

# HIV-INDUCED IMMUNE ACTIVATION – PATHOGENESIS AND CLINICAL RELEVANCE

SUMMARY OF A WORKSHOP ORGANIZED BY THE GERMAN AIDS SOCIETY (DAIG E.V.)  
AND THE ICH HAMBURG

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H. J. Stellbrink, S. Baldus, G. Behrens, J. R. Bogner, T. Harrer, C. Hoffmann, J. van Lunzen, J. Münch, P. Racz, C. Scheller, M. Stoll, K. Tenner-Racz, J. Rockstroh

## Abstract

This manuscript is communicated by the German AIDS Society (DAIG) ([www.daignet.de](http://www.daignet.de)). It summarizes a series of presentations and discussions during a workshop on immune activation due to HIV infection. The workshop was held on November 22<sup>nd</sup> 2008 in Hamburg, Germany. It was organized by the ICH Hamburg under the auspices of the German AIDS Society (DAIG e.V.).

## BACKGROUND

HIV infection is characterized by progressive CD4<sup>+</sup> T-cell depletion and chronic immune activation, resulting in polyclonal hypergammaglobulinaemia as well as increased turnover and apoptosis rates of T-cells [1-11].

Immune activation *per se* is a physiological response. The question has to be raised, however, if the persistence of HIV-induced immune activation over many years actually overstretches regenerative and homeostatic mechanisms, which are driven by increased cell turnover, and thereby leads to immune exhaustion.

Highly active antiretroviral therapy (HAART) reduces immune activation via a reduction of viral replication. It is as yet unclear, however, if it can be continued safely and effectively for decades in order to achieve a normal life expectancy. Furthermore, some patients retain elevated levels of immune activation despite successful HAART. A detailed analysis of the mechanisms and its consequences could therefore reveal important novel complementary approaches to HIV therapy, which could help to overcome the limitations of current therapies.

„IMMUNE ACTIVATION AND IMMUNE FUNCTION“  
(C. Scheller, Würzburg)

The immune system is based on innate and adaptive immune responses. Innate responses are older in evolution and are characterized both by immune cells such as macrophages and natural killer cells recogniz-

ing conserved structures of microorganisms, and the release of cytokines. Adaptive immunity comprises B-cell- and T-cell-mediated responses. Approximately 10<sup>12</sup> different binding specificities of cell surface receptors induce activation and differentiation to effector cells following antigen contact. A fraction of effector cells returns to a deactivated, resting „memory“ state within a couple of days, from which they can be awakened very quickly upon reexposure to the same antigen („acquired immunity“). The other activated effector cells then proceed to fulfil their function and undergo apoptosis after 2-3 weeks. Apoptosis is the natural consequence of activation. The absence of programmed cell death would lead to the accumulation of senescent, non-functional effector cells in the sense of a „super-leukemia“.

## - Chronic immune activation

Most infectious agents are cleared by the immune system after days to weeks. Some are not eradicated but controlled to the extent of a latent, clinically stable phase, and immune activation is reduced subsequently. In HIV infection, however, it persists. Recent studies suggest several reasons. CD4<sup>+</sup> T-cells are massively depleted from the gastrointestinal lymphatic tissue (GALT) during acute HIV infection, but also in later stages of the infection [12, 13]. This affects mainly the effector site (lamina propria), less so the inductive site (Peyer's plaques) [14]. It is estimated that half of the CD4<sup>+</sup> T-cells of the body reside in the GALT. They display the „memory“ phenotype and express CCR5, the dominant coreceptor of HIV in the early phase of infection.

The conditions for the first peak of virus replication are ideal within the gastrointestinal tract. As early as several days following infection, most of the cells are infected and succumb to the early burst of replication, most likely due to the viral cytopathic effect. This probably leads to an irreversible loss of a large proportion of the memory CD4<sup>+</sup> T-cell pool. Studies in subjects on antiretroviral therapy show that the number of CD4<sup>+</sup> T-cells in the GALT does not return to

normal even after years of successful treatment [15]. This feature of HIV infection, however, is also observed in non-pathogenic animal models, i.e. monkeys infected by SIV variants that do not cause disease in their natural host [16, 17] such as sooty mangabeys and African green monkeys.

Severe gastrointestinal CD4<sup>+</sup> T-cell depletion is subsequently associated with the translocation of microbial antigens from the gut lumen into the host tissue, leading to activation of innate and adaptive immune responses. Depending on the stage of HIV infection, levels of lipopolysaccharides (LPS) in the serum are increased, which stem from gram-negative bacteria in the gut [4]. Sooty mangabeys and African green monkeys, however, exhibit no increase of LPS translocation and immune activation. During anti-retroviral therapy LPS levels are reduced, but do not reach the levels of normal controls.

Moreover, as a consequence of the development of HIV-specific T-cell responses, stimulation and expansion of CD4<sup>+</sup> T-cells continuously provides new target-cells for HIV replication. The continuation of this state over many years apparently overstretches the regenerative capacities of the immune system, and AIDS develops.

#### *- Immune senescence*

Like all cells of the body, the immune system underlies ageing. In the process of T-cell receptor rearrangement during T-cell maturation in the thymus, excised T-cell receptor DNA persists in the cells (T-cell-receptor excision circles, or TRECs). As TRECs are not duplicated during mitosis, the content of TRECs in the blood is diluted with ongoing cell proliferation. Thus, TREC content reflects both thymic output and preceding rounds of T-cell proliferation with an accumulation of "older" T-cells. HIV infection leads to a shift in the average "immunological age" of peripheral blood T-cells: TREC content is markedly reduced in T-cells during HIV infection, as compared with an uninfected person. Telomere shortening during cell division serves as another marker of cell senescence. Both impaired thymic T-cell generation as well as chronic immune activation resulting in increased numbers of terminally differentiated T-cells probably contribute to this phenomenon and result in severe T-cell lymphopenia in the course of progressive AIDS.

