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Interleukin-12 as an Adjuvant for Induction of Protective Antibody Responses

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Abstract

Interleukin (IL)-12 is a pivotal cytokine that strongly stimulates Th1-associated cellular immunity. It is now recognized that IL-12 also activates humoral immunity to both T-dependent and T-independent antigens. This has to considerable interest in exploiting IL-12 as a vaccine adjuvant for protection against various bacterial and viral pathogens, particularly in the lung. Studies examining the efficacy of IL-12-mediated effects on protective antibody response are summarized in this review.

Keywords

interleukin-12; mucosal immunity; vaccine; bacterial infections; viral infections; antibody responses

Large numbers of microbes and microparticles enter the airways with every breath and the respiratory tract thus represents a major portal of entry for various viral and bacterial pathogens. The respiratory tract represents about 25% of the total 400 m² of mucosal tissue in the adult human; however, little is generally known about immune function in the airways and much of our current understanding of mucosal immunology is actually based upon information obtained from studies of the gastrointestinal system, despite the fact that many significant differences exist between lymphoid tissues in these two anatomic sites. Organized nasal-associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT) are present in the upper respiratory tract and the importance of these tissues in protection against infectious disease is well-accepted. However, the lung contains little, if any, organized mucosal tissue and the dominant population involved in innate defense in this organ appears to include alveolar macrophages rather than lymphocytes. In addition, M cells are sparse in the normal lung [1, 1]. Pulmonary lymphoid cells are generally low in numbers and not organized, although foci of inflammation can occur during asthma and in some cases, organized lymphoid areas have been reported to develop in the lungs of both humans and mice during inflammation [2–5] and viral infection [6].

Despite our general lack of knowledge regarding pulmonary immunity, there is continued interest in the effective induction of mucosal immunity for prevention of respiratory infections [7,8]. Intranasal vaccination is an attractive, noninvasive procedure that induces both mucosal and systemic immunity but is typically only successful for inactivated or protein/polysaccharide subunit vaccines when the antigen is co-administered with adjuvants. Cholera toxin (CT) has been found to be a potent mucosal vaccine adjuvant in animals [9,10].

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Unfortunately, CT is highly toxic to humans and variant molecules that have been engineered to cause reduced toxicity have still been found to induce significant neurological sequelae. Furthermore, CT induces primarily Th2 responses [11] that can exacerbate lung pathology rather than enhance protection [12]. Furthermore, CT suppresses IL-12 activity [13]. Although CT and other toxin-derived adjuvants have been found to be useful in mice, the reason for their effectiveness is largely unknown and this lack of knowledge concerning the requirements for a protective mucosal vaccine adjuvant has significantly hampered attempts to develop such adjuvants for use in humans.

This review summarizes work in animal and *in vitro* human models using interleukin-12 (IL-12) as a mucosal adjuvant for humoral immunity. IL-12 is a heterodimeric cytokine that has a m.w. of 75 kDa and is composed of disulfide-bonded 40 kDa and 35 kDa subunits. It is part of a larger family of cytokines that also comprises IL-23 and IL-27, is produced by a variety of antigen presenting cells including macrophages and dendritic cells, and was originally found to bind to receptors on activated T cells and NK cells. The ability of IL-12 to induce protective immunity has been thought to be due to its interrelationships with other cytokines and lymphoid cells. It has been shown to 1) enhance proliferation of T cells and NK cells, 2) increase cytolytic activities of T cells, NK cells, and macrophages, 3) activate T helper 1 (Th1) cells, and 4) induce production of IFN- γ and other cytokines [see reviews [14,15]].

IL-12 as an Adjuvant for Systemic Humoral Immunity against T-dependent (TD) and T-independent (TI) Antigens

