# IMMUNOLOGIC CONSIDERATIONS IN HIV-INFECTED CHILDREN

## I. INTRODUCTION

This chapter will review the pathogenesis and laboratory evaluation of immune defects associated with HIV infection in children. The role of potent ARV therapies and immune-based therapies on the reconstitution of the immune system also will be discussed. A glossary of immunologic terms used in this chapter can be found in Appendix A.

A competent immune system is essential for survival. The major cellular compartments of the immune system are made up of T and B lymphocytes, monocyte/macrophages, natural killer (NK) cells, and granulocytes. T cells are responsible for major histocompatibility complex (MHC)-restricted cytotoxicity cell-mediated immunity (CMI) and B cells for humoral (antibody-mediated) immunity. Monocytes/macrophages perform essential accessory cell and phagocytic functions. NK cells are important for non-MHC-restricted cytotoxicity. Granulocytes are necessary for phagocytosis and killing.

To function, each cell type relies on a series of interactions between receptors on the cells and extracellular ligands. These lead to a cascade of second messenger systems within the cell including transcriptional activation, and synthesis and secretion of a variety of factors, including cytokines and chemokines. In turn, cytokines and chemokines trigger and regulate cellular responses. The complexity of the immune system is further increased by the fact that development of some components of the immune system continues after birth, resulting in age-associated norms different from adult values. Appendices B and C offer further information regarding the T and B cell system, the role of the thymus, immunity to viruses, and the mechanism of HIV infection of specific target cells, including CD4 cells.

## II. IMMUNITY IN HIV INFECTION

As with any other infectious process, the rate of progression of HIV depends on complex interactions between the virus, the host, and the environment. Although protective immune responses are likely to play an important role in controlling disease progression, immune markers of protection are not yet well defined. Major components of the immune system that are thought to play a role in HIV-specific immunity are HIV-specific cytotoxic CD8 T cells, CD4 helper cells, and neutralizing antibody. In an attempt to dissect the immune response to HIV, considerable attention has been given to the relatively few individuals (1%-2%) who remain healthy for prolonged periods after being infected (long-term non-progressors) and to those who remain uninfected despite repeated exposure to HIV. In addition, investigators have carefully analyzed the immunologic changes following acute infection with HIV.

### A. Cytotoxic T Cells

Cytotoxic T cells have been shown to play an important role in host defenses against HIV by potentially eliminating infected cells and controlling the extent of infection.[1,2] Cytotoxic T cells "kill" the infected target cells in an MHC-restricted manner (i.e., MHC compatibility between target cells and effector cells is required), by mechanisms that require cell-to-cell contact and exocytosis of secretory molecules, such as perforin and granzyme, as well as engagement of death receptors on target cells. The presence of some HIV-specific T cells in HIV-exposed but uninfected individuals supports the role of cytotoxic T lymphocyte (CTL)

in immunoprotection.[3,4] Present at high levels in the early periods following infection, HIV-specific CTL precursors can be detected before seroconversion. The peak in CTL activity following acute infection coincides with the decrease of the initially very high viral load seen during acute infection, to the lower levels seen during chronic infection. In addition, associations between strong CTL responses and both delayed disease progression and low viral loads have been noted.[5,6] Importantly, progression to AIDS is marked by increased viral replication accompanied by the loss of the HIV-specific CTL response.[7]

### B. CD4 T Helper Response

A strong relationship has been demonstrated between CTL and HIV-specific CD4 T helper cell activity.[8] An emerging consensus is that decline in T helper cell function results in defects in other effector cells, including CTL.

### C. Humoral Response

Almost all HIV-infected individuals develop a humoral response to HIV, resulting in the production of antibodies directed against the envelope and core proteins. This serologic response forms the basis for serologic diagnostic testing for HIV infection. These antibodies, however, do not seem to offer any protection from HIV. Assays for identifying true neutralizing antibodies that could prevent cell-to-cell spread of virus are in development.

