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The role of Interleukin-15 in inflammation and immune responses to infection: implications for its therapeutic use

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Abstract

Interleukin-15 (IL-15) is a pleiotropic cytokine with a broad range of biological functions in many diverse cell types. It plays a major role in the development of inflammatory and protective immune responses to microbial invaders and parasites by modulating immune cells of both the innate and adaptive immune systems. This review provides an overview of the mechanisms by which IL-15 modulates the host response to infectious agents and its utility as a cytokine adjuvant in vaccines against infectious pathogens.

Keywords

IL-15; infectious diseases; vaccines; inflammation; molecular adjuvants

1. Introduction

Cells of the innate or natural immune system such as neutrophils, macrophages, dendritic cells (DC), natural killer cells (NK), eosinophils, basophils, and mast cells form the first line of defense to protect the host against microbial invaders and parasites [1]. To identify microbial invaders and to mount an appropriate inflammatory response, the innate immune system has evolved an elaborate system of pathogen and commensal microbial sensors in the form of germline encoded host cell receptors and their associated signaling pathways [2]. Of these, the best characterized sensors are several families of pattern recognition receptors (PRR) that detect invariant features of invading classes of microbes known as pathogen associated molecular patterns (PAMP) that include Toll-like receptors (TLRs), membrane-bound C-type lectin receptors (CLRs), cytoplasmic nucleotide-binding oligomerization domain (NOD) leucine-rich-repeat containing receptors (NLRs), and cytoplasmic retinoic acid-inducible gene 1 protein (RIG-1) helicase receptors [2]. Thus, the different cellular locations of the PRRs make it possible for the innate immune system to respond to both cell-invasive and non-invasive pathogens. Moreover, microbial pathogens are usually composed of several PAMPs and activate multiple PRRs on innate immune cells that act in concert leading to the activation of downstream signaling events and the secretion of inflammatory cytokines, chemokines, and antimicrobial peptides [2]. These responses cause recruitment of

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more innate immune cells to the site of infection leading to their activation, migration of activated antigen presenting DCs to draining lymph nodes to prime lymphocytes, and ultimately to the elimination of the pathogen or control of the pathogen until the adaptive immune response is enabled.

In addition to the rapid responses of the innate immune system to infections, vertebrates have also evolved a highly specific adaptive or acquired immune system comprised of B and T lymphocytes that responds later during the course of an infection than the innate immune response, to protect the host [1]. Unlike the germ line encoded PRR on innate immune cells, lymphocytes use RAG recombinase to generate a diverse number of antigen receptors through a series of site-specific, somatic DNA rearrangements that are collectively called variable-diversity-joining recombination [1]. Humoral immunity provided by B lymphocytes and their secreted antibodies protect against extracellular microbes and their toxins [1]. In contrast, cellular immunity mediated by T lymphocytes defends against intracellular microbes by killing infected cells, and/or the release of cytokines that inhibit growth of the microbes or affect their survival [1]. Elimination of invading microbes or neutralization of microbial toxins limits the injury caused to the host and leads to the resolution of inflammation. It also leads to the production of antigen-specific terminally differentiated effector memory T lymphocytes with relatively short life spans that migrate to the peripheral non-lymphoid and mucosal tissues and to a pool of long-lived central memory T lymphocytes within secondary lymphoid tissues [3]. While effector memory T lymphocytes display immediate effector activity by cytokine production or killing of infected cells when stimulated by antigen at tissue sites or at portals of microbial entry, central memory T lymphocytes lack immediate effector functions, but give rise to effector cells upon antigen stimulation [3]. Similarly, memory B lymphocytes in the secondary lymphoid organs do not actively secrete antibody, but when stimulated by microbial antigens rapidly divide and differentiate into antibody producing plasma cells [3]. Unlike innate immune responses mounted by a host that do not change on re-encounters with the same pathogen, memory T and B lymphocytes and their products provide a more rapid and robust recall response on re-exposure to the same pathogenic microbes, and prevent a re-infection or significantly reduce the severity of the disease. Moreover, recent advances have also highlighted the instructive role played by the innate immune system in programming the final outcome of the adaptive immune response to infections [4]. In addition, there is increased understanding of the roles played by anti-inflammatory regulatory T and B cells during chronic pathogenic infections to prevent immune-pathologies due to excessive inflammatory responses [5].

2. IL-15 expression and regulation

Mature human IL-15 is a 14–15 kDa glycoprotein and a member of the four α -helix bundle family of cytokines [6]. In both humans and mice cellular IL-15 production appears to be under stringent control through regulation of transcription, translation, translocation, and intracellular trafficking [7]. A variety of cell types constitutively express IL-15 mRNA, and these include monocytes, macrophages, DCs, keratinocytes, epidermal skin cells, fibroblasts, various epithelial cells, bone marrow stromal cells, and nerve cells [8,9]. In addition, IL-15 mRNA is also expressed in kidney, placenta, lung, heart, skeletal muscle, and brain tissues. Despite the widespread expression of IL-15 mRNA, except for monocytes, DCs, epithelial cells, bone marrow stromal cells, and fibroblasts, very few other cells and tissues secrete detectable levels of IL-15 proteins [9,10]. The paucity in secreted IL-15 protein can be explained by several structural features in IL-15 mRNA that impede IL-15 protein production. Translation of IL-15 is inefficient due to the presence of multiple AUG initiation sites in the 5'UTR of the IL-15 mRNA [7]. In humans and in mice alternative splicing also results in the production of two mature isoforms of the IL-15 protein that differ only in the length of their signal peptides [7,9]. Despite these differences both human and mouse

isoforms produce a 114-amino acids containing IL-15 protein. IL-15 with the long signal peptide follows the secretory pathway leading to the secretion of IL-15, whereas IL-15 with the short signal peptide is not secreted, but is stored intracellularly in the cytoplasm [7,9]. In contrast to other cytokines IL-15 also has an unusual mechanism of transport to the cell surface. While the high affinity IL-15 binding protein, IL-15 receptor α (IL-15R α) is not necessary for IL-15 to translocate into the endoplasmic reticulum, IL-15 is transported through the Golgi apparatus to the cell surface as a complex bound to IL-15R α , which also makes IL-15 less amenable to detection by standard methods for detecting secreted cytokines [11]. The IL-15 complexed to IL-15R α is then presented by membrane bound IL-15R α *in trans* by a mechanism now known as trans-presentation, to cells that express the intermediate affinity β (CD122) and γ (CD132) signaling chains [12]. Currently, although trans-presentation of IL-15 is thought to be the main mechanism of IL-15 delivery to the many IL-15 responsive cells such as NK cells and CD8⁺ T lymphocytes, more recent studies indicate that unlike mouse CD8⁺ T lymphocytes, human primary CD8⁺ T lymphocytes do not require trans-presentation of IL-15 for proliferation *in vitro* [13,14]. Therefore, these latter studies raise the possibility of species differences in the usage of trans-presentation as a mechanism for cellular IL-15 delivery and responses.

