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SURVEY

IL-12 and Viral Infections

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Interleukin-12 activates natural killer cells and promotes the differentiation of Th1 CD4+ cells; it is a critical factor in viral immunity. IL-12 is secreted by antigen presenting cells including dendritic cells, macrophages and astrocytes, both in tissues and in secondary lymphoid organs. Experimental studies have shown that administration of the cytokine rapidly activates both innate and specific immune responses; this results in enhanced host cellular responses and generally, promotes clearance of virus and host recovery from infection. The observations of many laboratories, studying viral immunity to both RNA and DNA based pathogens, are summarized.

Key words: Interleukin-12 • viral infection • signal transduction • innate immunity • acquired immunity.

INTRODUCTION

The elaboration of cytokines by distinct cell types and their activities on either themselves (autocrine) or on neighboring or more distant cells (paracrine and systemic, respectively) has been well documented as a part of both specific and innate immune responses. The cell surface receptors and the intracellular signal transduction pathways have been elucidated for many of these molecules [1–3]. The cytokines secreted in response to a variety of stimuli have been used to define distinct subsets (for example, CD4, Th2 cells are characterized to produce IL-4, IL-5, IL-10). However, almost exclusively, studies such as these have been performed using cells of the hematopoietic lineages (e.g. lymphocytes and macrophages). Many cytokines are produced or responded to by cells of other lineages. There is a growing body of literature of regulated cytokine gene expression in the CNS both by parenchymal cells in addition to inflammatory mononuclear cells [4]. This has been observed for autoimmune diseases such as Multiple Sclerosis and its animal model, experimental allergic encephalitis, as well as in response to bacterial and viral infections [5–7]. Cytokines may be elaborated to recruit and activate circulating mononuclear cells, but it also appears that resident parenchymal cells both synthesize cytokines and respond to them [4, 8, 9].

In order to respond to cytokines, cells must express receptors and also have the necessary signal transduction machinery for communicating receptor occupancy [1–3]. Subsequently, there must be a change in the gene expression of the cell, in response to the cytokine ligand-receptor binding. In some cases, cytokines deliver a differentiating or activating signal. Alternatively, in the case of TNF-z action for many cells, for instance, the response may be to initiate the apoptosis cascade [10–12]. There is abundant evidence of tissue pathology, including cell death, in the CNS associated with inflammatory cytokine synthesis [10, 13]. This review is focused on the effects of one cytokine, interleukin-12 (IL-12).

IL-12 IS AT THE CUSP OF INNATE AND SPECIFIC IMMUNITY

IL-12 is a 70 kD heterodimer of 35 and 40 kD peptides [14]. It is synthesized by antigen (Ag) presenting cells such as macrophages, dendritic cells, B-lymphocytes, and astrocytes in response to stimuli which may include bacterial cell wall products [14–17]. IL-12 was initially characterized as a Natural Killer (NK) cell activator [18] and promotes the production of Th1 CD4 effector cells from Th0 precursors [15].

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IL-12 has been shown to mediate a broad range of effects on both innate and acquired immunity. It induces IFN-γ production by NK, Th1 and CD8 cells, regulates T-cells proliferation, stimulates NK cell activity, and enhances CD8 CTL responses [18–20]. IL-12 has been shown to induce IFN-γ and TNF-α levels in serum and in brain tissue homogenates in mice [21–23]. Orange and co-workers showed that high levels of exogenous IL-12 induced strong acute phase responses and were associated with host toxicity through the activation of the hypothalamus-pituitary-adrenal axis [22], demonstrating an interaction of the immune and stress response systems.

IL-12 has been shown to serve as a direct chemotactic factor for NK cell infiltration and increases its binding to vascular endothelium cells in vitro [24]. NK cells infiltrate the virus-infected CNS before T-cell infiltration [25, 26]. NK cells have an important role in clearance of many viral infections that is independent of T-cell function [27, 28]. NK cells secrete IFN-γ, which participates in a positive feed-back loop amplifying IL-12 production.

IL-12 also induces Th1-specific immune responses by promoting the differentiation of TH1 cells from TH0 precursors at the expense of Th2 effector cell, inhibiting IL-4 production [29, 30]. The Th1 subset secretes IL-2, IFN-γ, and lymphotoxin (LT, TNF-β). Th1 cells are also cytolytic, recognizing MHC Class II and Ag. They mediate delayed-type hypersensitivity, and are thus involved in inflammation.