### D. Chemokines

In addition to CTL activity, CD8 T cells also mediate non-cytolytic HIV suppression by secretion of a cell-associated factor (CAF). Chemokines, such as the β-chemokines, can block the chemokine co-receptors and thus prevent infection of the target cells by HIV (see Appendix C).[9] Such activity is not MHC restricted and is not dependent on cell-to-cell contact between the target cells and effector cells. Although the data are controversial, most studies suggest that secretion of these non-cytolytic suppressor factors slows disease progression and plays a protective role in exposed, uninfected individuals.

## III. Immune Defects in HIV-Infected Patients

Immune defects in HIV infection have been attributed to the direct effects of infection of CD4 cells and to the indirect effects mediated by viral proteins and cytokine dysregulation. The major target cell for HIV infection is the CD4 T helper cell, which is considered central to the orchestration of the immune responses. Thus, HIV infection compromises general immune competence as well as the development of HIV-specific immunity. HIV activates cellular responses that are deleterious to the host and lead to qualitative and quantitative T cell deficiency involving CD4 and CD8 T cells.

Impaired lymphoproliferative responses to antigens are common and precede the loss of responsiveness to mitogens and CD4 depletion in HIV-infected children.[8-10] Such abnormal function correlates with the development of opportunistic and bacterial infections.[11,12]

Published studies evaluating responses to vaccine antigens in HIV-infected children were conducted prior to use of potent ARV therapy. Defects in vaccine responses were seen in children prior to CD4 depletion, demonstrating impairment of immunologic function in the presence of normal numbers of T cells. Even children who had protective antibodies did not achieve or maintain antibody levels comparable with those of healthy children. See Appendix D for further information.

## IV. Antiretroviral Therapy and the Immune System

**Recommendation:**

**For specific clinical recommendations, the clinician should refer to Chapter 4: *Pediatric Antiretroviral Therapy*.**

With potent ARV therapies, dramatic clinical benefits, including prolongation of life, suppression of HIV, and improvement in CD4 T cell counts, are being seen in children and in adults. Important questions have arisen as to whether the increase in CD4 T cells translates into immunologic reconstitution. A major effort is being directed toward evaluating the immune system following potent ARV therapy to determine immune status and find ways to correct remaining deficiencies.

Studies in adults suggest that early intervention with ARV therapy may be beneficial during acute HIV infection to preserve the immune responses. Studies of comparable intervention in infants are currently ongoing. See Appendix E for further information.

### A. Latent Infection

Recent studies in adults and in perinatally infected children who have maintained prolonged suppression of virus have demonstrated that replication-competent virus persists in a pool of long-lived resting memory lymphocytes, and perhaps in other cells as well, even when there is no detectable virus in the peripheral blood.[13,14] Although the size of the pool is small, it may serve as a source of lifelong persistence of infection, and current therapies are not expected to result in eradication of infection.

## V. Immunization of HIV-Infected Children

**Recommendation:**

**Immunizations to prevent infections should be used when available and safe** (see Table 1)**.**

Immunization strategies for HIV-infected children according to the American Academy of Pediatrics guidelines are summarized in Table 1. With the advent of potent ARV the role of IVIG in the treatment of HIV-infected children has been minimized. Children with recurrent bacterial infections, especially if they are hypogammaglobulinemic, may benefit from monthly prophylactic infusions of IVIG, although study results differ.[15,16]

**Table 1**
**Routine Immunization for HIV-Infected Children**

| Vaccine | Use and Precautions |
|---|---|
| Hepatitis B | YES |
| Diphtheria, tetanus, acellular pertussis | YES |
| Inactivated polio | YES; do not use oral polio vaccine; inactivated vaccine for household contacts |
| Measles, mumps, rubella | YES except in severely immunocompromised children [i.e., children in CD4 Immunologic Category 3 (see Table 2)] |
| *Haemophilus influenzae* type B | YES |
| Hepatitis A | CONSIDER, especially in children with hepatitis B and C and other liver dysfunction, and in travelers |
| Pneumococcal, polysaccharide, and conjugate | YES |
| Influenza | YES |
| Varicella | YES, ONLY to children who are asymptomatic and in CD4 Immunologic Category 1 (see Table 2); OFFER to uninfected, nonimmune household contacts |

