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Desired immune response characteristics in an RSV vaccine: What infants tell us

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Respiratory syncytial virus (RSV) represents an important target for vaccine development. The design of an effective vaccine for infants is hindered by a lack of knowledge of the nature of the protective immune response to RSV infection in this age group. To understand immune mechanisms in the infant lung, we examined respiratory samples from infants 0-6 months with severe RSV LRI, and compared these to uninfected infants. At the peak of RSV infection (but not in controls), activated macrophages (CD14+, CD16+, CD68+) and B lymphocytes (CD20+, IgA+, IgM+, IgG+) are prominent while dendritic cells (MHCII+), T lymphocytes and T cell associated cytokines (Welliver et al, 2007 J. Infect. Dis. 195:1126) are not. In nasopharyngeal secretions, polyreactive IgG, IgM and IgA recognizing RSV virions and apoptotic debris were readily detected. The presence of polyspecific local antibodies strongly correlated with mucosal expression of BAFF, a T-independent, B-cell activating cytokine, which localized in lung tissue to RSV-infected and apoptotic respiratory epithelium. Polyspecific local antibodies were associated with better oxygenation at presentation. In vitro, products of RSV infected epithelium, such as BAFF, TLR7 ligand, and type I IFN synergistically elicited Ig and cytokine production from cultured B lymphocytes, while virions alone had no such effect. Our data suggest that in infants, innate immune factors released from RSV infected epithelium elicit T independent, polyspecific B cell responses which have modest protective effects. Perhaps the lack of antigen presentation via dendritic cells in infant respiratory tissue impedes the development of T-dependent responses and contributes to severe disease susceptibility in this age group. RSV vaccine strategies tailored for infants should recruit and mature lung-associated macrophages and dendritic cells, in order to deliver antigen to elicit long-lasting, T-cell dependent immunity. Alternatively, an RSV vaccine that promotes local innate B lymphocyte responses might elicit short-term protection, bridging the gap between maternal antibody protection and the development of mature immune responses found in older children.

Keywords: respiratory syncytial virus; innate immunity; BAFF; mucosal antibody

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1. Main text

Respiratory syncytial virus (RSV) is the most important cause of severe lower respiratory infection (LRI) in infants. Virtually everyone is exposed to RSV within the first two years of life, resulting in approximately 100,000 infant hospitalizations and 400 infant deaths in the US each year, and one million deaths worldwide annually. There is currently no vaccination strategy to lessen the public health burden associated with RSV LRI of infancy.

B lymphocyte responses to RSV are thought to contribute to protection and immunopathology associated with primary infection. RSV-directed serum antibodies are detected at low levels in most infants one month after exposure, and persist for months post infection. Antiviral antibodies are also found in nasopharyngeal secretions of patients with acute infection, as early as one week post infection. The relationship between antiviral antibody responses and protection is unclear. Antiviral antibody titers are low in the youngest infants, possibly predisposing for more severe disease in this age group. High titer maternal antibodies are associated with protection against acute infection, and boosted antibody levels with re-exposure coincide with milder disease in older children. However re-infections with RSV are common, even in adults who have high anti-RSV antibody levels, suggesting that antiviral antibody is not completely protective. In rodent models of RSV infection, T helper type 2 (Th2) lymphocyte responses, which favor antibody production over cytotoxic responses, were associated with RSV persistence and immunopathogenesis. Clinical experience with a formalin-inactivated RSV (FI-RSV) vaccine also unfortunately demonstrated eosinophil and lymphocyte activation in blood and lung tissue after infection with wild type RSV, consistent with a Th2 skewed response. Antibodies elicited with FI-RSV vaccine were not protective and possibly contributed to immunopathogenesis when recipients were exposed to natural infection.

Recently we had the opportunity to examine secretions from a cohort of infants with upper versus lower respiratory RSV infection (Buffalo, NY), in addition to lung tissue obtained from infants with acutely fatal RSV LRI (Santiago, Chile). Although our goal was to confirm T lymphocyte contributions to severe disease, we instead observed that both CD4 and CD8 lymphocytes and their associated cytokines were nearly absent in RSV LRI. Our observations suggested that an inadequate adaptive immune response, rather than an exaggerated one, underlies severe RSV LRI in humans. However, weak T helper lymphocyte responses seemed at odds with reports of prominent mucosal antiviral antibody detection within days of initial RSV exposure. For this reason we extended our studies to characterize B lymphocyte responses during primary RSV disease in infants.