In vitro, IL-12 suppresses the synthesis of IgE by IL-4 stimulated B-cells [31]. In vivo, intraperitoneal injection of IL-12 resulted in the enhancement of IFN-γ and IL-10 gene expression, reduced levels of IL-3 and IL-4 gene expression, and increased serum IgG2a levels [32]. The in vivo effects of IL-12 on immunoglobulin isotypes were only partially mediated by IFN-γ. The administration of anti-IL-12 antibodies in vivo significantly blocked Th1 response to antigen, evaluated by either IFN-γ production or serum IgG2a antibody response [33, 34]. Thus IL-12 production may antagonize the differentiation of Th2 cells and their expansion of B-cells switching to the epsilon heavy chain [32]. IgE readily sensitizes mast cells, thus early expression of IL-12 during sensitization can inhibit the development of immediate hypersensitivity such as allergic rhinitis and asthma.
IL-12 knockout mice have been produced. It was also shown that IL-12-deficient mice are defective in IFN-γ production and type 1 cytokine responses [35]. These results suggest that IL-12 has a role in antigen-induced Th1 differentiation in vivo and its effects on Ig isotypes. However, in spite of the lack of IL-12 secretion in the knockout mice, Th1 cells are able to develop and are active for alloantigen responses [36] and for responses to the coronavirus, mouse hepatitis virus [37]. This suggests that other factors or cytokines may regulate Th1 cell differentiation, possibly IL-18 (reviewed in [38]).

IL-12 treatment of experimental animals has been found to modify the course of many infectious diseases and the response to tumors [39, 40]. In short, where an inflammatory delayed hypersensitivity (Type IV hypersensitivity) or cytolytic T-cell response is beneficial to the host, IL-12 treatment generally promotes recovery from the infection or tumor challenge. Where inflammatory responses are disadvantageous, such as in several autoimmune diseases, IL-12 treatment does not promote recovery [41, 42]. The effects of IL-12 on the host’s response to viral infection will be discussed in detail.

**IL-12 RECEPTOR AND ITS SIGNAL TRANSDUCTION PATHWAY**

The IL-12 receptor (IL-12R) has been cloned from both human and murine lymphoid cells [43–47]. They have termed the two chains β1 and β2, due to the sequence relatedness to other β chains of the hematopoietic growth factor receptor families. Th2 cells, which do not respond to IL-12, have been shown to express only one (β1) of the two IL-12R chains, which will bind IL-12 at low affinity [42, 44, 46]. The signal transduction pathway has been defined in lymphoid cells to include activation of Janus kinase family members Tyk2 and Jak2, which in turn activate Signal Transducers and Activators of Transcription (STAT)3 and STAT4 [48, 49]. A model of the IL-12 receptor interaction with its transducers is shown in the accompanying figure. For contrast, a very well characterized receptor, is included. IL-2R is known to react with Ras which phosphorylates Mitogen Activated Protein kinases (MAP kinases) which trigger nuclear factor kappa-B (NF-kB) activation and signaling also through the phosphoinositol-3 (PI-3) kinase pathway in addition to Jak3 and STATs 3 and 5.

STATs are inactive in the cytoplasm until a ligand-induced activation of the cell takes place. Receptor-mediated cascades lead to phosphorylation of STATs by members of the Jak/Tyk family of tyrosine kinases and subsequent homo- or hetero-dimerization. The dimers are able to translocate to the nucleus where transcription is initiated [1–3, 50].

Of all the cytokines and growth factors examined to date, STAT4 has uniquely been found to be phosphorylated by IL-12 [48]. Other stimuli are more promiscuous or overlapping in their STAT activation in lymphocytes [1–3]. Mice deficient in STAT4 have been developed and initially examined for functional responses [51]. They appear to be deficient in Th1 responses and sufficient in Th2 responses to specific Ags. STAT6 knockout mice are able to mount Th1 responses but not Th2, from which it was concluded that STAT6 is essential for the IL-4 response [52]. Mice deficient in Jak2 have recently been described to be embryonic lethal; they have impaired erythropoiesis, so that the impact of this deficiency on cytokine signaling in lymphocytes cannot easily be defined [53, 54].

**IL-12 AND VIRAL INFECTIONS**

Like bacterial infections of Ag presenting cells, viral infections rapidly induce IL-12 gene expression and immunoreactive material [55–57]. In the CNS, infection rapidly induces IL-12 expression and also IL-12 treatment augments this induction (Fig. 3), suggesting an autocrine pathway.

During infection, IL-12Rβ1 and β2 mRNA is also increased (Fig. 4), however, at this time, since RNA was prepared from tissue homogenates, we cannot determine whether this gene expression is on IL-12-producing cells or by inflammatory NK and Th1 cells.

There have been studies examining the role of IL-12 on the outcome from viral infection in many systems. In some experiments, investigators examined the change(s) in endogenous IL-12 gene expression. At times investigators have injected neutralizing Ab to IL-12 and in the majority of studies, IL-12 has been infused. The exact mechanisms by which IL-12 has its effects on the host may be distinct in each infection. We will attempt to put this into perspective.