Due to its type 1-stimulating properties, IL-12 is extremely potent in enhancing cell-mediated immunity to foreign pathogens. Thus, it might seem counter-intuitive to use IL-12 for enhancement of antibody production. Nevertheless, we and others have described a strong influence of IL-12 on T-dependent (TD) antibody responses, including increases in levels of both murine IgG2a and IgG3 isotypes [16–21]. Findings indicate that the effects of IL-12 on B cells occur through two steps: IL-12 initially stimulates Th1 and NK cells to secrete large amounts of IFN- γ which then causes B cells to switch to γ 2a and γ 3. IL-12 may further stimulate post-switched cells to produce increased amounts of antibody in an IFN- γ -independent manner (Fig. 1). Evidence for both processes has been obtained by *in vitro* analysis of Ig germline transcript formation, which correlates with isotype switching, and by *in vivo* immunization of IFN- γ knockout mice (hereafter referred to as GKO mice) [22]. The post-switch signal may be mediated by an intermediary cytokine other than IFN- γ or by direct stimulation of B cells. It has been shown that activated murine and human B cells bind IL-12 and express transcripts for both β 1 and β 2 chains of the IL-12 receptor [23–26]. Furthermore, it has been found that direct interaction of B cells with IL-12 leads to activation of the p50 and c-Rel NF- κ B family members [25], B cell proliferation and differentiation [27], IFN- γ production [25,28,29] and production of the type 2 cytokine, IL-4 [30]. Functional heterodimeric IL-12R in humans is absent on pre/pro-B cells, becomes expressed through all stages of peripheral B-cell differentiation, and is then upregulated at the plasmablast/plasma cell stage [31]. Interestingly, the IL-12R is silenced in chronic B-cell malignancies as a result of methylation of the CpG island [32].

The ability of IL-12 to enhance T-independent antibody responses has also been examined. The reasoning is that IL-12 may be capable of influencing humoral immunity in the absence of T cells either through activation of NK cells and subsequent secretion of IFN- γ , or by direct stimulation of specific B cells through binding to the B cell IL-12R. In fact, it has been clearly demonstrated that NK cells can activate B cells to produce IgG2a antibodies to TI antigens [33,34]. To determine the feasibility of this approach, adult mice were immunized in our laboratory with the model TI-2 antigen, dinitrophenyl-ficoll, and to vaccines composed of purified polysaccharides from pneumococci and meningococci.

It was found that IL-12 treatment at the time of immunization induced significantly elevated levels of IgG2a and IgG3 antibodies to all of the tested TI antigens [35]. The enhancement of IgG2a and IgG3 expression was accompanied by large increases in splenic IFN- γ but not IFN- α levels, both cytokines that are known to induce γ 2a switching [36–38]. Furthermore, in keeping with our previous results using TD antigens [18], there was no inhibition and in fact, even an enhancing effect of IL-12 on total antibody levels, including the Th2-associated IgG1 isotype. To determine the cell types and cytokines responsible for the enhancement, immunodeficient mice containing defined genetic disruptions were used. Mice lacking T cells (β - δ ⁻ double KO mice) or T and NK cells (CD3 ϵ transgenic mice) still showed enhancement of antibody responses to these antigens, demonstrating that such cells were not necessary for the observed IL-12 effects. Furthermore, the use of IFN- γ ^{-/-} mice showed that stimulation of TI antibody responses by IL-12 was only partially dependent on IFN- γ .

The above results demonstrate that IL-12 enhances TI antibody responses and suggest that this cytokine would be useful as a protective adjuvant for vaccination against polysaccharide-expressing encapsulated bacteria. Indeed, the ability of IL-12 to enhance anti-polysaccharide antibody responses was also tested using pneumococcal and meningococcal conjugate vaccines, which convert TI responses against these polysaccharides to TD responses. Examination of serum anti-meningococcal group C polysaccharide antibody levels 14 days after immunization with conjugate vaccine (polysaccharide conjugated to mutant diphtheria toxin CRM₁₉₇) showed that IL-12 treatment at the time of vaccination enhanced levels of total, IgG2a and IgG3 antibodies [39]. IL-12 had no effect on IgM or IgG1 antibody levels. Similar results were obtained in both BALB/c and C57Bl/6 mice. Evaluation of the effect of IL-12 on the CRM₁₉₇ carrier protein revealed that IL-12 treatment increased IgG2a anti-CRM₁₉₇ antibody levels but had no effect on total levels of antibody to CRM₁₉₇. Similar studies were performed using a pneumococcal conjugate vaccine consisting of pneumococcal capsular polysaccharide serotype 3 (PPS3) conjugated to CRM₁₉₇. It was found that IL-12 treatment enhanced production of IgG2a anti-PPS3 antibody, somewhat suppressed IgG1 expression and had no effect on levels of any other antibody isotype [40].

