BRIEF COMMUNICATIONS

A role for *Streptococcus pneumoniae* in virus-associated pneumonia

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Here we show, in a double-blind, randomized, placebo-controlled trial in 37,107 fully immunized infants in Soweto, South Africa, that a 9-valent pneumococcal conjugate vaccine, PncCV, prevents 31% (95% confidence interval = 15–43%) of pneumonias associated with any of seven respiratory viruses in children in hospital. These data suggest that the pneumococcus has a major role in the development of pneumonia associated with these viruses and that viruses contribute to the pathogenesis of bacterial pneumonia.

We previously showed that PncCV reduces invasive pneumococcal disease caused by vaccine serotypes by 72% and radiologically confirmed pneumonia by 17% in a population of both HIV-infected and HIV-uninfected African infants[1]. As the fraction of pneumonia attributable to pneumococcus may be reduced by seasonal respiratory syncytial virus (RSV) or influenza epidemics[2], we sought evidence of viral infection in these infants.

Ecological studies have shown that temporal associations have occurred between peaks of influenza and peaks of bacterial pneumonia, for example, in 1918 and 1957 (ref. 3); however, no randomized study has examined the hypothesis that bacteria and viruses are important copathogens in the etiology of pneumonia. Although there are no sensitive techniques available to diagnose pneumococcal pneumonia, the demonstration that, at least in children without HIV infection, PncCV prevents 85–97% of invasive disease caused by vaccine serotypes[1,4] provides a sensitive probe that can be used to explore the role of a bacterium (the pneumococcus) in the etiology of viral pneumonia.

Table 1 Percentage efficacy of pneumococcal conjugate vaccine by per protocol analysis in fully immunized infants

Clinical diagnosis	Vaccine $n = 18,245$	All children[e] Placebo $n = 18,268$	Efficacy (95% CI)	P value	Vaccine $n = 17,065$	HIV-uninfected children[f] Placebo $n = 17,086$	Efficacy (95% CI)	P value	Vaccine $n = 1,180$	HIV-infected children[f] Placebo $n = 1,182$	Efficacy (95% CI)	P value
Total number of pneumonia cases[a]	544	679	20 (10, 28)	0.00009	348	452	23 (11, 33)	0.0002	181	210	14 (−4, 28)	0.1
Pneumonia with alveolar consolidation[b]	251	303	17 (2, 30)	0.03	119	158	25 (4, 40)	0.02	128	140	8 (−15, 27)	0.5
Pneumonia without identified virus[c]	419	486	14 (2, 24)	0.03	252	299	16 (0, 29)	0.05	167	187	11 (−8, 26)	0.3
Any identified virus-associated pneumonia[d]	160	231	31 (15, 43)	0.0004	111	167	33 (15, 48)	0.0008	44	57	0.2 (−14, 47)	23
Influenza A	31	56	45 (14, 64)	0.01	21	32	34 (−14, 62)	0.1	9	21	57 (7, 80)	0.03
RSV	90	115	22 (−3, 41)	0.08	64	94	32 (6, 50)	0.02	22	17	−30 (−143, 31)	0.4
PIV types 1–3	24	43	44 (8, 66)	0.02	16	27	41 (−10, 68)	0.09	8	16	50 (−17, 78)	0.1
Adenovirus	14	15	7 (−94, 55)	0.9	9	13	31 (−62, 70)	0.4	5	2	−150 (−1,188, 51)	0.3

[a]First episodes are shown; thus, a child with episodes of pneumonia associated both with and without a virus are counted in that category for each first episode, but only once in the total number of pneumonia cases. [b]Alveolar consolidation (WHO-AC)[5]. [c]Includes episodes of pneumonia that tested negative for all of the respiratory viruses examined. [d]Includes the first episode of any identified virus-associated pneumonia including influenza B. [e]Includes children whose HIV status was unknown. [f]Although the number of children receiving vaccine or placebo are known, the denominators of HIV-infected and HIV-uninfected children are estimated as 6.47% and 93.53%, respectively (see **Supplementary Methods** online for the basis of this estimate).

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Table 2 Percentage efficacy of pneumococcal conjugate vaccine by intent-to-treat analysis

