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Utilizing IL-12, IL-15 and IL-7 as Mucosal Vaccine Adjuvants

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Abstract

In this paper we review and discuss three of the most exciting and promising cytokines for therapeutic intervention and immunomodulation of immune responses including those on mucosal surfaces. The main properties of IL-12, IL-15 and IL-7 are described and the studies utilizing these cytokines as immunomodulators and vaccine adjuvants discussed.

Keywords

IL-12; IL-15; IL-7; Mucosal; Vaccine

INTRODUCTION

The field of utilizing immunomodulators for therapeutic purposes and as vaccine adjuvants is rapidly growing. Of those, cytokines are some of the most interesting and most widely tested. Lately, three cytokines, IL-12, IL-15 and IL-7 because of their properties and effects on the immune system (summarized in Table 1) emerged as potentially useful therapeutic immunomodulators and mucosal vaccines adjuvants. We [1] and others [2] have previously reviewed the effects of IL-12 and IL-15 as vaccine adjuvants. In this paper we include the newest findings on IL-12 and IL-15 and we include another emerging candidate immunomodulator and vaccine adjuvant, IL-7.

INTERLEUKIN 12

Interleukin-12 (IL-12) is an important regulatory cytokine that has a central function in initiating and regulating cellular immune responses. This cytokine belongs to a large group of cytokines that fold into a bundle of four alpha helices. It is a heterodimeric protein of 70kDa that is composed of two disulfide-linked subunits, 35 and 40kDa in mass, which are coded by two separate genes that bear no apparent homology [3]. The gene encoding the p40 chain is mapped to chromosome 5q31–q33, a region that encodes many cytokines and cytokine receptors, and the gene encoding the p35 chain is located on chromosome 3p12–3q13.2. The murine counterparts of these genes contain 70 % (for p40) and 60 % (for p35) sequence homology to the human genes [4]. The IL-12 receptor is composed of two subunits, IL-12Rβ1 and IL-12Rβ2. IL-12Rβ1 is the subunit primarily responsible for binding IL-12. Its structure in humans and mice has been identified as most homologous to the leukemia inhibitory factor (LIF) receptor [5].

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Macrophages, monocytes, dendritic cells and B lymphocytes produce IL-12 in response to bacterial products and intracellular parasites. Subsequently, IL-12 stimulates production of IFN-γ and TNF-α from both NK cells and helper T cells. Because IL-12 induces production of IFN-γ, its immunological action might target primarily cells that are capable of producing IFN-γ ; activated T cells that also have the co-receptor CD30 present on their surface [6]. The production of IFN-γ has a very powerful effect in enhancing the ability of phagocytic cells to produce IL-12 and IL-12 also enhances the production of IFN-γ, creating a positive reinforcement loop. Also, as reported by Seder *et al*. [7], IL-12 reduces IL-4 mediated suppression of IFN-γ.

It has been reported that IL-12 plays an important role for induction of Th1 cells from naive Th0 cells and generation of CTL's [8]. However, as noted by O'Garra, repeated exposure to antigen and IL-12 is necessary to establish a stable Th1 response [9].

Early induction by IL-12 of IFN-γ expression is key to the initiation of innate immune response. Scott reported that IL-12 stimulates the production of IFNγ from T and natural killer (NK) cells [10] while Gazzinelli [11] found that it is required for T-cell-independent triggering of NK cells by intracellular parasites such as *T. gondii*.

IL-12 has been proposed as a treatment for HIV disease because it stimulates the Th1 subset of CD4 cells which may be important for preventing disease progression in HIV-infected people. Chehimi *et al*. reported that levels of interleukin-12 in HIV-infected people are significantly lower than those seen in uninfected age-matched controls [12]. Furthermore, as observed by Clerici *et al*. [13], IL-12 augmented the *in vitro* production of cytokines associated with Th1 responses (IL-2 and IFN-α) in HIV-infected individuals. When PBMCs from 40 HIV-positive individuals unable to produce IL-2 in response to envelope peptides of HIV-1 were Env-stimulated in the presence of IL-12, increased IL-2 production was observed in 28/40 (70%). Env-stimulated IL-2 production was not seen in PBMCs from nine HIV-negative individuals studied as controls. IL-12 has neither a direct inhibitory nor stimulatory effect on HIV replication *in vitro* [14].

Because of its immune properties, IL-12 has great potential as a vaccine adjuvant for promoting cell-mediated immunity and a Th1 cell response. Not only does immunization with IL-12 promote a long-term and stable Th1 response, it also enhances the primary Th1 response when given in conjunction with other adjuvants.

