infection. For some pathogens, such as HCV, a proportion of people can clear the infection either spontaneously or with treatment. Others, like HIV, appear to always persist for life.

trigger an acute-phase reaction, causing the liver to produce acute-phase proteins such as C-reactive protein (CRP), fibrinogen, and plasminogen.

At the local level, these chemicals induce physiological changes, including blood vessel dilation and increased permeability (leakiness), leading to the classic inflammatory signs of redness, swelling, heat, and pain. They also play a role in coagulation (blood clotting) and tissue repair.

On a systemic level, pro-inflammatory signals act on the brain and elsewhere in the body, causing fever, loss of appetite, fatigue, and other flu-like symptoms. An extreme version of this reaction, known as a "cytokine storm," has proven fatal in clinical trials of experimental therapies and has been proposed as an explanation for the high death rate during the 1918 influenza pandemic.

Early responders release additional cytokines, including interferon-gamma, that promote longer-term immune ac-

adaptive branch of the immune system responds to specific threats.

T-cells—key players in adaptive immune response—mature and differentiate into CD4 and CD8 cells in the thymus, an organ in the chest; B-cells are produced in the bone marrow. New T-cells and B-cells are naive, meaning they are able to respond to newly encountered antigens. After an immune response, a subset of these cells evolve into long-lived memory cells that remember a specific pathogen and are primed to respond rapidly if it appears again.

Chief among the lymphocytes are CD4 ("helper") T-cells—the primary target of HIV—which manage the overall immune response. CD8 ("killer") T-cells destroy virus-infected and malignant cells. CD8 T-cells, macrophages, and natural killer cells are responsible for *cell-mediated immunity*, in which immune cells themselves attack pathogens or compromised cells. B-cells produce antibodies, the basis of *humoral immunity.*

In contrast with localized acute inflammatory responses, chronic inflammation may be systemic, affecting the entire body. The overall effect is persistent immune activation, but it is more accurately thought of as immune *dysregulation,* characterized by a shift in leukocyte activity. During chronic inflammation, neutrophils become less active, while T-cells and other lymphocytes take on a larger role.

Persistent activation of T-cells accelerates their maturation and progression through the cell cycle of growth and division. Eventually, T-cells burn out prematurely and may undergo apoptosis (programmed cell death, or "cell suicide") or lose their ability to divide (replicative senescence).

Long-term immune activation and sustained high levels of pro-inflammatory cytokines can cause damage throughout the body, and chronic inflammation is increasingly recognized as a common denominator underlying a host of progressive and age-related diseases.

HIV AND INFLAMMATION

Many experts are now convinced that chronic inflammation and immune activation contribute to the higher rates of cardiovascular disease and other non-AIDS conditions seen in people living with HIV. Yet the physiological processes underlying this association remain poorly understood, and likely involve multiple overlapping mechanisms.

As Deeks discussed in a recent issue of the International AIDS Society (IAS) journal *Topics in HIV Medicine,* these mechanisms include residual HIV replication, leakage of bacteria from the gut, chronic coinfections, loss of cells that regulate immune response, reduced thymus function, and damage to other lymphoid tissues.

DIRECT IMMUNE ACTIVATION

Given that HIV suppresses immune function, how can it also cause excessive immune activation and inflammation?

The answer to this paradox lies in the complexity of the immune response. As Peter Hunt and colleagues

from Deeks' team at UCSF explained in a poster at CROI, "HIV has its foot on the accelerator and the brake at the same time." While late-stage HIV/AIDS is indeed characterized by profound immune deficiency, earlier stages are marked by ongoing immune activation and dysregulation.

In the November 2009 issue of the *Journal of HIV Therapy,* devoted to the topic of inflammation, Sonia Fernandez and colleagues explained that during primary and early chronic infection, HIV causes substantial CD4 cell depletion and disrupts lymphoid tissues, especially in the gut; the second phase of chronic infection is characterized by a state of persistent immune activation. Although ART reduces this activation, residual immune dysfunction persists.

How does HIV promote inflammation? Viral proteins, including Tat, Nef, Vpr, and gp120, appear to directly trigger immune response, probably by altering cytokine signaling. Two elements of the early innate immune response—NF-kB and toll-like receptors—in turn facilitate HIV replication, creating a vicious cycle.

Even as HIV infects and kills CD4 helper T-cells that coordinate overall immune activity, it also activates CD8 killer T-cells. HIV positive people typically show an inversion of the normal CD4 cell to CD8 cell ratio, a shift from naive to memory T-cells, and an increase in cells carrying senescence or apoptosis markers.

Hunt has reported that up to half of peripheral CD8 T-cells are activated in individuals with long-term HIV infection, compared with less than 10% in healthy HIV negative people. Increased T-cell activation has been linked to higher risk of death in untreated HIV positive individuals and to poor CD4 cell recovery in people on ART.

One clue pointing to the role of inflammation is that the amount of virus and the absolute number and percentage of infected cells do not seem adequate to explain the extent of immune dysregulation and over-

all poor health among people with HIV. A majority of CD4 T-cells in the blood and lymph nodes of people with chronic infection do not harbor the virus, and people on fully suppressive ART only have residual virus thought to be released from latent cells. But it apparently does not take much virus or many infected cells to sustain a self-perpetuating inflammatory state.

As further evidence, African green monkeys infected with SIV (a primate virus related to HIV) show little evidence of immune activation, maintain normal T-cell counts, and do not develop progressive immunodeficiency disease. In contrast, SIV-infected macaque monkeys develop persistent immune activation and CD4 cell depletion, and rapidly progress to AIDS. Similarly, people with HIV-2, a strain concentrated in western Africa, typically show both less ongoing immune activation and less aggressive immune deficiency progression than those with the more common HIV-1.

Researchers have also looked at disease progression among "elite controllers," the small proportion of HIV positive people who naturally control the virus long-term without treatment. The UCSF researchers and others have shown that while elite controllers typically maintain an extremely low viral load (detectable only with ultrasensitive assays), they nevertheless show signs of stepped-up immune activation, are at higher risk for cardiovascular disease and other non-AIDS conditions than HIV negative people, and may eventually experience CD4 cell decline.

GUT BACTERIA

HIV infection can also contribute to inflammation in less direct ways.

The virus establishes itself in gut-associated lymphoid tissue in the gastrointestinal tract—the body's largest source of HIV-susceptible CD4 T-cells—at the earliest stages of infection.

As Jason Brenchley and colleagues described in *Nature Medicine* in 2006, HIV infects these T-cells and damages the epithelial lining of the intestines,

which becomes more permeable and allows bacteria that naturally reside in the gut to escape, a process known as microbial translocation. When these bacteria and the substances they produce enter the bloodstream, they spark a strong systemic immune response. The bacterial endotoxin lipopolysaccharide (LPS), in particular, triggers an immune signaling cascade involving pro-inflammatory cytokines such as IL-6 and TNF-alpha.

People with HIV typically have elevated LPS levels associated with systemic immune activation. Wei Jiang and colleagues reported last year that elevated plasma levels of bacterial DNA are also associated with T-cell activation and poor CD4 cell recovery in people with HIV. LPS levels are typically higher in individuals with greater viral load, decrease (but do not completely normalize) with suppressive ART, and rebound during treatment interruption.