We next tested whether passive transfer of antisera from mice that had been vaccinated with conjugate vaccine in the presence or absence of IL-12 would confer increased protection from bacterial infection. The advantage of the passive transfer model is that it directly assesses the protective efficacy of antibodies induced by vaccination. Briefly, 2.3×10^4 cfu of *S. pneumoniae* and a 1:10 dilution of immune serum were injected intraperitoneally into naïve BALB/c mice which were then monitored for survival. Bacteremia levels were also measured by plating serial dilutions of blood and spleen homogenates on blood agar plates. The identity of the pneumococci in the blood of septic and moribund mice was confirmed by testing for sensitivity to optochin. It was found that mice receiving immune serum from animals vaccinated with conjugate vaccine in the absence of IL-12 lived approximately 24 hr longer than those which received serum from mice vaccinated with CRM₁₉₇ alone [39]. However, overall survival in this group was only 12.5% compared to 87.5% in the group that received immune serum from animals treated with vaccine plus IL-12. Furthermore, 48 hr after infection, there was a major difference in the degree of bacteremia between the two groups – the group receiving immune serum from mice treated with vaccine and PBS vehicle had high levels of bacteria in the spleen whereas all but one mouse given immune serum from IL-12-treated animals showed absolutely no evidence of bacteremia and appeared to be perfectly healthy. Thus, the use of IL-12 as a vaccine adjuvant can dramatically increase survival rates after systemic infection with pneumococci.

IL-12 as a Mucosal Adjuvant for Induction of Protective Humoral Immunity against Invasive Disease

IL-12 has also been shown to be very effective as a vaccine adjuvant for enhancement of protective antibody responses against pulmonary bacterial and viral infections, and is particularly effective when administered locally to mucosal surfaces, *i.e.*, intranasally (i.n.), together with vaccine. We and others have repeatedly found that i.n. treatment of mice with IL-12 leads to increases in expression of both Th1 and Th2 antibodies not only in the serum and but particularly, in the lung. In a typical experiment, mice are inoculated i.n. with vaccine only or vaccine + IL-12. Bronchoalveolar lavage (BAL) fluids and sera are then collected on Day 35 and titers are determined by isotype-specific ELISAs. It was found that inclusion of IL-12 during vaccination induces significant levels of both IgG and IgA antibodies that are not expressed after exposure to vaccine alone. Protection against lethal viral and bacterial infections include those induced with influenza virus [41], *Streptococcus pneumoniae* [40, 42,43], *Francisella tularensis* [44], and *Yersinia pestis* (D. Kumar and D.W. Metzger, unpublished results).

In experiments with lethal influenza virus infection [41], it was found that all mice given only PBS 35 days before viral challenge succumbed to influenza infection by Day 9 and that i.n. immunization with only influenza subunit vaccine consisting of purified hemagglutinin (H1) and neuraminidase (N1) provided only partial protection. Combining vaccine with i.n. IL-12 inoculation, however, enhanced levels of both lung and spleen IFN- γ and IL-10, as well as serum and BAL IgG2a anti-H1N1 antibody. In addition, it enhanced expression of secretory IgA anti-H1N1 antibody in BAL fluid. Importantly, mice immunized i.n. with H1N1 and IL-12 exhibited decreased weight loss and dramatically increased survival rates after lethal challenge with live influenza virus compared to animals inoculated with vaccine in vehicle only. Protection was dependent on B cells as demonstrated using μ MT KO mice, and could be transferred to naïve mice by inoculation of either serum or BAL fluid from IL-12-treated mice. IL-12 administration alone had no effect on survival.

As discussed above, the major effect of IL-12 on humoral immunity is induction of IgG2a, which happens to be the murine isotype that is most effective at mediating complement fixation and opsonization through high affinity binding to Fc γ RI on phagocytic cells. To examine the precise role of Fc γ R during IL-12-mediated protection, the influenza virus infection model was again used in BALB/c Fc γ chain KO mice. These mice have a genetic disruption in expression of the common γ chain that is shared by Fc γ RI and III, as well as by Fc ϵ RI and in humans, Fc α R, which remains undefined in mice. WT and Fc γ chain KO mice were immunized with influenza H1N1 subunit vaccine in the presence or absence of IL-12. The mice were then challenged i.n. 5 weeks later with 2×10^3 PFU of A/PR/8/34 influenza virus and monitored daily for mortality. It was found that WT and KO mice behaved identically with regard to cytokine and antibody production, yet the KO mice were significantly more susceptible to lethal influenza infection [45]. The results indicated for the first time that control of influenza infection by IgG2a occurs through Fc γ R-mediated clearance rather than simply antibody neutralization.