Several laboratories have reported that the cellular immune responses to DNA vaccines in mice can be enhanced by co-delivering DNA plasmids expressing specific immune modulators with IL-12 considered to be one of the most exciting in the mouse system. When researchers from Dr. Weiner's laboratory [15] sought to compare these molecular adjuvants in a primate model system, animals co-vaccinated with the IL-12 molecular adjuvant demonstrated enhancement of the CD4 compartment and the highest induction of IFN-γ producing CD8 effectors cells. In addition, the IL-12 plasmid expanded antigen-specific granzyme B production two fold over pSIVGag. Importantly, the combination of IL-12/IL-15 dramatically enhanced the CD8 antigen-specific granzyme B response induced to pSIVGag vaccine [15]. Similarly, Egan *et al*. [16] reported that priming with plasmid DNAs expressing IL-12 and simian immunodeficiency virus (SIV) gag enhances the immunogenicity and efficacy of an experimental AIDS vaccine based on recombinant vesicular stomatitis virus (VSV). Macaques receiving the combination plasmids showed increased SIVgag-specific cell-mediated and humoral immune responses and significantly lower viral loads post challenge with SHIV89.6P.

More recently Yoshida *et al*. [17] investigated the immunogenicity and protective efficacy of DNA vaccine combinations expressing mycobacterial heat shock protein 65 (Hsp65) and IL-12 using gene-gun bombardment and the hemagglutinating virus of Japan (HVJ)-liposome

method. In this mouse model a single gene gun vaccination with the combination of Hsp65 DNA and mIL-12 DNA provided a remarkably high degree of protection against challenge with virulent *Mycobacterium tuberculosis*; bacterial numbers were 100-fold lower in the lungs compared to BCG-treated mice. The HJV-liposome method improved the protective efficacy compared to gene gun vaccination; Hsp65+mIL-12/HJV vaccination resulted in a greater degree of protection than that evoked by BCG. This efficacy was associated with the emergence of IFN-γ secreting T cells and activation of proliferative T cells and cytokine (IFN-γ and IL-2) production upon stimulation with Hsp65 and antigens from *M. tuberculosis*, suggesting that Hsp65+mIL-12/HJV could be a promising candidate for a new tuberculosis DNA vaccine, which is superior to BCG vaccine.

However, systemic administration of IL-12 has been shown to have numerous toxic effects. In June 1995, a clinical trial testing it as a treatment for kidney cancer was stopped after ten participants were hospitalized and one died. The toxicity of IL-12 was due to the rate and frequency of the dosing. Trials of IL-12 as a treatment for cancer and HIV are now continuing using a weekly dose initially given at 100 mg/kg and gradually increased to 300 mg/kg [18].

When delivered intranasally, IL-12 induces less systemic IFN-γ production and fewer pathological tissues changes, yet is efficacious, as indicated by enhanced CD3+ T cell activation and increased production of Th1-associated immunoglobulins [19]. Thus IL-12 can be delivered safely and effectively by the intranasal route and effectively modulates antigen-specific immune responses. As described by Marinaro *et al*., IL-12 administered by mucosal routes (nasal or oral) could also redirect Th2-type responses induced by the mucosal adjuvant cholera toxin towards a Th1-type immune response [20], [21]. In more recent studies performed by the same group, IL-6 and IL-12 were co-delivered intranasally with the protein vaccine tetanus toxoid (TT). IL-12 administered nasally with TT not only induced sharp increases in TT-specific serum IgG (mainly IgG1 and IgG2b) and IgA, but also elevated mucosal S-IgA Ab responses. Co-administration of IL-6 and IL-12 with TT did not enhance the mucosal or serum Ab responses over those seen with IL-12 alone. [22].

These observations were supported by other studies where mice intranasally immunized with dinitrophenyl conjugated to OVA (DNP-OVA) and cholera toxin subunit B (CTB) were found to have elevated levels of IFN-γ and IL-10 mRNA transcripts in both lungs and spleen if immunization was followed by administration of IL-12. A significant increase in serum IgG2a, IgG2b and IgG3 anti-DNP antibody and fecal IgG2a but decrease in IgA antibody titers was observed in the mice where IL-12 was administered intranasally [23]. Another study used co-delivery of IL-12 in combination with CTB as adjuvants for intranasal administration of recombinant 89.6 gp120 or 89.6 gp140 and found that IL-12 and CTB act synergistically to enhance both systemic and local mucosal antibody responses to the HIV-1 glycoproteins. Moreover, significant levels of IgA antibody were obtained only in mice vaccinated in the presence of both IL-12 and CTB [24].