In addition to immune senescence, HIV infection leads to release of inflammatory and pro-inflammatory cytokines that initiate mechanisms known from physiological ageing, such as osteoporosis, arteriosclerosis, and HIV dementia („inflammaging“).

#### THE ROLE OF VIRAL PROTEINS, OR: WHAT CAN WE LEARN FROM MONKEYS ? (J. Münch, Ulm)

The AIDS epidemic is clearly recognized as a viral zoonosis [18]. More than 30 primate species in Africa have been shown to be infected with simian immunodeficiency viruses (SIV). The causative agent of AIDS, HIV-1, arose by transmission of simian immunodeficiency virus (SIV) from a subspecies of chimpanzees (SIVcpz) into the human population in the first half

of the last century [19-21]. HIV-2, that is less pathogenic in humans than HIV-1, has its closest ancestors in SIVs found in sooty mangabeys (SIVsm) [22]. Interestingly, almost all SIVs do not cause immunodeficiency or AIDS like symptoms in their natural African primate host despite high viral loads in some species. The only exception to date is SIVcpz that has recently been demonstrated to be associated with an increased mortality and AIDS-like immunopathology in wild SIVcpz infected chimpanzees [23]. Thus, HIV-1 and its ancestors from chimpanzees cause immune dysfunction whereas HIV-2 and remaining SIVs are less or non-pathogenic in their respective hosts [24]. Elucidating the differences in pathogenic versus non-pathogenic infection in humans and monkeys is thus a key to understand AIDS pathogenesis, it has become clear that one major difference to non-pathogenic SIV infection is the chronic generalized activation of the immune system in progressive AIDS in humans. Hyper-immune activation causes increased T-cell turnover and might ultimately result in the loss of CD4<sup>+</sup> T-cells, the hallmark of AIDS. High plasma viremia, a viral cytopathic effect for CD4<sup>+</sup> T-cells, and the early depletion of CD4<sup>+</sup> T-cells from the gastrointestinal tract occur similarly in both the pathogenic and the apathogenic condition.

#### *- HIV-1 Nef – a master manipulator of the immune response*

All SIVs and HIVs encode for the multifunctional Nef protein. Nef is expressed early in the viral life cycle and helps the virus to evade the immune system by interfering with the cellular activation state and modulating the expression levels of several cell surface factors [25]. For example, Nef from all immunodeficiency viruses down-regulates MHC class I molecules from the surface of the infected cell, thereby preventing lysis by cytotoxic CD8 T-cells [26, 27]. Interestingly, expression of HIV-1 Nef alone in T-cells results in a hyper-responsiveness to activation [28]. This "priming" for activation of infected cells could create an ideal environment for HIV replication and could thus contribute to the increased immune activation and T-cell apoptosis in chronic HIV-1 infection. Analysis of Nefs derived from pathogenic HIV-1/SIVcpz and non-pathogenic HIV-2/SIVs showed differences in their ability to induce a cellular hyper-responsive state [29]. Nef alleles from the great majority of primate lentiviruses, including HIV-2, are able to down-modulate the T-cell receptor (TCR)-CD3 complex from infected T-cells [29]. Since CD3 is crucial for T-cell activation, HIV-2/SIV Nef expressing cells did not respond to T-cell activation whereas nef alleles from HIV-1 and SIVcpz fail to down-regulate TCR-CD3. HIV-1 infected cells hyper-respond to activation and die due to activation-induced cell death. Thus, Nef-mediated suppression of T-cell activation by down-regulating CD3 is a fundamental property of non-pathogenic primate lentiviruses that likely evolved to maintain viral persistence in the context of an intact host immune system [29]. Pathogenic SIVcpz and HIV-1 lost the ability to down-modulate CD3, thereby resulting in higher rates of T-cell activation and cell death - the hallmarks of AIDS [29].

However, Nef alone does not account for all observed differences in disease progression in humans or naturally infected primates. Other viral proteins like Env or Tat have also been discussed to be implicated in driving a chronic immune activation, but are investigated less thoroughly. Host factors play a central role and determine disease outcome as well. For example: infection of Asian rhesus macaques with SIVsm that is able to down-regulate CD3 results in an AIDS-like disease, whereas the same virus is non-pathogenic in sooty mangabeys and moderately pathogenic in humans (HIV-2) [30]. In summary, immune activation plays an important role in AIDS pathogenesis. The underlying mechanisms are multifactorial in nature and involve complex interactions between host and viral factors. Future studies aimed to understand chronic immune activation in detail are central for a better understanding of AIDS disease progression that could lead to new treatment options.

#### IMMUNE ACTIVATION BY HIV: IMPACT ON NATURAL HISTORY

(J. Rockstroh, Bonn)

The natural course of HIV infection is characterized by primary infection, consecutive progressive CD4<sup>+</sup> T-cell loss, an increasing plasma viremia, and by the onset of clinical complications. There is strong evidence that HIV-1 itself has a direct impact on immune activation: it reaches its highest level during acute infection. When antiretroviral therapy is started, immune activation markers decrease within days and reincrease when plasma viremia increases following treatment interruption.

##### - Association with morbidity and mortality

Before the era of modern antiretroviral therapy, HIV-infected hemophiliacs were shown to have increased  $\beta$ 2-microglobulin and IgA levels, which correlate with survival [31].