Similar effects were seen for pneumococcal lung infection [42]. BALB/c mice were inoculated i.n. with 1 μ g of the pneumococcal surface protein A (PspA) and IL-12. One month later, the IL-12-treated mice were found to have enhanced levels of total and IgG2a anti-PspA antibodies in both serum and BAL. Upon transfer of sera to naïve recipients and challenge with a lethal dose of *S. pneumoniae*, all mice that had received normal mouse serum succumbed to infection within 5 days while mice that had received serum from animals vaccinated with PspA alone showed no significantly increased protection. Strikingly, every mouse that had received serum from animals treated with both PspA and IL-12 survived the infection. In addition, i.n.

vaccination with PspA and IL-12 provided increased protection against nasopharyngeal carriage. Protection was due to passively transferred antibody since it was lost by pre-adsorption of the sera before transfer with anti-Ig-coated beads.

More recent experiments have focused on the use of IL-12 as a mucosal vaccine adjuvant for protection against biothreat agents that could be used for biowarfare and bioterrorism. *F. tularensis* is a gram-negative facultative intracellular bacterium that is ingested by and multiplies within macrophages, and that causes the pulmonary form of tularemia in humans [46]. Because of its extreme infectivity, ease of dissemination, and substantial capacity to cause illness and death, the Working Group on Civilian Biodefense considers *Ft* to be a dangerous potential biological weapon and has classified it as a Category A Select Agent. Vaccination with the live vaccine strain (LVS) of *F. tularensis* can partially engender protection against lethal respiratory tularemia, but use of inactivated or subunit forms of the bacterium has been found to be only poorly effective. A probable reason for the lack of efficacy observed with inactivated LVS is the fact that *F. tularensis* is an intracellular pathogen. Inactivated organisms and soluble proteins typically induce Th2-like immunity, while live, attenuated organisms are more effective at inducing cell-mediated Th1-like immunity that is believed to be required for protection against intracellular pathogens. Thus, we hypothesized that establishment of a lung Th1 environment during vaccination with inactivated LVS would induce protection against challenge with fatal doses of LVS. Indeed, it was found that i.n. vaccination with inactivated LVS in the presence of IL-12 resulted in 90–100% survival after lethal challenge while mice receiving only vaccine or only IL-12 all succumbed to infection [44]. Survival was correlated with reductions in bacterial burden, inflammation, and production of immunomodulatory cytokines (IFN- γ , TNF- α , and IL-6) in the lung as well as in the liver and spleen. While NK cells were primarily responsible for the production IFN- γ in the lungs of unvaccinated animals, vaccinated mice showed increased levels of lung IFN- γ^+ CD4 T cells. These results demonstrated that i.n. vaccination with inactivated *F. tularensis* LVS combined with IL-12 can result in protection from respiratory tularemia.

The role of antibodies in protection against respiratory infection with the intracellular bacterium *F. tularensis* is not clear. To investigate the ability of antibodies to clear bacteria from the lungs and prevent systemic spread, studies were conducted similar to those described above for pneumococcal infection. Immune serum was passively administered i.p. to naïve mice before i.n. *F. tularensis* LVS infection. It was found that immune serum treatment provided 100% protection against lethal challenge while normal serum or Ig-depleted immune serum provided no protection [47]. Protective efficacy was correlated with increased clearance of bacteria from the lung and required expression of Fc γ R on phagocytes, including macrophages and neutrophils. However, complement was not required for protection. *In vitro* experiments demonstrated that macrophages were more readily infected by antibody-opsonized bacteria but became highly efficient in killing upon activation by IFN- γ . Consistent with this finding, *in vivo* antibody-mediated protection was found to be dependent upon IFN- γ . SCID mice that lack T cells but contain normal or even elevated numbers of NK cells were not protected by passive antibody transfer, suggesting that T cells but not NK cells serve as the primary source for IFN- γ . These data indicate that a critical interaction of humoral and cellular immune responses is necessary to provide sterilizing immunity against *F. tularensis*. Of considerable interest was the finding that serum antibodies were capable of conferring protection against lethal respiratory tularemia when given 24 to 48 hr post-exposure. Thus, this study provided the first experimental evidence for the therapeutic use of antibodies in *Francisella*-infected individuals.