**Table 2**
**Immunologic Categories for HIV-Infected Children Based on Age-Specific CD4 T-Lymphocyte Counts and Percentage of Total Lymphocytes**

| Immunologic category | Age | | |
|---|---|---|---|
| | **<12 months cells/mm³ (%)*** | **1-5 years cells/mm³ (%)*** | **6-12 years cells/mm³ (%)*** |
| No evidence of suppression | ≥1,500 (>25) | ≥1,000 (>25) | ≥500 (>25) |
| Evidence of moderate suppression | 750-1,499 (15-24) | 500-999 (15-24) | 200-499 (15-24) |
| Severe suppression | <750 (<15) | <500 (<15) | <200 (<15) |

Adapted from *MMWR Morb Mortal Wkly Rep* 1994;43:RR-12.
* Percentage of total lymphocytes.

## VI. Immunologic Laboratory Evaluation of HIV-Infected Children

**Recommendations:**

**A baseline evaluation should be completed and should include quantitative assessment of the cellular immune system, including lymphocyte immunophenotyping, to determine absolute numbers and percentages of CD4 and CD8 T lymphocyte subsets.**

**Lymphocyte subsets should be obtained at baseline (preferably in replicate), and 1 to 2 months after initiation of a new ARV regimen as a measure of response to treatment. They should subsequently be repeated every 3 to 4 months to make sure immune function is being maintained, or more frequently in children with clinical deterioration or rapid decline in CD4 count.**

Although abnormalities of both CD4 and CD8 lymphocytes counts are seen during HIV infection, for routine follow-up of patients, monitoring of the CD4 lymphocyte count alone is usually sufficient. Clinicians may consider monitoring CD8 lymphocyte counts at less frequent intervals for further evaluation of the patient's immune status.

CMI can be assessed by skin testing to evaluate for delayed-type hypersensitivity (DTH) and by functional assays for T cells to determine their ability to proliferate in response to various stimuli. These tests are generally not part of routine care. Similarly, assays for cytokine secretion and effector functions such as cytotoxic activity are specialized and currently not recommended for routine assessments. See Appendix F for further information on these advanced assays.

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## Further Reading


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# APPENDIX A

# GLOSSARY OF IMMUNOLOGIC TERMS USED

**TABLE A-1**
**GLOSSARY OF IMMUNOLOGIC TERMS USED**

| Term | Definition |
|---|---|
| Antigen-presenting cell (APC) | A cell that displays peptide fragments of protein antigens, in association with major histocompatibility complex (MHC) molecules, on its surface, which permits specific recognition by T-cell receptor (TCR) and activation of specific T cells. APCs must also express co-stimulatory molecules to optimally activate T lymphocytes. |
| Apoptosis | A process of cell death characterized by DNA cleavage, nuclear condensation and fragmentation, which leads to phagocytosis of the cell without inducing inflammatory response. |
| B lymphocyte (CD 19+) | The only cell type capable of producing antibody molecules and the central cellular component of humoral immune response. |
| CD molecules | Cell surface molecules expressed on various cell types in the immune system that are designated by the "cluster of differentiation" or CD number. |
| CD4 (T4, Leu-3) | Molecule expressed on a subset of lymphocytes, monocytes, and macrophages, and the primary receptor for HIV. It acts as a signaling and adhesion co-receptor in class II MHC-restricted antigen-induced T cell activation (binds to class II MHC molecules). |
| CD8α (Cytolytic T cells, T8, Leu-2,) | Molecule expressed in a subset of lymphocytes. It acts as a signaling and adhesion co-receptor in class I MHC-restricted antigen-induced T cell activation. |
| CD19 (B4) | Molecule expressed in most B cells. |
| CD38 (T10) | Expressed on early and activated B cells, plasma cells, activated T cells. |
| CD45R (forms of CD45 with restricted cellular expression) | CD45RO: Expressed on memory cells, subsets of B cells, monocytes, macrophages.<br>CD45RA: Expressed on naïve T cells, B cells, monocytes. |
| CD95 (Fas antigen, APO-1) | Binds Fas ligand, mediates signals leading to activation-induced cell death. |
| CD4+16+ CD+56+ | Natural killer cells (NK cells). |
| Cell-mediated immunity (CMI) | The form of adaptive immunity that is mediated by T lymphocytes and serves as the defense mechanism against microbes that survive within cells. CMI responses include CD4+ T cell-mediated activation of macrophages that have phagocytosed microbes as well as CD8+ cytolytic T lymphocyte (CTL) killing of infected cells. |
| Chemokine receptors | Cell surface receptors for chemokines that transduce signals stimulating the migration of leukocytes. These receptors are members of the seven transmembrane α-helical, G protein-linked family of receptors. |
| Chemokines | A large family of structurally homologous, low molecular weight cytokines that stimulate leukocyte movement and regulate the migration of leukocytes from the blood and tissues. |
| Costimulator | A molecule on the surface of or secreted by an APC that provides a stimulus (or second stimulus) required for the activation of naïve T cells, in addition to antigen. The best-defined co-stimulators are the B7 molecules on professional APCs that bind to the CD28 molecule on T cells. |