During primary RSV LRI, antiviral antibodies have been reported in the respiratory tract, but the source of these antibodies is not clear. Mucosal antibodies may be transported from serum into lung through transudation and/or active transport. Alternatively antiviral antibodies may be locally produced by B lymphocytes in lung tissue. To help determine the source of antibody in RSV exposed infants, we identified B cells by immunohistochemistry (IHC) in a panel of lung tissues obtained from nine infants with acutely fatal RSV LRI (Santiago, Chile). CD20+ B lymphocytes were abundant in lung tissue of infants with RSV LRI, and localized to alveolar and perivascular spaces, where RSV antigen was also prominently detected. In contrast, CD20+ cells were nearly absent in control lung tissues obtained from infants who died of asphyxia. CD4+ cells were rarely identified in either RSV LRI or control lung tissues, as previously observed. In RSV LRI, the lung associated B lymphocyte population included plasma cells, based on strong cytoplasmic detection of IgA, IgG and IgM, while almost no IgA, IgG, or IgM was detected in age-matched control lung tissue.

Because a vigorous B lymphocyte response was apparent in lung tissue of infants with acute RSV LRI, while T lymphocytes were rare, we considered the possibility of T independent B lymphocyte antibody production. We therefore examined lung tissue for expression of B cell stimulating factors by IHC. Strong expression of CD40-L related factors BAFF and APRIL was observed in bronchiolar epithelium of RSV exposed infants, but not lung tissue of uninfected infants. Both BAFF and APRIL have been implicated in CD40 independent Ig production and class switching. Similarly, vasoactive intestinal peptide (VIP), which has also been implicated in T-independent B lymphocyte activation, was detected in lung epithelium of infants with RSV LRI and not in control lung tissue. Epithelial detection of VIP, BAFF and APRIL coincided with RSV antigen along and type I interferon induced proteins OAS-1 and and MxA. Together these data suggest that RSV infected respiratory epithelium is a potentially important source of B cell directed factors.

To further evaluate primary antiviral antibody responses, nasopharyngeal secretions (NPS) from 33 surviving infants with acute LRI (22 RSV, 10 influenza, 1 adenovirus) were collected for analysis. As has been previously reported, IgA, IgG and IgM antibodies reactive with RSV antigens were robustly detected in secretions of most

infants with LRI at the time of presentation, and were significantly correlated with total Ig. Infants presenting with RSV LRI had significantly greater RSV directed IgM and RSV directed IgA compared with infants with non-RSV LRI. However, RSV-directed Ig was also detected in influenza and adenovirus LRI cases. Because virtually all infants have been exposed to RSV prior to age 2, the observation of anti-RSV antibodies in infants presenting with influenza or adenovirus infection could reflect previous RSV experience. In fact we did identify one infant presenting with influenza LRI who had received outpatient care for RSV LRI one week previously. However, another possibility is that respiratory antibodies are poorly diversified and polyspecific. Respiratory infection is associated with the accumulation of apoptotic epithelial cell debris and the release of replicative viral intermediates. We hypothesized that such debris, laden with pathogen associated molecular patterns (PAMPs), presents shared epitopes associated with highly conserved intracellular structures such as nucleic acids, nucleosomes, and virosomes. To address the question of polyspecific antibody production, we tested the nasopharyngeal aspirates for reactivity with a preparation of human nuclear and nucleosomal antigens, derived from apoptotic U937 cells. Ig reactive with nuclear and nucleosomal antigens of apoptotic cells was present in NPS, with IgA detection greater than IgG or IgM. However no reactivity was observed against BSA or a lysate prepared from uninfected Hep-2 cells, the cell line in which the RSV antigens were prepared. Reactivity with apoptotic epitopes was highest in infants with RSV LRI, compared with influenza or adenovirus LRI. Thus the recovery of Ig reactive with apoptotic cell epitopes appeared to be linked to antiviral Ig recovery, raising the possibility that factors present in infected lung tissue might be responsible for promoting both.