3. IL-15 signaling

IL-15 binds to the IL-15-specific high affinity binding protein IL-15R α and signals through a β chain and a γ chain signaling complex, leading to the recruitment of Janus kinase (JAK) JAK1 by the β chain and activation of JAK3 that is constitutively associated with the γ chain [8]. Activated JAK1 and JAK3 then phosphorylate signal transducer and activator of transcription (STAT) proteins STAT3 and STAT5 respectively, to mediate IL-15 effects in T lymphocytes [15]. While IL-15 specificity is provided by binding to the unique IL-15R α protein, IL-15 shares the β chain of the signaling complex with IL-2 and the γ chain with cytokines IL-2, IL-4, IL-7, IL-9, and IL-21 that together with IL-15 form the common γ chain family of cytokines [16]. Because of the shared $\beta\gamma$ signaling complex with IL-2, IL-15 shares some functions with IL-2, but also has immunomodulatory properties that are distinct from IL-2 by targeting a wider range of cells and tissues than IL-2, thereby leading to the systemic effects seen with IL-15 compared to the more local effects of IL-2 [8,9]. Similar to IL-2, IL-15 is also able to bind to the intermediate affinity $\beta\gamma$ signaling complex without the requirement for the IL-15R α high affinity binding protein, and signal through the recruitment of other non-receptor tyrosine kinases such as Lck, Fyn, Lyn, Syk and cross talk with signaling proteins of the PI3K and MAPK pathways [9]. Like IL-15 mRNA, IL-15R α mRNA is also widely expressed in many cell types and various tissues such as T and B lymphocytes, macrophages, thymic and bone marrow stromal cells, activated vascular endothelial cells, and in liver, heart, spleen, lung, and skeletal muscle tissues [9]. Moreover, IL-15R α mRNA levels can be increased in response to IL-2, anti-CD3 antibody, and phorbol-myristate acetate in T lymphocytes, and by interferon (IFN)- γ in macrophages [10]. To add another level of complexity to IL-15 regulation and signaling, in humans and in mice proteolytic cleavage of membrane bound IL-15R α results in the production of a soluble form of IL-15R α [12]. The presence of soluble IL-15R α could affect the free IL-15 pool by competing with membrane bound IL-15R α or alternatively it could prolong the availability of IL-15 by regulating the slow release of IL-15 and protracting the responses. Both antagonistic and agonistic activities have been detected with IL-15/soluble IL-15R α complexes [12]

4. Role of IL-15 on innate immune cells

4.1. NK cells

NK cells are bone marrow-derived large granular lymphocytes that play a key role in immune defense against viral infections, bacterial infections, and tumor cells. IL-15 plays a crucial role in the development, differentiation, and survival of NK cells. [7,15]. The critical importance of IL-15 and its signaling in NK cell development was indicated by the absence of NK cells in IL-15-deficient, IL-15R α -deficient, and IL-2/IL-15 β -deficient mice [17–19]. Although NK cells are not producers of IL-15, resting NK cells express IL-15R α , and the $\beta\gamma$ signaling complex [15]. Despite the presence of IL-15R α on NK cells, studies in mice demonstrated a requirement for NK-cell-independent IL-15R α expression for the maintenance of peripheral NK cells, while IL-15R α expression on NK cells was not required for this function [20]. The demonstration that *in vivo* development and maintenance of NK cells required IL-15 transpresented by CD11c⁺ DC, and that CD11c⁺ DC transpresented IL-15 also induced the differentiation of NK cells by up regulating the activating and inhibitory Ly 49 receptors, further confirms the dependence on transpresented IL-15 as a source of IL-15 by NK cells [21]. In addition, IL-15 also induced the development of CD56⁺ NK cells from bone marrow derived CD34⁺ hematopoietic progenitor cells [22]. These CD56⁺ NK cells were potent producers of IFN- γ , and produced moderate amounts of TNF- α and GM-CSF when stimulated with IL-15 and IL-12 when compared to human peripheral blood NK cells [22]. In addition, in humans distinct subsets of NK cells perform different functions. Although cytolytic activity is mostly performed by the CD56^{dim} CD16⁺ subset, these cells can also be stimulated by IL-15 in combination with IL-2 and IL-12 to rapidly produce IFN- γ , whereas cytokine production is generally seen in CD56^{bright}CD16^{+/-} cells [23]. IL-15 also increased the cytotoxicity of NK cells via up-regulation of NKG2D and MICA, the expression of cytotoxic effector molecules TRAIL and perforin, and the phosphorylation of signaling molecules STAT1 and ERK1/2 [24]. IL-15-primed human NK cell function is also regulated by suppressor of cytokine signaling 2 via control of phosphorylated Pyk2 [25]. Mature NK cells in addition to being critically dependent on IL-15 for their survival, are also dependent on IL-15 for their homeostatic proliferation when in an NK cell deficient environment [26]. On examining the impact of transient and prolonged *in vivo* stimulation of NK cells by preformed IL-15/IL15R α complexes, transitory stimulation increased the number of activated NK cells and significantly enhanced their effector functions, whereas prolonged stimulation by IL-15/IL15R α complexes led to a marked accumulation of mature NK cells with impaired functions and proliferative activity [27].

4.2. Monocytes/macrophages, and DC

Circulating monocytes recruited from the bloodstream to inflamed tissues give rise to macrophages, and together with DCs play a pivotal role as antigen presenting cells and thereby shape the adaptive immune responses to pathogens [28]. IL-15 mRNA is expressed by mouse monocytes and macrophages when primed with IFN- γ and triggered with various microbial agents and this expression is not down regulated by inhibitory cytokines such as IL-13 and TGF- β [29]. IL-15 is also constitutively expressed as a membrane-bound biologically active protein in human monocytes and monocytic cell lines (MONO-MAC-6, THP-1, and U937) and is further up regulated by IFN- γ [30]. Furthermore, as seen in mouse monocytes/macrophages, inhibitory cytokines IL-4 and IL-13 failed to down regulate both constitutive and induced cell-surface expression of IL-15. In human monocytes, IL-15 is able to induce the production of IL-8 and monocyte chemotactic protein 1, two chemokines that attract neutrophils and monocytes respectively to sites of infection [31]. In macrophages IL-15 also functions as a potent autocrine regulator of proinflammatory cytokine production

by these cells, with high concentrations of IL-15 favoring TNF- α , IL-1, and IL-6 production, whereas very low concentrations of IL-15 favor IL-10 production [32].

DCs produce IL-15 and these cells also differentiate in response to IL-15 [33]. DCs produce IL-15 in response to type I IFN, double-stranded RNA, or lipopolysaccharide and are then activated to up regulate co-stimulatory molecules, and increase production of IFN- γ and develop an enhanced capability to stimulate T lymphocytes [34]. In both humans and in mice IL-15 is able to modulate the cellular arm of the adaptive immune response by regulating DC production of IL-2, a major T lymphocyte growth factor [35,36]. IL-15 also enhances the proliferation of human follicular DCs and chemokine secretion by these cells and plays a crucial role in the development of follicular DC networks during the germinal center reaction [37]. The IL-15 captured by IL-15R α on follicular DC promotes germinal center B lymphocyte survival and proliferation [38]. These functions lead to the development of high affinity antibodies against infectious microbes by antigen stimulated B lymphocytes within the germinal center [37]. In addition, mature DCs require IL-15 and type I IFN to induce tumoricidal activity of NK cells [39]. Furthermore, IL-15 in combination with GM-CSF is able to induce human monocytes cultured short-term in their presence, into immature DCs [40]. Monocytes and DCs are also the highest cellular expressors of IL-15R α , which enable these cells to transpresent IL-15 [41]. Moreover, IL-15 acts in an autocrine manner in DCs to prevent apoptosis and thereby promotes their survival. Since transfer of DCs from wild-type mice into IL-15-deficient mice resulted in the appearance of CD44^{high} CD8⁺ T lymphocytes in IL-15-deficient mice, it is likely that DCs also mediate the effects of IL-15 on these memory T cells [41].

