IMMUNE RESTORATION IN THE CONTEXT OF HAART

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The advent of highly active antiretroviral combination therapy (HAART) has resulted in significantly improved survival of patients and a reduction in the progression of human immunodeficiency virus (HIV) infection.[1]

At the root of these clinical benefits is a dramatic reduction in HIV viral load. HAART induces a sustained effective suppression of HIV replication in most patients and leads to a preservation or restoration of immune function.

The restoration of an impaired immune system is assessed by clinical parameters, immunological changes which can be measured in the laboratory and a marked reduction in the morbidity and mortality of patients. To understand the features of immune restoration it is necessary to review the immunopathogenesis of HIV infection and highlight areas where improvement of function occurs with HAART.

THE IMMUNOPATHOGENESIS OF HIV INFECTION

It is important to remember that the destruction of the immune system is related to the kinetics of viral replication. In most cases increased viral replication is associated with a more rapid destruction of the immune system and a more rapid progression of HIV disease.

The hallmark of HIV infection is a loss of CD4+ lymphocytes. The loss of lymphocytes occurs for a variety of reasons, among them direct destruction of CD4+ cells, immunologically mediated CD4+ loss and apoptosis (programmed cell death), which is related to the intense and ongoing immune activation invariably present throughout the course of HIV disease. In early disease the loss of CD4+ cells is compensated for by an increased output and the CD4+ lymphocyte homeostasis is largely maintained. This situation continues for the first few years, but progressive events occur in lymphoid tissue which compromise the output of CD4+ lymphocytes.

MECHANISM OF CD4+ CELL LOSS

- ◼ Direct cytotoxic effect of viral replication
- ◼ Immunologically mediated destruction
- ◼ Apoptosis mediated by immune activation
- ◼ Bone marrow stem cell failure
- ◼ Thymic failure of CD4+ cell programming

IMMUNE RESPONSES TO HIV INFECTION

Cytotoxic T cells (CTLs)

Studies in persons with chronic HIV-infection have detected both cellular and humoral immune responses to HIV.[2] As in other chronic viral infections cytotoxic T lymphocytes are generated in response to infections that inhibit virus replication by at least two mechanisms. In the first instance direct killing of cells infected with HIV occurs. Small peptide fragments form complexes with class 1 HLA molecules and these are presented on the cell surface to trafficking CTLs. The presence of a viral peptide (usually 9 - 10 amino acids in length) within the peptide-binding cleft of a class 1 molecule is a signal to the immune system that a foreign pathogen is present within that cell. This then triggers the CTL to kill the infected cell through a direct recognition mediated by the T-cell receptor (TCR) on the CTL. The killing of the cell results from a production of perforins and granzymes secreted by the CTL.

The second mechanism exerted by the CTL is the release of soluble antiviral factors via the activated CTL. These include the beta-chemokines (RANTES, MIP-1alpha and MIP-1beta). There is also a further soluble factor, which is in the process of being defined. This factor, termed the CD8+ antiviral factor (CAF), is thought to suppress viral replication at the transcriptional level. This occurs in a non-major histocompatibility complex (MHC) manner.[3]

Each HLA class 1 allele is slightly different and therefore there is variation in the viral peptides that are able to bind
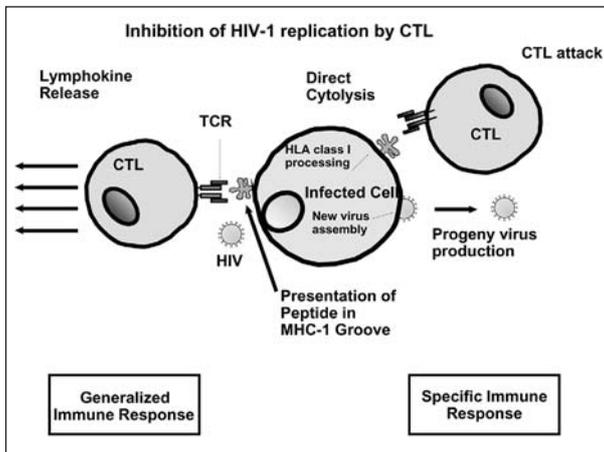
Fig. 1. Inhibition of HIV-1 replication.

Fig. 2. Virus-specific T-helper cells mechanism.

to a specific allele. As over 50 class 1 alleles have now been defined it can be seen that many different viral peptides can be bound and presented, and consequently there is a considerable variation between individuals with regard to their immune response. The importance of CD8+ CTLs is seen throughout the course of HIV disease. It is an important mechanism to curtail HIV replication at primary infection, and high levels of CD8+ cells are especially seen in persons who are slow progressors (so called long-term non-progressors).