All of these studies point to IL-12 as one of the most promising cytokines to use as a mucosal vaccine adjuvant.

IL-15

IL-15 belongs to the family of the 4 α-helix bundle cytokines that includes IL-2, IL-3, IL-6, IL-7, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and human growth factor [25,26], [27]. It is similar in its activity to IL-2 and utilizes its receptors. While the α receptor of IL-2 (IL-2Rα) is not involved, both β and γ receptors are required for IL-15 binding [28]. Giri *et al* [29] have cloned a distinct murine IL-15 receptor alpha chain (IL-15Rα), which alone displays a high affinity for IL-15 and is structurally similar to IL-2Rα.

The distribution of IL-15 and IL15Rα mRNA suggests that IL-15 may have biological activities distinct from IL-2. Also, unlike IL-2 that is produced by T cells, IL-15 is produced by macrophages, dendritic cells, keratinocytes and epithelial cells as reviewed in Weng *et al*. [30]. IL-15 is highly expressed in skeletal muscle [31], kidney epithelium, activated monocytes and macrophages and intestinal epithelial cells [32]. IL-15Rα mRNA has been observed to have a wide cellular distribution, such as in T cells, B cells, macrophages, thymic cells, skeletal muscle, lung, and liver [28]. This widespread distribution of the IL-15R system suggests that IL-15 mediates pleiotropic effects on multiple cell types, including cells of nonlymphoid origin [33].

IL-15 induces T cell proliferation and cytokine production, stimulates locomotion and chemotaxis of normal T cells [25], [28], [34], [35], [36], [37], [38] and protects them from apoptosis [39]. It enhances NK cell cytotoxicity and antibody-dependent cell-mediated cytotoxicity, up-regulates NK cell survival and production of NK cell-derived cytokines such as IFN-γ, GM-CSF, and TNF [40], [41], and induces B cell proliferation and isotype switching [42]. IL-15 has recently been found to promote CTL memory [43], [44], [45].

IL-15 was implicated in the pathogenesis of celiac disease (reviewed in [46]). The new paradigm implies a genetically determined primary defect underlying the epithelial sensitivity to gluten and/or other exogenous factors (e.g., microorganisms) inducing mucosal hypersecretion of IL-15 in celiac patients. In turn, IL-15 upregulates natural killer receptor (NKR) variants on intraepithelial lymphocytes (IELs) that recognize non-classical MHC-I molecules such as MIC. This could lead to further activation of IELs through two possible mechanisms: (1) the reduction of the activation threshold of IELs and their recognition of low-affinity self-antigens and (2) the acquisition by IELs of natural killer-like activity. Hyperactivated IELs can then damage the epithelium by cytotoxicity and IFN-γ secretion [46].

In the intestinal tract, similarly to IL-2, IL-15 is able to stimulate proliferation of intestinal epithelial cells [32] and in contrast to IL-2 it can also stimulate proliferation of mast cells [33].

Production of IL-15 by intestinal epithelial cells may be influencing the lymphocytic composition within lamina propria [32] similarly to its effects on antigen-dependent T and B cells shown *in vitro* [42], [25], [28]. In addition, IL-15 has been shown to be chemotactic for T lymphocytes [34]. IL-15, much like IL-2, upregulates expression of TGFβ in epithelial cells, a cytokine that when produced by intestinal epithelial cells regulates the expression of αEβ7. The integrin αEβ7 anchors the IELs to intestinal epithelial cells through its interaction with E-cadherin [47]. While the IELs of the intestinal mucosa are both αβ and γδ, the γδ T cells are the prevalent T cells in the intestinal and other mucosal epithelia of most vertebrates and it is believed to be the source of secreted factors necessary for tissue maintenance [48], [49], [50]. IL-15 regulates the generation of the restricted TCR variable γ -region repertoire of IELs [51]. Its effects might not be exclusive to the γδ IEL subset as analysis of the IELs from IL-15 −/− [52] and IL-15Rα−/− [53] mice revealed substantial decrease in the proportion of CD8αα- bearing cells expressing either TCRαβ or TCRγδ. In experiments done *in vitro*, IL-15 promoted survival of the CD8αα+ subset in the presence of TCR stimulation and promoted survival of both CD8αα and CD8αβ in the absence of TCR stimulation. In addition, unlike exogenous IL-2, exogenous IL-15 did not support the effector functions of either IEL subset, including IFN-γ production, IL-4-induced Th2 cytokine production, and anti-TCR mAb-redirected cytotoxicity [54].