COINFECTIONS

Viral and bacterial coinfections also have a place in the HIV inflammation picture. Waning immune function related to HIV infection—even while CD4 counts are still quite high—can lead to loss of control of other pathogens in the body.

For example, many people (with and without HIV) harbor latent viruses in the herpes family, including herpes simplex viruses (HSV-1 and HSV-2), varicella zoster virus (cause of chickenpox and shingles), and cytomegalovirus (CMV). While these viruses do not directly target CD4 cells as HIV does, they do trigger immune activation and release of pro-inflammatory cytokines. Hepatitis B and C viruses primarily cause inflammation of the liver, but they too contribute to systemic immune dysregulation.

HIV positive people with chronic active viral coinfections on average have higher plasma HIV viral load, lower CD4 cell counts, and faster progression to AIDS. Priscilla Hsue and colleagues, also with the UCSF group, found that HIV positive people

with greater CMV-specific CD8 T-cell responses had higher levels of inflammation biomarkers such as CRP and greater intima-media thickness, an indicator of atherosclerosis.

Hans Rempel and colleagues reported at CROI that HIV/HCV coinfected people showed more monocyte activation than those with HIV alone, who in turn had more than uninfected individuals. In related work described in the March 15, 2010, *Journal of Infectious Diseases,* Andrea Kovacs and colleagues found that among HIV/HCV coinfected women with detectable HCV, those with the most activated CD8 T-cells had three times the risk of progression to AIDS as those with the fewest (a relationship not observed in women with HIV alone).

METABOLIC EFFECTS

The relationship between inflammation, body composition, and metabolic abnormalities associated with HIV and its treatment is complex and not fully understood. In a chicken-and-egg fashion, metabolic changes can trigger inflammation, and inflammatory changes in turn can affect metabolism.

Inflammation is associated with metabolic syndrome, characterized by abdominal obesity, high blood pressure, insulin resistance, and a high-risk blood lipid profile with high low-density lipoprotein (LDL) "bad" cholesterol and low high-density lipoprotein (HDL) "good" cholesterol.

Body fat, or adipose tissue, is not just a passive depot for storing energy, but rather has extensive metabolic and endocrine activity. Fat cells and macrophages in fat tissue release pro-inflammatory cytokines including IL-6 and TNF-alpha, as well as specialized hormones called adipokines that influence appetite and regulate metabolism.

Among the adipokines, leptin exerts an overall pro-inflammatory effect, while adiponectin generally suppresses inflammation. Studies have shown that genetically modified mice that do not produce leptin are protected from inflammatory and autoimmune diseases.

Excess body fat—especially visceral fat around the internal organs—leads to an inflammatory state characterized by greater secretion of pro-inflammatory chemicals, reduced production of inflammation-dampening signals, and insulin resistance; fat loss has the opposite effect.

Work by Andrew Carr's group in Sydney showed that HIV positive men on ART had an inflammatory profile similar to that of obese, insulin-resistant HIV negative men, despite having less body fat and greater insulin sensitivity. HIV positive people with lipodystrophy (peripheral fat loss plus abdominal fat accumulation) showed even greater systemic inflammation, with higher CRP and lower adiponectin levels.

Much remains to be learned about the links between metabolic abnormalities and the inflammatory process, but inflammation may explain at least in part how metabolic changes related to HIV infection and ART contribute to cardiovascular disease and other non-AIDS conditions.

ROLE OF ART

Antiretroviral therapy may play a role in inflammation, but this appears to be mostly indirect, through its effects on metabolism. For example, protease inhibitors as a class are associated with elevated levels of total and LDL cholesterol and triglycerides, as well as insulin resistance and abdominal fat accumulation. It is not clear, however, that antiretroviral drugs directly cause inflammation.

Some studies, including the large SMART treatment interruption trial (described below), have found higher levels of inflammatory and coagulation biomarkers among people taking abacavir (Ziagen, also in the Epzicom and Trizivir coformulations), which could potentially help explain the higher rate of heart attacks seen in some studies. Others, however, did not see this effect, including the HEAT trial comparing Epzicom versus Truvada (the tenofovir/emtricitabine coformulation).

Overall, the most consistent find-

ing—regardless of drug regimen—is that ART *reduces* inflammatory and cardiovascular biomarkers compared with no treatment. Today, using antiretroviral drugs to reduce HIV viral load as much as possible is perhaps the quickest and most effective way to reduce inflammation and immune activation.

A NEW PARADIGM

At the 5th IAS Conference on HIV Pathogenesis, Treatment and Prevention in July 2009, Waafa El-Sadr from Columbia University, principle investigator with the SMART trial, discussed inflammation as a "new paradigm" for thinking about HIV pathogenesis.

A growing body of evidence indicates that the medical complications seen in people with HIV are attributable not only to the detrimental effects of the virus on the immune system, but also to the immune system's response to the virus.

Traditionally, the common understanding of the natural history of the disease included a long period of clinical latency, during which HIV was present in the body but had not yet killed enough CD4 cells to cause serious harm. But the idea that the virus has little impact on health during this period appears to have been a misperception.

"We have assumed that after somebody is infected they have...many years when they're infected but are actually doing well, or appear to be doing well," said El-Sadr. But research now shows that "during this long period when the person appears to be quite well, there are ongoing processes in their bodies—due to HIV itself—that may be causing some unseen damage that may ultimately have a very large impact on a person's survival and wellness."

While inflammation is currently a hot topic in HIV medicine, the idea that immune activation influences disease progression is not new. Researchers have long recognized that HIV triggers immune activation from the moment it first enters the body.

To review briefly, the virus primarily infects CD4 T-cells circulating in the blood and residing in lymphoid tissues such as the lining of the gut. It replicates rapidly during early infection, leading to high viral load. In addition, HIV integrates its genetic material into the DNA of long-lived latent host cells. Current antiretroviral drugs can suppress HIV in the blood, but cannot reach this sequestered provirus. Eventually, however, these cells can "wake up" and begin releasing infectious virus, which is why even fully suppressive ART does not eradicate HIV.

HIV antigens trigger an adaptive immune response, mainly by CD8 T-cells that target virus-infected cells for destruction. While this natural immune response typically lowers viral load temporarily after acute infection, it is not adequate to control HIV over the long term in the vast majority of people. But this chronic immune activation does seem to be enough to cause problems throughout the body over time.

Since the early years of the epidemic, researchers have reported that various inflammatory compounds are elevated in people with HIV, and that rising levels correlate with worsening disease. These include NF-kB; neopterin (a compound release by activated macrophages); pro-inflammatory cytokines such as IL-6 and TNF-alpha; and chemokines, signaling molecules that spur immune cell migration, or chemotaxis.

Soon after the advent of effective combination ART in the mid-1990s, researchers with the Multicenter AIDS Cohort Study (MACS) reported that shorter survival of people with HIV was more strongly associated with CD8 T-cell activation than with plasma viral load. And Hunt and others found that immune activation predicted poor CD4 cell recovery despite sustained viral suppression on therapy. A recent study described in the January 15, 2010, *Journal of Infectious Diseases* found that CD4 and CD8 T-cell activation even during early infection (median 225 days after seroconversion) predicted faster disease progression later on.