Clinical diagnosis	All children[e]				HIV-uninfected children[f]				HIV-infected children[f]			
	Vaccine $n = 19,922$	Placebo $n = 19,914$	Efficacy (95% CI)	P value	Vaccine $n = 18,633$	Placebo $n = 18,626$	Efficacy (95% CI)	P value	Vaccine $n = 1,289$	Placebo $n = 1,288$	Efficacy (95% CI)	P value
Total number of pneumonia cases[a]	975	1,162	16 (9, 23)	0.00003	566	681	17 (7, 26)	0.0006	379	446	15 (5, 24)	0.004
Pneumonia with alveolar consolidation[b]	356	428	17 (4, 28)	0.01	169	212	20 (3, 35)	0.03	182	209	13 (−4, 28)	0.1
Pneumonia without identified virus[c]	726	845	14 (5, 22)	0.002	385	448	14 (2, 25)	0.03	341	397	14 (3, 24)	0.01
Any identified virus-associated pneumonia[d]	274	353	22 (9, 34)	0.001	195	250	22 (6, 35)	0.009	70	91	23 (−4, 43)	0.09
Influenza A	42	71	41 (13, 60)	0.006	25	41	39 (0, 63)	0.05	15	26	42 (−8, 69)	0.08
RSV	184	208	12 (−8, 27)	0.2	141	161	12 (−10, 30)	0.2	36	40	10 (−40, 42)	0.6
PIV types 1–3	31	55	44 (3, 64)	0.01	18	32	44 (0, 68)	0.05	13	22	41 (−17, 70)	0.1
Adenovirus	16	16	0.0 (−100, 50)	1	10	14	29 (−61, 68)	0.4	6	2	−200 (−1,382, 39)	0.3

[a–f]See **Table 1** for an explanation of the footnotes.

Details of the demographics of the study population have been reported[1]. Of 39,836 children, 18,245 received all three doses of study vaccine and 18,268 received placebo, according to the per protocol analysis (see **Supplementary Methods** online for the trial method).

We showed previously that children without HIV who received PncCV had 25% less pneumonia with alveolar consolidation, as assessed by the World Health Organization definition (WHO-AC)[1,5]. We now extend those data to show a 20% reduction in all-cause first episodes of clinical pneumonia among all children (95% confidence interval (CI) = 10–28%, $P = 0.00009$) (**Table 1**) with a similar reduction (14%; 95% CI = 2–44%) in pneumonias with which no virus was identified. **Table 1** also shows similar reductions in all-cause pneumonias among HIV-infected (14%; 95% CI = 4–28%) and HIV-uninfected (23%; 95% CI = 11–33%) children. In all children, PncCV also reduced pneumonias associated with any of the identified viruses by 31% (95% CI = 15–43%; $P = 0.0004$), with similar point estimates of efficacy and CI associated with influenza A virus (45%; 95% CI = 14–64%), parainfluenza viruses (PIVs) types 1–3 (44%; 95% CI = 8–66%) and RSV (22%; 95% CI = −3 to +41%).

The results of intent to treat analyses are shown in **Table 2**. The frequency of *Streptococcus pneumoniae* isolated from blood associated with viral pneumonia is shown in **Supplementary Table 1** online. No differences were found in the frequency of all-cause or virus-specific bronchiolitis between children who received the vaccine and those who received placebo (data not shown).

This study provides quantitative evidence of the importance of *S. pneumoniae* superinfection in virus-associated pneumonias in children in hospital and underscores the limited value of blood cultures to identify this association. The reduction in pneumonias associated with RSV, influenza A and PIV types 1–3 in children without HIV (**Table 1**) suggests that most of the pneumonias associated with these viruses in hospitalized children are due to concurrent bacterial infections. Conversely, most vaccine-preventable pneumococcal pneumonias in hospitalized children may require a viral respiratory infection.

Although the 9-valent PncCV provides coverage against 87% of serogroups of pneumococci in the study community[6], the vaccine cannot be expected to protect against bacterial pathogens such as *Staphylococcus aureus*. All children received *Haemophilus influenzae* type B conjugate vaccine, and serotype replacement carriage with non-vaccine pneumococcal serotypes occurs in this population[7]. Our data thus provide a minimum estimate of the burden of virus-associated pneumonia that may be due to bacteria.

Abundant epidemiological and biological evidence indicates that respiratory viruses contribute to bacterial infections (reviewed in ref. 8) through viral destruction of respiratory epithelium, viral upregulation of bacterial adhesion molecules such as the PAF receptor and (for influenza and PIV) the effect of viral neuraminidase on bacterial adhesion[8]. In addition to the viruses examined in our study, rhinovirus may upregulate pneumococcal adherence to respiratory epithelial cells[9], and it is possible that coronavirus and human metapneumovirus (an important cause of virus-associated pneumonia in this population[10]) may be also involved in the pathogenesis of bacterial pneumonia. The effect of whole-cell killed, split virus, or live attenuated influenza vaccines, in addition to PncCV, on pneumonia in children deserves study. We have shown that PncCV reduces pneumonia associated with respiratory viral infections, presumably by preventing superimposed bacterial coinfection.

Thus, pneumonia after acquisition of a new pneumococcal serotype during an upper respiratory viral infection may be prevented by opsonophagocytic antibody induced by conjugate vaccine. During the 7 d before the pneumococcal capsule-induced antibody response, there may be a temporary increase in susceptibility to virus-associated pneumococcal pneumonia, the mechanism of which remains speculative[1]. We would caution that our data supporting empirical antibiotic use for virus-associated pneumonia apply only to infants in hospital, most of whom would be already receiving antibiotics. Conjugate vaccine has been shown to reduce antibiotic use in outpatient settings[11]. The lesser impact of PncCV on virus-associated pneumonias in HIV-infected children may be due to the high frequency of concurrent

Pneumocystis jiroveci infections (42%) and bacterial infections other than pneumococcal among these children[12].