Similar studies are now being performed using inactivated *Y. pestis* for i.n. vaccination in the presence of IL-12. Curiously, it is believed that killed *Y. pestis* is incapable of providing protection against the pneumonic form of plague initiated by the respiratory route of infection

even though several groups are actively attempting to identify subunit targets for protection of mucosal surfaces against the pathogen [48]. In our hands, inactivated *Y. pestis* inoculated i.n. together with IL-12 can provide complete protection against pulmonary challenge with the highly virulent CO92 *Y. pestis* strain.

3. IL-12 as a Neonatal Mucosal Adjuvant

Neonatal animals show generally poor responsiveness to foreign antigens, especially to encapsulated microbes that bear TI polysaccharide antigens. Furthermore, neonates are known to display polarized expression of type 2-like cytokines and antibody responses. We have shown that newborn mice have greatly reduced expression of IL-12 in the periphery [49], which could explain the overwhelming Th2 bias on neonates. Attempts to overcome this Th2 bias and overcome poor responsiveness to vaccination were performed by immunization and treatment with IL-12 within 24 hr of birth. This tactic resulted in elevated levels of IFN- γ and IL-10 mRNA in the spleens of mice compared to animals exposed to antigen only. Moreover, such animals showed significant enhancement of IgG2a and IgG2b antibody levels upon adult challenge compared to animals primed with antigen alone. IgG1 levels, a measure of type 2 activity, were unaffected or even somewhat enhanced by neonatal IL-12 treatment. Furthermore, animals vaccinated and simultaneously treated with IL-12 at birth displayed enhanced survival after lethal challenge with infectious influenza virus as adults compared to infected animals that had been primed with vaccine alone. This augmented protection required B cells and could be transferred to naive mice by immune serum. Collectively, the results provide evidence that IL-12 administration at birth induces a type I-like cytokine response and causes priming for heightened protective memory antibody responses.

Otitis media (OM) induced by *S. pneumoniae* is the leading bacterial cause of acute OM in young children and often produces invasive disease. Typical intramuscular routes of vaccination are poorly protective against development of OM. To examine the effectiveness of i.n. vaccination in the presence of IL-12 to prevent neonatal OM, 1 week-old mice were i.n. inoculated with pneumococcal polysaccharide conjugate vaccine and IL-12 as a mucosal adjuvant. The protective efficacy of this treatment was tested by challenging immunized infant (3 week-old) mice with bacteria to induce OM and invasive disease. The vaccination procedure was found to enhance levels of specific antibodies, mostly IgA antibodies, in middle ear (ME) washes and sera from wild-type mice (but not IFN- $\gamma^{-/-}$ mice). Immunization in the presence of IL-12 also resulted in enhanced clearance of *S. pneumoniae* from the ME. Furthermore, opsonization of bacteria with ME wash fluids or sera from the immunized mice mediated increased bacterial clearance from the ME of naïve mice. Finally, immunized mice demonstrated 89% survival after OM-induced invasive pneumococcal infection, compared to 22% survival in unvaccinated mice. These results indicate that i.n. vaccination of neonatal mice in the presence of IL-12 is able to enhance IFN- γ dependent ME mucosal and systemic immune responses to pneumococci and efficiently protect against both OM and invasive infection [50].

4. Lack of Toxicity with IL-12 Delivered i.n

In human trials, IL-12 was found to be highly toxic when administered parenterally, daily over several days [51]. This toxicity was felt to severely limit its usefulness for *in vivo* therapy of humans. We thus conducted a detailed study to compare toxicity of IL-12 administered directly to a mucosal site versus subcutaneously (s.c.) to mimic the human therapeutic strategy. Groups of mice were given various amounts of IL-12 in vehicle i.n. or s.c. for six (6) consecutive days and survival was monitored. It was found that s.c. injection of IL-12 was uniformly lethal at doses of 0.5 and 1.0 $\mu\text{g/day}$ [52]. This toxicity correlated with increased IFN- γ expression, decreased serum glucose levels, and altered histological responses in the duodenum. On the