Activation of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, as assessed by the expression of CD38, is associated with decreased survival in advanced infection (<50 CD4<sup>+</sup> T-cells/ $\mu$ l), independent from plasma viremia and viral coreceptor tropism [32]. Increased CD8<sup>+</sup> T-cell activation, as assessed by CD38 expression, and increased expression of the apoptosis marker PD-1 have been identified as prognostic markers for disease progression, independent from CD4<sup>+</sup> T-cell count, but probably coupled to virus replication [33-39]. Serum RANTES as another marker of immune activation correlates with CD8<sup>+</sup> T-cell activation [40]. Recently, a loss of regulatory CD4<sup>+</sup> T-cells has been suggested as a possible factor in the maintenance of immune activation [41].

Low-level plasma viremia in treated patients (3- 400 copies/ml) activates the immune system to a similar extent as detectable plasma viremia (>400 copies/ml). Apparently, treatment does not completely normalize immune activation, especially if low-level replication continues [42].

The results of a large trial investigating continued versus intermittent therapy (SMART study) have reinforced the discussion about immune activation: patients in the treatment interruption arm, apart from

being at higher risk for HIV-related complications, also had higher rates of non AIDS-associated complications such as cardiovascular, renal, and hepatic events than subjects in the continuous therapy arm [43]. The increased risk of death during treatment interruption was paralleled by increased D-dimer, IL-6, and hs-CRP levels, indicating a link between virus replication, inflammation, and risk of progression.

##### - Different hypotheses to explain CD4<sup>+</sup> T-cell depletion

Plasma viremia in HIV-infected and in HIV/HCV-coinfected patients is positively associated with apoptosis rate; when antiretroviral therapy is started, apoptosis is reduced [44]. The upregulation of different immunological effector mechanisms prevents a simplistic conclusion regarding the importance of a single mechanism for CD4<sup>+</sup> T-cell depletion.

Explorative gene expression analyses performed on intestinal tract biopsies show an upregulation of interferon, chemotaxis, cell cycling, and apoptosis in acutely HIV-infected subjects, and a downregulation of factors affecting lipid and glycogen metabolism [45].

A variety of mechanisms for immune hyperactivation with subsequent apoptosis are discussed in addition to those mentioned before: bystander activation, homeostatic proliferation, T-cell responses against autoantigens or against intestinal microorganisms, and a progressive loss of regulatory T-cells. As a current hypothesis based on primate animal models it is suggested that activation of the toll-like receptor TLR-7 leads to the release of proinflammatory cytokines and subsequently to the activation of T-cells [46].

#### IMMUNE ACTIVATION IN LYMPHOID TISSUE

(K. Tenner-Racz, Hamburg)

Physiological immune responses require a strict coordination of spatially separated immunological compartments. In the secondary lymphatic tissue (lymph nodes, spleen, and mucosa-associated lymphoid tissue [MALT]), antigen-dependent activation and differentiation of naïve T and B-cells into effector and memory populations takes place. This requires an interaction between B-cells, different groups of T-cells, and antigen-presenting cells as well as migration of lymphocytes into the T-cell- and B-cell dependent zone. Such migration is essential for a normal immune function. Within 12 days, lymph nodes turn over the equivalence of their own weight in circulating lymphocytes [47]. This high turnover is facilitated by a high blood flow (minimum of 1 ml/min/g of lymph node tissue) and a high circulation of lymph node fluid (10 ml/hr/g lymph node tissue). In case of an antigenic stimulus, this flow can be up-regulated by two- to threefold.

Here we summarize some aspects of the activation of the B-cell dependent zone. It is important to keep in mind that germinal centres (GC) play a central role in HIV disease pathogenesis as an important reservoir for HIV (summarized in [48]). Importantly, GC support the differentiation of memory B-cells and long-lived antibody-secreting cells during infection or upon vaccination. This function requires the help of T-cells. According to their effector function and cytokine production profile, the T-cells are classified as Th1 (IFN- $\gamma$ , IL-

2) and Th2 (IL-4, IL-5, IL-10) helper cells, and non-polarized T-cells (no IL-4 and IFN- $\gamma$  production, but efficient induction of immunoglobulin production).

The secondary follicles consisting of a mantle zone and a GC are composed of B-cells, follicular dendritic cells and tingible body macrophages, and CD4<sup>+</sup> T-cells. A few bone marrow-derived dendritic cells are also present [49].

The CD4<sup>+</sup> cells that contribute approximately 10% to the cell pool within the GC, is distinct from the CD4<sup>+</sup> cells in peripheral blood or the T-cell-dependent zone of the lymphoid tissue. They represent follicular T helper (T<sub>FH</sub>) cells. These cells are only present in the secondary lymphoid tissue and do not circulate in the peripheral blood [50]. They are non-polarized, thus, they produce neither IFN- $\gamma$  nor IL-4. Nevertheless, T<sub>FH</sub> cells are very efficient in stimulating Ig production [50-53]. Nearly 60-75% of the cells express CD57.

T<sub>FH</sub> already had contact with an antigen and express CD69, but not CD25. A characteristic feature of these cells is the upregulation of CXCR5 which is the receptor for CXCL13 ligand (CXCL13). In mice lacking CXCL13 or its receptor CXCR5, the architecture of the follicles and the differentiation of T and B-cells are disturbed. CXCR5-dependent T-cell positioning is important not only in the formation and expansion of GC, but also for the induction of immunoglobulin isotype class switching and for the development of long-lived antibody-secreting cells [54].

CXCL13 is a „B-cell attracting chemokine 1“and facilitates the follicular homing of B and T-cells. Freshly isolated CD4<sup>+</sup>CD57<sup>+</sup> T<sub>FH</sub> cells show a high expression of CXCL13 mRNA. Without persistent T-cell activation in culture, however, they are unable to produce CXCL13 [55]. This chemokine is an important contributor to the localization of T<sub>FH</sub> cells to the primary follicles as well as to GC formation [51, 52, 54, 56]. The chemokine CXCL13 promotes follicular homing by B and T-cells during chronic inflammation. It is a component of the *de novo* formation of lymphatic tissue [57-60]. Although HIV-1 infection is associated with B-cell abnormalities and severely altered lymph node histology, including the B-dependent zone, no attention has been paid to the tissue distribution of this chemokine.