4.3. Neutrophils and eosinophils

Neutrophils are one of the first innate immune cell types to arrive at sites of inflammation and rapidly engulf infectious pathogens such as extracellular bacteria and fungi, and induce antimicrobial activities for killing pathogens [42]. Human neutrophils constitutively express IL-15R α and the $\beta\gamma$ signaling complex and respond to IL-15 [43]. IL-15 promotes neutrophil cytoskeletal rearrangement by increasing actin expression, the phagocytosis of opsonized sheep erythrocyte, and delays apoptosis of neutrophils, thus promoting neutrophil movement and prolonging neutrophil functions [44]. IL-15 also stimulates the production and secretion of IL-8 by neutrophils through the activation of the NF- κ B transcription factor, leading to further recruitment of neutrophils to the inflammatory site and amplifying the inflammatory reaction [45]. Interestingly, downstream activation of STAT signaling proteins was not detected in these IL-15 stimulated neutrophils unlike in T lymphocytes [45]. IL-15 was also able to induce the expression of major histocompatibility complex class II molecules on neutrophils providing neutrophils with the capability of acting as antigen presenting cells [46]. Moreover, in response to IL-15, neutrophils also increased the expression of CD14, a receptor for bacterial cell wall lipopolysaccharide and CD64, a high affinity receptor for IgG, thereby greatly augmenting their ability to respond to Gram negative bacterial infections and to bind to pathogen-specific antibodies [46]. In addition, IL-15 mediates antigen-induced neutrophil migration by triggering IL-18 production [47].

4.4. Mast cells

Mast cells are dispersed throughout most tissues but are also crucially located at the host's interfaces with the environment and increasing evidence indicates that mast cells protect the host from pathogenic infections caused by not only parasites, but also bacteria, and viruses [48]. In mast cells instead of the classical IL-15R α , an alternative IL-15 receptor known as IL-15TX is used to signal IL-15 responses without the requirement for the $\beta\gamma$ signaling complex used by T lymphocytes and NK cells [7]. IL-15-induces mast cells proliferation in the absence of IL-15R α and the β chain, with the recruitment of JAK2 and STAT5 in place

of JAK1, JAK3, STAT3 and STAT5 that are classically activated in T lymphocytes [7]. Mouse mast cells express both constitutive and lipopolysaccharide-inducible IL-15 and store it intracellularly [49]. This intracellular IL-15 acts as a specific negative transcriptional regulator of a mouse mast cell chymase needed for effective antibacterial responses [49]. In another study intracellular IL-15 was found to control mast cell survival, because IL-15 deficiency in bone marrow derived mouse mast cells resulted in increased susceptibility to apoptosis mediated by endogenous acid sphingomyelinase or growth factor deprivation [50]. Moreover, IL-15 was found to prevent mouse mast cell apoptosis through STAT6-mediated Bcl-xL expression providing a mechanism for its anti-apoptotic effects [51]. IL-15 was also able to induce the migration of mouse and human mast cells with a potency of response that was similar to those induced by other well-established mast cell chemoattractants [52]. In addition, the IL-15-induced migration of both mouse and human mast cells was dependent on the expression of a pertussis toxin-sensitive receptor, activated in response to IL-15 [52].

4.5. Other innate immune cells

NK1.1 and T cell receptor- α/β expressing NKT cells and CD8- α/α expressing intestinal intraepithelial lymphocytes (IELs) require IL-15R β chain for their development, and IL-15 also preferentially promotes the proliferation of these lymphocyte subsets [53]. NK1.1 T cells are a lymphocyte subset that shares common features with both NK cells and conventional T cells. This cell lineage expresses NK markers including NKR-P1, Ly-49, and IL-2R $\beta/15R\beta$, as well as an invariant V α 14J281TCR- α chain in combination with V β 8, V β 7, or V β 2. The majority of TCR- α/β and TCR- γ/δ intestinal IEL express CD8- α/α homodimers. The evidence supporting the importance of IL-15 for the development of NK1.1 T cells and TCR- γ/δ IEL was provided by genetically targeted mice deficient in IL-15, IL-15R α , and signaling components IL-15R β chain and JAK-3, as these mice exhibited a deficiency or reduction in these cell types [15]. Moreover, mice deficient in the transcription factor interferon regulatory factor 1 (IRF-1) showed impaired IL-15 expression in bone marrow cells indicating that IRF-1 regulates IL-15 gene expression [53]. Additionally, in these mice NK1.1 T cells were severely reduced and within the CD8 α/α intestinal IEL subset, both TCR- α/β and TCR- γ/δ cells were equally affected, once again highlighting the requirement for IL-15 by these cells [53]. Furthermore, trans-presentation of IL-15 by intestinal epithelial cells was found to be involved in the development of CD8 α/α IELs and IL-15 also induced the preferential differentiation of Thy1^{low}CD8 α/α IELs [54].

5. Role of IL-15 on adaptive immune cells

5.1. T lymphocytes

Lymphocytes are not usual producers of IL-15, except for some T cell lines that express IL-15 mRNA. The effects of IL-15 on CD8⁺ and CD4⁺ T lymphocyte subsets differ according to their developmental and activation stage as described below. The critical importance of IL-15 in maintaining memory CD8⁺ T lymphocytes was provided by IL-15- and IL-15R α -deficient mouse strains, where both types of mice displayed a dramatic reduction in the number of CD44^{high} memory CD8⁺ T lymphocytes [17,18]. Furthermore, by using mice deficient for IL-15 and IL-15R α , it was also found that IL-15 and IL-15R α are required to support the survival of CD8⁺ T lymphocytes at all development stages by increasing the expression of anti-apoptotic molecule Bcl-2 [55]. Other studies identified additional anti-apoptotic molecules induced by IL-15 to play a crucial role in promoting CD8⁺ T lymphocyte survival. In one study, IL-15 induced the upregulation of anti-apoptotic Bcl-2 in naïve CD44^{low} cells and both Bcl-2 and Bcl-x_L in memory CD44^{high} CD8⁺ T lymphocytes [56]. Moreover, IL-15 was able to inhibit IL-2-induced activation induced cell death of effector lymphocytes [57]. A regulatory role for IL-15 on bone marrow CD69 expressing memory CD4⁺ and CD8⁺ T lymphocytes was revealed when it was determined

that these cells interacted with IL-15-producing bone marrow cells found in their vicinity to maintain their heightened activation state [58]. Although IL-15 was first identified as a cytokine able to support the proliferation of an IL-2-dependent CTLL-2 mouse CD8⁺ T cell line [7], conflicting results are found regarding the role of IL-15 in primary CD8⁺ T lymphocyte expansion and memory CD8⁺ T lymphocyte generation following a viral infection. In one study following infection of IL-15- and IL-15R α -deficient mice with lymphocytic choriomeningitis virus, the primary CD8⁺ T lymphocyte response and the memory lymphocyte generation were found to be similar to those seen in wild-type mice indicating that IL-15 was not necessary for these responses [59]. However, in another study using similar mice, IL-15 was found to be required for the primary expansion of vesicular stomatitis virus-specific CD8⁺ T cells and also played a role in memory cell generation [60]. Despite these discrepant observations IL-15 is required to support antigen-independent basal homeostatic proliferation of memory CD44^{high} CD8⁺ T lymphocytes to replenish their numbers [61]. These memory lymphocytes also express higher levels of the signaling IL-15R β chain compared with naïve CD8⁺ T lymphocytes reflecting the greater ability of memory CD8⁺ lymphocytes to respond to IL-15 [62]. In contrast to the requirement for IL-15 for basal homeostatic proliferation of memory CD8⁺ T lymphocytes, either IL-15 or IL-7 is sufficient for acute homeostatic proliferation of memory lymphocytes in lymphopenic environments for maintaining homeostasis of peripheral T lymphocyte numbers [61]. Furthermore, IL-15 is a chemo-attractant for T lymphocytes but not for B lymphocytes [63].