A recent study, however, demonstrated that IL-15 dramatically increases *in vivo* proliferation of rhesus macaque (RM) CD4 (+) and CD8 (+) T (EM) cells with little effect on the naive or central memory T (T (CM)) cell subsets [55].

The finding that IL-15 is pivotal in the development of long-lasting immunological memory and the maintenance of an immune response has provided the scientific basis for the incorporation of IL-15 into molecular vaccines (reviewed in [1]). Indeed, co-injection of IL-15 gene with HIV DNA immunogens vaccine increased CTL responses [56]. A recent study from Xin *et al*. reported that an IL-15 expression plasmid increased cell mediated immunity induced by a DNA vaccine [57]. In addition, administration of HIV vaccinia virus vaccine expressing IL-15 induced robust, long lasting, CD8+ cytotoxic T-lymphocyte-mediated immunity. The IL-15 cytokine milieu at the time of immunological priming had persistent long-term effects on the character of the memory CD8+ T cells [58]. Similarly, immune response to DNA immunization with HIV Env and Nef vectors was the most potent if co-administered with IL-15, IL-12 or IL-2 DNA plasmids [59]. Enhanced immune responses to plasmid DNA vaccination by IL-15 was also reported against hepatitis B surface antigen (HBsAg) [60], the herpes simplex virus type 1 (HSV-1) glycoprotein B (gB) [61], influenza A PR8/34 hemagglutinin [62], chicken *Eimeria* DNA vaccine carrying the 3-1E parasite gene (pcDNA3-1E) [63] and the viral vector SV40-delivered HIV envelope antigen [64].

CD8+ T cells from mice infected with the vaccine strain of *Toxoplasma Gondii* show increased Ag-specific responses and are protective against *Toxoplasma* challenge. However, over time these responses decline and mice become susceptible to *Toxoplasma Gondii* infection. Their function is completely restored if mice are pretreated with IL-15 two weeks prior to challenge, indicating an important role of IL-15 in maintaining long-term CD8+ T cell memory responses [65].

Systemic administration of IL-15 is capable of augmenting the primary $CD8^+$ T cell response to vaccination. This was evaluated in an experiment where naive CD8+ (OT-1) T cells were first adoptively transferred into mice and then mice were immunized with peptide-pulsed dendritic cells. The immunization induced modest expansion of OT-1 cells but addition of systemic IL-15 for the following 7 days resulted in significant increase in the expansion of responding T cells in peripheral blood, spleen and lymph nodes that were cytotoxic and maintained a Tc1-biased phenotype [66].

It has recently been shown that IL-15 plays an important role in intravaginal herpes simplex type 2 infection (HSV-2) [67]. Mice lacking IL-15 are significantly more susceptible to HSV-2 infection. Also, intravaginal HSV-2 infection induced IL-15 in vaginal washes and IL-15-positive cells were detected in the submucosa and vaginal epithelium following intravaginal HSV-2 infection. Local delivery of recombinant IL-15 to the genital mucosae resulted in significant reductions in HSV-2 titers in genital washes and 60% survival following intravaginal HSV-2 challenge [67]. The important role of IL-15 in mucosal immune responses was also ascertained in a recent study that examined the ability of plasmid-encoded IL-15 (pIL-15) to induce and maintain the mucosal B and T cell responses to DNA vaccine against Herpes Simplex Virus [68]. Mice were immunized intranasally and immune responses assessed in the spleen and vaginal mucosa. IL-15 co-application supported generation and long-term maintenance of Ag-specific CD8+ T cells in spleen but the enhanced CD8+ T cell responses in vaginal mucosa were not maintained for a long time and were lost. IL-15 induced four-fold higher levels of Ag-specific IgA at the vaginal mucosa that remained stable for a long period of time. Humoral and cellular responses in the intestinal mucosa were unfortunately not assessed in this study. This study confirmed the findings published by Hiroi *et al*. that IL-15 is a critically important cytokine for the differentiation of both sIgM1, IgA2 and sIgM2sIgA1 B-1 cells expressing IL-15R into IgA-producing cells in mucosal tissues [69]. In addition,

mucosal IgA levels were found to be inhibited by anti-IL-15 mAb treatment *in vivo*, but enhanced by administration of rIL-15, while serum IgA levels remained unaffected [69].