We investigated lymph nodes from HIV-1-infected patients and HIV-negative individuals. Previous reports have indicated follicular dendritic cells (FDC) and follicular stromal cells as a source of CXCL13 [56, 59, 61]. In agreement with these findings, we also detected CXCL13 protein in a pattern resembling the FDC network. However, only a few GCs contained this chemokine in both HIV-1 samples and controls. As we reported recently [62], we found that the main expression of CXCL13 was localized in immature CD1a<sup>+</sup>DCs of the T-dependent zone. The expression was less prominent in CD83<sup>+</sup> mature DCs. This could suggest that the CXCL13 protein might be lost during DC maturation. The numbers of DC expressing CXCL13 in lymph nodes from HIV-negative cases were higher than in HIV-positive subjects. The overall lower content in CXCL13 in HIV-positive patients was

not influenced by six months of HAART. The relationship between CXCL13 producing DC and the pathogenesis of HIV disease, however, remains uncertain. Additional *in situ* and *in vitro* investigations are required in order to further characterize functions of these cells.

#### CHEMOKINES, CYTOKINES, HIV – WHAT A MESS?

(G. Behrens, Hannover)

Chemokines play a central role for the migration and activation of immune cells. At least 20 different chemokine receptors and approximately 50 different chemokines have been described as their ligands [63]. These inflammatory chemokines differ from homeostatic chemokines in that the latter are active during normal physiological conditions.

#### - Complex interplay

The complex interplay of chemokines is characterized by redundancy, i.e. by many ligands binding to different receptors, and these interactions are directed by compartmentalisation, combinatory interactions and alterations in activity by matrix metalloproteases. Chemokines are present as monomers, dimers, tetramers, and other oligomers. Different chemokine receptors of the two major forms CCR and CXCR are associated with different diseases.

Chemokine receptors are expressed by dendritic cells (DC) and T-cells [64]. Immature DCs exhibit a wide range of chemokine receptors that are also important for their migration.

The contact with a pathogen provides the impulse for immature DCs to become active („licensed DC“). In the process of maturation and activation CCR7 is expressed, which lets the cell migrate along a CCL16 and CCL21 gradient into the lymph node, where the DC matures completely. Subsequently, co-stimulatory molecules are upregulated, as well as MHC class I and II, and T-cells are activated.

It is hypothesized that immature DCs promote T-cell tolerance, whereas regulatory T-cells and mature DCs lead to protective T-cell responses.

Chemokine receptors are also used by pathogens. *Toxoplasma gondii* evades immune responses by binding to CCR5. Due to the lower efficacy of a Th1 response against *T. gondii*, this results in a balance, and the parasite can persist in the host. HIV also activates DCs, with subsequent T-cell activation and proliferation, which in turn provides HIV with potential target cells for replication. Chemokines also regulate effector cells and regulatory T-cells which display different chemokine receptors.

#### - CCL3L1 blocks CCR5

The Chemokine-Ligand 3-like 1 (CCL3L1) is a ligand of the CCR5 receptor. The gene for CCL3L1 occurs in several copies, and the number of duplications is different between populations and individuals.

In a recent cohort study, persons with a specific combination of CCR5 and CCL3L1 displayed a reduced immune response and a lower CD4<sup>+</sup> T-cell number [65]. As a consequence, a lower expression of receptors, although in principle protective with respect

to HIV infection, could alter the migration of immune cells. In turn, an altered expression of the ligand could stimulate the decay of the receptor and thereby limit HIV replication, as well as increasing the migration of effector cells. In this cohort analysis the CCL3L1 concentration had a greater impact on HIV prognosis than the presence of a functional CCR5 receptor.

#### IMPACT OF IMMUNE ACTIVATION ON THE PROGNOSIS OF HAART

(M. Stoll, Hannover)

It is frequently assumed that the increase of CD4<sup>+</sup> T-cell counts during HAART-mediated suppression of virus replication totally mirrors the reversion of HIV-induced immunodeficiency. However, this view does not take the consequences of immune activation into account. The results of the SMART study showed that deaths due to opportunistic events and other, not classically HIV-associated events occurred more frequently in subjects randomized to the treatment interruption arm as early as 16 months into the study, as compared with subjects with continued therapy [43]. Treatment interruption was associated with a higher risk of opportunistic disease and death independent from CD4<sup>+</sup> T-cell count. A sub-analysis of the SMART study showed that the increase of several inflammatory serum markers with an established prognostic role for cardiovascular events (hs-CRP, interleukin-6, amyloid A and P, D-dimers and the prothrombin fragments 1 and 2 (F1.2)) was associated with increased mortality and, of note, also with cardiovascular events [66].

Meanwhile, immune activation is considered in the model to explain the effects of HAART. It is postulated that, in addition to reducing virus replication and thereby increasing CD4<sup>+</sup> T-cell counts, HAART also reduces immune activation and the risks associated with chronic inflammation.

Of note, a variety of organ systems appear to be affected by chronic inflammation. As a consequence, the reduction of immune activation by HAART could prevent non-opportunistic events such as arteriosclerosis, myocardial infarction, liver diseases, and impairment of renal function. For these events, however, other risk factors have to be taken into account, such as age, HAART toxicity, smoking, illicit drugs, alcohol, nutrition, level of physical activity, and genetic disposition.

There is no doubt as to whether HAART reduces immune activation. This was demonstrated several years ago [67]. It is not clear, however, if immune activation is completely normalized by HAART.