For the basal homeostatic proliferation of CD4⁺ lymphocytes under normal conditions both IL-15 and IL-7 are essential [64]. But for survival, memory CD4⁺ lymphocytes are less dependent on IL-15 than memory CD8⁺ T lymphocytes, and these findings correlate with the levels of IL-15R β chain expression by these cells, with memory CD4⁺ T lymphocytes expressing far less of the IL-15R β chain than memory CD8⁺ T lymphocytes [64]. In addition, the responsiveness of mature CD4⁺ T lymphocytes to IL-15 was found to be dependent on the activation status of these cells. In the absence of concomitant TCR triggering, IL-15 induces a quiescent phenotype and down regulates the expression of CD25, CD71, and CD95 [65]. However, this IL-15 induced quiescence of CD4⁺ T cells is reversed in the presence of concomitant TCR engagement resulting in robust proliferation of these cells while becoming resistant to TCR-induced cell death [65]. Furthermore, IL-15 was able to induce CD154 (CD40 ligand) on previously activated CD4⁺ T lymphocytes without requiring additional signals from CD3 and the costimulatory molecule CD28, thereby increasing the ability of these cells to interact with antigen presenting cells [66]. Interestingly IL-15 was able to prevent human peripheral blood derived CD4⁺ and CD8⁺ T lymphocytes from the suppressive effects of natural CD4⁺CD25⁺Foxp3⁺ regulatory T cells through activation of the PI3K pathway. Conversely, IL-15 also promotes the acquisition of regulatory functions by CD4⁺CD25⁻ T cells in the presence of TGF- β when these cells were stimulated with anti-CD3 and anti-CD28 antibodies [67]. While prevention of suppressive effects by regulatory T cells could lead to transient pro-inflammatory immune responses against pathogens, these effects could potentially become deleterious to the host during chronic infections with protracted IL-15 over expression.

During aging of the immune system in humans there is a loss of naïve T cells and an accumulation of T cells that have lost CD28 expression [CD28(null)], affecting mainly CD8⁺ T cells but also involving CD4⁺ T cells leading to a diminished immune response to infection and vaccination. Treatment with IL-15 resulted in the preferential proliferation of CD4⁺CD28(null) T cells compared to CD4⁺CD28⁺ T cells [68]. In addition, IL-15 augmented the cytotoxic properties of CD4⁺CD28(null) T cells by increasing mRNA and the storage of granzyme B and perforin, thereby increasing the functional properties of these

cells [68]. Thus, IL-15 could play a role in stimulating immune responses to infections in the elderly.

5.2. B lymphocytes

The absence of B lymphocyte abnormalities in IL-15 and IL-15R α deficient mice, led to the belief that IL-15 has no role or a limited role in B lymphocyte functions. While IL-15 has no stimulatory effects on resting B lymphocytes there is evidence to indicate IL-15 co-stimulates proliferation of B lymphocyte in response to phorbol ester or immobilized anti-IgM [69]. In addition, IL-15 in combination with recombinant CD40 ligand was also able to potentially induce polyclonal IgM, IgG1, and IgA secretion, without causing the production of IgG4 or IgE [69]. Furthermore, while IL-15 and CpG oligonucleotides induced the proliferation of class switched human memory B cells, anti-Ig in the presence of IL-15 and CpG induced the proliferation of naïve B cells [70]. In contrast to the direct effects of IL-15 on T lymphocytes some of the effects of IL-15 on B lymphocytes are indirect. For example as previously described, follicular DCs produce IL-15 leading to trans-presentation of membrane-bound IL-15 to germinal center B lymphocytes that express the IL-15 receptor $\beta\gamma$ signaling chains, leading to augmented B lymphocyte proliferation and ultimately the production of higher affinity antibodies against various pathogens through the germinal center reaction [37,38].

6. Role of IL-15 in viral infections

6.1. Human immunodeficiency type I virus (HIV-1)

After viral transmission, an acute-phase reaction to HIV-1 infection is followed by a steep rise in the viral load that coincides with the production of inflammatory cytokines by multiple activated cell types including DCs, monocytes, macrophages, NK cells, and T lymphocytes [71]. As the disease progresses HIV-1 destroys and dysregulates CD4⁺ T lymphocytes and also induces immunologic dysfunction of CD8⁺ T lymphocytes, B lymphocytes, NK cells, and nonlymphoid cells [72]. The importance of DCs and NK cells as critical effectors in the immune response to HIV-1 infection was indicated when conventional DCs from HIV-1 infected individuals were found to secrete reduced levels of IL-15 in addition to IL-12 and IL-18, resulting in reduced NK cell activation [73]. In another study, addition of IL-15 was able to reverse HIV-1 infection associated functional impairment of NK cell cytotoxicity to levels seen in healthy donor cells, by demonstrating an increase in NK cell cytotoxicity and an increase in the expression of cytotoxic granules seen in PBMCs isolated from HIV-1 patients on anti-retroviral therapy when treated with IL-15 [74]. In the same study IL-15 also induced the proliferation of PBMCs from HIV-1 infected patients and rescued them from spontaneous apoptotic death. Furthermore, by analyzing isolated PBMC-derived CD4⁺ T lymphocyte subsets (naïve, effector memory, and central memory) from antiretroviral naïve HIV-1 infected patients, IL-15 and its superagonist RL1 were found to specifically induce the proliferation of CD4⁺ effector memory T lymphocyte subset, in addition to inducing CD8⁺ T lymphocyte proliferation [14]. Although these studies indicate that IL-15 could be an effective immunotherapeutic in restoring NK cell functions and in increasing CD8⁺ lymphocyte proliferation, along with increasing the CD4⁺ effector memory T lymphocyte subset, one has to be cautious that this approach also could potentially risk increasing viral replication and disease progression since these cells are a target of HIV-1 infection.

Upon examining IL-15 production in HIV-1 patients, IL-15 produced by peripheral blood mononuclear cells (PBMCs) was significantly decreased in antiretroviral naïve patients and in those with treatment failure, whereas in patients responsive to antiretroviral therapy, IL-15 production was comparable to healthy donors and IL-15 was able to stimulate HIV-1

positive monocytes to produce IL-8 and MCP-1 [75]. In another study involving a cohort of U.S. women, the group with untreated progressive HIV-1 infection was associated with decreased serum levels of IL-15 and IL-12, while IP-10 and TNF- α levels were increased. Whereas the antiretroviral treated group had cytokine profiles resembling those of uninfected women [76]. These studies also indicate a potential use for IL-15 in combination with antiretroviral therapy to treat HIV-1 infected patients who fail to respond to antiretroviral therapy. An additional protective role for IL-15 against HIV-1 infection was provided when high concentration of IL-15 present in human breast milk was associated with protection against postnatal HIV-1 transmission [77]. In an earlier study while the presence of HIV-1-specific CD8⁺ T lymphocytes in human breast milk of infected women was reported, in a later study the majority of CD8⁺ T lymphocytes in breast milk were identified as effector memory cells [78,79]. These studies suggest that IL-15 in breast milk induces HIV-specific effector memory CD8⁺ T cell generation and/or proliferation that allows infants fed with breast milk by HIV-1 infected women to escape infection. But despite these studies indicating a protective role for IL-15 against HIV-1, an *in vitro* study suggests that IL-15 might also permit infection of resting T lymphocytes by this virus [80].

