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Broad antibody and T cell reactivity induced by a pneumococcal whole-cell vaccine

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Abstract

Injecting mice with killed cells of non-capsulated strain RM200 adsorbed on Al(OH)3 (pneumococcal whole-cell vaccine; WCV) reduces nasopharyngeal colonization by capsular serotype 6B and prevents fatal aspiration pneumonia by serotype 3 or serotype 5 strains. To further examine the potential for omni-strain immunity, we here examined a panel of clinical isolates and a library of capsule-switch variants in the TIGR4 background. IgG binding to these bacteria in sera of rabbits injected with WCV or Al(OH)3 alone was assayed by ELISA without and with adsorption with cell-wall polysaccharide, a species-common antigen. The examined strains were 23 primary isolates including at least 10 different MLS types and 13 serotypes; 15 of these strains were invasive isolates, subsequently mouse-passed. Additionally, to investigate the effect of capsulation, TIGR4 strain constructs with the capsulation genes of 20 different serotypes was evaluated. In ELISA all strains showed a large difference in IgG binding due to the immunization, of which most of the antibody typically was not adsorbed and presumably directed to exposed protein antigens. Increased binding of IgG in the WCV-immunized serum to the 20 isogenic capsule-switch strains was shown also by flow cytometry. Further, all these 20 strains elicited IL-17A in T cells of WCV-vaccinated mice, a cytokine known to accelerate pneumococcal clearance. Thus WCV induced both humoral and T_H17 cell-mediated immunity against all tested strains.

Keywords

Streptococcus pneumoniae; vaccine; colonization; sepsis; IL-17; serotype-independence; species-common antigen

Introduction

Prevention of disease due to *Streptococcus pneumoniae* continues to represent a global health priority, with pneumococcal infection accounting for approximately one million childhood deaths per year [1]. The majority of pneumococcus-associated mortality and

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morbidity occurs in the developing world. Pneumococcal conjugate vaccines, though highly effective at reducing vaccine-type carriage and disease, have several disadvantages, namely limited coverage against >90 known pneumococcal serotypes, replacement in carriage and disease prevalence by non-vaccine serotypes, and high cost of manufacture [2]. For these reasons, many laboratories have been investigating surface-expressed proteins common to all serotypes of the pneumococcal species, with the goal of inducing serotype-independent protection from pneumococcal disease and/or carriage and to be of lower cost. Over twenty such proteins with protective potential have been discovered [3].

An economical approach we have been investigating is immunization with killed cells of a capsule-negative strain, which would present many surface proteins in native configuration, un-occluded by capsule. We hypothesized that this whole-cell antigen (WCA), due to redundancy in protective antigen expression, would elicit immune responses to pneumococci of varying capsular type, isolation site, and genetic background. WCA strain RM200 was constructed with the added features of deletion of the lytA autolysin and expression of a pneumolysin variant with attenuated hemolytic activity [4, 5]. Immunization of mice with WCA, designated whole-cell vaccine (WCV) when administered with appropriate adjuvant, has demonstrated multi-serotype protection in the models tested thus far. For example, when adsorbed to Al(OH)3 and given subcutaneously, WCV protects C57BL/6 mice from fatal aspiration-sepsis with serotypes 3 and 5 and reduces nasopharyngeal colonization with serotype 6B [5].

We are currently testing the protective effect of active WCV immunization in several other mouse challenge models using different serotypes and routes of inoculation; however, the number of serotypes that can be used to infect mice is limited and will not allow for a comprehensive assessment the serotype coverage of WCV-induced immunity. Therefore to more broadly test the potential coverage, we examined the effect of WCV immunization against a panel of selected strains in several assays *in vitro*. Based on what is understood about immunity to pneumococcal disease and carriage, we chose several techniques to assess the cross-serotype immune responses elicited by WCV. In preclinical development studies, WCV-induced serum antibodies had been shown to be sufficient for protection from fatal pneumonia and sepsis, since anti-WCA IgG titers above a certain value correlated with protection, immunity could be transferred with serum, and WCV-immunized animals treated with anti-CD4+ antibodies were still protected [5]. Here we therefore evaluated the IgG titers in WCV-immunized sera against live clinical isolates varying in serotype, MLS type, and isolation site, and against a library of 20 isogenic capsule-switch strains generated from a TIGR4 parent strain.

