# Part I

# Scientific basis of pediatric HIV care

# 1 The scientific basis of pediatric HIV care

Combined from the following chapters in the *Textbook of Pediatric HIV Care: Normal development and physiology of the immune system* Sherilyn Smith and Ann Melvin. *HIV basic virology for clinicians* Steven L. Zeichner. *The immunology of pediatric HIV disease* Elizabeth McFarland. *The clinical virology of pediatric HIV disease* Paul Palumbo. *The natural history of pediatric HIV disease* Grace Aldrovandi

## Normal development and physiology of the immune system

### Components and function of the immune system

The immune system can be divided into two components. The "innate arm" of the immune system provides a rapid, non-specific pathogen response. It acts as a surveillance system and initiates the antigen-specific phase of the immune system. The major components of innate immunity include physical barriers, complement and other opsonins, the spleen, phagocytes and NK (natural killer) cells. Many responses are triggered by the Toll-like receptor (TLR) with the molecules they bind.

The antigen-specific phase of immunity is directed at specific pathogen antigens. The inducible portions of the immune system include cellular and humoral immune responses. These components control infection and form long-term immunity. Table 1.1 summarizes the major functions of the immune system and the infections that can result from its dysfunction.

#### Innate immune system

**Barriers**

The initial defense against microbes is an intact physical mucosal and epithelial barrier. Specialized cells (including ciliated respiratory epithelia), and localized chemical barriers (stomach acid, mucus layers in respiratory and gastrointestinal tracts, and skin

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**Table 1.1.** The immune system: functions, developmental aspects and infections associated with dysfunction

| Immune system component | Function | Developmental differences | Infections associated with dysfunction |
|---|---|---|---|
| Innate | | | |
| Epithelial barriers/ mucosal defense | • Impede entrance of microorganisms<br>• Present antigen<br>• Sample environment | • Epithelial barriers decreased in premature infants<br>• Decreased IgA–adult levels by 6–8 years | • Low virulence organisms: coagulase negative staphylococcus opportunistic gram negative bacteria fungi |
| Complement/opsonins | • Amplify the immune response<br>• Facilitate phagocytosis<br>• Chemoattractants | • Terminal complement levels decreased in neonates | • Encapsulated organisms<br>• Recurrent infections with *Neisseria* species<br>• Recurrent/recalcitrant skin infections |
| Phagocytes | • Engulf and kill microorganisms<br>• Present antigens to T-cells (macrophages)<br>• Elaborate immune active substances including cytokines and chemotactic factors | • Monocytes: decreased chemotaxis, decreased cytokine production–adult function by 6 years<br>• Neutrophils: decreased bone marrow pool in neonates, decreased chemotaxis–adult levels by 1 year | • *S. aureus*<br>• Low virulence organisms: other staphylococci gram negative opportunistic bacteria fungi |
| Spleen | • Filters intravascular organisms<br>• Aids with opsonization<br>• Antibody formation | | • Encapsulated organisms (*S. pneumoniae*, Salmonella, *H. influenzae*)<br>• Develop severe or recurrent infections |

| | | | |
|---|---|---|---|
| Natural killer (NK) cells | • Lyse cells presenting "non-self" antigens (e.g., tumor or viral proteins) | • Decreased ADCC, decreased cytolytic activity | • Recurrent/severe viral infections with members of the Herpesvirus family |
| Dendritic cells | | | |
| | • Capture and present antigens to lymphocytes | • Decreased ability to present antigen | ? |
| Antigen specific | | | |
| T-cells | • Cell-mediated immunity<br>• Elaboration of cytokines<br>• Regulation of the immune response<br>• Cytolysis<br>• Increases the efficiency of B-cell function by providing "help" | • Increased absolute numbers – decline to adult levels by late childhood<br>• Naive phenotype in neonate (90%) decreased cytokine production, costimulatory molecule expression, and ability to provide "help" to B-cells – normalizes throughout infancy with antigenic exposure | • Infections with "unusual" organisms: intracellular bacteria (listeria, mycobacteria)<br>• Fungi (aspergillus, candida)<br>• Viruses (esp HSV, VZV, CMV, HHV-8)<br>• Protozoa (giardia, *Pneumocystis carinii*) |
| B-cells | • Humoral immunity (formation of antibody to specific antigens) | • Unable to respond to polysaccharide antigens until ~ 2 years of age | • Encapsulated organisms<br>• Enteroviral infections<br>• Recurrent GI or sinopulmonary infections<br>• Inability to respond to vaccines |

fatty acids and cerumen) impede pathogen entry. Breaches in these barriers may result in infection by low virulence organisms.