| Table A-1<br>Glossary of Immunologic Terms Used (Cont'd.) | |
|---|---|
| **Term** | **Definition** |
| Delayed-type hypersensitivity (DTH) | An immune reaction in which T cell-dependent macrophage activation and inflammation causes tissue injury. A DTH reaction to the intradermal injection of antigen is often used as assay for CMI, e.g., the purified protein derivative skin test for immunity to mycobacterium tuberculosis. DTH is a frequent accompaniment of protective CMI against microbes. |
| Dendritic cell | Bone marrow-derived immune accessory cells found in epithelial and lymphoid tissues that are morphologically characterized by thin membranous projections. Dendritic cells function as APCs for naïve T lymphocytes and are important for initiation of adaptive immune responses to protein antigen. |
| Epitope | The specific portion of a macromolecular antigen to which an antibody binds. In the case of a protein antigen recognized by a T cell, an epitope is the peptide portion that binds to an MHC molecule for recognition by TCR. |
| Fas (CD95) | A member of the tumor necrosis factor (TNF) receptor family that is expressed on the surface of T cells and many other cell types and initiates a signaling cascade leading to apoptotic death of the cell. |
| Idiotope | A unique determinant on an antibody or TCR molecule, usually formed by one or more of the hypervariable regions. Idiotypes may be recognized as "foreign" in an individual because they are usually present in quantities too low to induce self-tolerance. |
| Major histocompatibility complex (MHC) Class I MHC Class II MHC | Polymorphic, heterodimeric membrane proteins that bind and display peptide fragments of protein antigens on the surface of APCs for recognition by T lymphocytes. Class I MHC molecules usually display peptides derived from the cytoplasm of the cell. Class II MHC molecules display peptides derived from extracellular proteins that are internalized into phagocytic-endocytic vesicles. |
| MHC restriction | The characteristic of T lymphocytes that they recognize a foreign peptide antigen only when it is bound to a particular allelic form of an MHC molecule. |
| Naïve lymphocyte | A mature B or T lymphocyte that has not previously encountered antigen, nor is the progeny of antigen-stimulated mature lymphocyte. Naïve lymphocytes have surface markers and recirculation patterns that are distinct from previously activated lymphocytes. |
| Natural killer cells (NK cells) | A subset of bone marrow-derived lymphocytes, distinct from B or T cells, that function in innate immune response to kill microbe-infected cells by direct lytic mechanisms and by secreting IFN-$\gamma$. |
| Provirus | A DNA copy of the genome of a retrovirus that is integrated into host cell genome from which viral genes are transcribed and the viral genome is reproduced. HIV proviruses can remain inactive for long periods and represents a latent form of HIV infection that is not accessible to immune defense. |
| Double-positive thymocyte | A subset of developing T cells (thymocytes) in the thymus at intermediate developmental stages that express both CD4 and CD8 molecules. |
| T cell receptor (TCR) | The clonally distributed antigen receptor on CD4 and CD8 T lymphocytes that recognizes complexes of foreign peptides bound to self-MHC molecules on the surface of antigen presenting cells (APCs). $\alpha\beta$ TCR is the most common form of TCR and recognizes peptide antigen bound to an MHC molecule. $\gamma\delta$ TCR is distinct from the more common $\alpha\beta$ TCR and is expressed on a subset of T cells found mostly in epithelial barrier tissues. |