However, reduction in pneumococcal carriage in WCV-immunized animals has been found to be antibody-independent and occurs in a CD4+ T cell-dependent and IL-17A-mediated manner [6, 7]. In light of this, we additionally measured the IL-17A production *in vitro* by WCV-primed splenocytes stimulated with the 20 capsule-switch variants.

MATERIALS AND METHODS

Whole-cell vaccine preparations

Pneumococcal strain RM200 derived from Rx1 is capsule-negative, autolysin-negative, and expresses a non-hemolytic pneumolysoid as described [4, 8]. Cells were centrifuged, washed and killed using beta-propiolactone (BPL) as described [5]. Protein concentrations were determined by bovine serum albumin standardized Total Protein Kit (Sigma) and the antigen, designated WCA, was frozen in aliquots at −80°C until further use. Three hours prior to immunization, aliquots were thawed, diluted in sterile saline (B. Braun Medical Inc., Bethlehem, PA) with Al(OH)3 (aluminum hydroxide) from Brenntag North America (2%

Alhydrogel), and gently mixed at 4°C until use; these preparations were designated whole-cell vaccine (WCV).

Immunization of animals

C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were used. Animals were allowed to acclimate to our animal facility for 2–3 days prior to first immunization at age 4–6 weeks. Unanesthetized and gently restrained animals were injected 3 times at 2-week intervals in the lower back. Each injection contained 100 μg of WCA and 240 μg of Al(OH)3. Immunized mice were euthanized by CO2 inhalation 2–4 weeks following last immunizations, and spleens were harvested.

For antibody titers determined by enzyme-linked immunosorbent assay (ELISA), antiserum was generated at MPI (Mattawan, MI) by immunizing rabbits with Al(OH)3-adsorbed WCA (500 μg of WCV) as previously described [5] with Al(OH)3-immunized rabbit serum used as a control. For use in flow cytometry, hyper-immune rabbit serum was generated by Cocalico Biologicals Inc. (Reamstown, PA) by injecting animals subcutaneously with WCA, 1mg/dose, mixed with Freund's adjuvant in 4 doses (days 0, 14, 21 and 49) according to Cocalico's custom antibody protocol.

Preparation of bacteria for immunological assays

Isogenic capsule-switch variants were generated as described [9, 10]. For use as the capture coat for antibody ELISAs, bacteria were grown in Todd Hewitt Broth with 0.5% yeast extract (THY) (BD, Franklin Lakes, NJ) to mid-log phase (OD_{600} = 0.6) and used to coat plates as described below. For use as stimuli on splenocytes from WCV-immunized mice, bacteria were grown in THY (1ml thawed starter culture in 49ml THY) to OD_{600} = 1.0 at 37°C with 5% CO_2 and then resuspended to 3×10^9/ml prior to BPL-killing as previously described [5].

IgG binding detected by flow cytometry

Isogenic capsule-switch strains grown in 5ml THY were aliquoted in 1-ml volumes, and cells were pelleted by centrifugation and resuspended in 1ml PBS. Given the requirement that only non-viable bacteria be used in the cytometer, cells were heat-killed in a 58°C heat block for one hour. The cells were pelleted and resuspended in 1ml PBS 1%BSA and rotated overnight at 4°C for blocking. Cells were again pelleted and resuspended in 1 ml of diluted serum from the same rabbit either pre- or post-4X-WCV immunization (1:1000 dilution in PBS/0.5%Tween/1%BSA (PBST/BSA)) and incubated on a rotator for one hour. After two washes in PBST/BSA, pelleted cells were resuspended in 250 μl of Alexa Fluor®488 goat anti-rabbit IgG (Invitrogen, Carlsbad, CA) at a 1:50 dilution and rotated in the dark for one hour. The cells were washed twice, resuspended in 500 μl sterile PBS, and kept in the dark until analysis. Flow cytometry was performed using a Cytomation MoFlo (Beckman Coulter). At least three separate samples of each capsular type were prepared and analyzed and at least 10,000 events were included in each mean fluorescence intensity measurement.