When the effect of IL-15 on viral replication was closely examined, IL-15 alone was found to have no major effect on HIV-1 replication in naturally infected peripheral blood mononuclear cells (PBMCs) stimulated with phytohemagglutinin (PHA) or with soluble anti-CD3 [74,75]. In contrast, the ability of IL-15 to induce viral replication in PHA-stimulated PBMCs from healthy donors or HIV-1 seronegative individuals infected with HIV-1 *in vitro* depended on the strain of the virus, with some dual-tropic and T tropic HIV-1 strains inducing marginal to undetectable viral release, while other dual tropic strains such as 89.6 inducing detectable levels [74,75]. Our own study using peripheral blood lymphocytes from normal volunteers has shown that IL-15 promotes the entry and replication of macrophage-tropic HIV in T lymphocytes, by inducing the expression of chemokines and their receptors on these cells [81]. Moreover, the simultaneous treatment of PBMCs with IL-2 and IL-15 also resulted in additive to synergistic effects on viral replication [74,75]. In addition to the studies described above, using different experimental approaches numerous other investigators have determined the effects of HIV-1 infection on cytokine production *in vivo* and *in vitro* by different cell types, including PBMCs, T lymphocyte subsets, monocytes, and monocyte-derived macrophages from HIV-infected individuals, as well as cultures infected with HIV-1 *in vitro* [82]. These studies indicated that during the course of an HIV-1 infection, secretion of T-helper type 1 cytokines was decreased, whereas production of T helper type 2 cytokines and proinflammatory cytokines was increased [82]. Additionally, numerous other *in vitro* studies have also determined the effects of individual cytokines on HIV-1 replication, with TNF- α , TNF- β , IL-1, IL-6, stimulating HIV-1 replication in T lymphocytes and monocyte-derived macrophages and IL-2, IL-7, and IL-15 inducing HIV-1 replication in T lymphocytes [82]. In contrast, IFN- α , IFN- β , IL-10, IL-13, and IL-16 were found to suppress HIV replication, with IFN- γ , GM-CSF, and IL-4 inducing or suppressing HIV-1 replication depending on the state of differentiation of the cells used for the studies. But by studying the effects of individual cytokines on HIV replication *in vitro*, the effects of the complex interplay between IL-15 and other cytokines *in vivo* leading to viremia may be lost. This issue was addressed by evaluating the early cytokine response in individuals with primary HIV infection from prior to plasma virus detection until HIV-1 sero-conversion [83]. From this study it was found that an increase in viremia was associated with an ordered sequence of increases in plasma levels of multiple cytokines and chemokines, with an initial rapid and transient increase in IL-15 and IFN- α , a large increase in inducible protein 10 (IP-10) levels, rapid and more sustained increase in TNF- α and MCP-1, more slowly initiated elevations in levels of proinflammatory cytokines IL-6, IL-8, IL-18, and IFN- γ , and a late peaking increase in IL-10 [83]. Therefore, this study suggests IL-15 as one of the earliest cytokines to be rapidly

and transiently produced during an acute HIV-1 infection that is likely to impact viremia and viral set point. Collectively, these data suggest that IL-15 plays a dual role in HIV disease with IL-15 promoting disease during the acute phase by inducing viral replication either alone or by having an additive or synergistic effect on other HIV-1 replication promoting cytokines such as TNF- α that are induced early and switching to a more protective role once the disease becomes more chronic as seen in HIV-1 infected patients responding to antiretroviral therapy.

6.2. Human T cell lymphotropic virus type I (HTLV-1)

HTLV-1 is the causative agent of two diverse disease syndromes; the neurological disorder HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia/lymphoma [84]. Although a minority of individuals infected with HTLV-1 develops these two diseases, the majority of individuals infected with the virus remain as asymptomatic carriers [84]. On examining PBMCs from HAM/TSP patients, these cells were found to undergo spontaneous proliferation in the absence of additional exogenous cytokines in *ex vivo* cultures [85]. This spontaneous proliferation was due to the production of IL-15, in addition to the expression of IL-2 by HTLV-1-infected T lymphocytes, since IL-15 expression was elevated in HAM/TSP PBMCs when compared to those of normal donors. The HTLV-1 encoded Tax transcriptional activator induces IL-15 over expression via a mechanism involving NF- κ B transcription factors. In addition, the extraordinarily high frequency of HTLV-1 Tax regulatory protein-specific CD8⁺ T lymphocytes with mostly memory phenotype markers present in both peripheral blood leukocytes and cerebrospinal fluid (CSF) of HAM/TSP patients, and the fact that IL-15 selectively stimulated memory phenotype CD8⁺ cells implicate IL-15 in the long term survival of these cells *in vivo* [85]. The involvement of IL-15, IL-2 and their receptors in the spontaneous proliferation seen in PBMCs from HAM/TSP patients was confirmed when blocking antibodies against IL-2, IL-15, or their receptors inhibited this spontaneous proliferation. Furthermore, IL-15 expression was shown to be up-regulated by TNF- α and IFN- γ , two cytokines that are known to be elevated in the blood and CSF of HAM/TSP patients. In addition, IL-15 expressing CD14 bearing HTLV-1 infected mononuclear phagocytes in patients with HTLV-I-associated neurologic disease mediate spontaneous degranulation of IFN- γ expressing HTLV-1 specific CD8⁺ T lymphocytes, suggesting that virus-infected or activated mononuclear phagocytes may be an important but under recognized reservoir of HTLV-I particularly in HAM/TSP patients [84]. Thus, collectively these findings argue that targeting IL-15 and its receptors could be of therapeutic benefit in treating HAM/TSP patients.

6.3. Herpesviruses

There are eight known genetically stable human herpes viruses that have coevolved with the human species for millions of years, where virus and host have struck a fine balance, allowing their mutual coexistence [86]. Generally, upon herpes virus transmission to a naive host, the virus first amplifies through replicative infection in a permissive cell type, and then persists for the life of the host as an asymptomatic latent infection in a second cell type, with occasional reactivations into lytic cycle producing infectious virions transmissible to a new host [86]. Human cytomegalovirus (HCMV) causes a clinically benign primary infection and the virus then establishes itself causing a persistent subclinical chronic infection that is controlled by a large number of virus-specific CD8⁺ T lymphocytes [87]. Although *in vitro* expansion of memory CD8⁺ T lymphocytes isolated from PBMCs of HCMV-seropositive blood donors was completely dependent on the presence and function of helper CD4⁺ T lymphocyte, IL-15 was able to substitute for helper CD4⁺ T lymphocytes in this experimental system [87]. In addition, mice infected with murine CMV, the accumulation/survival of proliferating NK cells required IFN $\alpha\beta$ /STAT1 induced endogenous IL-15,