| Table A-1<br>Glossary of Immunologic Terms Used (Cont'd.) | |
|---|---|
| **Term** | **Definition** |
| $T_H1$ cells | A functional subset of helper T cells that secretes a particular set of cytokines, including INF-γ, and whose principal function is to stimulate phagocyte-mediated defense against infections, especially with intracellular microbes. |
| $T_H2$ cells | A functional subset of helper T cells that secretes a particular set of cytokines, including IL-4 and IL-5, and whose principal function are to stimulate IgE and eosinophil/mast cell-mediated immune reactions and to downregulate TH1 responses. |
| TRECs, or T cell receptor excision circles | Formed during intrathymic T cell differentiation and diluted as T cells start multiplying in the periphery, they are a marker for T cells recently released from the thymus and thus serve as a measure of thymic function. |

Adapted from Cellular and Molecular Immunology, 4th ed, Abbas AK, Lichtman AH, Prober JS, pp 468-499, Copyright (2000), with permission from Elsevier Science.

# APPENDIX B

# THE T AND B CELL SYSTEM AND ROLE OF THE THYMUS

The maturation of T cells occurs in the thymus, an organ that plays a pivotal role throughout life in generating T cells of different antigenic specificities so that all possible invaders can be confronted and dealt with appropriately. The thymus, which is largest at birth and during the first few months of life, involutes with age. By adolescence, the thymus is greatly diminished in size, although recent studies indicate that thymic remnants are functionally active well into the fifth and sixth decade of adulthood.

As committed progenitor cells enter the thymus, they undergo a series of differentiation steps by interaction with the thymic microenvironment constituted predominantly by thymic epithelium and a variety of secretory products, including cytokines.

During maturation in the thymus, T cells develop T cell receptors (TCR) and mature into the two major T cell subsets, CD4- and CD8-expressing T cells. The T cell receptor has an α and a β chain, and each chain has a variable (V) and a constant (C) region. The diversity of the immune response is constituted by the TCR β-chain variable region (Vβ) because it determines the specificity for antigen recognition. Thus far, 24 Vbeta "families" have been identified, each endowed with multiple antigen specificities. At birth, the TCR repertoire appears to be fully developed. Cells that emerge from the thymus are "naïve" cells (i.e., they have not encountered any foreign antigen). When an antigen is encountered, naïve cells undergo proliferation to become effector cells to mediate the function that they are programmed to perform. On completion of this action, the majority of the cells undergo death by apoptosis. However, some cells, known as memory cells, revert to a resting stage, primed to go into action if the same antigen is again encountered. Memory cells can be distinguished from naïve cells by characteristic surface markers (i.e., CD45RO for memory cells and CD45RA for naïve cells). Recently, a new marker has been described for T cells that identifies them as recent thymic emigrant cells. These cells are identified by presence of T cell receptor excision circles (TRECs), which are formed during intrathymic T cell differentiation and are diluted as T cells start multiplying in the periphery. Research laboratories are evaluating TRECs in peripheral blood samples as a possible measure of thymic function.

There are normal age-related changes in lymphocyte subpopulations. CD4 T lymphocyte counts are highest at birth and in the neonatal period and decrease to adult values by 6 to 7 years of age. The CD4:CD8 ratio in young children can be three times the CD4:CD8 ratio in adolescents or adults. Thus, T cell values in pathologic situations such as HIV infection should be evaluated in relation to age-associated normal values. In a newborn, most T cells bear the phenotype of naïve cells. Presumably, as a result of exposure to antigens, cells with the memory phenotype expand gradually after birth with the functional role of T cells being to provide a defense system against intracellular pathogens such as mycobacteria, fungi, and most viruses. Severe deficiencies in the T cell system result in failure to thrive and susceptibility to opportunistic infections.

The second component of the lymphoid system is made up of B lymphocytes. Dependent on the presence of an appropriate microenvironment, progenitors of B cells also undergo a series of differentiation steps. The major function of B cells is to produce antibodies and provide a defense system against high-grade bacterial pathogens by secreting antibodies. For many antigens, such as protein antigens, the B cell response is dependent on adequate help from T cells.