Virus–specific T–helper cells

In addition to CTLs the cellular immune response is also associated with the generation of virus-specific T-helper cells. These cells are required for the maintenance of CTL function. CD4+ T-helper cells recognise viral proteins that have been taken up in lysosomes of antigen-presenting cells (APCs). There they are processed to smaller peptides and then presented at the cell surface within the peptide-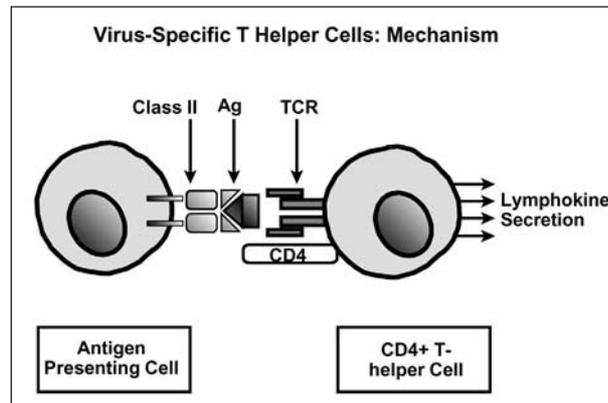binding groove of class 2 HLA molecules. The presence of a viral peptide in the class 2 binding group elicits a CD4+ helper response, which in turn orchestrates an overall immune response. The CD4+ helper effect is mediated by direct cell-to-cell interactions and the release of a number of cytokines. The viral peptides that service targets for the T-helper response tend to be larger than those involved in CTL recognition (12 - 15 amino acids in length).

Antibodies

Neutralising antibodies are detected in HIV infection and are targeted against a number of different epitopes. These include antibodies directed against portions of the envelope protein that are involved in virus entry to the cell (V3 loop antibodies) and in CD4+ binding (CD4+ binding site antibodies).[4] One of the limitations of neutralising antibody responses is that they are typically type-specific. This means that antibodies generated against one particular virus may not cross-react with others. This has implications for immune control since viruses continue to mutate within the infected host and are constantly escaping immune detection.

IMMUNE ACTIVATION

Positive consequences	Negative consequences	Immunodeficiency
■ Expression of activation antigens – HLA-DR, CD38	■ Promotes reverse transcription	■ Chronic immune system dysfunction
■ Activation of monocytes	■ Viral integration	■ Reduction of function of macrophages, neutrophils, dendritic cells and NK cells leading to decrease in chemotaxis, microbial killing, altered cytokine expression and antigen presenting function
■ Lymph node hyperplasia	■ Upregulates viral replication	
■ Increased cytokine expression	■ Induces expression in latently infected cells	
■ Antiviral effects	■ Spread to activated cells	
■ Removal of infected cells	■ Anergy of immune cells	
.... normal vigorous immune response	■ Perturbation of T-cell repertoire	■ Decreased CD4+ responses to recall antigens, allo-antigens and mitogens
	.... chronic immune activation	■ Decreased cytokine production especially IL-2
		■ Decreased CD8 responses and CTL functions
		.... immune exhaustion

IMMUNE ACTIVATION

One of the hallmarks of HIV infection is a chronic immune activation, which persists for years. Immune activation is a necessary component in any immune response to infecting pathogens, but after the acute event the immune activation usually subsides. In HIV disease the persistent generation of both humoral and cellular escape mutants means that in effect the immune system is constantly facing a new pathogen, and this leads to a persistent state of immune activation. The negative consequences of immune activation are induction of activation of cells, which in turn leads to productive infection of latently infected cells and also makes uninfected cells more susceptible to infection. Immune activation also promotes apoptosis of uninfected CD4+ lymphocytes and thus contributes to the overall CD4+ cell loss.

Both CD4+ and CD8+ cells all have surface expression of molecules such as CD38 and HLA-DR that are associated with immune activation. Recent studies[5] have shown that high-level expression of CD38 on CD8+ cells is a powerful predictor of HIV disease progression. CD38 expression also correlates with lymphocyte susceptibility to programmed cell death.

IMMUNE PHENOTYPIC CHANGES

The CD4+ lymphocyte compartments consist of two functional subsets separated on the basis of expression of certain cell surface markers. Naïve cells express CD45RA and CD62L whereas memory cells express CD45RO. In principle, naïve cells are newly generated through a selection process in the thymus. Naïve cells have the potential to generate immune responses to newly encountered antigens but are not particularly capable of cytokine expression or of effector cell activity. After exposure to antigenic peptides expressed on cell surface class 1 MHC molecules (CD8+ cells) or those on cell surface class 2 MHC molecules (CD4+ cells), naïve cells evolve into effector cells and will eventually express the memory phenotype. These cells are capable of an array of cytokine expression and also possess effector cytolytic activity. After antigenic exposure the majority of effector cells die by apoptosis and a minority revert to the memory state. These cells are capable of rapid responses to previously encountered antigens providing the classic secondary or anamnestic response to previously encountered antigens. In healthy adults approximately 50% of circulating cells are of the naïve phenotype and 50% of memory cells.