**Mucosal immunity**

Mucosal-associated lymphoid tissues are located at sites close to the environment. These lymphoid aggregates (e.g., Peyer's patches) sample the environment, allowing for early initiation of antigen-specific responses [1, 2]. Secretory IgA, synthesized in these tissues, adds to the local mucosal defense.

**Opsonins**

Opsonins are proteins that bind to pathogen surfaces, facilitating phagocytosis. They include acute phase reactants (C-reactive protein, fibronectin), complement and antibody. Complement is a protein that is sequentially activated by proteases. Complement plays an important role in the killing of invasive bacteria. Two pathways activate complement: the classical pathway (antibody binds bacterial antigen which then is complexed with C1, a complement component), which begins a series of proteolytic reactions activating additional complement components, and the alternate pathway (bacterial antigen directly binds the C3b complement component). Both pathways produce a complex that lyses bacteria [3].

**Spleen**

The spleen efficiently filters opsonized bacteria and is an antibody production site. Absence or dysfunction of the spleen predisposes to overwhelming infection with encapsulated organisms.

**Macrophages**

Macrophages and monocytes clear invading microbes. Macrophages migrate to sites of infection and phagocytose foreign substances. Macrophages elaborate many cytokines and growth factors that modify evolving immune responses [4].

**Neutrophils**

Neutrophils are blood phagocytes that migrate to sites of infection and phagocytose pathogens, notably immunoglobulin- or complement-coated microbes, including bacteria and fungi. Neutrophils kill phagocytosed pathogens via the respiratory burst (reactive oxygen metabolites generation) or by degranulation with release of substances that directly kill pathogens.

**Natural killer (NK) cells**

NK cells are specialized lymphocytes that recognize non-self proteins, important in early responses to viral infections. Viruses often down-regulate host major histocompatibility complex molecules (see below) on infected cells surfaces, which causes NK cells to recognize them as foreign, making them targets for lysis.

**Dendritic cells (DC)**

DC capture antigen and present it to lymphocytes. There are three major DC populations: (a) Langerhans cells (interdigitating cells) reside in tissues and migrate to T-cell areas of lymphoid organs after antigen uptake; (b) myeloid DCs (interstitial or dermal DCs), which become germinal center DCs in lymphoid follicles; and (c) plasmacytoid DCs, which reside in T-cell lymphoid tissues areas [5]. Immature DCs take up and process antigen. Migrating to lymphoid tissues, they mature to become antigen-presenting cells [5]. Different populations of DCs have different functions. Langerhans cells activate CD8+ cytotoxic T-cells [6] and promote T-helper type 1 (Th1) responses in CD4+ T-cells. Myeloid DCs (known as DC1 cells) also promote Th1 responses; plasmacytoid DCs (DC2 cells) induce Th2 responses [7].

**Toll-like receptors**

Toll-like receptors are a family of transmembrane proteins that help initiate the innate immune response. Ten Toll-like receptors (TLR1–10) have been cloned. They serve as an "early warning system" for recognition of microbial antigens and molecules produced by microbes, such as lipopoly saccharides, CpG DNA, and double-stranded RNA. Activation releases chemokines and other inflammatory mediators from dendritic cells and macrophages and modulates expression of chemokine receptors on dendritic cells. Several toll-like receptors can probably act in concert [8].

## Antigen-specific immunity

### Cell-mediated immunity

**T-cells**

T-lymphocytes (thymus-dependent lymphocytes) mediate delayed-type hypersensitivity reactions, regulate the development of antigen-specific antibody responses, and provide specific defense against many organisms. Distinct T-lymphocyte subpopulations express different cell surface proteins (see Table 1.2).

**T-cell receptor complex**

T-cells bear antigen-specific T-cell receptors (TCR), required for foreign antigen recognition and binding. TCRs have either $\alpha$- and $\beta$-chains or $\gamma$- and $\delta$-chains. Each chain has a variable amino-terminal involved in antigen recognition and constant carboxy-terminal regions. As T-lymphocytes mature, the TCR genes rearrange [9], creating unique TCRs within T-cells with specific antigen recognition capacity, generating TCR diversity to recognize many antigens. Lymphocytes with $\alpha/\beta$-chain TCRs ($\alpha/\beta$-cells) locate in lymphoid organs and peripheral circulation, those with $\gamma/\delta$ TCR chains ($\gamma/\delta$ T-cells) locate in mucosa.