Enzyme-linked immunosorbent assay (ELISA)

Live clinical isolates and isogenic capsule-switch variants in the TIGR4 background were suspended at 2×10^8 cells/ml in sterile PBS. Immulon®2 96-well flat-bottom plates were coated with 50 μl cell suspension/well (approximately 1×10^7 cells per well) overnight at 4°C and then blocked with 100 μl/well PBS 1% bovine serum albumin (BSA; Sigma Aldrich, St. Louis, MO) for one hour at room temperature. Rabbit serum dilutions were prepared 2-fold in PBST; Al(OH)3-immunized sera were diluted from 1:25 to 1:800, and WCV-immunized sera from 1:200 to 1:6400.. Pneumococcal cell-wall polysaccharide

(CWPS; Statens Serum Institut, Copenhagen, Denmark) was added, where indicated, to dilute sera to a final concentration of 0 or 10 μg/ml and incubated for one hour at room temperature. Fifty μl of non-adsorbed and CWPS-adsorbed sera dilutions were then added to the blocked cell-coated plates and incubated for 2 hours. Donkey anti-rabbit IgG HRP (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at 1:20,000 dilution was used to detect bound antibody, followed - after washing - with Sureblue TMB substrate (KPL, Inc., Gaithersburg, MD). Reactions were stopped with 2N sulfuric acid, and optical densities were determined at 450 nm.

Assay of IL-17A from stimulation of splenocytes of WCV-immunized mice

Spleens from WCV-immunized mice were harvested and processed as previously described [7]. BPL-killed isogenic capsule switch TIGR4 variants were used as stimuli. All strains were resuspended to 3×10^9/ml prior to BPL killing. All killed preparations were diluted in splenocyte stimulation medium (DMEM/F12 with L-glutamine supplemented with 10% fetal calf serum, 50 μM 2-mercaptoethanol, and 10 μg/ml ciprofloxacin) to a final concentration of 1×10^6 pneumococcal cells in 250μl total volume in the well containing 1×10^6 splenocytes. After 72 hours at 37°C in 5% CO_2, IL-17A was measured from supernatants of cell culture after stimulation (R&D Systems ELISA kits, Minneapolis, MN).

Statistical analysis

Data analysis and graphing were performed using PRISM (version 4.0, GraphPad Software, Inc.). The methods to quantify the *in vitro* neutrophil killing efficiency of the isogenic capsule-switch variants were previously described [9]; non-parametric Spearman correlation was used to evaluate the association between these values and the median IL-17A elicited by these capsule-switch strains in WCV-immunized splenocytes.

RESULTS

Choice of strains and assays

The cross-strain immunogenicity of WCV was evaluated with a variety of strains described in Table 1 including a) 23 clinical isolates representing both carriage (isolated nasopharyngeally) and invasive (isolated from blood) strains and b) a library of TIGR4 strains isogenic except for the capsule loci expressing 20 different serotypes. All the strains were tested in ELISA with live bacteria as capture antigen, determining the IgG antibody titer in sera of rabbits immunized with WCV or co-housed rabbits injected with Al(OH)3 alone as control. While a constant concentration of live cells was used as the capture layer in the ELISA, consistent coating from strain to strain could not be assured. Thus while the titers are not strictly comparable among strains, the control versus immunized titers are comparable for a given strain. The titers were determined without and with adsorption with cell wall polysaccharide (CWPS), a species-common antigen, thus the titer in the adsorbed serum shows presumed protein-specific antibody. In addition, to avoid possible artifacts from the coating process, the TIGR4 capsule-switch panel was examined for WCV-elicited antibody binding by flow cytometry. Further, this panel was tested for the IL-17A produced in culture when the bacteria were used to stimulate splenocytes from WCV-immunized mice.

Effect of WCV-immunization of rabbits on IgG titers to the various strains in capture ELISA

Against all strains tested, higher titers were measured in WCV-immunized serum over alum-control serum, both without and with adsorption by CWPS. With adsorption (the more important measurement; Table 2) the fold-rise against 22 of 23 of the clinical isolates ranged from 19–300, while the often sub-cultured serotype 3 strain WU2 (fold-rise = 10) was an

outlier. The range against the 20 isogenic capsule-switch variants was less variable at 26–43. As a control for the effect of capsulation *per se*, an isogenic TIGR4 strain with the capsule locus deleted (Δcapsule) was included and showed a fold-rise of 43. In ELISA without CWPS adsorption somewhat higher titers were found in both control and WCV-immunized sera. With the clinical strains, non-CWPS IgG represented 42–72% of the total measured IgG in WCV-immunized sera; for isogenic TIGR4 capsule variants, non-CWPS IgG represented 68–94% of the total.