As treatment improved enough to allow most patients with good adher-

ence to maintain undetectable plasma viral load, experts began to express hope that sustained suppression of HIV replication could restore health and allow a normal life expectancy.

But the enthusiasm was tempered by growing concern about long-term adverse effects of treatment. By the turn of the millennium, people with HIV began to develop unexpected symptoms such as body fat changes, abnormal blood lipids, and insulin resistance. And as they lived longer, they experienced rising rates of cardiovascular disease, liver and kidney disease, and some non-AIDS cancers, exceeding those seen in the HIV negative general population.

Many blamed these complications on antiretroviral drugs, prompting a shift toward later ART initiation and exploration of structured treatment interruption. A large treatment interruption trial known as SMART would bring inflammation back to center stage.

LOOKING AT BIOMARKERS

SMART (Strategies for Management of Antiretroviral Therapy) enrolled more than 5,000 HIV positive adults at more than 300 sites worldwide starting in 2000. Participants started the study with a CD4 count above 350 cells/mm³, and were randomly assigned either to stay on continuous ART or to suspend treatment when their CD4 cell count was above this level, resuming when it fell below 250 cells/mm³.

The treatment interruption arm was discontinued ahead of schedule in January 2006 after an interim analysis showed that participants in that group had higher rates of illness and death than those receiving continuous therapy.

While the increase in OIs and AIDS-related death was not surprising—given that these people spent more time with lower CD4 counts—the investigators also found that participants who periodically stopped treatment had a higher rate of serious non-AIDS conditions, including heart, liver, and kidney disease. This was unexpected, since the researchers had hypothesized that people who spent

less time on therapy should have lower rates of complications attributed to antiretroviral drugs.

Presenting the data at the XVI International AIDS Conference the following summer, Jens Lundgren from the University of Copenhagen suggested there must be some "missing link" behind the excess risk of death among participants in the treatment interruption arm.

The SMART findings spurred an intensive search for an explanation, one that would usher in a new way of looking at HIV disease. The diversity of manifestations in this and other studies—ranging from heart and liver disease to cancer and neurocognitive impairment—suggests a common denominator underlying the "badness" resulting from chronic HIV infection even in the absence of advanced immune suppression.

This search led researchers to look at various biomarkers, or compounds in the blood that offer clues to inflammation, coagulation, and dysfunction of the endothelial cells lining the blood vessels (see sidebar on page 13 for a list of some commonly used biomarkers).

At the 2008 CROI, Lewis Kuller from the SMART team reported that elevated levels of the inflammation biomarker IL-6 and the coagulation marker D-dimer at study entry were strongly associated with increased cardiovascular and all-cause mortality; high-sensitivity CRP had a weaker correlation. IL-6 and D-dimer rose along with viral load after treatment interruption, while remaining stable in people on continuous therapy.

In a published report of these findings in *PLoS Medicine,* the investigators noted that people with the highest CRP values were twice as likely to die as those with the lowest levels. Those with the highest IL-6 levels had an eight-fold greater risk of death, while the highest D-dimer values were associated with a 12-fold increase. This pattern, the researchers wrote, "suggests that HIV infection results in activation of coagulation and inflammatory pathways that may impact multiple organs."

In follow-up reports, SMART investigators revealed that elevated biomarker levels in the treatment interruption group decreased but did not normalize after resuming therapy, and that the link between elevated baseline inflammation biomarkers and death persisted after more than two years of follow-up.

Another treatment interruption trial presented at CROI in 2008 also demonstrated a link between viral suppression and biomarkers of inflammation and endothelial dysfunction. Alexandra Calmy and colleagues measured blood biomarkers from 145 participants in STACCATO, the Swiss-Thai-Australia Treatment Interruption Trial. Compared with SMART, these participants had more advanced HIV disease, fewer traditional cardiovascular risk factors, and resumed therapy when their CD4 count fell below 350 rather than 250 cells/mm³.

After starting ART, levels of D-dimer, soluble vascular cell adhesion molecule 1 (VCAM-1), P-selectin, monocyte chemoattractant protein 1 (MCP-1), and leptin decreased as HIV was suppressed. Levels rose following treatment interruption, but did not fall back to baseline levels when therapy resumed. Conversely, anti-inflammatory biomarkers including IL-10 and adiponectin increased as viral load declined and fell during treatment breaks. In this cohort, however, there were no significant changes in IL-6 or CRP; because no participants experienced cardiovascular events or died, the researchers could not determine whether there was a link between biomarkers and clinical outcomes.

Another large trial looked at biomarker differences between people with and without HIV. Jacqueline Neuhaus and colleagues compared SMART participants versus HIV negative individuals in two large population-based cardiovascular studies, MESA (Multi-Ethnic Study of Atherosclerosis; 5,386 participants aged 45–76 years) and CARDIA (Coronary Artery Risk Development in Young Adults; 3,231 participants aged 33–44 years).

Overall, people with HIV had significantly higher levels of IL-6, high-sensitivity CRP, and D-dimer (all 50% to > 100% higher), as well as cystatin C, a marker of kidney impairment. Biomarker levels were higher among SMART participants both on and off ART relative to the HIV negative groups, and the pattern held after adjusting for traditional risk factors.

In the years since the initial SMART report, more and more studies have measured biomarkers of inflammation, coagulation, and endothelial dysfunction. In the January 15, 2010, *Journal of Infectious Diseases,* for example, Jason Baker and colleagues reported that inflammation is apparent in HIV positive people with CD4 cell counts high enough that they do not yet require treatment. Untreated people with HIV had IL-6, D-dimer, and soluble intercellular adhesion molecule-1 (ICAM-1) levels 65%–70% higher than those of uninfected individuals.

Inflammation is also evident in people on stable ART with consistently suppressed viral load. Michael Boger and colleagues recently reported that HIV positive people with undetectable HIV RNA and low Framingham cardiovascular risk scores still had elevated CRP; half, in fact, had CRP levels greater than 3 mg/dL, suggesting a high risk of future cardiovascular events despite an absence of traditional risk factors.

EFFECTS OF INFLAMMATION ON THE BODY

In order to understand the implications of ongoing inflammation in people with HIV, it is useful to look at some specific conditions in more depth. Much of the research associating inflammation with disease has been carried out in HIV negative populations, but can offer insights for HIV positive people as well.

AIDS AND CD4 RECOVERY

As noted above, research from early in the epidemic showed that immune activation markers were associated with

progression to AIDS, and a similar link has been demonstrated for inflammation biomarkers such as CRP. But correlation does not prove that inflammation causes disease progression or vice versa.

Relatively few studies have assessed the connection between inflammation and OIs. But a SMART analysis presented by Alison Rodger and colleagues at the 2009 CROI showed that elevated CRP and IL-6 levels predicted development of opportunistic disease as well as non-AIDS conditions, independent of CD4 cell count and HIV viral load.