establishing a critical role for IL-15 in the maintenance of NK cells during this infection [88]. Similarly, the development of multiple severe herpes viral infections in an adolescent with a total absence of NK cells, underscores the critical role played by NK cells in mediating protection against primary herpes virus infections in humans [89]. Moreover, infection of PBMCs with human Herpesvirus-6 led to a rapid upregulation of NK cell cytotoxicity and the NK cell activity was inhibited by monoclonal antibodies against IL-15, suggesting that IL-15 secreted in response to the viral infection was responsible for the observed effect and the importance of early activation of NK cells in the control of viral infections [90]. Epstein-Barr virus (EBV) a ubiquitous human lymphotropic gamma herpesvirus is associated with certain human lymphoproliferative disorders and malignancies, and is the etiological agent of infectious mononucleosis [86]. In an *in vitro* study by examining the effect of IL-15 on EBV infected PBMC cultures, it was determined that IL-15 mediated its anti-EBV effects by first activating NK cells and subsequently inducing cytolytic NK-T cells leading to the elimination of EBV-transformed immortalized cells in PBMC cultures [91]. In a study where EBV-specific total T lymphocyte populations and NK cells from infectious mononucleosis patients were followed from the time of acute infection through convalescence into asymptomatic carrier state, there was a loss of IL-15R α expression by these cells following an acute infection [92]. This IL-15R α loss was not specific to EBV-specific CD8 $^{+}$ T lymphocyte populations, but affected the whole peripheral T- and NK-cell pool of activated cells and lasted for many years [92]. Moreover, in an *in vitro* assay when the IL-15 responsiveness was assessed, the IL-15R α deficit in these cells resulted in defective IL-15-induced STAT5 phosphorylation in not only EBV-specific lymphocytes but also other CD8 $^{+}$ T lymphocytes, suggesting a critical requirement for surface IL-15R α expression on human CD8 $^{+}$ T lymphocytes to respond to IL-15, unlike in mice where IL-15 is transpresented by antigen presenting cells as a surface IL-15R α -bound complex to CD8 $^{+}$ T lymphocytes [92].

In studies examining the responses to Herpes simplex virus type 2 (HSV-2), IL-2R α -deficient mice developed abnormal memory phenotype CD44 $^{+}$ CD69 $^{-}$ CD8 $^{+}$ T cells and these cells protected the mice from a systemic infection with HSV-2 by increasing the production of IL-15, whereas IL-2/IL-15R β -deficient mice were susceptible to systemic HSV-2 infection [93,94]. Taken together these studies suggest that IL-15 by signaling through the IL-2/IL-15R β chain mounts protective immune responses against a systemic HSV-2 infection. In addition, the protection against genital HSV-2 infection was critically dependent on the presence of IL-15, NK and NK T cells in a mouse model of genital herpes infection [95]. Surprisingly, despite IL-15 transgenic mice having higher numbers of NK cells in their genital mucosa and more CD8 $^{+}$ T lymphocytes than control mice, HSV-2 immunized IL-15 transgenic mice were susceptible to genital HSV-2 infection following HSV-2 challenge. This finding was attributed to a reduction in HSV-2-specific CD4 $^{+}$ T lymphocytes due to competition from the increased numbers of CD8 $^{+}$ lymphocytes produced by excess IL-15 for space and ligands [96]. Whereas similar susceptibility to genital HSV-2 infection was seen in another study with IL-15 transgenic mice, this result was attributed to aberrantly high levels of TGF- β 1 and decreased levels of IFN- γ being produced by the intraepithelial cells of the uterus/vagina, in addition to impaired production of IFN- γ by T lymphocytes in these mice [97]. A role for IL-15 in the control of HSV-1 that causes mild primary infections early in life was also found to be due to enhancement of NK cell activity that suppresses viral replication [98].

6.4. Hepatitis B virus

Primary Hepatitis B virus (HBV) infection in susceptible children are mostly asymptomatic rather than symptomatic, whereas most primary infections in adults, whether symptomatic or not are self-limited, with clearance of virus from blood and liver and the development of

lasting immunity to reinfection [99]. HBV being not very cytopathic, the hepatic injury in persistently infected individuals is primarily driven by immune-mediated mechanisms. Chronically infected subjects have a risk of developing hepatocellular carcinoma that is 100 times as high as that of for noncarriers. In a temporal analysis of early immune responses in patients with acute HBV infection, neither IL-15 nor type I IFN or IFN- λ 1 (IL-29) was detected during peak viremia [100]. However, in a recent study Zhang et al [101] reported that in patients with active disease, hepatic infiltration of NK cells displaying activated phenotype occurs that coincides with *in situ* over-expression of IL-12, IL-15, and IL-18.

6.5. Hepatitis C virus

Hepatitis C virus (HCV) causes persistent infection in susceptible hosts after parenteral or percutaneous transmission [102]. Although majority of the infected become chronic carriers and most have mild disease, up to 20% of those infected with HCV may develop complications including cirrhosis, liver failure, or hepatocellular carcinoma [102]. Extrahepatic disease is also common and affects an estimated 40% of patients with chronic HCV infection. The major site of HCV replication is the liver and other cell types reported to contain HCV RNA include B lymphocytes, DCs, monocytes, lymph node cells, and cells of the digestive tract [102]. In chronic HCV patients the expression of MHC class I-related chain A and B (MICA/B), ligands for NKG2D was severely impaired on monocyte-derived DCs in response to IFN- α limiting their ability to activate resting NK cells through MICA/B-NKG2D interactions [103]. In a subsequent study by the same group, the expression of MICA/B on monocyte-derived DCs from HCV infected individuals was found to be IL-15 and type I IFN-mediated, since incubation of DCs in the presence of anti-IFN- α/β or anti-IL-15R α resulted in the loss of MICA/B expression [104]. In addition, IL-15 stimulated monocyte-derived DCs to produce IFN- α/β which acted in an autocrine/paracrine manner to express MICA/B on the IL-15-stimulated DCs. By serially analyzing serum cytokine levels in patients diagnosed at various stages of the disease [asymptomatic HCV carriers, patients with chronic hepatitis (CH), patients with liver cirrhosis (LC), or patients with hepatocellular carcinoma (HCC)] from the time of diagnosis and during the course of IFN- α treatment, increased IL-15 levels were found in patients with CH, LC, and HCC compared with normal controls, with the highest levels seen in patients with HCC [105]. Moreover, IFN- α treatment in CH patients resulted in suppression of serum IL-15 levels with a corresponding decrease in serum aminotransferase. These results suggest that serum IL-15 levels were closely associated with the progression of chronic liver disease in chronic HCV infections and reflect the degree of inflammation in the liver and the development of HCC. Although the cellular sources of IL-15 were not identified, a pathogenic role for IL-15 in disease progression was implied [105]. In contrast to the above study by Kakumu et al, during acute HBV infection an increase in serum IL-15 levels were not seen although delayed increases in levels of other cytokines and chemokines were observed [83].