We next attempted to clarify a role for T-dependent versus T-independent processes in the generation of total, RSV-directed, or anti-nuclear/nucleosomal antibodies recovered from infant NPS. T lymphocyte associated cytokines including IL-2, IL-4, and IL-10 have been implicated in CD40-dependent immunoglobulin secretion and class switch to the IgA isotype. Low levels of IL-2 (mean 33.7±7.2 pg/ml), IL-4 (mean 59.4±11.3 pg/ml), and IL-10 (mean 50.5±9.3 pg/ml) were detected in most secretions analyzed. IL-4 positively correlated with RSV-specific and anti-nuclear/nucleosomal IgA, but no other immunoglobulin measures. IL-2 also tracked with higher RSV-specific and anti-nuclear/nucleosomal IgA although these relationships failed to reach statistical significance. On the other hand, IL-10 predicted higher levels of total immunoglobulin recovery. IL-10 was associated with higher RSV IgG, but did not correlate with RSV specific IgM or IgA, or with anti-nuclear/nucleosomal antibody levels.

In contrast with the low levels of T lymphocyte dependent cytokines observed in NPS, we recovered higher quantities of VIP (5201.8±1559.4 pg/ml), BAFF (108.0 ±20.9 pg/ml) and APRIL (1518±635.4 pg/ml) in most NPS samples. The strongest predictors of mucosal Ig were VIP and BAFF, both of which also correlated with IL-10 recovery. APRIL was strongly correlated with RSV-directed and anti-nuclear/nucleosomal IgA, and also predicted RSV-directed IgM. Surprisingly, APRIL alone positively correlated with better oxygen saturation at the time of presentation, suggesting a protective role. RSV directed IgM and IgA were associated with better oxygen saturation; however RSV IgG was not. We were not able to detect IFN-α or IFN-β consistently by ELISA, but did observe that the interferon-induced protein CXCL10 (IP-10) was among the highest expressed cytokines in NPS of infants with LRI (mean 2053.8±297.5 pg/ml), perhaps consistent with type I IFN induction in lung tissue. IFN-γ recovery was much lower (31.4±6.0 pg/ml) and not correlated with IP-10. Together these data suggest that innate immune factors, produced locally in large quantities, shape the B cell response to primary RSV infection in concert with IL-4.

Both T lymphocyte-dependent and -independent stimuli may influence the mucosal antibody repertoire in infants with RSV LRI. To determine how lung-localized B cells might integrate these disparate signals, we incubated human peripheral blood B cells with mixtures of type I IFN, TLR7 agonist imiquimod, and BAFF, along with anti-IgM, anti-CD40, and IL-4. As has been previously observed, B cells costimulated with type I IFN plus the TLR7 agonist imiquimod secreted IgM, IgG, and IgA, plus IL-6 and IL-10, in a dose-dependent manner. The addition of BAFF further enhanced both Ig secretion and cytokine release in the absence of T lymphocyte derived factors. We found that the combination of TLR7 agonist plus type I IFN could synergize with anti-IgM, either with or without anti-CD40 and IL-4, to augment secretion of Ig, in the absence of T cell help. As has been previously observed with influenza virus, incubation of B lymphocytes with RSV virions, either alone or in combination with anti-IgM, anti-CD40, or IL-4, had minimal impact on immunoglobulin release. These data indicate that innate stimuli associated with RSV infected cells, such as type I IFN, BAFF and TLR7 ligand, may synergize to elicit robust B cell responses even in the absence of T cell help.

T lymphocyte dependent antibody production is inefficient in infants. Neonatal T lymphocytes exhibit deficits including low expression of TCR, adhesion molecules, and CD40L which result in blunted T cell help for B lymphocytes. Nevertheless, an antiviral antibody response in NPS of infants is present early during primary RSV LRI. In this study we observed that B lymphocytes, including plasma cells, are heavily recruited to infant lung at the peak of RSV illness, while CD4+ T lymphocytes were not abundant. We also showed that B cell detection in tissue coincides with local expression of B cell tropic factors including BAFF, APRIL and VIP, localized primarily to infected respiratory epithelium. Similarly, specific and total Ig levels in secretions of surviving infants with RSV LRI coincided with and correlated with BAFF, APRIL and VIP recovery, suggesting a causative relationship. Assuming similar disease pathogenesis in Chilean and US infants, these data implicate T lymphocyte independent processes in shaping the primary antiviral antibody response, in a setting where cognate T cell help is suboptimal.