In conclusion, PncCV not only prevents invasive disease and radiologically confirmed pneumonia[1], but also reduces all-cause clinically diagnosed pneumonia. The data in **Table 1** suggest that the population-based effect of the vaccine on total pneumonia, including virus-associated pneumonias, should be considered in terms of total cases prevented and the cost-effectiveness of the vaccine. We have also shown that the vaccine is a useful probe that has established, for the first time to our knowledge, that a significant fraction of viral pneumonia is attributable to bacterial coinfection and is preventable by a bacterial vaccine. Because immunization of children has been shown to reduce invasive pneumococcal disease in adults[13], our data suggest that studies should be developed to investigate the strategy of infant immunization with pneumococcal conjugate vaccine to reduce morbidity and mortality associated with influenza and other viral pneumonias in both children and adults.

Note: Supplementary information is available on the Nature Medicine website.

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Vascular endothelial growth factor receptor-3 mediates induction of corneal alloimmunity

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There are no studies so far linking molecular regulation of lymphangiogenesis and induction of adaptive immunity. Here, we show that blockade of vascular endothelial growth factor receptor-3 (VEGFR-3) signaling significantly suppresses corneal antigen-presenting (dendritic) cell trafficking to draining lymph nodes, induction of delayed-type hypersensitivity and rejection of corneal transplants. Regulating the function of VEGFR-3 may therefore be a mechanism for modulating adaptive immunity in the periphery.
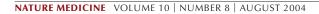
The accessible location and transparent nature of cornea make it an optimal site for lymphatic studies. In its center, the normal cornea is devoid of mature dendritic cells, which are capable of stimulating T cells[1], and lymphatic vessels, which allow efficient trafficking of antigen-presenting cells (APC) to lymphoid organs[2]. By contrast, the inflamed cornea eventually acquires both of these factors[1] to induce immunogenic inflammation that can jeopardize vision. To investigate the relationship between corneal inflammation and lymphangiogenesis and to determine the functional relevance of VEGFR-3 (refs. 3,4) to corneal immunity, we induced corneal inflammation using a mouse model of corneal hemangiogenesis and lymphangiogenesis[1] (**Fig. 1a**). After 7–14 d, we collected eyes for immunofluorescence confocal microscopic studies[1]. A sharp increase in VEGFR-3 expression was evident in the stroma of these inflamed corneas. Expression was present on both stromal dendritic cells[5] and newly developed lymphatics in the center of these inflamed corneas (**Fig. 1b**). Staining with lymphatic vessel endothelial hyaluronan receptor[6] (LYVE-1) confirmed the growth of new lymphatic vessels (**Fig. 1c**), which also stained positive for VEGFR-3 (**Fig. 1d**), into the periphery of the normally lymphatic-free corneas. Flow cytometric studies showed that 90% of cultured corneal dendritic cells[1] expressed VEGFR-3 (**Fig. 1e**), similar to these cells' acquisition of major histocompatibility class II expression as they mature in culture[1]. Control stromal fibroblasts (keratocytes)[7] did not show expression of VEGFR-3 (**Fig. 1e**). Our results from the transwell chemotaxis assay[8,9] demonstrated that these dendritic cells migrated in response to VEGF-C, a principal VEGFR-3 ligand, in a dose-dependent manner (**Fig. 1f**). This migration was blocked by a VEGFR-3/immunoglobulin (Ig) chimeric molecule that prevents VEGFR-3 ligation (**Fig. 1g**).

To test whether VEGFR-3 expression mediates APC trafficking to draining lymph nodes *in vivo*, corneal transplantation[1,10] was carried out between two fully (MHC and minor H) allo-disparate mouse strains, C57BL/6 (H-2I-A[b], donors) and BALB/c (H-2I-A[d], recipients). Corneal transplantation is a particularly suitable model because it allows for quantification of graft-derived APC trafficking to regional lymph nodes by identification of donor type (H-2I-A[b]) MHC class II cells[1]. Our data revealed that local (ocular) blockade of VEGFR-3 reduced corneal dendritic cell trafficking to draining lymph nodes of the host in a dose-dependent fashion (**Fig. 2a**). To confirm this effect by flow cytometry, bilateral submandibular lymph nodes were harvested for quantification of cornea-derived cells. Such cells were readily seen in ipsilateral, but not contralateral, nodes after transplantation under cover of control Fc/Ig (**Fig. 2b**). However, administration of blocking VEGFR-3/Ig led to a significant suppression in graft-derived cell flow (**Fig. 2b**) to ipsilateral lymph nodes.

To determine whether the dendritic cell flow blockade following VEGFR-3/Ig administration was due primarily to its effect on lym-

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