In contrast, there is an extensive body of research looking at the hallmark of HIV infection, CD4 T-cell depletion. In 2008, Hunt and the UCSF team presented two studies at CROI, the first showing that among untreated individuals with chronic HIV infection, increasing T-cell immune activation (indicated by CD38/HLA-DR expression) was strongly correlated with falling CD4 counts. The second, looking at elite controllers with naturally suppressed viral load, found that greater CD4 and CD8 T-cell activation was again strongly associated with lower CD4 counts—even though the median remained close to 700 cells/mm³.

Studies have also consistently shown that T-cell activation increases the likelihood of poor CD4 cell recovery despite combination ART that fully suppresses plasma HIV replication, a phenomenon known as discordant response. Hunt and colleagues found this to be true both in their San Francisco cohort and in a group of patients starting ART in Uganda.

IMMUNOSENESCENCE

Chronic HIV infection is characterized by premature immunosenescence, or loss of immune cell function. T-cells and other leukocytes normally proliferate in response to pathogens, but cells have a built-in maximum number of divisions; once this limit is reached, they can no longer multiply in response to threats.

As Seema Desai and Alan Landay

explained in the February 2010 issue of *Current HIV/AIDS Reports,* ongoing immune activation and inflammation due to constant stimulation by HIV or other chronic infections accelerates the process of T-cell exhaustion that occurs during the normal course of aging. T-cell homeostasis—the balance between cell production and cell death—may also be disrupted by changes in cytokine levels and premature CD4 and CD8 cell apoptosis.

Reduced T-cell regeneration is thought to be attributable in part to atrophy or shrinkage of the thymus; large numbers of new T-cells mature in the thymus from late fetal life through puberty, but the organ is typically mostly inactive in older adults. In addition, other lymphoid organs and tissues that can produce T-cells undergo fibrosis, or replacement of functional cells with scar material.

Middle-aged people with HIV show evidence of immunosenescence resembling that of HIV negative individuals two decades older. This includes low naive-to-memory cell ratio, reversal of the normal CD4-to-CD8 cell ratio, reduced T-cell proliferation, more cells with apoptosis markers, and more exhausted immune cells expressing CD57 without CD28. Once it occurs, T-cell senescence fails to normalize even with suppressive ART.

ACCELERATED AGING AND FRAILTY

The immune system is not the only thing that ages prematurely in people with HIV. The types of chronic progressive conditions seen with greater frequency among HIV positive individuals—heart disease, cancers, kidney disease, bone loss, cognitive impairment—are the same ones that plague aging HIV negative people.

However, these conditions typically occur sooner in the setting of HIV disease, helping explain why HIV positive people as a group still do not achieve a normal lifespan. Various studies have shown, for example, that HIV positive people have blood vessel function resembling that of HIV negative coun-

terparts ten or more years older, while neurological activity is similar to that of people 15–20 years older.

As researchers in medical fields ranging from cardiology to oncology have discovered, all of these conditions are linked to inflammation, leading Claudio Franceschi of the University of Bologna to coin the term "inflamm-aging."

Numerous studies over many years have shown that elderly people (usually defined as age 65–70 or older) have elevated levels of inflammation biomarkers compared with younger individuals; among women, a more pro-inflammatory pattern emerges after menopause.

Elderly people not only are more prone to specific organ diseases, they also can experience "frailty" characterized by unintentional weight loss, exhaustion, weakness, motor slowness, and a low level of physical activity. The MACS investigators reported that people with HIV were about 5–10 times more likely to develop frailty than uninfected individuals of the same age, and studies in the HIV negative population have linked frailty to inflammation.

Given that half of HIV positive people in the U.S. will be over age 50 by 2015, age-related complications and their causes will increasingly be a central aspect of HIV management (see "HIV and Aging," *BETA,* Summer/Fall 2009).

CARDIOVASCULAR DISEASE

In the HIV negative general population, the role of inflammation has been most clearly demonstrated for cardiovascular disease. This link may be even stronger for people with HIV, who face the triple threat of increased inflammation, ART toxicities, and higher frequency of traditional risk factors.

Atherosclerosis ("hardening of the arteries") is a progressive process in which artery walls thicken and lose their elasticity as they fill up with plaques made up of accumulated lipids, immune cells, calcium, and scar tissue. Over time, this leads to impaired blood flow, which can ultimately result in a heart attack or stroke.

Over the past two decades, it has become increasingly clear that atherosclerosis is not just a matter of cholesterol passively building up in the arteries, but rather involves an active inflammatory process. This begins with damage to endothelial cells lining the blood vessels, which dilate and constrict the vessels to accommodate physiological demands. HIV proteins may directly contribute to endothelial dysfunction by altering cell-signaling pathways; CRP may also have a direct effect on endothelial function.

The earliest stage of plaque formation involves accumulation of "fatty streaks" of lipids including LDL cholesterol on the endothelial lining; this happens especially at artery forks or bifurcations where blood flow dynamics change. As lipids oxidize, they attract immune cells, primarily macrophages, which release pro-inflammatory signals. T-cells arrive, and in turn produce their own cytokines. The resulting inflammatory cascade releases "downstream" molecules such as CRP that can be measured in circulating blood.

The inflamed endothelial lining around these lesions expresses adhesion molecules—including VCAM-1, ICAM-1, and selectins—that enable leukocytes to move along and bind to the endothelial lining. Lipids and immune cells eventually breach this lining and build up in the intima, or innermost layer of the artery wall.

There, scavenger macrophages continue to ingest oxidized LDL, becoming foam cells. Signals released by activated immune cells promote fibrogenesis, or production of extracellular structural material such as collagen that forms a fibrous cap over the lipid core of a plaque; around the outer edges, plaques accumulate calcium and harden.

But other inflammatory chemicals act to weaken the cap, causing it to rupture. This triggers formation of blood clots (assisted by fibrinogen), furthering narrowing the artery. Clots are broken down by plasminogen, releasing D-dimer as a byproduct. Pieces of plaques or clots can break away and become lodged in smaller vessels, causing complete blockage.

As plaques build up in the coronary arteries supplying the heart muscle, they deliver less oxygen (known as ischemia), which can cause angina, or chest pains. Complete blockage of the coronary arteries leads to myocardial infarction, or heart attack. Blockage of the carotid arteries supplying the brain leads to strokes.

Inflammation helps explain the effects of traditional cardiovascular risk factors. Cigarette smoke, for example, is a toxin that promotes inflammation. Excess body fat produces pro-inflammatory compounds, as discussed above. LDL cholesterol builds up in artery walls and triggers inflammatory responses, while HDL cholesterol removes LDL from the arteries and has anti-inflammatory effects.

Several large general population studies have shown that circulating blood levels of compounds involved in atherosclerosis—including IL-6, VCAM-1, selectins, fibrinogen, D-dimer, and especially CRP—can predict future cardiovascular events. Not coincidentally, these are the same markers linked to cardiovascular events and death in SMART and other studies of people with HIV.

People with chronic HIV infection—even those on suppressive ART—are more likely than HIV negative individuals to have signs of endothelial dysfunction and subclinical atherosclerosis. These include decreased flow-mediated dilation (how well arteries respond to changes in blood flow), reduced arterial distensibility (ability to dilate), increased arterial stiffness, and greater intima-media thickness (IMT), or width of the artery wall.