Previous studies have focused on the DC as the prime regulator of antiviral Ig responses in adult systems, either indirectly through T cell stimulation, or through IFN-dependent release of IL-6 and BAFF. DCs obtained from infants exhibit multiple deficiencies, including limited responses to in vitro stimuli, and low expression of MHC class II and costimulatory molecules. Thus while mature DCs can clearly make enormous contributions to B cell activation and Ig production, it is unclear whether immature DCs in infants can play this role. Most studies which implicate DCs in antibody production measure serum levels of Ig. In fact, in infants younger than six months, serum IgM and IgA responses to RSV are almost undetectable during the first month, while the antibody response in secretions appears within days and is similar qualitatively and quantitatively in infants and older children with acute RSV LRI. This dissociation between mucosal and systemic Ig responses in infants may highlight a differential role for DCs in these two anatomic sites. In the context of inefficient DC function, local epithelium may provide an alternative source of B lymphocyte directed stimuli to maintain mucosal antibody levels. In support of this point, recent in vitro studies show that RSV infection of respiratory epithelial cells results in TLR3 activation, IFN-β release, and IFN-β dependent BAFF and APRIL expression in cultured respiratory epithelium. Our data confirm prominent epithelial expression of VIP, BAFF and APRIL in vivo during RSV LRI. Colocalization of IFN-induced proteins such as MxA and OAS in RSV infected alveolar epithelium, and high levels of CXCL10 in NPS, imply that type I IFN is probably also locally expressed. Together, these data point to RSV infected epithelium as an important source of innate immune factors, particularly during uncontrolled respiratory infection, and suggest that these cytokines may be sufficient to induce T lymphocyte independent antibody production if DC functions are immature.

BAFF and APRIL overexpression have been previously implicated in the generation of self-reactive antibodies in patients with autoimmune disease. Here we report antibodies reactive with apoptotic cell epitopes in NPS during primary RSV LRI. Anti-nuclear/nucleosomal antibodies in NPS were strongly correlated to BAFF and APRIL, suggesting that similar mechanisms of B lymphocyte activation could be present in SLE and infant respiratory infection. In acute RSV LRI, airways are occluded with dense apoptotic cellular debris, arising from massive infection of respiratory epithelium. Accumulation of apoptotic cellular debris is also a cardinal feature of SLE, in which a failure in macrophage clearance of debris is implicated in pathogenesis. The generation of antibodies recognizing cellular debris may be a conserved, protective response. Autoreactive antibodies are proposed to have a beneficial role in removing immune-stimulating components of dying cells, thus avoiding inflammatory cell recruitment and tissue destruction. Supporting this idea, APRIL and anti-nuclear/nucleosomal antibodies of IgA isotype were associated with better oxygen saturation values in this cohort of infants with bronchiolitis, suggesting a role for APRIL and/or APRIL induced IgA in providing some degree of protection in air exchange tissue. IgA is thought to provide anti-inflammatory and tolerizing signals in the mucosa, and may antagonize pro-inflammatory actions of IgG such as DC maturation. The ability to generate local polyspecific IgA may facilitate the clearance of cellular debris without augmenting inflammation, preserving lung tissue and respiratory function.

Together these data provide some insight into the challenges associated with an RSV vaccination approach for infants. In hospitalized infants, the T cell response to RSV is blunted, and mucosal antibody response to RSV appears linked to factors elaborated by infected epithelial cells. Thus, antiviral responses are initiating too late, after virus has already established a foothold in respiratory tissue. Novel adjuvant strategies, in which T cell -independent and -dependent stimuli work in synergy to optimally stimulate lung-associated B lymphocytes, may enhance locally-administered vaccines. Alternatively attenuated live viruses may be utilized to stimulate sentinel macrophages and DCs in neonates, and trigger more prompt T and B lymphocyte collaboration after wild type virus challenge. Engaging the unique contributions of both innate and adaptive antiviral responses may be required for a successful RSV vaccine in neonates.