7. Role of IL-15 in bacterial infections

7.1. Mycobacterium tuberculosis

M. tuberculosis infection in humans is now viewed as a continuum of host-pathogen interactions resulting in a spectrum of responses with marked heterogeneity in host immune responses, rather than two separate and distinct states of latent and active tuberculosis [106]. Control of the infection is dependent on innate immune cells such as macrophages and DCs that play a key role in engulfing and eliminating the intracellular pathogen, while NK cells and granulocytes also play a protective role [107]. In addition, adaptive immune responses also play a crucial role in the control of infection with the activation of phagocytes by IFN- γ and TNF- α produced by CD4⁺ T lymphocytes and the production of cytotoxic granules by CD8⁺ T lymphocytes, with help from other CD4⁺ T lymphocyte subsets such as those that

produce IL-17 [106]. When the effect of IL-15 on survival was examined in BALB/c mice infected with *M. tuberculosis*, IL-15 was found to promote the survival of infected mice only when administered 3 weeks post-infection and not when administered at the time of infection suggesting that IL-15 could potentially be used to treat this disease [108]. In another study when the response to primary *M. tuberculosis* infection was examined in IL-15-deficient mice, there was no evidence of increased apoptosis, defect in proliferation of effector CD8⁺ T lymphocytes or in their cytotoxic responses, although recall T lymphocyte responses were impaired. These IL-15-deficient mice were also unable to clear *M. tuberculosis* infection in the presence of antibiotics suggesting an unidentified role for IL-15 in immune protection against this bacillus [109]. In a similar study of experimental tuberculosis with IL-15-deficient mice, while CD4⁺ T lymphocytes were not affected, the production of IFN- γ and cytotoxicity of CD8⁺ T lymphocytes were impaired, leading to an increase in the mortality of mice during the chronic phase of infection, thus implying that IL-15 is necessary for inducing effector mechanisms in CD8⁺ T lymphocytes which were found to be NKG2D-dependent [110]. In addition, in an *in vitro* co-culture of human Vgamma9Vdelta2 T lymphocytes and *M. tuberculosis* infected immature DCs, addition of exogenous IL-15 enhanced bi-directional signaling between the cell types leading to activation of DCs and full differentiation of lymphocytes to effector memory cells capable of reducing the viability of the intracellular pathogen [111]. IL-15 also induced human NK cells to produce IL-22 through a mechanism that involved IL-15 signaling and an increased expression of the adaptor protein DAP10, and the IL-22 produced then led to the enhancement of phagolysosomal fusion in *M. tuberculosis* infected macrophages leading to the inhibition of intracellular growth of *M. tuberculosis* [112].

In contrast to a protective role for IL-15 against tuberculosis in the above studies, mice infected with the H37Rv strain of *M. tuberculosis*, higher levels of IL-15, IL-12, and IFN- γ mRNA were found in the lungs and spleens of mice with slowly progressive pulmonary tuberculosis, whereas mice with latent disease showed decreased expression levels of these cytokines leading to the conclusion that IL-15 may play an important role in mediating active disease during tuberculosis infection [113]. Alternatively, it is possible that rather than mediating active disease, the increase in IL-15, IL-12, and IFN- γ expression may indicate a protective immune response to progressive infection, with an increase in these cytokines needed to control the infection. Moreover, by stimulating PBMC from infected patients and control subjects with purified protein derivative and assaying culture supernatants for an array of cytokines, increased levels of IFN- γ were seen in PBMCs of both active and latently infected compared to controls. Additionally IL-15, TLR-10, and IKK- β mRNA expression was down-regulated in PBMC from latently infected individuals, but not in PBMC from individuals with active tuberculosis [114]. Furthermore, by evaluating 25 biomarkers in the supernatant of whole blood samples from patients with active, latent or no evidence of tuberculosis following overnight stimulation with a peptide cocktail from ESAT-6, CFP-10 and TB7.7 (p4) proteins, the expression levels of IL-15 and MCP-1 were found to be significantly higher in the active tuberculosis group compared to the latent tuberculosis group [115]. These studies indicate the difficulty in assigning a cause or an effect to any specific factor especially in human studies due to the differences in the time and frequency of exposure, strain of bacillus, inoculum, and coinfections that can affect the interpretation of results [107].

7.2. Other bacterial pathogens

By using a combination of approaches such as adenovirus vectored overexpression of IL-15 in mice, *in vivo* depletion of NK1.1 cells from wild-type mice, and the use of IL-15-deficient mice, IL-15 and NK 1.1⁺ cells were found to provide innate immune protection against acute *Salmonella enterica* serovar Typhimurium infection by preventing

colonization in the gut and in systemic tissues targeted by the pathogen [116]. Accordingly, IL-15-deficient mice that lack NK cells, serovar Typhimurium extensively colonized the gut of these mice. Similarly, in wild-type C57BL/6 mice depleted of NK1.1 cells prior to infection, even in the absence of antibiotic pretreatment to reduce colonization resistance, there was enhanced dissemination of *Salmonella* from the gut, although the colon and cecum of these mice did not show overt inflammation [116]. In assessing the serum cytokine levels and T cell responses of patients with *Salmonella* infection, it was found that serum IL-15 and IL-18 levels were significantly elevated and prolonged in patients with the systemic form of salmonellosis, than in those patients with the gastroenteritis form of infection, and activated $\gamma\delta$ T cell numbers correlated with increased IL-15 levels and these cells expressed higher levels of IFN- γ , than $\gamma\delta$ T cells from healthy patients [117]. Although no correlations were found between increased serum IFN- γ levels and total activated T cell numbers in salmonellosis, it was concluded that IL-15 together with IL-12, and IL-18 were involved in total T cell activation and $\gamma\delta$ T cell expansion resulting in IFN- γ production and protection against salmonellosis infection [117]. In contrast to the protective role of IL-15 seen against salmonellosis in both humans and in mice, IL-15 is implicated in the pathogenesis of human inflammatory bowel diseases, such as Crohn's disease, ulcerative colitis, and in celiac disease [8]. In another study using IL-15 transgenic mice, memory phenotype CD44⁺ CD8⁺ T lymphocytes were found to be involved in the early protection against a primary infection with the intracellular bacterium *Listeria monocytogenes* [118]. Moreover, IL-15 was found to protect mice in several mouse models of sepsis including sepsis caused by cecal ligation and puncture, pneumonia induced by *Pseudomonas aeruginosa*, or intraperitoneal injection of a lethal dose of *Escherichia coli* into IL-15 transgenic mice [119,120]. In the cecal ligation and puncture model of sepsis, IL-15 improved the survival of these mice by the blockade of apoptosis in a number of cell types including NK cells, DCs, CD8⁺ T lymphocytes, and gut epithelial cells by increasing anti-apoptotic protein Bcl-2 expression, with a concomitant decrease in proapoptotic proteins Bim and PUMA [119]. However, no improvement in survival was seen by treating septicemic RAG-1 null mice that lack mature T and B lymphocytes with IL-15, indicating that lymphocytes are required for the beneficial effects of IL-15 seen in sepsis. In the gram negative *Escherichia coli*-induced endotoxic shock model using IL-15 transgenic mice, IL-15 acted as a potent inhibitor of TNF- α -mediated apoptosis of peritoneal cells and also cells in multiple organs such as liver, spleen, and lung, without affecting the levels of TNF- α produced or the bacterial burden in organs [120]. Because the majority of patients with sepsis survive the initial insult, but develop sepsis induced multi-organ dysfunction weeks later and exhibit sepsis-induced immunosuppression, including a shift in inflammatory Th1 to anti-inflammatory Th2 cytokines, apoptosis of lymphocytes and gastrointestinal epithelial cells, along with demonstrable anergy, it is conceivable that IL-15 with its anti-apoptotic effects and its immunostimulatory effects on a number of cell types of both the innate and adaptive systems will be able to reverse the immunosuppressive effects seen during sepsis thus having value as a potential therapeutic modality in the treatment of generalized sepsis [121].