other hand, inoculation of 0.5 μg of IL-12 i.n. had no effect on survival and 1.0 μg still led to 50% survival. Further experiments revealed that s.c. IL-12 had an LD_{50} of between 0.125 - 0.25 $\mu\text{g}/\text{day}$, compared to i.n. IL-12 which had a 4–8-fold higher LD_{50} (1 $\mu\text{g}/\text{day}$). Furthermore, when delivered i.n., IL-12 induced less systemic IFN- γ and fewer pathological changes, yet was efficacious, as indicated by enhanced levels of Th1-associated immunoglobulins (*i.e.*, IgG2a). Others have similarly seen lack of i.n. IL-12 toxicity, even after administration of very high doses of IL-12, and have also observed no neurological inflammation, which is a significant problem with the use of CT as an adjuvant (P. Boyaka, personal comm.). Thus, it appears that introduction of IL-12 i.n. is a very effective and safe route of inoculation. Nevertheless, when combined with vaccination, it may allow sufficient local inflammation to provide a means for IgG transudation across mucosal barriers (see below).

5. Role of IgA in IL-12 induced Protection

To investigate the role of IgA in IL-12-mediated protection, we examined the efficacy of i.n. vaccination in mice lacking mucosal IgA. The importance of IgA for protection at mucosal surfaces remains unclear and in fact, it has been reported that IgA deficient mice have fully functional vaccine-induced immunity against several bacterial and viral pathogens. The role of respiratory antibody in preventing colonization by *S. pneumoniae* was examined using pIgR^{-/-} mice, which lack the ability to actively secrete IgA into the mucosal lumen. I.n. vaccination with the Prevnar polysaccharide conjugate vaccine elicited serotype-specific anti-capsular polysaccharide antibody locally and systemically in wild type mice. However, pIgR^{-/-} mice had approximately 5-fold more systemic IgA and 6-fold less nasal IgA antibody than wild-type mice due to defective transport into mucosal tissues. Wild-type but not pIgR^{-/-} mice were protected against infection with serotype 14 *S. pneumoniae*, which causes mucosal colonization but does not induce systemic inflammatory responses in mice. The relative importance of secretory IgA (SIgA) in host defense was further shown by the finding that i.n. vaccinated IgA^{-/-} mice were not protected from colonization. Although SIgA was found to be important for protection against nasal carriage, it does not appear to have a crucial role in immunity to systemic pneumococcus infection, because both vaccinated wild-type and pIgR^{-/-} mice were fully protected from lethal systemic infection by serotype 3 pneumococci. The results indicate that parenteral vaccination to induce serum antibodies is not sufficient to protect against nasal carriage and that mucosal-derived IgA antibody is necessary to clear this noninflammatory condition in which there is likely to be no leakage of serum antibody into the upper respiratory tract. However, serum antibodies are sufficient to provide protection against inflammatory, invasive disease [43].

As mentioned above, different laboratories have obtained disparate results and made contrasting conclusions regarding the importance of IgA in protection of the respiratory tract from infectious agents. After reviewing the role of IgA in respiratory immunity and surveying various results in the field, it becomes clear that the experimental model used and the amount of inflammation induced by a particular infection can influence the apparent need for protective IgA antibody [53]. During infections that induce little inflammation, such as pneumococcal nasal carriage discussed above, IgA has been found to be critical for protection, but in other cases in which inflammation exists that, in turn, allows leakage of serum antibody into the mucosal site of infection, IgA has been found to be dispensable and IgG is fully protective. This pattern holds true for both viral and bacterial mucosal infection models. It thus appears that IgA antibody provides an important first line of defense against infections of the respiratory tract. If these infections progress to a point such that significant inflammation occurs, especially in the lung, epithelial and endothelial barriers will become compromised, transudation of serum IgG antibody will occur and this IgG will provide a second line of defense to prevent systemic, lethal spread of the infection.

6. Summary

Extensive studies over the past 10 years have established the ability of IL-12 to enhance antibody responses to foreign antigens including protein and polysaccharide subunit vaccines, and to markedly increase protective immunity to respiratory pathogens such as pneumococci, influenza virus, and other biothreats. In addition, it has been shown that IL-12 can serve as a potent adjuvant for protective neonatal immunity. The findings provide a solid foundation to continue to investigate the efficacy of IL-12 as a vaccine adjuvant and to examine the mechanisms responsible for the induced protection against common microbial pathogens in mucosal tissues.

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Figure 1. A model for the actions of IL-12 on humoral immunity

IL-12 induces Th1 and NK cells to secrete large amounts of IFN- γ which then causes B cells to switch to $\gamma 2a$ production. Secondly, IL-12 binds to receptors expressed on plasmablasts and enhances production of the switched isotypes.