**MHC molecules**

Antigen-presenting cells present antigen to T-cells as short peptides complexed with major histocompatibility complex (MHC) molecules. These cell surface molecules were

**Table 1.2.** Lymphocyte function and phenotype

| Lymphocyte type | Function | Type of antigen receptor | Common cell surface markers |
|---|---|---|---|
| T-lymphocytes | • Regulation of the immune response | αβ T-cell receptor | CD3+,CD4+, CD8− |
| Helper | • Development of "memory" response to antigens | | |
| Th1 | • Cell mediated immunity – control of intracellular pathogens, DTH response<br>• Activates macrophages via cytokine elaboration (IFN-γ and IL-2) | αβ T-cell receptor | CD3+,CD4+, CD8− |
| Th2 | • Stimulates B-lymphocyte differentiation and proliferation (humoral immunity)<br>• Elaborates cytokines involved primarily in the allergic response (IL-4, IL-5, IL-10) | αβ T-cell receptor | CD3+,CD4+, CD8− |
| Cytotoxic | • Lysis of tumor cells, virus-infected cells<br>• Stimulates cell-mediated immunity via cytokine production | αβ T-cell receptor | CD3+, CD4–, CD8+ |
| B-lymphocytes | • Production of antigen-specific immunoglobulins (humoral immune response) | Immunoglobulin molecules (IgG, IgM, IgE, IgA) | Fc receptors, MHC II molecules CD20, CD19 |
| Natural killer (NK) lymphocytes | • Lysis of virus infected cells and tumor cells lacking MHC class I; antibody-dependent cellular cytotoxicity | | CD16, CD56 |

initially identified as major antigens involved in transplant rejection. Cells expressing different MHC molecules are recognized as "non-self" and rejected. When foreign antigens are complexed with MHC molecules, the complex is recognized as non-self, initiating an immune response [10]. Class I MHC molecules are expressed on the surface of most cells and present intracellular antigens (e.g., antigens derived from infecting viruses). Class II MHC molecules exist primarily on "professional" antigen presenting cell (monocyte, macrophage, dendritic, and B-cell) surfaces and present proteins originating outside or within the cell (e.g., phagocytosed bacterial protein). CD4+ (helper/inducer) T-cells recognize exogenous antigen bound to class II molecules, and CD8+ (cytotoxic) T-cells recognize endogenous antigen bound to class I molecules [11].

**Antigen presentation**

The initiation of specific immune responses begins when the T-cell TCR recognizes short peptides processed and bound to an antigen-presenting cell (APC) MHC molecule. The TCR-associated CD3 molecule transduces a signal into the cell. Proper antigen recognition requires the TCR/CD3 complex. Other T-cell accessory molecules (CD4 and CD8) must also interact with the APC [12]. They bind the invariant regions of class I or class II MCH molecules. Other molecules, including CD28 and integrins, act as costimulatory signals to induce certain immune responses. TCR–MHC–antigen interaction produces T-cell activation and differentiation, initiating the response. T-cell surface markers change once the T-cell TCR encounters specific antigen. A subset of peripheral CD4+ T-cells (CD45RA+ $CD29^{low}$) includes naïve cells that have not encountered specific antigen, forming the pool of cells responding to novel antigens. After an initial antigen encounter they develop into memory T-cells (CD45RO+$CD29^{hi}$) [13]. These memory T-cells rapidly proliferate and produce cytokines when rechallenged with previously encountered antigens, yielding rapid, expanded secondary responses.

**CD4+ T-cells**

Most peripheral $\alpha/\beta$ T-cells express CD4 or CD8 antigens. CD4+ T-cells (helper/inducer cells) help regulate the immune response. CD4+ T-cells help B-cells to produce antigen-specific antibody. B-cells process antigen and present self-MHC-bound antigen fragments, activating CD4+ T-cells. During interactions between B- and CD4+ T-cells, membrane molecules that increase the efficiency of the interaction are upregulated [14]. CD40 ligand appears on the activated T-cell surface, which acts on B-cells, promoting humoral immune response [15]. CD4+ cells activate B-cells into antibody-secreting cells and help generate CD8+ T-cell cytotoxic and suppressor functions (see below). Memory T-cells (see above) are CD4+ T-cells.

**$T_H1$ vs. $T_H2$ T-cells**

Two functionally distinct CD4 cell subsets are distinguished by their cytokine expression [16]. $T_H1$ cells produce interferon-$\gamma$ and IL-2, and enhance cellular immunity

**Table 1.3.** Selected cytokines, cell source and principal effects

| Cytokine | Cell source | Target cell/principal effects |
|---|---|---|
| IL-2 | T-cells | T-cells: proliferation and differentiation; activation of CTL and macrophages |
| IL-3 | T-cells, stem cells | Cell colony stimulating factor |
| IL-4 | T-cells | T/B-cells: B-cell growth factor, isotype selection |
| IL-6 | T/B-cells | B-cells/hepatocytes: B-cell differentiation, acute phase reactant production |
| IL-8 | Monocytes | Granulocytes, basophils, T-cells: chemotaxis, superoxide release, granule release |
| IL-12 | Monocytes | T-cells: induction of TH1 cells |
| IFN-$\gamma$ | T-cells, NK cells | Leukocytes, macrophages: MHC induction, macrophage activation and cytokine synthesis |
| TNF-$\alpha$ | Macrophages, mast cells, lymphocytes | Macrophages, granulocytes: activation of monocytes, granulocytes, increase adhesion molecules, pyrexia, cachexia, acute phase reactant production |