#### - Do we have to start HAART earlier ?

The pendulum of treatment indication currently appears to swing back towards an earlier start of therapy, resulting in the recommendation to start HAART at a CD4<sup>+</sup> T-cell count of less than 350/ $\mu$ L in most guidelines.

The leading argument for starting treatment earlier is the prevention of opportunistic infections and death, but the prevention of non-opportunistic events is increasingly being recognized as another argument

for an early start. Starting HAART at even higher CD4<sup>+</sup> T-cell counts (>500/ $\mu$ L) is currently supported only by a recent cohort analysis [68], which has some methodological issues. A prospective clinical trial (START study) is under preparation and will likely resolve the issue.

The impact of treatment-emergent long-term toxicities on the rate of events and the balance between those and the beneficial effect of HAART on these event cannot be fully judged at present. The risk of treatment interruption requires preparedness of the patients for life-long HAART, especially when treatment is started early.

#### - Late HAART: how urgent is it ?

In patients with late initiation of HAART („late presenters“), it should not be delayed, with the possible exception of subjects with concomitant mycobacteriosis. The untoward effect of a possible immune reconstitutive inflammatory syndrome (IRIS) and adverse drug interactions does not outweigh the improvement of prognosis if treatment is started 2 weeks after diagnosis. This recommendation is based on the prospective ACTG A5164 study, which did not include patients with mycobacteriosis [69], and the evaluation of a South-African cohort, in which IRIS occurred in all patients who initiated HAART within 30 days after diagnosis of tuberculosis. A detailed analysis of the 160 subjects in this cohort revealed that 19 of them developed IRIS, two of them fatal. However, ten deaths occurred prior to the initiation of HAART [70].

A larger Brazilian cohort study based on 963 incident tuberculosis cases among more than 15000 HIV-infected subjects showed only a small difference in survival when HAART was initiated within 60, 61-180, or >180 days after the start of tuberculostatic therapy [71].

#### - Arguments against immediate HAART for late presenters

The data that argues for an immediate initiation of HAART is based on studies from countries in which HIV care is restricted by economic constraints. De-masked opportunistic infections which manifest as IRIS show the detrimental potential of untreated opportunistic infections after HAART initiation.

Further studies should provide a basis for active risk management, by which patients at high risk for IRIS can be identified beforehand.

Preliminary data suggests an increased risk with certain TNF polymorphisms, cytokine receptor polymorphisms, an association with HLA antigens and with certain patterns of immune activation (i.e. disturbances in the relationship between Th1 and Th2 cells).

The optimal clinical approach to this basically physiological response of the immune system is not well-defined and requires further investigation.

It is conceivable that in the future immunomodulatory or transient immunosuppressive approaches will be utilized in order to prevent or mitigate IRIS. They could be limited to subjects at high risk identified by novel parameters yet to be defined.

ABACAVIR AND MYOCARDIAL INFARCTION: IS IT  
IMMUNE ACTIVATION ?  
(Th. Harrer, Erlangen)

The relationship between HAART and cardiovascular risk cannot be explained solely and mechanistically by the mode of action of therapy. Despite an equivocal effect of a HAART regimen that includes abacavir on the reduction of immune activation, the prospective D:A:D study on 33.347 HIV-infected patients from eleven cohorts found an association between the current or recent use of abacavir and myocardial infarction or equivalent cardiovascular events. This also applies to didanosine, although to a lesser extent [72]. A total of 517 subjects (1,6%) suffered an episode of myocardial infarction. Recent or current abacavir use was associated with a 90% risk increase. Recent didanosine use showed a 47% risk elevation. There was no association with stroke.

The risk increase was highest for subjects with a pre-existing cardiovascular risk elevation. There was no increase of risk for past use of abacavir or didanosine<sup>1</sup>.

The possibility of selection bias (i.e. a preference for abacavir by physicians due to its favourable lipid profile) cannot be fully excluded, although it appears improbable in the context of similar results from other studies. An analysis of the SMART study revealed an increased risk of cardiovascular events of any severity for patients on abacavir without didanosine at baseline (22.4%; n = 259). The risk for myocardial infarction was also elevated (n = 19). There was no association with didanosine use in this analysis [73].

A retrospective analysis of 54 prospective clinical studies performed by GlaxoSmithKline, which included 14.683 therapie-naïve and pretreated patients, did not show this association [74]. A major difference between these studies and cohorts is the underrepresentation of patients at high risk of cardiovascular events.

*- Abacavir and inflammatory markers*

The mechanism of increased risk is still unclear. The SMART study showed an elevation of hs-CRP and interleukin-6 in patients on abacavir, independent from continuous therapy or treatment interruption. Cardiovascular risk in the continuous therapy arm depended on the presence of at least five coronary risk markers.

A retrospective analysis of the HEAT study, in which abacavir+3TC was compared with tenofovir + FTC, both combined with lopinavir/r, did not show a difference in the plasma levels of inflammatory parameters. These markers declined until week 96 in both arms [75].

The increased risk associated with abacavir use appears not to be a general immune-activating effect or a lesser reduction of HIV-associated immune activation.

MRI studies of the long-term effects on coronary artery sclerosis performed on 48 patients in Erlangen revealed coronary artery calcifications in 28 patients

without abacavir, 21 on abacavir, and in 12 control subjects (Ropers et al., unpublished observation). There were no differences in the extent of calcification of coronary arteries, aortic valve, and mitral valve. CD4<sup>+</sup> and CD8<sup>+</sup> cells and viral load as well as plasma levels of TNF-alpha, IL-6, and of hs-CRP were also comparable. This would be compatible with a short-term effect.

*- Do abacavir-associated cardiovascular events represent a hypersensitivity reaction ?*

The question has to be raised if the increased cardiovascular risk represents a reaction similar to the well-known inflammatory reaction associated with the presence of the HLA-B\*5701 antigen.