At this year's CROI, Hsue's group reported that HIV positive people in the San Francisco SCOPE cohort experienced more rapid atherosclerosis progression than HIV negative individuals over two years, as determined by measuring IMT at the bifurcation of the carotid artery. This was seen in people with undetectable viral load on ART and even elite controllers. IMT progression was associated with inflammation as indicated by elevated CRP levels.

When the investigators looked at flow-mediated dilation of the brachial (upper arm) artery, they again found that HIV positive people had significantly worse measurements than uninfected people. Elevated CRP was a stronger predictor of impaired dilation than older age in the HIV positive group, while the opposite was true in the HIV negative group.

Robert Kaplan and colleagues reported at CROI that increased carotid artery IMT and reduced distensibility correlated with greater CD4 and CD8 cell activation and T-cell senescence among HIV positive participants (but not among HIV negative women) in the Women's Interagency HIV Study.

What about clinical events? In the July 2009 *Journal of Acquired Immune Deficiency Syndromes,* Virginia Triant and colleagues reported that in a retrospective analysis of patients seen at Massachusetts General and Brigham and Women's Hospitals in Boston, HIV infection and elevated CRP both more than doubled the risk of acute myocardial infarction. These factors had an additive effect, so HIV positive people with high CRP had a four-fold higher risk relative to HIV negative people with normal CRP.

Summing up her research, Hsue said these studies support the "common theme that independent of drug therapy, independent of viremia and viral load, there is some kind of badness associated with HIV infection in terms of cardiovascular risk."

LIVER DISEASE

Liver disease has become a major cause of illness and death in people with HIV in the combination ART era. This is often due to hepatitis B or C coinfection, but drug toxicity may also play a role. Viral hepatitis, drug toxicity, heavy alcohol use, and other causes of injury promote liver inflammation signaled by elevated levels of

alanine transaminase (ALT) and other liver enzymes.

Over time, chronic liver inflammation can lead to fibrosis and cirrhosis, as the liver attempts to heal itself by producing extracellular scar material. The associated altered chemical signaling and oxidative stress can promote development of hepatocellular carcinoma, a form of liver cancer.

The impact of systemic immune activation and persistently elevated pro-inflammatory cytokines on liver disease is not fully understood. TNF-alpha and IL-6, for example, appear to have paradoxical effects on the liver, promoting some types of liver injury while protecting against others.

Like HIV, HBV and HCV can directly trigger immune activation and alter cytokine signaling. Stepped-up immune activation from fighting two viruses at once may help explain the more rapid liver fibrosis progression—and possibly worse HIV disease progression—seen among HIV/HCV and HIV/HBV coinfected people.

Liver disease was one of the non-AIDS conditions that increased among participants undergoing treatment interruption in the SMART trial. HIV/HBV or HIV/HCV coinfected people had a higher rate of death due to non-opportunistic causes even after controlling for liver cancer.

At this year's CROI, Lars Peters and colleagues showed that elevated hyaluronic acid (a component of extracellular material used as a biomarker for liver fibrosis) was associated with higher levels of CRP, IL-6, and D-dimer in HIV/HBV and HIV/HCV coinfected SMART participants, predicting a four- to six-fold increase in the risk of non-AIDS death. They suggested that coinfected patients with impaired liver function are "particularly in a pro-inflammatory state" that might be exacerbated by ART interruption.

KIDNEY DISEASE

People with HIV are susceptible to HIV-associated nephropathy (a form of chronic kidney failure) as well as kidney toxicity due to certain antiretroviral drugs (see "Renal Complications of HIV/AIDS," *BETA,* Summer 2007). Kidney disease was also among the non-AIDS conditions that increased during ART interruption in SMART.

Kidney disease plays a role in the nexus of metabolic abnormalities linked to inflammation. Studies have shown that compounds involved in inflammation, coagulation, and endothelial dysfunction (including CRP, cell adhesion molecules, MCP-1, and fibrinogen) are elevated in people with kidney impairment.

As Dena Rifkin and Michael Sarnak explained in the April 2009 *American Journal of Kidney Disease,* glomerulosclerosis—the hardening of capillary webs that filter blood in the kidneys—in many ways resembles atherosclerosis, featuring accumulation of cholesterol, migration of monocytes, production of lipid-laden macrophages, and fibrosis, suggesting that the same inflammatory processes are likely involved.

COGNITIVE IMPAIRMENT

Despite widespread ART use, people with HIV continue to develop a spectrum of cognitive, motor, and psychological manifestations ranging from asymptomatic neurocognitive impairment to HIV dementia (see "HIV and the Brain," *BETA,* Summer/Fall 2009). As with other non-AIDS conditions, neurocognitive impairment improves with ART, but does not completely normalize.

HIV enters the brain soon after initial infection, hiding in macrophages to cross the protective blood-brain barrier. Within the central nervous system (CNS), the virus does not infect neurons (the cells that transmit electrical impulses responsible for thought and movement), but mainly targets specialized brain macrophages called microglia. Once inside these long-lived cells, it can remain latent for an extended period.

HIV typically does not directly kill brain cells in large numbers, but rather sets off a cascade of damaging inflammatory changes; in addition, HIV Tat and gp120 proteins have direct neuro-

toxic effects. Immune cells activated to fight the virus produce pro-inflammatory cytokines such as TNF-alpha and interferons, which activate astrocytes (brain support cells) and attract more leukocytes. This inflammatory cascade disrupts cellular communication channels and leads to oxidative stress.

The extent of the inflammatory response in the brain does not appear to closely correspond to the amount of HIV present; even residual virus in people on suppressive ART may be enough to sustain a self-perpetuating inflammatory response. In addition to the direct effects of chemicals in the brain, broader metabolic abnormalities and vascular changes linked to inflammation also contribute to cognitive impairment.

It is increasingly clear that these factors also play a role in Alzheimer's disease and other types of age-related cognitive decline. This suggests that as HIV positive people age, they may be prone to additive neurocognitive impairment due to multiple processes.

CANCER

Several large studies indicate that people with HIV have higher rates of cancers not traditionally considered AIDS-related. Some, including anal cancer and liver cancer, have known infectious causes, but others do not. While cancer risk increases with lower CD4 counts, there appears to be excess risk even among people with well-preserved immune function.

Outside the HIV field there has been extensive research looking at the link between immune activation, inflammation, and cancer. In June 2004, the U.S. National Cancer Institute (NCI) held a meeting on inflammation and cancer, one of a series of cancer biology think tanks.

"It is generally accepted that chronic inflammation—triggered by toxins, microbes, or autoimmune reactions—plays a major role as a tumor promoter," the participating experts summarized.

The immune system fights disease that originates within the body as well

as invasions coming from outside. CD8 T-cells and natural killer cells maintain surveillance of cells throughout the body and destroy those that show signs of abnormal growth, or malignant transformation. But the signaling cascades that direct the normal processes of cell growth and tissue repair can go awry, resulting in uncontrolled cell proliferation and invasion of healthy tissue.