For an immune response to be initiated, antigen recognition is an essential first step that occurs via T cell receptors on T cells and surface Ig molecules on B cells. CD4 helper T lymphocytes recognize foreign antigens bound to class II MHC molecules on professional antigen-presenting cells (i.e., B lymphocytes, macrophages, dendritic cells, Langerhans cells, and endothelial cells). By contrast, CD8 T cells recognize foreign antigens bound to class I MHC molecules found on nearly all nucleated cells except neurons.

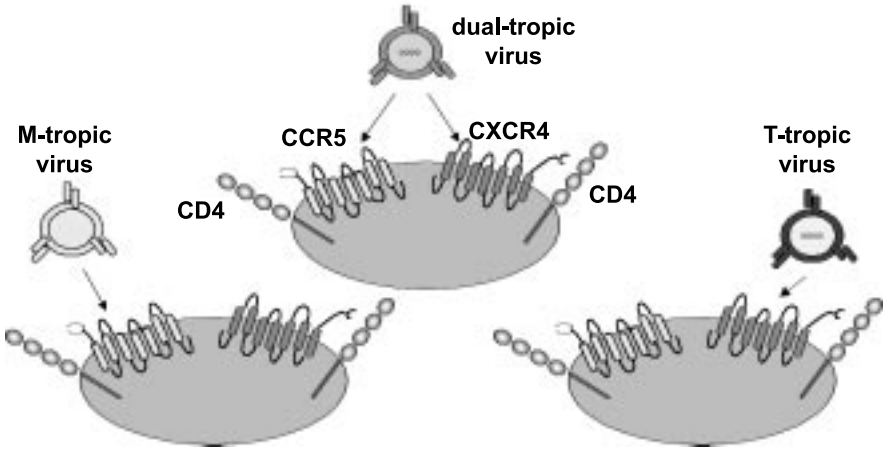
# Appendix C

# Mechanism of Infection of Target Cells by HIV

HIV needs two sets of receptors to enter its target cells: the CD4 molecule and a chemokine receptor. For many years after the discovery of HIV, it was believed that CD4 was the primary and possibly the only receptor used by HIV to infect target cells. The discovery in 1996 that HIV utilized a co-receptor has broadly influenced our understanding of the mechanism of infection, cellular tropism of different types of HIV, the genetic resistance to HIV, and the role of the natural ligands that bind these receptors in protection from infection and disease progression. The major co-receptors are CCR5 and CXCR4 viruses that utilize CCR5 co-receptor for entry into the target cell are known as macrophage tropic (M tropic), and those which utilize CXCR4 are known as T cell tropic (T tropic) viruses (see Figure C-1). These terms are somewhat misleading, as the expression of these chemokine receptors is not restricted to T cells and macrophages and has a broader distribution involving other cells as well. CD4 T cells, for example, can express CCR5 as well as CXCR4, whereas monocytes/macrophages express mainly CCR5, and T cell lines express mainly or exclusively CXCR4. Most HIV transmission, including transmission from mother to child, occurs with predominantly CCR5-utilizing virus strains. It has been shown that a genetic defect involving 32 base pair deletion mutation in CCR5 confers resistance to HIV. Individuals who are homozygous for the deletion and who have repeatedly engaged in high-risk behavior have remained uninfected, leading to the term "exposed-uninfected" individuals. With disease progression, CXCR4-utilizing strains or dual tropic strains of HIV emerge. The natural ligands for the chemokine receptors are small molecules of 8-12kD, previously known for their role in leukocyte trafficking. Based on the arrangement of cysteine residues, the chemokines are divided into subgroups: CXC chemokines, such as Stromal-derived factor (SDF-1) bind to CXCR4, and CC or β-chemokines (Rantes, MIP-1 α and MIP-1 β) bind to CCR5. These chemokines are considered to be "natural" blockers of the HIV co-receptors and thus have stimulated interest in their role in HIV disease progression and in therapeutic approaches aimed at blocking HIV infection.

Although CD4 helper T cells are the principal target of HIV, many different cells can be infected with HIV, including cells of the monocyte/macrophage lineage, megakaryocytes, peripheral blood dendritic cells, follicular dendritic cells, epidermal Langerhans cells, astrocytes, oligodendroglia and microglia, CD8 cells, cervical cells, rectal mucosal cells, renal epithelial cells, cardiac myocytes, and retinal cells.