CYTOKINES–TH1/TH2 AXIS

T-helper lymphocytes consist of two distinct subsets designated TH1 and TH2. The differentiation into the subsets is based on the cytokine profiles elaborated by the individual subsets. Cytokines are soluble factors produced by immune cells that subserve immunological functions. The TH1-subset is associated with the production of interleukin-2 (IL-2) and gamma interferon (IFN-γ). The TH1-response promotes cell-mediated immunity and enhances CD8+ lymphocyte function. This is seen in early disease and is also part of immune restoration after HAART is initiated. The TH2-response is associated with the production of IL4, IL5, IL6 and IL10. The TH2-response is linked to an enhanced humoral activity with activation of B cells. There is a cross-regulation between the two functional subsets (Fig. 3). During progression of HIV infection there is a switch from a TH1 to a TH2 cytokine profile, which is often reversed following the increase in CD4+ cell numbers and function with HAART.
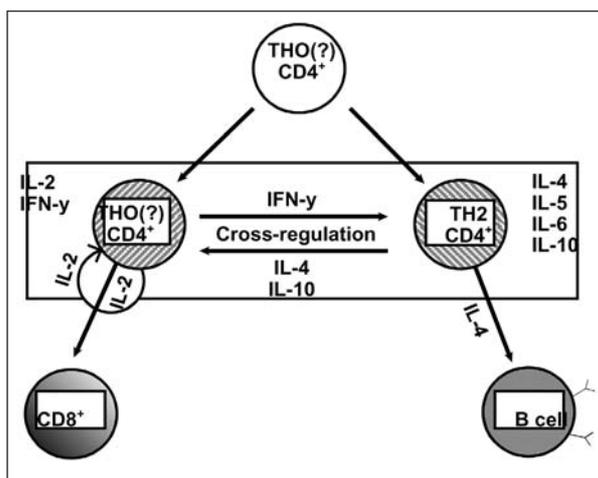

Fig. 3. TH1 and TH2 model.

THE T–CELL REPERTOIRE

An enormous diversity of foreign antigens, approximately 10^{13} to 10^{15}, are recognised by T cells through the T-cell receptor to antigen (TCR). This comes about following intrathymic maturation by the diversity of recombination in the gene fragments that encode the TCR, with each TCR providing the specificity for a given antigen. The overall diversity of the T-cell repertoire decreases with age and also following chronic exposure to antigens. This process is accelerated throughout the course of HIV infection because of the state of immune activation and also because thymic output decreases once the thymus gland itself becomes infected. The introduction of HAART improves the diversity of the TCR repertoire via both a thymic regeneration of naïve cells and a decrease in immune activation, which is associated with consumption of T-cells.

THYMIC FUNCTION

It is a long-held view that thymic function decreases with age and is not present at all in adults. This view has been challenged in recent times.[6] It has been observed in HIV-infected persons that thymic responses occur as measured

by increase in thymic size in patients on treatment and also by an increased thymic output of naïve cells.

IMMUNOLOGICAL CHANGES WITH DISEASE PROGRESSION

■ Decreased CD4+ lymphocyte count
■ Increased CD8+ lymphocyte count (from sero-conversion to late-stage disease)
■ Decreased naïve CD4+ lymphocytes (CD45RA)
■ Decreased memory CD4+ lymphocytes (CD45RO)
■ Increased activation markers
    Increased HLA-DR on CD8+ lymphocytes
    Increased CD38+ on CD8+ lymphocytes
■ Decreased HIV–specific CD4+ lymphocytes
■ Decreased TCR repertoire diversity
■ Switch from TH1 to TH2 cytokine profile

IMMUNE RESTORATION FOLLOWING HAART

The benefits of HAART can be shown not only in the clinical context but also by following certain laboratory parameters that relate to immune function. The introduction of HAART has led to a marked decrease in the incidence of opportunistic infections and it has also been shown that certain categories of opportunistic disease may disappear without specific therapies. Complete or partial resolution has been reported in the following conditions:

■ progressive multifocal encephalopathy
■ Kaposi's sarcoma
■ oral hairy leukoplakia
■ oral/oesophageal candidiasis
■ cryptosporidiosis/microsporidiosis.