It is hypothesized that abacavir is metabolized within antigen-presenting cells, and that a metabolite is presented in the context of HLA-B\*5701 as an antigen [76].

The resulting secretion of cytokines would then lead to an inflammatory syndrome, which could propagate myocardial infarction in the presence of pre-existing risk factors.

In the cohort analyses mentioned above, HLA-B\*5701 testing was not performed routinely. Therefore, it appears conceivable that an "abortive" hypersensitivity reaction actually precipitated myocardial infarction.

An analysis of the Erlangen cohort so far was unable to confirm this hypothesis: 28 (7.8%) of 358 HLA-typed patients were HLA-B\*5701-positive. Seven of nine subjects in the cohort who had received a stent or had experienced myocardial infarction had received abacavir. However, only one subject was HLA-B\*5701-positive. So far the relationship between inflammatory reactions and abacavir use in HLA-B\*5701-negative individuals remains speculative. It has to be assessed in prospective clinical studies such as ASSERT, which are currently ongoing.

HIV AND CARDIOVASCULAR RISK  
(S. Baldus, Hamburg)

HIV-infected subjects show a 75% higher incidence of cardiovascular events than an HIV-negative control group, which increases even further with age [77]. In HIV infection, coronary heart disease is characterized by an increased proliferation of fibroblasts, increased thrombocyte activation, decreased vascular relaxation, direct viral effects and effects of antiretroviral therapy.

*- HIV accelerates coronary atherosclerosis*

It is currently believed that acute coronary occlusion in the setting of coronary atherosclerosis is a highly dynamic process with plaque rupture and subsequent thrombus formation, rather than a slowly progressive occlusion of the vessel. A vulnerable plaque with a high risk of rupture due to a thin covering layer cannot be identified or predicted by angiography, in contrast to plaques with a thick layer, which are less likely to rupture [77, 78].

HIV has a high affinity to endothelium and locally induces the production of the cytokines interleukin-1 $\beta$  and interleukin-6, which activate lymphocytes and

<sup>1</sup> A recent updated analysis showed a significant risk increase for cumulative exposure, as well, although to a much lesser extent. Lundgren J et al. 16<sup>th</sup> Conf Retrovir Opportun Infect 2009, abstract no. 44LB.

granulocytes. Moreover, HIV can induce increased cell membrane permeability. The virus attracts monocytes, which invade into the vascular endothelium together with LDL particles. Residing macrophages („foamy cells“) subsequently oxidize the LDL and take up lipids together with monocytes. These cells also release cytokines, which stimulate the proliferation of smooth muscle cells and accelerate the growth of the plaque. If fibroblast or leukocyte metalloproteinases react in the vicinity of the thin covering layer, it can rupture and lead to the adhesion of thrombocytes.

#### *- Vascular function as a prognostic parameter*

Vascular function represents an essential parameter for the prediction of cardiovascular risk. Leukocyte and thrombocyte adhesion, proliferation of smooth muscle cells and fibroblasts, and the expression of adhesion molecules on the endothelial surface are regulated by signal molecules which are produced in the endothelium. The most important and best characterized anti-inflammatory signal molecule is nitric monoxide (NO). It is produced in the endothelium by an NO synthase and is responsible for the downregulation of the processes mentioned above. Due to its long half-life, the radical can permeate into smooth muscle tissue, where it activates additional signalling cascades which lead to the relaxation of smooth muscle cells.

Meanwhile, the measurement of NO bioavailability by ultrasound or invasive methods is established for determining cardiovascular risk, especially in patients with cardiovascular diseases.

NO as a surrogate parameter for cardiovascular risk might be better than inflammatory markers. Its bioavailability is probably regulated by increased oxidation or inactivation of NO by superoxide and peroxidases, especially myeloperoxidase, from monocytes, macrophages, and neutrophils. The relevance of NO depletion by superoxide and myeloperoxidase is illustrated by animal experiments, in which the humoral and structural integrity of the endothelium was altered by these two factors.

Endothelial vasoreactivity provides an important prognostic information. The blood flow-dependent dilatation of the brachial artery is easy to determine: Occlusion of the vessel for five minutes leads to ischemia. The increased vascular blood flow following ischemia is NO-mediated. HIV-infected subjects show a dilatation of the brachial artery of only 3.5%, as opposed to 10% in healthy controls [79].

#### *- Cardiovascular risk factors in HIV infection*

In addition to direct effects of HIV on the endothelium, changes in the cardiovascular risk profile impact on the risk of cardiovascular events: The prevalence of hypertension, diabetes and dyslipidemia is increased in HIV infection [77, 78]. It has to be stated clearly that especially a diabetic patient has to be viewed like a patient with proven coronary heart disease, as their risks are equivalent.

#### *- HAART accelerates coronary atherosclerosis*

The D:A:D cohort showed a 16% increase of cardio-

vascular risk per year of protease inhibitor therapy, resulting in a doubling of risk over five years [80]. A small cross-sectional study in 83 HIV-positive children showed an impaired endothelial function in the PI-treated group compared with children not receiving a PI [81]. A study on carotid intima media thickness in 141 HIV-infected patients showed an increase with PI treatment [82]. Several factors contribute to this difference: insuline resistance, arterial hypertension, and the alterations in the lipid profile with the resulting dyslipidemia and lipodystrophy. The increases in LDL cholesterol lead to more LDL oxidation and uptake by macrophages, thereby contributing to plaque progression.

#### *- HIV-infected patients are a high-risk group*

HIV-infected patients, like diabetics, belong to a cardiovascular high-risk group. Risk factors such as hypertension, diabetes, hyperlipidemia, and smoking should be reduced. Moreover, investigations of vascular function, the measurement of inflammatory markers (e.g. CRP), and statin treatment with its lipid-lowering and anti-inflammatory effects could be used to assess and manage cardiovascular risks.