**Figure C-1**
**HIV Infection of Target Cells Is Dependent on CD4 and Chemokine Receptors**



# APPENDIX D

# IMMUNE DEFECTS IN HIV-INFECTED PATIENTS

HIV downregulates the major histocompatibility complex (MHC) class I expression in infected cells, providing a mechanism to evade immune surveillance.[1,2] This contributes to the inability of CD8 T cells to efficiently eliminate HIV *in vivo*. The reduction in the expression of class I antigens seen several days after infection has been attributed to the effects of viral gene products—Nef and Vpu.[3-7] Nef is produced in abundance early in infection, and induces rapid endocytosis of CD4 molecules and MHC class I molecules, followed by their degradation in lysosomes. Vpu protein interferes with early events in the assembly of class I molecules. HIV also affects the MHC class II pathway on professional antigen-presenting cells (APC) in *in vitro* model systems, resulting in the loss of class II expression and inhibition of exogenous antigen processing. Aberrant immune activation is a hallmark of HIV infection, resulting in expression of activation markers on T cells (DR, CD38, CD95), secretion of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and increased lymphocyte apoptosis. Lymphocyte apoptosis (also termed "activation induced cell death") in HIV disease is believed to result from direct and indirect effects of HIV and from the ensuing heightened state of lymphocyte activation. Both CD4 and CD8 T cells exhibit increased apoptosis, and effective ARV therapy is associated with reversal of this defect.[8,9] Collectively, the immune suppressive effects, the aberrant immune activation, and increased lymphocyte apoptosis result in decrease in CD4 T cells, impaired T cell function, phenotypic imbalance of CD4 and CD8 lymphocytes, dysregulated cytokine production marked by a deficiency in interleukin-2 (IL-2) production, functional abnormalities of cells of the monocyte/macrophage lineage, abnormalities in B cell function, and defects in natural killer (NK) function.[10-12]

In HIV-infected adults, decreased IL-2 production is an early event that does not correlate with CD4 counts.[13] Regardless of disease state, decreased production of IL-2 in infected children has been shown.[14] The addition of IL-2 can correct defects in *in vitro* antigen-specific T cell proliferative responses to tetanus toxoid.[15] IL-2 is also important for B cell proliferation and differentiation. Defects in B cell differentiation have been observed in studies of patients with advanced HIV disease as well as in asymptomatic infection in both children and adults.[16,17]

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# APPENDIX E

# ANTIRETROVIRAL THERAPY AND THE IMMUNE SYSTEM

Until recently, most of the studies evaluating the immune system have been performed in adults. These studies have shown that with antiretroviral (ARV) therapy, CD4 cells increase with the rise first occurring in memory cells and later in naïve T cells. Studies of T cell receptor excision circles (TRECs) and T cell receptor (TCR) repertoire have yielded encouraging but inconclusive results.[1,2] Lymphocyte proliferation assay (LPA) responses to recall antigen *Candida* recover first, but tetanus responses are slower to recover. Even with ARV therapy, HIV-specific immune responses to the core antigen do not improve; this suggests that HIV vaccines may be required to boost HIV-specific immune responses. These questions are currently under investigation in pediatric patients; recent data are encouraging and suggest that return of CD4 T cells is more vigorous in children than in adults. In contrast to the immune recovery in adults, in children naïve cells return first followed by memory cells, indicating a faster recovery of the thymus in children.[3-5] Humoral immune responses to vaccine antigens wane in HIV-infected children, and the extent of recovery following potent ARV therapy is under investigation. With normalization of the T cell repertoire and the active production of new T cells, it is expected that re-immunization will lead to an effective immune response.

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# APPENDIX F

# IMMUNOLOGIC LABORATORY EVALUATION OF HIV-INFECTED CHILDREN: ASSAYS

## I. QUANTITATIVE ANALYSES

Lymphocyte immunophenotyping (CD subsets) is an important tool to assess the level of general immune function. Absolute counts and percentages of CD4 helper T cells are determined. Based on their CD4 cell counts, children of different ages can be categorized into different immune categories defining the degree of immunosuppression. Other lymphocyte subsets that are sometimes quantitated include CD8 T lymphocytes, natural killer (NK) cells, and B lymphocytes. The ratio of CD4 T:CD8 T lymphocytes provides an idea of the degree of lymphocyte imbalance; normally the ratio is >1 but may be much higher in young infants. In the setting of HIV infection, this ratio is reversed.