**EMC**

Administration of a small, 20 ng dose of IL-12 protected mice from a lethal encephalomyelocarditis virus infection. This effect was not seen in mice deficient in the IFN-γR, and appeared to be acting through induction of endogenous IFN-γ secretion by NK and T cells [58].

**Influenza**

Endogenous IL-12 was induced during influenza pneumonia. The IL-12 attracted and activated NK cells, which secreted IFN-γ, inhibiting viral replication. In addition, there was a modest enhancement of the CD8 T cell response in response to IL-12 [59]. There was, however, no sensitivity of influenza to another downstream pathway [23].

**LCMV**

IL-12 administration was found to be either efficacious (at low doses) or quite toxic to mice infected with lymphocytic choriomeningitis virus. Low doses (1–10 ng) inhibited viral replication and enhanced host CD8 responses; in contrast, high doses (up to 1 mg) resulted...
in high levels of serum TNF-α, increased doses of virus and inhibited CD8 responses [21]. The toxicity, including thymic atrophy, of IL-12 was shown to be mediated by TNF-α and glucocorticoids [22].

**VSV**

IL-12 treatment promotes clearance of vesicular stomatitis virus from neurons in the CNS and survival of infected mice. This is accompanied by induction of GFAP, mac-1, MHC I and II, IFN-γ, TNF-α, NOS-1-2 and -3. IL-12 [57] (Fig. 3) and IL-12R [Fig. 4] [23, 60–62]. IL-12 activity is not dependent on either IFN-γ or TNF-α in the IFN-γ-deficient mice [63]. However, IL-12 activity appears to require intracellular activity of NOS-1 in neurons for clearance of virus (not shown) and for host survival [23] (Fig. 5). Nitric oxide (or its reaction product peroxynitrite) is a potent antiviral in many systems [64].

Most investigators have administered IL-12 either prior to or beginning on the day of infection. To be practical, however, if it were to be administered during human infections to viruses, IL-12 should be efficacious after infection has started, when symptoms are becoming apparent to the patient. IL-12 does have recovery-promoting activity even after the start of a lethal VSV encephalitis infection (Fig. 6).

**MHV**

In mice deficient of IFN-γR, mouse hepatitis virus infection results in increased susceptibility to liver injury and did not upregulate IL-12 mRNA. Exogenous IL-12 treatment of the IFN-γR knockout mice did not restore their resistance to MHV infection. However, normal mice could be protected by either IL-12 or by IFN-γ treatment [65]. IL-12 p40 or p35 knockout mice responded to MHV infection with a Th1 response like wild type mice, which was unexpected [37]. This suggests that IL-12 function may have been complemented by another cytokine or that viral infection served as a co-factor for IFN-γ-
mediated activities. MHV is sensitive to IFN-γ and to another pathway, in vitro [23], but not in vivo [66].

**Measles**

Cell mediated immune suppression associated with measles virus infection was attributed to an inhibition in IL-12 production by infected macrophages. Measles virus receptor, CD46, also a complement receptor, is expressed on these monocytes; cross-linking of the cell surface molecule was found to diminish IL-12 production [75].

**MAIDS**

Murine acquired immunodeficiency syndrome, caused by infection with LP-BM5 virus, is associated with splenomegaly and lymphadenopathy as well as polyclonal B-cell activation. Treatment of MAIDS-infected mice with IL-12 resulted in diminished lymphoproliferation. This beneficial effect was not seen in IFN-γ deficient mice [67].

**HIV**

Co-incubation of HIV gp 120 and human macrophages/monocytes resulted in an IFN-γ-dependent production of IL-12. PBMC from HIV-infected patients were found to be deficient in production of IL-12, but not TNF-α, IL-1-β and IL-10 [20]. This led to the hypothesis that HIV-infected patients should receive IL-12 to supplement their responsiveness, overcoming the depletion of Th1 cells due to infection-related apoptosis. In addition, co-administration of genetic vaccines for HIV gp 160 and IL-12 resulted in enhanced cell mediated responses in mice [68].

**HBV**

Hepatitis B virus does not naturally infect mice. However, Chisari has developed a model of transgenic mice, which express virus in hepatocytes and develop immunopathology. IL-12 treatment induced IFN-γ, TNF-α, and IFN-α/β, and inhibited HBV replication in liver and kidney [69].

**PRV**

Th1 Pseudorabies virus vaccine responses were augmented by IL-12 administration in immunocompetent mice. However, mice which lacked IFN-γR were unresponsive to the IL-12 treatment and did not develop resistance to viral challenge [70].

**MCMV**

For many herpes virus infections, an early NK response is essential in recovery from infection [27]. For Murine Cytomegalovirus infection, NK-cell derived IFN-γ was shown to be required for the host’s response. This was augmented by IL-12 administration, increasing NK activity, increasing IFN-γ production, and diminishing viral titer [71].