The clear-cut effect of statins on cardiovascular events was demonstrated in the JUPITER trial: Almost 18000 healthy subjects without diabetes or cardiovascular disease and an LDL cholesterol level of <130 mg/dl as well as an hs-CRP level of 2-5 mg/l received rosuvastatin or Placebo. In these subjects with low-level inflammation, rosuvastatin reduced the cumulative rate of cardiovascular events by 44% [83].

It is currently unclear if CRP is more than just a surrogate parameter, i.e. that it acts as a mediator of inflammatory processes. Markers such as hs-CRP, measures of endothelial function and vascular imaging techniques require further validation in HIV-infected patients.

#### IMMUNE ACTIVATION: GENETIC FACTORS (J. Bogner, München)

When compared with death due to cardiovascular events or malignant tumours, death due to infection has the strongest association with genetics [84]. The strong association between severity of disease and genetic background of the individual also applies to HIV infection.

The most important areas for which genetic polymorphisms have been identified that influence HIV pathogenesis: cytokines that interfere with immune regulation and activation, the control of viral entry into the host cell, and polymorphisms that influence adaptive immunity.

#### *- Severity of disease*

TNF-alpha is known to be a cofactor for AIDS dementia, with some data pointing at an impact of genetic polymorphisms on its manifestation: the TNF-alpha-308 A allele is more frequent in patients with AIDS dementia than in controls (0.28 versus 0.007; OR 5.5; 95% CI 1.8-17.0) [85]. Patients with sepsis and IRIS also show an over-representation of the TNF-alpha-308 A allele.

#### - Immune activation

A naturally occurring inhibitor of IL-1-IL-1ra antagonizes IL-1 receptor binding competitively. Two different alleles (IL-1RN\*1 with 4 tandem repeats and IL-1RN\*2 with 2 tandem repeats) have been described. Homozygosity is rare and is associated with an increased pro-inflammatory response. IL-1ra reduced HIV replication in vitro. HIV plasma viremia is significantly lower in IL-1RN\*2 homozygous women, and this polymorphism is more frequent in caucasian than in non-caucasian women [86]. Meanwhile, 13 different polymorphisms have been described in a French cohort, with their different distribution being associated with differences in the course of HIV infection [87]. Virologic treatment response was associated with the presence of the IL-1alpha-Allels IL1A889\*2 allele in an Australian cohort; however, the IL-1a-genotype had no impact on CD4<sup>+</sup> T-cell count [88].

#### - T-cell regeneration

IL-7 plays a pivotal role in T-cell thymopoiesis, with an inverse correlation between IL-7 plasma level and CD4<sup>+</sup> T-cell count. The expansion of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells is stimulated by IL-7 [89]. A three base-pair deletion in the IL-7 gene increases IL-7 expression and results in a different course of CD4<sup>+</sup> T-cells [90].

#### - Immune reconstitution

IRIS as a special state of immune activation is also influenced by genetic factors. Different genetic profiles such as the major histocompatibility complex alleles HLA-A2 and HLA-B44 were found to be associated with CMV end-organ disease as IRIS manifestation, and increased IL-6 levels as well as increased CCR5 expression were associated with an increased occurrence of IRIS [91].

The regeneration of immunity during antiretroviral therapy is influenced by a variety of genetic polymorphisms. The multivariate analysis of data obtained from 873 treatment-naïve patients yielded 137 Single Nucleotide Polymorphisms (SNPs) in 17 genes, all of which had a measurable impact on the magnitude of CD4<sup>+</sup> T-cell increase [92].

#### IMMUNE ACTIVATION AND TUMOUR RISK (C. Hoffmann, Hamburg)

The risk of malignancy in AIDS patients showed clear trends in the time-period between 1996 to 2002: the standardized incidence ratios in comparison to the general population decreased for Kaposi's sarcoma and – albeit to a lesser extent – for Non Hodgkin's lymphoma (NHL). In contrast, the incidence of cervical cancer and non AIDS-defining malignancies (non-ADM) remained essentially unchanged, with the exception of Hodgkin's lymphoma. Kaposi's sarcoma, NHL, and non-ADM all represent a third of all malignant tumours diagnosed in HIV-infected patients [93]. Of note, the increase of Hodgkin's disease in the HAART era was observed in several cohorts. In the German AIDS-related lymphoma (ARL) cohort more patients were diagnosed with Hodgkin's disease than with NHL [94]. It is hypothesized that the increase in Hodgkin's disease is not a direct effect of HAART,

but that the HAART-associated increase in CD4<sup>+</sup> T-cell counts provides a suitable milieu with growth signals for proliferation and survival of the neoplastic Reed-Sternberg cells, which represent only about 1% of all cells in Hodgkin tissue [95].

In 2005 16% of all deaths in patients with HIV infection were due to non-ADM, more than the observed 11% in 2000 [96]. Tumour-related mortality (non-ADM and ADM) is currently higher than mortality due to AIDS-related infectious complications, with approximately one third of all fatalities in HIV-infected patients being due to malignancy.

Once antiretroviral therapy is started, the likelihood of Kaposi's sarcoma and NHL is clearly reduced [97].

Mortality in the SMART study [43] was mainly due to non-ADM, with no difference between the study arms. AIDS-defining malignancies (ADM), however, occurred significantly more frequently in the treatment interruption arm [98]. The risk of fatal ADM and non-ADM in the D:A:D-Studie was inversely correlated with CD4<sup>+</sup> T-cell count [99].