Initially, inflammation contributes to cancer development by causing oxidative stress and DNA damage. Over time, chronic inflammation appears to promote cancer cell survival and proliferation. In another vicious cycle, tumor cells produce compounds that attract immune cells, which in turn secrete chemicals that stimulate further cancer proliferation. In addition, cancer cells can co-opt chemokines, cell adhesion molecules, and other inflammatory mediators (intermediate chemicals) to facilitate their migration.

Several large general population studies have linked a variety of cancers to elevated levels of inflammation markers, including CRP. Since the SMART trial, investigators have started to search for these associations in people with HIV, and evidence to date suggests a similar link.

MORTALITY

Finally, chronic inflammation has been shown to have an effect on mortality itself, as evidenced by studies presented at this year's CROI. Hunt's team reported that in their study of people starting ART in Uganda, patients with greater CD8 T-cell activation before starting treatment had shorter survival times than those with less activation, despite good HIV viral load suppression.

Phyllis Tien and fellow investigators looked at the relationship between biomarkers and mortality among more than 900 participants in the FRAM Study (Fat Redistribution and Metabolic Change in HIV Infection) over five years. After adjusting for HIV status and traditional cardiovascular risk factors, baseline levels of both CRP and fi-

brinogen were strongly associated with death during follow-up. "Our findings suggest that even in those with apparent restoration of CD4 cells, inflammation remains an important risk factor for mortality," they concluded.

Investigators with the INSIGHT collaboration looked for a similar association among participants in the FIRST study, which compared three types of first-line antiretroviral regimens in people with advanced HIV/AIDS. Participants who progressed to AIDS, immune reconstitution inflammatory syndrome (IRIS), or death within 12 months of starting ART had higher baseline biomarker levels. "Elevated levels of biomarkers associated with inflammation (CRP, IL-6), coagulation (D-dimer), and tissue fibrosis (hyaluronic acid) in ART-naive patients who have good virologic response may be useful in identifying patients at risk for AIDS or death during the first year of ART," the researchers concluded.

MANAGING INFLAMMATION

The increased focus on inflammation and its resulting "badness" in people with HIV has spurred a search for management strategies, dovetailing with ongoing work in other fields of medicine. This is no simple task, however, and there is a risk of unintended consequences when interfering with complex cell-signaling pathways that are not fully understood.

The original focus of HIV medicine was on managing OIs and simply keeping people alive; then, during the early combination ART era, it shifted to suppressing HIV replication and managing drug side effects and complications. Today, the emphasis is on improving overall health and enabling people with HIV to live as long as their HIV negative counterparts.

Approaches for managing inflammation generally fall into three broad areas: reducing T-cell activation, altering production or activity of cytokines and inflammatory mediators, and changing underlying risk factors through lifestyle modification.

OPTIMIZING ART

Combination ART is today the most effective strategy for reducing excessive immune activation and inflammation. Numerous studies have shown that suppressing HIV viral load decreases the number of activated T-cells and reduces blood levels of several inflammation biomarkers; conversely, stopping treatment can worsen inflammation.

Inflammation and its consequences are worse in individuals who sustain more extensive immune system damage before starting ART. This adds to the growing body of evidence supporting earlier therapy, which last year led U.S. and European experts to raise the threshold for ART initiation in their treatment guidelines (see "When to Start HIV Treatment: A Changing Equation" BETA, Summer 2008).

But while T-cell activation and inflammation typically decrease after ART initiation, they do not normalize completely. According to Deeks, it remains to be seen whether this might be achieved with very early treatment while immune function is still mostly intact, a prospect being explored in the START trial (see "Open Clinical Trials," BETA, Summer/Fall 2009).

If current ART decreases inflammation somewhat, might better or additional antiretroviral drugs reduce it more? Several recent studies have explored this type of treatment intensification.

One study presented at this year's CROI found that adding the integrase inhibitor raltegravir (Isentress) to an existing suppressive ART regimen normalized CD8 T-cell activation in the blood. Another observed a modest (but highly variable) decrease in CD8 cell activation in the blood and other sites, while a third saw no significant effects on CD4 or CD8 T-cell activation in the blood or gut lymphoid tissue. (Better results were seen with maraviroc intensification, as described below).

Experts agree that drawbacks due to potential pro-inflammatory effects of antiretroviral drugs related to metabolic abnormalities are far overshadowed by the overall benefits of ART in reducing

viral load and increasing CD4 count.

However, regimen choice can make a difference for some patients. As a class, protease inhibitors cause more metabolic changes linked to inflammation and cardiovascular risk than NNRTIs. On the other hand, protease inhibitors also tend to produce larger CD4 cell gains, which may be particularly important for people with high-level immune activation.

Among the protease inhibitors, atazanavir (Reyataz) causes fewer lipid abnormalities. But in one small study, people who switched from their current protease inhibitor to atazanavir experienced no change in levels of CRP or D-dimer, even though they saw the expected lipid reductions.

CORECEPTOR BLOCKERS

One antiretroviral class, the CCR5 antagonists—currently consisting of a single approved drug, maraviroc (Selzentry)—may have additional anti-inflammatory effects.

HIV can use two different cell surface coreceptors, CCR5 and CXCR4, to enter CD4 T-cells. CCR5 antagonists are designed to block one of these gateways. In addition to acting as a coreceptor for HIV, CCR5 is also a chemokine receptor that plays a role in immune cell signaling.

CCR5's role in immune response is not fully understood, but some clues come from people with a natural genetic variant known as CCR5 delta-32. People with two copies of this variation are resistant to HIV infection and, if they do become infected, may be elite controllers; those with one copy are typically slow progressors. However, people with this mutation appear more susceptible to West Nile virus and may experience worse progression of hepatitis C and rheumatoid arthritis.

Clinical trials of maraviroc have found that while its ability to suppress HIV was no better than that of other standard-of-care drugs, it did appear to produce larger CD4 cell gains in both treatment-experienced and treatment-naive patients. Even some people with

CXCR4-tropic HIV who did not benefit from viral suppression showed CD4 cell increases. Given its apparent influence on immune function, researchers asked whether using maraviroc might reduce immune activation and inflammation.

At the 2009 CROI, Nick Funderburg and colleagues reported data from a subanalysis of the treatment-naive MERIT trial showing that both maraviroc and efavirenz recipients experienced decreases in CD4 and CD8 T-cell activation and levels of IL-6 and D-dimer, but the maraviroc group had a drop in CRP while the efavirenz group saw a gain.

At this year's conference, two teams reported that maraviroc treatment intensification reduced immune activation. In the ACTG 5256 pilot trial, 34 participants with suboptimal CD4 cell recovery despite a year on suppressive ART added maraviroc to their existing regimen. After 24 weeks, they showed decreased CD4 and CD8 T-cell activation; they did not, however, experience a significant CD4 cell gain.

A European maraviroc intensification study of nine participants with suppressed viral load for two years and CD4 counts above 350 cells/mm^3 found a significant decrease in CD4 T-cell activation at week 12, but the reduction in CD8 cell activation did not reach statistical significance. Another study found that maraviroc intensification did not reduce CD4 or CD8 cell activation in gut lymphoid tissue.