Finally, while utilizing IL-15 as a mucosal adjuvant awaits further investigation, the data published so far certainly indicate that its co-administration with immunogens induces stronger cellular and humoral responses systemically and mucosally.

IL-7

Interleukin-7 (IL-7) was first described in 1988 as a potent hematopoietic growth factor of B-cell progenitors [70] but was soon shown to be a potent costimulus for both murine and human, immature and mature T cells [70].

This cytokine is constitutively secreted by non-hematopoietic stromal cells in bone marrow, thymus, lymphoid organs, mucosal lymphoid tissues, skin and liver [71], [72], [73]. Even though IL-7 mostly targets developing B and T lymphocytes and mature T cells, those cells do not produce it.

IL-7 plays an important role in early lymphocyte development by regulating V(D)J rearrangement [74], and promoting thymocyte proliferation and survival [75]. Mutations of the IL-7Rα result in severe T-cell deficiency confirming its unique role in thymopoesis [76].

An anti-apoptotic effect *via* up-regulation of bcl-2 family members, exerted on mature T-cells has also been described [77]. IL-7 is a crucial regulator of the proliferation of naive CD4+ and CD8+ T cells, mainly in settings of so-called “homeostatic expansion” occurring in the periphery [78]. It decreases the threshold for activation and therefore is required for T cell proliferation induced by interactions with low-affinity antigens.

Although the generation of primary effector cells from naive $CD4^+$ cells and their dissemination to nonlymphoid tissues are not affected by IL-7 deficiency, memory cells fail to subsequently develop in either the lymphoid or nonlymphoid compartments. [79]. And while it plays a similar role for memory CD8+ T cells, it may be replaced by IL-15 in this action [80].

IL-7 also has an important role in regulating the immune responses following an antigen entry. Although this cytokine is dispensable in the initiation phase of antigenic responses, it regulates the generation of memory CD4+ and CD8+ T cells [81] and appears to be a key-regulator of transition of effector T cells to memory T cells, the process critically dependent on IL-7/IL-7R signaling [82].

Altogether, numerous studies confirmed the role of IL-7 as an important lymphoid growth factor and it has become a natural candidate for immunostimulatory therapies, mostly in the context of enhancing immune reconstitution during lymphopenia. Thus far, no data describing the effects of IL-7 administration in humans is available. The safety of recombinant human IL-7 is currently being evaluated in a Phase I dose escalation trial at the National Cancer Institute, Bethesda, MD. However, the effects of IL-7 administration have been assessed in a number of animal studies. IL-7 has been shown to increase thymopoiesis as assessed by the enumeraton of TRECs (thymocytes positive for a TCR excision circle) [83]. Interestingly, this effect has been mainly seen in irradiated or young mice but not in normal mice [84], [85]. Administration of IL-7 also exerts profound effects on mature T cells. IL-7 therapy induced widespread T-cell cycling and increase of total lymphocyte counts in both CD4+ and CD8+ subsets [86]. In primates, supra-physiological doses of IL-7 affected equally naive, central memory and effector subsets, and, interestingly, it led to the conversion of naive cells to

memory-like phenotype [87]. Importantly, the effects mentioned above were accompanied by the increase in bcl-2 expression in CD4+ and CD8+ lymphocytes.

As CD4+ depletion remains one of the cardinal features of HIV infection, IL-7 was suggested as a crucial cytokine with therapeutic potential for that disease. An inverse correlation between IL-7 serum levels and the levels of lymphopenia was described in HIV-infected patients [88], [89]. However, despite increased levels of endogenous IL-7 in SIV-infected primates, the treatment with recombinant cytokine stimulated T-cell proliferation and peripheral expansion [86], [90], [91]. The use of IL-7 in settings of HIV/SIV infection brought some controversies after IL-7 was shown to stimulate virus replication and increase susceptibility of naive T-cells to HIV *in vitro* [92]. However, the studies of Fry *et al*., Nugeyre *et al*. and Beq *et al*. with the use of IL-7 in SIV-infected primates did not demonstrate increased viral replication [86], [90], [91].

The persistence of HIV-1 in virally suppressed infected individuals on highly active antiretroviral therapy (HAART) remains a major therapeutic problem. The use of cytokines has been envisioned as an additional therapeutic strategy to stimulate latent proviruses in these individuals. In a recent study, IL-7 was found to be significantly more effective at enhancing HIV-1 proviral reactivation than either IL-2 alone or IL-2 combined with phytohemagglutinin (PHA) in CD8-depleted PBMCs and in resting CD4(+) T lymphocytes from HIV-1-infected patients on suppressive HAART [93]. This could be used to potentially deplete HIV-1 reservoirs and lead to the rational design of immune-antiretroviral approaches.