A meta-analysis of cancer incidence in HIV-infected patients (7 studies with 444.172 patients) compared with patients with renal transplants (5 studies with 31.977 patients) showed an increased occurrence of many tumours in both groups [100]. When compared with HIV-infected patients, renal transplant recipients showed a higher incidence of colorectal carcinoma, melanoma, bladder carcinoma, lymph node cancer, renal cancer, vulva- and vaginal carcinoma and oral cancer). The incidence of malignancy was higher only for a few kinds of tumours: Hodgkin's lymphoma, NHL, and Kaposi's sarcoma.

#### - Direct impact of immune activation on lymphoma

Apart from HIV infection itself, other mechanisms such as oncogenic viruses, age, alcohol, and nicotine might contribute to increased lymphoma rates. Immunodeficiency, however, is the major risk factor for ADM as well as for non-ADM. There is some evidence for a role of immune activation in lymphomagenesis, but there is no direct proof of B-cell activation as a cause of lymphoma. The clinical manifestation of lymphoma is relatively homogeneous, with 60-80% of diagnoses made in late stages, 70-80% extranodal manifestations, more than 90% high-risk subtypes with high proliferation rates, low remission rates, high probability of relapse, and an unfavourable diagnosis without HAART.

The underlying pathogenetic mechanisms leading to lymphoma, however, are probably heterogeneous. This is reflected in differences in CD4<sup>+</sup> T-cell counts at manifestation, e.g. around 300 cells/ $\mu$ L for Burkitt-like lymphoma, as opposed to less than 20 cells/ $\mu$ L for primary central nervous system lymphoma (PC-NHL). Moreover, malignant transformation appears to occur at different stages of cellular differentiation. The association with Epstein-Barr Virus (EBV) is also different: the closer the association, the more profound is immune deficiency.

B-cell stimulation appears to be a risk factor for NHL. This view is supported by several different observations. NHL cases had higher serum globuline levels cases compared with controls before the onset



of lymphoma [101]. Moreover, lymphoma patients in the MACS cohort had a higher expression of CD30, which is expressed by type 2 T-cells and stimulates the release of B-cell-stimulating cytokines [102]. Furthermore, the activation-induced cytidine deaminase (AID) is increased in Burkitt-like lymphomas as compared with other lymphomas [103]. This APOBEG-like enzyme is expressed in germinal center B-cells and is essential for somatic hypermutation in the process of affinity maturation of B-cells. AID is induced by oncogenic viruses and B-cell stimulation. There is insufficient data for other activation markers like IL-10, CD44, CD27, and CD23, which were mostly investigated on frozen samples from the MACS cohort.

Cumulative viremia during HAART appears to contribute to the risk of Burkitt-like lymphoma, probably because it drives B-cell activation [104]. In Castleman's disease the level of HHV-8 viremia correlates tightly with IL-6, IL-10, and CRP. HHV-8 produces a viral IL-6, which has a 25% amino acid homology with human IL-6 and binds to the human IL-6 receptor. The overproduction of viral IL-6 and the activation of subsequent events in the inflammation cascade leads to a massive activation of the immune system [105].

#### SUMMARY OF THE DISCUSSION

The question to which extent immune activation due to HIV infection is more than just a reflection of a physiological process can not be answered clearly to date. The answer could actually be very individual, with genetic and environmental factors modulating the extent of immune activation and its sequelae, as well as preexisting factors such as cardiovascular risk factors. However, comparisons of non-pathogenic SIV infections in non-human primates with HIV-1 in humans have confirmed the clear association of immune activation with pathogenesis. Especially in the SIV lineage that gave rise to HIV-1, the viral protein Nef appears to play a pivotal role in persistent immune activation.

The relevance of HIV-induced immune activation as a major driving factor of pathogenesis is reflected in several cohort analyses and clinical studies investigating interruptions of antiretroviral therapy, which suggest that, in addition to classical HIV-related clinical events such as opportunistic infections, resurgence of virus replication also promotes non AIDS-defining conditions such as coronary heart disease, osteoporosis, hepatic and renal events and certain types of non-Hodgkin's lymphoma.

Moreover, in addition to HIV itself, persistent or repeated coinfections such as CMV or concurrent sexually transmitted diseases might act as triggers for immune activation. The most extreme example of a physiological reaction against concurrent infections that turns into a potentially detrimental phenomenon would be IRIS.

The persistence of increased levels of immune activation in some individuals with virus suppression on HAART raises the question if HIV pathogenesis continues even in the absence of measurable levels of active virus replication. The association of "residual" immune activation with a persistently increased risk of

infections and tumours during HAART in cohort studies should be investigated, but currently remains hypothetical. It is also unclear if it just represents a consequence of prior extensive CD4<sup>+</sup> T-cell loss, as reflected by incomplete recovery of CD4<sup>+</sup> T-cells in the gastrointestinal tract and elevated LPS levels or persistent functional T-cell defects, or if it plays an independent pathophysiological role. The prolonged persistence of viral antigens on follicular dendritic cells in the lymph node despite successful HAART known to exert immunomodulatory functions, induce T-cell loss or drive immune activation could also help to explain persistent immune activation.

Any clinical events propagated by such effects will be overlapped by the dramatic reduction of clinical progression by HAART. As a consequence, large and well-controlled cohort studies and prospective clinical trials are required in order to characterize their importance.

The natural course of SIV infection in pathogenic and apathogenic monkey models suggests that immune activation plays a pivotal role in the pathogenesis of HIV infection. In view of the uncertainty regarding the feasibility of life-long antiretroviral therapy with currently available drugs, novel treatment approaches should be investigated which are not merely directed towards virus replication but towards its consequences for the host organism.

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*Address for correspondence:*

Prof. Dr. Hans-Jürgen Stellbrink  
 Infektionsmedizinisches Centrum Hamburg/ICH  
 Grindelallee 35  
 20146 Hamburg  
 Germany  
 E-mail: stellbrink@ich-hamburg.de