Looking at a different type of inflammatory measure, researchers at Sapienza University in Rome reported that in a laboratory study, maraviroc downregulated the chemotactic activity of neutrophils, dendritic cells, and macrophages taken from healthy HIV negative donors, thereby inhibiting immune cell migration.

Other chemokine receptors involved in inflammatory pathways might also be modifiable. At CROI, Calvin Cohen presented the first data on Tobira Therapeutics' investigational drug TBR-652, which blocks both CCR5 and CCR2. In a pilot trial with

54 participants, TBR-652 demonstrated potent anti-HIV activity and increased levels of MCP-1 (demonstrating CCR2 activity), and was generally well-tolerated, positioning it for Phase 2b trials.

Found on monocytes, dendritic cells, and some T-cells, CCR2 naturally binds with MCP-1, promoting monocyte migration into tissues; it plays a role in inflammatory diseases ranging from atherosclerosis to rheumatoid arthritis. As with CCR5, however, immunological processes involving CCR2 are not fully understood, and interference might have unexpected negative effects.

ANTI-INFLAMMATORY THERAPIES

Multiple drug classes have anti-inflammatory effects, distinguished by what aspect of the immune response they target.

Nonsteroidal anti-inflammatory drugs (NSAIDs) include aspirin and ibuprofen as well as the stronger COX-2 inhibitors, of which celecoxib (Celebrex) is the only one currently available in the U.S. These agents stop the release of prostaglandins during early stages of immune response, reducing pain, swelling, and fever.

The COX-2 inhibitors demonstrate the risk of interfering with cell-signaling pathways. In addition to inhibiting release of prostaglandins, the drugs are now thought to have a similar effect on another mediator molecule that helps prevent blood clotting. The widely used COX-2 inhibitor rofecoxib (Vioxx) was withdrawn from the market in 2004 after studies showed it increased the risk of heart attacks and strokes; another, valdecoxib (Bextra), was likewise withdrawn the following year.

Other anti-inflammatory agents work by altering the synthesis, secretion, or activity of various cytokines. For example, chloroquine—best known as a treatment for malaria—inhibits release of the pro-inflammatory cytokine IL-6 in response to bacterial LPS, while also promoting production of the anti-inflammatory IL-10. A clinical trial looking at the drug's effects on CD4 and CD8 T-cell activation in people with HIV is currently recruiting (see

"Open Clinical Trials," page 52).

Pentoxifylline (Trental), which inhibits TNF-alpha and also appears to have other immune-modulating effects, is currently under study for reducing endothelial dysfunction in people with HIV, both on and off ART.

At the 2009 International Workshop on Adverse Drug Reactions and Co-Morbidities in HIV, investigators reported that salsalate, an inhibitor of the NF-kB pathway, did not improve endothelial function in HIV positive patients not taking ART, although other studies have shown it did so in HIV negative people; furthermore, it raised levels of IL-6 and D-dimer.

A different approach involves monoclonal antibodies that act against pro-inflammatory cytokines. Approved agents of this type include adalimumab (Humira) and infliximab (Remicade), both of which target TNF-alpha and are used to treat autoimmune conditions including rheumatoid arthritis and Crohn's disease.

Other anti-inflammatory agents that have been evaluated for people with HIV include the TNF-alpha antagonists etanercept (Enbrel, approved to treat autoimmune conditions) and CPI-1189 (under study for HIV-related neurocognitive impairment); the platelet-activating factor antagonist lexipafant (also for neurocognitive impairment); the matrix metalloproteinase inhibitor CTS-1027 (also being studied with interferon alpha in people with hepatitis C); and theophylline (most widely used as a treatment for asthma).

Because the inflammatory cascade is so complex, it might take a combination of multiple agents with different mechanisms to achieve the desired effect. Furthermore, some of the compounds that are elevated in the blood during inflammation are not the underlying cause, so therapies aimed solely at reducing biomarker levels are unlikely to be effective.

IMMUNE SUPPRESSANTS

Drugs with stronger immune-modulating effects are typically classified as immunosuppressants rather than anti-inflammatory agents, even though some of them work by similar mechanisms. Cyclosporine, for example, interferes with T-cell production and interleukin release.

Imunosuppressants are typically used to prevent organ rejection after a transplant or to manage autoimmune diseases. They include corticosteroids such as prednisone, as well as a variety of other agents, including mycophenolate, sirolimus (also known as rapamycin), and tacrolimus. Again, some are monoclonal antibodies; daclizumab (Zenapax), for example, targets IL-2, which is important for T-cell production.

In 2002, Italian researchers reported results of a small study showing that people with primary HIV infection who were treated with ART plus cyclosporine for eight weeks demonstrated reduced T-cell activation. More recently, however, Martin Markowitz from the Aaron Diamond AIDS Research Center and colleagues reported that adding cyclosporine to ART during acute or early infection did not reduce immune activation or improve CD4 counts. Likewise, the ACTG 5138 trial found that cyclosporine plus ART for two weeks provided no sustained immunologic benefit for people with chronic HIV.

Needless to say, caution is warranted when using immunosuppressant drugs in people with a disease characterized by both immune deficiency and excessive immune activation. Even in HIV negative people, these drugs can suppress immune function enough to cause OIs, and the risk is even higher for HIV positive people.

STATINS

Statins (also known as HMG-CoA reductase inhibitors) are an example of drugs primarily used to treat other conditions that also have anti-inflammatory "side effects." Developed and marketed as lipid-lowering agents, they significantly reduce the risk of atherosclerosis and cardiovascular events including heart attacks.

Researchers recently demonstrated that statins can also have beneficial effects for people without elevated lipids. The large JUPITER trial followed nearly 18,000 participants age 50 years or older in 26 countries who had normal cholesterol levels but elevated CRP. After about two years of follow-up, people taking rosuvastatin (Crestor) had close to a 50% reduction in the rate of myocardial infarction, stroke, or death due to cardiovascular events, suggesting an anti-inflammatory effect.

After JUPITER, some experts proposed that everyone should have their CRP measured and start statin therapy if it was elevated. Currently, however, the American Heart Association and Centers for Disease Control and Prevention (CDC) recommend CRP monitoring only for people with moderate cardiovascular risk, for whom knowing their CRP level might influence treatment decisions.

Statins have been successfully tested in HIV positive people for the purpose of lowering lipids and reducing the risk of cardiovascular disease. Some antiretroviral drugs can interact with statins because they are metabolized by the same CYP450 liver enzymes. The older drugs lovastatin (Mevacor) and simvastatin (Zocor) are contraindicated with protease inhibitors, while rosuvastatin and pravastatin (Pravachol) are preferred.

The anti-inflammatory action of statins is thought be related to interference with T-cell activity (among other mechanisms), suggesting they may be useful for decreasing immune activation and inflammation in people with HIV; research to evaluate this prospect is currently underway.

However, a report published in the March 2010 issue of the *Journal of Leukocyte Biology* revealed that simvastatin appeared to suppress macrophage activity against bacteria in laboratory studies and in mice, potentially increasing the risk of infections—again illustrating the potential for unintended outcomes.

TREATMENT OF COINFECTIONS

Given the effect of coinfections on immune activation in people with HIV, treatment to control other pathogens could have benefits with regard to inflammation.