IL – interleukin; IFN – interferon; TNF – tumor necrosis factor.

and macrophage activity. $T_H1$ cells regulate delayed-type hypersensitivity, granuloma formation, and intracellular pathogen killing. $T_H2$ cells produce IL-4, IL-5, and IL-10, and regulate humoral immunity, which mediates the development of allergic diseases: IL-4 promotes IgE production; IL-5 induces eosinophil proliferation and differentiation [12, 17]. Differentiation of naïve CD4+ T-cells into $T_H1$ or $T_H2$ cells depends on the cytokine milieu, antigen dose, and the specific antigen.

**CD8+ T-cells**

CD8+ (cytotoxic/suppressor) T-cells act as cytotoxic T-cells and can suppress immune responses [11]. Class I MHC-bound antigen activates CD8+ T-cells to generate antigen-specific cytolytic activity. Cytolytic T-lymphocytes (CTL) respond to viral infection of most host cells [12]. CD4+ cells help CD8+ T-cells to develop a CTL response by producing several cytokines, particularly IL-2 [18].

**Cytokines**

Cytokines are soluble proteins that modulate immune responses. They interact with specific membrane receptors. Different cytokines may perform similar functions and affect multiple cell types. Cytokine functions include (a) regulating lymphocyte growth and differentiation, (b) mediating inflammation, and (c) regulating hematopoesis. Cytokines affecting T-cells include the interleukins (IL), interferons, growth factors, and tumor necrosis factor (TNF) [12] (Table 1.3).

**Table 1.4.** Selected chemokines, their receptors and target cells

| Chemokine | Receptors | Target cell |
|---|---|---|
| MIP-1α | CCR1 – 7 | Eosinophils, monocytes, activated T-cells, dendritic cells, NK cells |
| MIP-1β | CCR1 – 7 | Monocytes, activated T-cells, dendritic cells, NK cells |
| RANTES | CCR1 – 7 | Eosinophils, basophils, monocytes, activated T-cells, dendritic cells, NK cells |
| Fractalkine | $CX_3CR1$ | Monocytes, activated T-cells, NK cells |
| SDF-1 | CXCR4 | Monocytes, resting T-cells, dendritic cells |
| MIG | CXCR3 | Activated T-cells, NK cells |
| IL-8 | CXCR1 and 2 | Neutrophils |
| IP-10 | CXCR3 | Activated T-cells |
| MCP-1 | CCR2 and 5 | Monocytes, activated T-cells, dendritic cells, NK cells |
| Eotaxin-1 | CCR1-3 | Eosinophils, basophils |

MIP – macrophage inflammatory protein; SDF – stromal-cell derived factor; MIG – monokine induced by interferon gamma; IL – interleukin; IP – interferon inducible protein; MCP – monocyte chemoattractant protein
Adapted from [19].

**Chemokines**

Chemokines are a family of cytokines that regulate chemotaxis [19]. Over 40 chemokines are grouped into four families, including the α- and β-chemokines. β-chemokines have two adjacent cysteine residues (CC); α-chemokines have one amino acid separating the first two cysteine residues (CXC). Almost all cell types produce chemokines, particularly in response to inflammation. Proinflammatory cytokines (IL-1 and TNF-α), lymphokines (INF-γ and IL-4), and bacterial LPS and viral infection stimulate chemokine production. Chemokines bind specific target cell receptors. Most chemokine receptors bind more than one chemokine; however, CC chemokine receptors bind only CC chemokines and CXC receptors bind only CXC chemokines. Different leukocyte types express different chemokine receptors. Some receptors are restricted to specific cell types; others are expressed widely (Table 1.4).

Infiltrating inflammatory cells are determined partly by chemokines in affected tissue. Chemokines link innate and adaptive immune systems. Dendritic cells internalize antigens in tissues to carry them to lymph nodes, where naïve B- and T-cells are activated. Activated cells traffic back to inflammation sites. Chemokines regulate DC and lymphocyte trafficking [19].

## Humoral immunity

**Immunoglobulins**

Immunoglobulins (antibodies) are proteins that bind antigen with high affinity and specificity. An immunoglobulin molecule is made up of two heavy and two light chains,