A number of recent studies, described also in this part of the review, demonstrated that IL-7 could become an attractive candidate as a vaccine adjuvant. The brief summary of rationales for that perspective is the following: IL-7 stimulates T-cell proliferation, affects the transition of effector cells into memory cells, prevents T-cell death, and, importantly, decreases the threshold for induction of proliferation of T-cells specific for low-affinity antigens. Recruitment of CD8+ T-cells specific for low-affinity antigens would be of particular benefit as one of the biggest failures of current vaccines is the small diversity of generated effector pools.

Thus far, IL-7 was demonstrated to be an effective adjuvant in settings of vaccinations with tumor cells, tetanus-toxoid and herpes simplex virus [94], [95], [96]. Recently, the adjuvant effect of IL-7 was examined by means of concomitant administration of IL-7 and vaccination as described in an excellent study by Melchionda *et al.* [97]. Combination of IL-7 and vaccine significantly increased effector responses directed not only against dominant but also subdominant antigens. Moreover, IL-7 administration resulted in a long-term survival of newly generated CD8+ memory cell pool. Those data for the first time provided strong evidence that IL-7 as an adjuvant may effectively increase the size and breadth of vaccine-induced T-cell responses.

As some chronic infections (i.e., HIV infection) tend to localize in mucosa of gastrointestinal and genitourinary tract, some researchers pursued the effects of mucosal application of T-cell stimulating vaccines. In fact, Belyakov *et al*. demonstrated that intrarectal peptide immunization was more efficient than subcutaneous immunization in generating mucosal virus-specific CTL responses and decreasing viral loads [98]. Because of the great adjuvant potential of IL-7 as previously described, this cytokine becomes also a natural candidate for direct mucosal application. Importantly, potential responsiveness to such a treatment would be warranted as IL-7R is expressed by human intestinal mucosal lymphocytes [72]. IL-7 itself is locally released by epithelial cells in the human gut mucosa and exhibits different modulatory effects on mucosal T cells, i.e. regulates the proliferation of mucosal lymphocytes and their responsiveness to anti-CD3 stimulation [72]. Moreover, dysregulation of IL-7/IL-7R-mediated

immune responses leads to development of chronic intestinal inflammation in mice [99]. Targeting IL-7/IL-7R pathway with the use of anti-IL-7R mAb and subsequent elimination of IL-7R+ cells was shown to abrogate murine chronic colitis [100]. In addition, IL-7 cooperates with IL-12 and IL-15 in expanding the IFN-γ related mucosal immune responses [101]. These findings clearly demonstrate an important role of IL-7 in regulation of the intensity of intestinal inflammation.

Altogether, the immunomodulatory features of IL-7 described above provide a strong rationale for the use of this cytokine in further studies exploring the new strategies for more effective vaccinations.

The three cytokines described above are some of the most promising naturally occurring adjuvants to use for mucosal vaccines. The toxic effects often seen with systemic administration appear not to be an issue when they are applied to mucosal surfaces. Yet, the effects are as strong and promising. The number of published studies utilizing these cytokines as mucosal adjuvants is still very limited and the final evaluation of their usefulness would happen as more data accumulates.

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Table 1

Main producers and Effects of IL-12, IL-15 and IL-7

Cytokine	Produced by	Effects
IL-12	Macrophages Monocytes Dendritic cells B lymphocytes T lymphocytes	-increased secretion of IFN-γ and TNF-α by NK and Th cells -induction of Th1 from Th0 and generation of CTLs -increased secretion of IL-2 -elevated serum IgG and mucosal IgA
IL-15	Macrophages Dendritic cells Keratinocytes Epithelial cells	-induces T cell proliferation -protects from apoptosis -promotes CTL memory -stimulates proliferation of intestinal epithelial cells -chemotactic for T lymphocytes -induces differentiation of sIgM1, IgA2 and sIgM2sIgA1 B cells into IgA-producing cells in mucosa
IL-7	Non-hematopoetic stromal cells of bone marrow, thymus, lymphoid organs, mucosal lymphoid tissues, skin and liver	-development, proliferation and survival of memory CD4+ and CD8+ T cells -key-regulator of transition of effector T cells to memory T cells -long term survival of CD8 memory T cells