**HSV**

Experimental corneal Herpes simplex virus infection induces the production of IL-12 p40 mRNA both in cornea and in draining lymph nodes. This may result in initiating inflammation to the site of infection [56], resulting in immunopathology. However, IL-12 exhibits potent antiviral activities for HSV, induced IFN-γ, and protected mice from lethal infection [72]. IL-12 treatment also protected mice from thermal injury and increased susceptibility to HSV-1 morbidity and mortality [73].

**H. Sam.**

Like Epstein–Barr virus transformation of human B-lymphocytes, Herpes saimiri virus can immortalize human γδ T cells from peripheral blood. Treatment of transformed T-cells with IL-12 led to their activation, induced synthesis of perforin and granzyme B, and enhanced their CTL activity [74].
Figure 3. IL-10 receptor mRNA expression induced by IL-12 treatment during viral infection. (A) Male BALB/cAnTac mice were untreated, uninfected, treated with media, uninfected and treated with 200 ng IL-12/mouse for 3 days, infected for 3 days with VSV and treated with media, or infected for 3 days with VSV and treated with IL-12 (lanes 1–5 respectively). Neuroblastoma cell lines NB41A3 (ATCC), N18 (Drs P Tucker and D Griffin, Johns Hopkins), and OBL21a (Dr M Buchmeier, Scripps Institute) (lanes 8–10) were stimulated with 5 ng IL-12/ml for 19 h. Total RNA was collected from CNS homogenates or cultured cells using Ambion kits. A ribonuclease protection assay was performed on 50 µg samples of total RNA using probes obtained in plasmid form from Dr Louise Showe of the Wistar Institute. Yeast tRNA (lanes 5 and 6) was used as a control. (B) 50 µg of total RNA isolated from OBL21a a neuroblastoma cell line and RAW a mouse macrophage cell line stimulated for 19 h with 5 ng IL-12/ml (lanes 1 and 2 respectively), yeast tRNA (lane 3). The data show the expression of IL-12Rβ1 and β2 subunits both in vitro and in vivo as a result of IL-12 treatment.

CONCLUSIONS

IL-12 treatment of experimental hosts generally has a profoundly beneficial outcome on the response to viral infection. Whether IL-12 acts directly or indirectly through IFN-γ or TNF-α and their downstream responses (RNAase L, NOS, caspases, for instance), as shown in Fig. 1, the general effects are (1) the recruitment and induction of NK cells, (2) cytokine release by innate immune and T-cells, (3) facilitating the differentiation of Th1 cells which are both inflammatory and cytolytic as well as helpers, (4) amplifying CD8 responders, (5) enhancing the production of neutralizing IgG2a Ab, and ultimately (6) the inhibition of viral replication and clearance of virus from host cells. These activities appear both in peripheral and in CNS infections. Table 1 illustrates some of the cytokine-inducible proteins and their activities in virally infected cells.

Since IL-12 works both at the time of infection, and also after symptoms and viral replication has begun (Fig. 6), it may be efficacious in treating humans. IL-12 treatment also enhances responses to vaccination, whether by co-administration of cytokine at the time of inoculation of protein Ags, or by genetic vaccination [68].

The cytokine is already in Phase II clinical trials for renal carcinoma and trials are under way for Mycobacterium tuberculosis infections, both long-term treatments; therefore, it may be relatively easy to develop...
Delayed administration of IL-12 is efficacious. BALB/cAnTac male mice were infected on day zero and divided into four different groups of 10 mice: media treated beginning on the day of infection, 199 ng IL-12 on the day of infection, IL-12 on day 0 and IL-12 beginning on day 1 post infection. Survival was noted over the course of a 15 day observation period. These data represent the means of three replicate experiments.

Table 1. Effects of IL-12 on virally infected cells

| Action    | Effector | Effector action                  | Example(s)                                                                 | Outcome                                                                 |
|-----------|----------|----------------------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Direct    | IL-12    | IL-12 inducible genes            | NOS 1, 2, 3, TNF-α, IFN-γ others?                                         | Inhibition of viral infection, chemotaxis for NK cells                  |
| Indirect  | IFN-γ    | IFN-γ-inducible genes            | IRF-1, Mx, GTPases, RNAse L, 2-5'A Synthase, RNA-dependent Protein Kinase (PKR), NOS 1, 2, 3, MHC, IP-10/crg-2 | Cytostasis, viral inhibition, increases sensitivity to CTL lysis, chemotaxis and angiostatic activities |
| Indirect  | TNF-α    | TNF-α-inducible genes and pathways | Caspases, NOS 1, 2, 3                                                     | Apoptosis of virally infected cells, viral inhibition                   |

clinical trials from human encephalitis (picornavirus, rabies), for Herpes infections, and for HIV. It may not be a magic bullet alone, but may substantially augment antiviral drug therapies.

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