Levels of one or more classes of immunoglobulins (IgG, IgM, IgA, IgE) are usually increased in HIV-infected children and are indicative of excessive B cell stimulation. With control of HIV infection, these levels decrease to normal or near normal levels. In rare cases, hypogammaglobulinemia has been reported to occur in association with HIV infection.

## II. FUNCTIONAL ANALYSES

Skin testing for delayed-type hypersensitivity (DTH) uses one or more antigens to which the child has been exposed. The most commonly used antigens are diphtheria and tetanus, which almost all children have been exposed to through vaccination, and *Candida*, a common infection during infancy. Generally, approximately 60% to 90% of healthy children respond well to diphtheria and tetanus antigens but have age-dependent responses to *Candida* antigen. Testing involves intradermal injection of the antigen with the site assessed for induration after 48 hours.

*In vitro* mitogen and antigen stimulation are used as relatively crude measures of cellular immunity. These tests may be useful if assessment of cellular function is needed. This testing is not available in all laboratories. In *in vitro* mitogen and antigen stimulation for lymphoproliferation, the patient's peripheral blood mononuclear cells are cultured with mitogens, such as phytohemagglutinin, concavalin A, pokeweed mitogen, and other antigens to which the patient has been exposed, including tetanus toxoid and *Candida albicans*. A stimulation index is then calculated.

Functional analyses of B cells include the determination of humoral responses following vaccinations; however, at the current time, there are no specific guidelines concerning routine use. Production of antibody responses to vaccine antigens, such as tetanus, diphtheria, *Haemophilus influenzae* type b, hepatitis B, measles, mumps, rubella, and pneumococcal antigens, can be tested 1 month following vaccine administration for the determination of a primary response. Follow-up testing can be considered every 5 years to determine the presence of long-term humoral immunity and to guide decisions regarding revaccination.

*In vitro* B cell function tests are not readily available and are not recommended.

## III. NEWER TESTS

Recently, five tests have been developed and are currently being evaluated in research settings. (see Table F-1). These tests hold promise for the future assessment of immunologic reconstitution in HIV-infected children on potent ARV therapy.

| TABLE F-1<br>NEWER TESTS FOR IMMUNOLOGIC RESPONSE TO ANTIRETROVIRAL THERAPIES | |
|---|---|
| **Tests** | **Description** |
| Thymic rearrangement excision circles (TRECs) | This test enumerates output of new T cells from the thymus and is considered to be important for the regeneration of the immune system that was adversely affected by the virus. |
| Tetramer binding cells | This assay quantitates cytotoxic T cells that are specific for a particular antigen epitope. Such an assay can provide estimated numbers of antigen-specific cells (e.g., numbers of HIV-gag binding cytotoxic T cells).[1] Fluorescently labeled tetrameric complexes of host MHC and a peptide antigen are presented to CD8 T cells. If the T cells recognize the antigen, this will result in binding of the complexes to the T cells. The presence of tetramer-T cell binding can be measured using a flow cytometer. |
| Antigen-induced cytokine production | HIV antigen-induced interferon gamma secreting CD8 T cells (surrogate markers for cytotoxic T cells) and interferon or interleukin-2 secreting CD4 T cells (for antigen-specific T helper cells) can be determined by flow cytometry assays for intracellular cytokines or by their secreted cytokines in "ELISPOT" assays. |
| T cell receptor Vbeta repertoire in CD4 and CD8 T cells | This test provides an assessment of T cell diversity as well as information on expansions and deletions in particular Vbeta families.[2,3] The goal of immune reconstitution is for these perturbations to be corrected and for the T cell repertoire to normalize. |
| Lymphocyte apoptosis by flow cytometry-based assay | This assay determines the extent of lymphocyte apoptosis, which is expected to be corrected with reduction in viral load.[4,5] |

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