ENHANCED IMMUNE FUNCTION

After commencement of HAART there are at least two phases of changing numbers of circulating lymphoid cells. The first-phase increase in the CD4+ cells is composed of memory cells that lack markers of cell proliferation, suggesting that these cells have been redistributed from other lymphoid sites into the general circulation. This comes about as a result of suppression of viral replication in lymphoid tissue and CD4+ lymphocytes are thus 'freed' from being sequestered in these sites. The early (often impressive) CD4+ lymphocyte increases are therefore mainly composed of memory cells. This is supported by measuring the expression of the KI67 antigen, which is selectably expressed in proliferating cells. This nuclear antigen was only found to be present after 2 - 3 months on HAART, suggesting that new lymphocyte proliferation begins at this time.[7]

The rapid initial increase of peripheral blood CD4+ lymphocytes is of the order of 1 to 5 cells/µl/day. Since the peripheral blood lymphocytes represent only 2% of the total lymphocyte compartment a minimal virus reduction in lymph tissue following HAART mobilises enough CD4+ cells to substantially increase the peripheral numbers. This phenomenon is more noticeable when treatment is commenced during late-stage disease.

The second-phase increase in T-cells is a much slower process and involves an increase in naïve cells, which require programming in the thymus gland and also possibly in some, as yet undefined, extra-thymic sites. These cells therefore represent a true and meaningful restoration of the immune system. Direct evidence for a thymic participation is the T-cell regenerative process provided by the detection in CD45RA+ T cells of DNA circles produced by TCR rearrangements during thymic maturation. These T-cell receptor excision circles (TRECs) can be quantified by polymerase chain reacton (PCR) and are a good measure of thymic output of naïve cells. Another measure of active thymus involvement involves a measure of thymic size by imaging, utilising a CT scan.
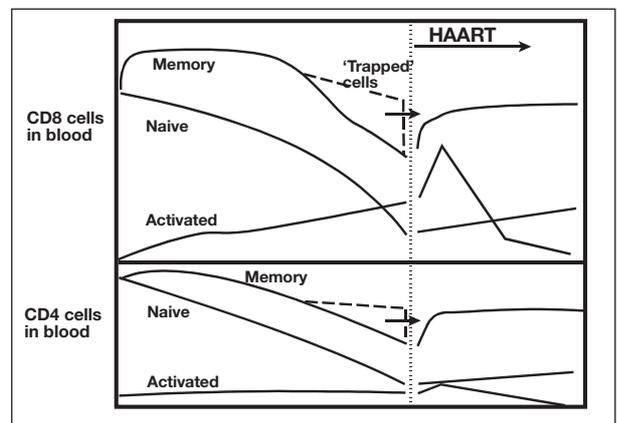

Fig. 4. HIV immune reconstitution.

Another consequence of the introduction of HAART is a decrease in the high level of immune activation. This can be demonstrated by a reduction and expression of the memory HLA-DR and CD38 activation markers on both CD4+ and CD8+ T cells. A reduction in the expression of Fas was also observed.[8] Fas is an expression marker of cells undergoing apoptosis. There is also a return of markers indicating immune competence and function of T-helper cells such as CD28 and CD7. The return of memory CD4+ cell reactivities against pathogens such as CMV and *Mycobacterium tuberculosis* can lead to an immune reconstitution inflammatory syndrome.

Ultimately the reconstitution of the immune response can lead to the discontinuation of primary or secondary chemoprophylaxis for opportunistic pathogens. One of the disappointing features of immune restoration is the lack of increase of CD4+ helper cells specific for HIV. In a few patients treatment initiated at the time of primary

infection can preserve this important cell group. These cells, it is to be emphasised, are the key to a co-ordinated immune response and their loss leads to significant immune disregulation.

MARKERS OF IMMUNE RECONSTITUTION AFTER HAART

- Increase in CD4+ memory cells (CD45RO)
- Increase in naïve CD4+ cells (CD45RA62L+)
- Increase in CD8+ naïve cells (CD45RO)
- Decrease in activation markers (CD38 and HLA-DR)
- Increase in TCR repertoire
- Increase in T-cell receptor excision circles (TRECs)
- Cytokine profile — TH1-response restored.
- Improved lymph node architecture
- Renewed responses to antigens

IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME (IRIS)

Within the first few weeks of commencing ART, the immune system begins to recover and may respond to certain infections that have been present in tissues in a dormant form. With the recovery of the immune system the antigens become recognised and an acute inflammatory response ensues.

This situation is seen when patients begin treatment when their CD4 counts are very low, usually < 50 cells/μl. For patients with tuberculosis, the syndrome is characterised by fevers, lymphadenopathy, worsening pulmonary lesions and expanding CNS lesions. These reactions are commonest in the intensive phase of treatment and are typically self-limiting.

IRIS has been described in the following situations:
- cytomegalovirus (CMV) retinitis
- mycobacterial disease
- hepatitis B or C
- cryptococcal meningitis.

It has been noted in the clinical situation that 15 - 20% of new AIDS diagnoses occur in the first few months after commencement of HAART in patients whose CD4 counts are < 50/μl. It is important that HAART should not be discontinued just because the patient develops an immune reconstitution syndrome, and steroids may be useful to suppress the acute inflammatory response.

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