Hunt and colleagues, having previously observed that HIV positive people with a high percentage of activated CD8 T-cells are usually coinfected with CMV, designed a small pilot study to test whether daily treatment with the anti-herpes drug valganciclovir might reduce activation. Looking at 30 CMV antibody-positive patients with poor CD4 cell recovery after more than a year on ART, the researchers found that valganciclovir for eight weeks led to undetectable CMV viral load and significantly decreased CD8 T-cell activation; inflammation biomarker levels were similar overall in the valganciclovir and placebo arms, however, as were CD4 cell counts.

"CMV (and possibly other herpesviruses) appears to be a major determinant of CD8 T-cell activation during antiretroviral therapy," the investigators concluded. "Given the potential impact of inflammation and immune activation on clinical outcomes, and the potential role of CMV in cardiovascular disease, T-cell senescence, and aging, strategies to reduce CMV replication in HIV-infected individuals are worth pursuing in larger trials."

Treatment of chronic HBV and HCV infection are other potential avenues. Both hepatitis B and C can be treated with interferon-alpha—a manufactured version of a natural pro-inflammatory cytokine—which promotes a stronger antiviral immune response.

Hepatitis B is more commonly treated with directly targeted anti-HBV drugs. HIV positive people are routinely tested for HBV, and treatment is fairly easily accomplished in HIV/HBV coinfected people given that tenofovir, 3TC (lamivudine; Epivir), and emtricitabine (Emtriva) are effective against both viruses.

In contrast, many HIV positive people—assuming they are not at risk—have never been tested for HCV, and interferon-based therapy has difficult side effects, leading to low treatment rates for HIV/HCV coinfected patients. New directly targeted anti-HCV agents currently in the pipeline may be more effective and better tolerated. The most advanced of these—the HCV protease inhibitor telaprevir—is now starting trials in coinfected individuals (see "Open Clinical Trials," page 53).

Turning to escaped gut bacteria, effective ART does appear to improve the health of the gut and reduce microbial translocation, though not completely. Researchers have also explored other therapies, including bovine colostrum (fluid produced by cows prior to milk), which may improve gut immune function; sevelamer, a phosphate-binding agent that also appears to bind to bacterial LPS; and toll-like receptor antagonists that interfere with LPS binding to receptors that trigger immune response.

LIFESTYLE MODIFICATION

Given that inflammatory response and immune activation are so closely intertwined with metabolism, it is not surprising that lifestyle changes that improve metabolic status can help decrease chronic systemic inflammation.

Cigarette smoking has pro-inflammatory as well as carcinogenic effects (see "Smoking and Your Health: How to Quit (and Why You Should)," *BETA*, Winter 2008). Smoking increases production of inflammatory mediators and generates reactive oxygen forms. Conversely, smoking cessation reduces inflammation. A study published last year found that HIV negative women who stopped smoking showed decreased levels of CRP, IL-6, TNF-alpha, and VCAM-1 within weeks after quitting.

Obesity, too, contributes to inflammation. Achieving and maintaining a healthy weight is beneficial, but the composition of the diet is also important, as some foods promote inflammation while others dampen it. Total calories consumed is also an important consideration. Studies in animals have shown that drastic calorie restriction is associated with reduced inflammation and apparent slowing of the aging process, including immunosenescence.

Foods and supplements that act as antioxidants—including vitamin C, vitamin E, and glutathione—may help prevent or reverse some of the damaging effect of inflammation on cells and tissues. Omega-3 fatty acids have immune-modulating and anticoagulant properties; studies have shown that omega-3s (and one good source, fish oil) reduce the risk of clinical events related to cardiovascular disease in both HIV positive and HIV negative people.

Many immune cells carry receptors for vitamin D, which triggers a mix of pro-inflammatory and immunosuppressive effects. Vitamin D deficiency has been linked to inflammatory conditions including cardiovascular disease; and at the recent CROI, researchers from the U.S., Europe, and Africa reported that vitamin D deficiency was widespread among people with HIV (see "News Briefs," page 5).

Other supplements with potential anti-inflammatory properties include probiotics (organisms such as *Lactobacillus* that improve the health of the gut and may also have more direct anti-inflammatory effects), curcumin (a component of the spice turmeric), and plant flavonoids such as resveratrol (a compound in red wine).

Exercise has anti-inflammatory effects, too, though it is not clear how much this is due to fat loss and metabolic improvements. A recent U.K. study showed that over three years, HIV negative people who were lean and physically fit had significantly lower levels of CRP and IL-6 compared with overweight unfit participants.

Exercise does not have to be strenuous to be beneficial. A recent study by researchers at Ohio State University showed that HIV negative women who practiced yoga regularly had lower blood levels of IL-6. The benefits of yoga may also come from stress reduction; the study found that

women who did yoga experienced smaller IL-6 increases when subject to stress than non-practitioners.

Several studies have linked chronic stress and elevated levels of inflammation biomarkers in HIV negative individuals. At the 2009 IAS meeting, Carmina Fumaz and colleagues reported similar findings from a small study of people with HIV. Looking at 21 mostly male participants on ART with undetectable viral load for at least one year, individuals facing greater stress (determined using the standardized Perceived Stress Scale) had significantly higher levels of IL-6 and TNF-alpha.

Adequate sleep also plays an important role in overall good health, and even modest sleep deprivation can adversely affect cytokine levels. One study, for example, found that sleep restriction in a controlled experiment led to 40%–60% average increases in IL-6 and 20%–30% increases in TNF-alpha.

CONCLUSION

Inflammation may prove to be the key that unlocks some of the mysteries of HIV disease, and advances in the HIV/AIDS field can contribute to the development of anti-inflammatory therapies that will also benefit people with a host of other diseases. The state of knowledge about the role of inflammation in the pathogenesis of HIV disease and non-AIDS conditions has advanced remarkably in just a few years, but much remains to be learned.

Researchers have already begun routinely collecting data about biomarkers and other indicators of inflammation and immune activation in their studies, and clinicians are starting to think about how such measures could be applied in real-world patient care.

Today, the state-of-the-art approach to managing inflammation, excessive immune activation, and their consequences is optimized ART that durably suppresses viral load and

aggressive management of modifiable risk factors using both medical therapy and lifestyle changes.

But numerous investigators—both within the HIV field and in other areas—are exploring countless potential anti-inflammatory therapies, including repurposing agents usually used for other indications.

"I think people should really be creative and look at what's on the shelf, and what's been used before and discarded, and really try to go outside the box thinking of other drugs that we haven't thought of as anti-HIV drugs," El-Sadr said in her IAS presentation last summer.

As Don Smith of the Albion Street Centre in Sydney explained in an editorial in the *Journal of HIV Therapy* inflammation issue, it has long been recognized that HIV disease is characterized by a mix of immune suppression and immune activation. To date, the emphasis has been on fixing the suppressive side, because this has caused most short-term disease and death. But with improved antiretroviral treatment and longer survival, the focus has shifted.

"While our patients are predominantly controlling HIV replication and have heartening recoveries in CD4 cell counts, we might think that the battle is over," he cautioned. "However, we have succeeded in only one part of a dual-faceted disease."

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