8. Role of IL-15 in parasitic infections

IL-15 was implicated in enhancing both innate and adaptive immune responses to blood stage malaria infection in an experimental model of *Plasmodium chabaudi* AS infection in mice, since IL-15-deficient mice showed delayed clearance of the infection, lower type 1 cytokine production, impaired DC and NK cell functions, and lower titers of malaria specific antibodies than wild-type mice [122]. In addition, in another study peripheral and placental plasma IL-15 concentrations were also significantly higher in uninfected women than in women infected with *Plasmodium falciparum*, reflecting the early activation of the innate immune response in the uninfected women, which is implicated to be protective against plasmodial infection through the lysis of infected erythrocytes by activated NK cells and the

ability of IL-15 to function with IL-2 to enhance the capacity of $\gamma\delta$ -T cells to inhibit parasite replication [123]. IL-15 also activates human NK cells to eliminate the intestinal intracellular protozoan *Cryptosporidium* by increasing the activating receptor NKG2D on CD3⁻CD16^{high}CD56⁺ cytolytically active cells [124]. *Leishmania infantum* the causative agent of visceral leishmaniasis replicates in macrophages and IL-15 induces the killing of these parasites in PMA-activated human monocyte-derived macrophages by increasing IL-12 in a manner similar to IFN- γ [125]. Moreover, a protective role for IL-15 against the obligate intracellular parasite *Toxoplasma gondii* has been suggested as mice immune to the parasite when given soluble IL-15R α prior to a secondary challenge with the parasite, there was exacerbation of intracellular parasitic infection with a blockade of the development of memory CD8⁺ T cell responses as determined by a reduction in IFN- γ released and target cell lysis [126]. In contrast to the protective role of IL-15-induced memory CD8⁺ T lymphocyte responses against *Toxoplasma gondii* infection, another study using IL-15-deficient mice found impaired DCs in these IL-15-deficient mice, leading to sub-optimal priming of CD4⁺ T lymphocytes and down-regulation of IFN- γ production by these sub-optimally primed CD4⁺ T lymphocytes in response to the parasite, which resulted in reduced gut necrosis and increased survival of infected mice [127].

9. IL-15 as a vaccine adjuvant

The goal of an effective vaccination strategy against infectious diseases is to induce a robust pathogen-specific T- and B-cell memory response as well as long-lived plasma cells. Unlike the most successful vaccine in humans, the smallpox vaccine that was developed against the variola virus with a stable genome, the current challenge is to develop vaccines to protect against pathogens that display extensive genetic variability, such as HIV, HCV, influenza and malaria. Although several strategies are being employed to develop vaccines with improved effectiveness against these pathogens, these vaccines must be more effective than natural infections since the natural infections of these pathogens do not confer protection against reinfection. One such strategy that is being explored is the use of immune enhancing cytokines as adjuvants [128]. Numerous studies including ours have indicated the effectiveness of IL-15 as a vaccine adjuvant in increasing both cellular and/or humoral immune responses against infectious pathogens employing multiple techniques to target the pathogen including DNA vaccines, virus vector-based vaccines, with different methods to deliver IL-15 [128]. In a direct comparison of the effectiveness of IL-15 and IL-2 as vaccine adjuvants in enhancing the immune responses to a vaccinia virus-based HIV gp160 vaccine in mice, IL-15 was found to induce robust long-lived, antigen-specific CD8⁺ T lymphocytes, whereas the immunity induced by IL-2 adjuvanted vaccine was short-lived, while both IL-15 and IL-2 induced strong and long-lasting antibody-mediated immunity [129]. Similarly, IL-15 was found to be superior to IL-2 in the generation of long-lived antigen specific memory CD4⁺ and CD8⁺ T cells in rhesus macaques vaccinated with tetanus toxoid and influenza vaccines [130]. When a simian immunodeficiency virus (SIV) DNA vaccine was administered to chronically SIV infected macaques on antiretroviral therapy, followed by a boost with the SIV-DNA vaccine plus IL-15 or IL-12 as a molecular adjuvant, the macaque group that received IL-15 triggered both CD4⁺ and CD8⁺ effector memory T cell subsets, while only CD8⁺ effector memory T cells were induced by the IL-12 group [131]. In another study, a combined DNA-IL-15 vaccine against *Brucella abortus* in mice induced a robust humoral response and a predominantly protective CD8⁺ T cell response, although CD4⁺ T cells also played a contributory role in the protection against infection [132]. In addition, co-administration of a plasmid DNA encoding IL-15 improved long-term protection of a genetic vaccine against *Trypanosoma cruzi* by inducing antigen-specific CD8⁺ memory T lymphocytes in mice [133]. Furthermore, using our strategy of vaccinia virus-based vaccines with IL-15 incorporated as a molecular adjuvant, we have demonstrated the generation of robust sustained humoral and polyfunctional T lymphocyte immune responses against

tuberculosis, malaria, H5N1 avian influenza, and in a dual vaccine against smallpox and anthrax [134].

Interestingly, the ability of IL-15 to enhance the proliferation of memory CD8⁺ T lymphocytes to concurrent infection in mice is found to be at the expense of increased erosion of pre-existing memory due to homeostatic mechanisms that limit excessive lymphocyte expansion, and this erosion of preexisting memory has been observed during both heterologous bacterial and viral infections [135].

10. Preclinical evaluation of IL-15 in non-human primates

As a prelude to human clinical trials, clinical grade recombinant non-glycosylated human IL-15 (rhIL-15) produced in an *E. coli* system according to the current good manufacturing practices (cGMP) was evaluated in rhesus macaques for safety (toxicity), pharmacokinetics, immunogenicity, and impact on elements of the normal immune system [136]. Animals given daily doses of rhIL-15 for 12 days intravenously did not develop anti-rhIL-15 antibodies or any of the undesirable side effects seen with IL-2 such as the capillary leakage syndrome, activation induced cell death, or increased numbers of regulatory T cells. Some animals that received the maximum dose developed grade 3/4 neutropenia as the only meaningful hematologic abnormality, presumably caused by the redistribution of neutrophils from blood to tissues [136]. In addition, the peripheral blood of these animals exhibited a dramatic expansion of NK cells, central memory and effector memory CD8⁺ T lymphocytes, with increased NKT cells and a modest increase in non-functional CD4⁺CD25⁺Foxp3⁺ regulatory T cells [136,137]. In contrast to this study, rhIL-15 produced in mammalian cells and administered to macaques subcutaneously daily for 5 to 14 days and evaluated for toxicity and immunological effects, developed severe neutropenia, anemia, weight loss, and a generalized skin rash [138]. No toxicity was observed in macaques when the rhIL-15 was administered intermittently every 3 days, although the increases in NK cell proliferation, and CD8⁺ effector memory and central memory T lymphocyte numbers were lower than those seen in animals that received daily doses [138]. The discrepancies between the two studies might be related to the glycosylated versus nonglycosylated rhIL-15 used in the studies which might affect biologic activity and clearance. Currently, recombinant human IL-15 is being evaluated in a phase I trial involving patients with either metastatic malignant melanoma or metastatic renal cell cancer (NCT01021059; ClinicalTrials.gov).

11. Conclusions

IL-15 is now recognized as a cytokine with potent survival and immunomodulatory effects on cells of both the innate and adaptive immune systems that play a central role in defense mechanisms against pathogens. Although IL-15 shares the $\beta\gamma$ signaling complex and some functions with IL-2, IL-15 does not induce many of the toxicities elicited by IL-2. The ability of IL-15 to activate and expand NK cells, NKT cells, CD8⁺ effector memory and central memory T lymphocytes, makes IL-15 an excellent cytokine adjuvant to be incorporated into vaccine strategies against infectious diseases to improve their effectiveness or to use as an immunotherapeutic modality to treat disorders or infections that are likely to benefit from enhanced activities these effector cell populations. In contrast to protective immune responses that defend the host against infectious agents, IL-15 is also implicated in the development of several autoimmune and chronic inflammatory diseases that this review has not addressed.

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