Assessing the effect of WCV-immunization by flow cytometry

To assess IgG binding without possible complications from adherence of the pneumococcal cells to polystyrene ELISA wells, heat-killed isogenic capsule-switch variants were reacted with WCV-immunized rabbit or control sera, and binding was detected by flow cytometry. Isogenic TIGR4 strains of 20 different capsule types and an unencapsulated variant were each grown to mid-log phase and then heat-killed. After incubation with WCV-immunized rabbit sera, or separately with pre-immune sera from the same animal, the washed cells were reacted with secondary fluorophore and mean fluorescence intensity (MFI) of each preparation was measured. As shown in Figure 1, the MFI of bound IgG from the immune serum ranged from 171–702 and the MFI of the unencapsulated cells was 755. Pre-immune serum demonstrated minimal binding: the MFI ranged from 2.9–4.9.

Measuring the IL-17A immunogenicity of isogenic capsule-switch variants in WCV-primed splenocytes

To investigate the IL-17A immunogenicity of the non-capsular antigens presented in pneumococci expressing varying capsular types, we studied the response of WCV-immunized splenocytes to stimulation with the isogenic capsule-switch strains. Spleens from 10 WCV-immunized mice were processed and stimulated separately; splenocytes were not pooled. IL-17A in each stimulation supernatant was measured; individual and median IL-17A responses for each capsular-type whole cell antigen are shown in Figure 2. The median response to medium alone was 30 pg/ml of IL-17A. Median IL-17A responses to capsule-switch whole cell antigens ranged from 900 (type 8) to 4800 (type 14) pg/ml. For comparison, the isogenic strain with capsule locus deleted (ΔCPS) was used as stimulus and the median IL-17A response was 3800 pg/ml.

Assessing the correlation between a serotype's in vitro IL-17A immunogenicity and resistance to surface phagocytosis

Previous work identified several biochemical and physical features of the polysaccharide capsule of pneumococcus that correlate with strain prevalence in carriage rates and with *in vitro* assessments of survival from surface phagocytosis [9]. Based on the demonstrated role of IL-17A in enhancing neutrophil-mediated clearance of mucosally colonizing pneumococci [7], we hypothesized that the capsular types that elicit the highest IL-17A levels systemically would be most efficiently killed by surface phagocytosis. Using the phagocytosis survival values obtained in the cited study, we found that median IL-17A levels elicited from WCV-immunized splenocytes by varying capsular type whole-cell antigens correlated inversely with survival from surface phagocytosis (Figure 3) with a Spearman $\rho = -0.6545$ ($p = 0.03$).

DISCUSSION

The effects of the 7-valent pneumococcal conjugate vaccine (PCV) on reduction of invasive disease rates in the US [11] and of a 9-valent PCV in developing nations [12, 13] demonstrate the possibility for global pneumococcal disease prevention if broad enough serotype coverage were provided. While expanded-valency PCVs represent one approach to

extending this coverage, the cost and complexity of these vaccines to manufacture may limit their accessibility worldwide. In addition, serotype replacement and serotype-specific changes in pneumococcal ecology following implementation of PCV programs may impact the longstanding effect of PCV at reducing pneumococcal disease [2, 14, 15]. The various species-common protein projects under development represent an approach to address these problems, with the aim to either include protein antigens that confer protection against colonization and invasive disease, or incorporate these proteins in existing PCVs [3]. Our WCV approach might work more simply and economically by conferring immunity to a range of noncapsular pneumococcal species antigens that are well conserved across varying serotypes and sequence types.

Some of the typical correlates of immunity that are reliably assessed in PCV efficacy trials, such as vaccine-induced anticapsular IgG levels, will not apply to evaluation of WCV-induced (or for that matter, protein-induced) immunity, and this could pose a challenge for licensing strategies for this approach [16]. Additionally, pneumococcus is an essentially human pathogen; the paucity of pneumococcal carriage and invasive disease animal models limit comprehensive evaluation of serotype coverage via active immunization studies. For these reasons, we are evaluating other methods of assessing the serotype coverage of the immunity elicited by WCV administration. The *in vitro* assays described herein were chosen because they represent immune correlates that apply to what is known about acquired immunity to various phases of pneumococcal infection.

The role of anticapsular antibodies in acquired immunity to pneumococcal disease has been largely corroborated by the efficacy of PCVs in reduction of both carriage and invasive disease rates due to vaccine-types. There is less known about the role of noncapsular antibodies, but studies with several pneumococcal species proteins suggest that these entities can protect against invasive disease, albeit with higher titers needed than of capsular antibody; for this reason vaccination with mixtures of several such proteins is being tested (reviewed in [3]). Hypothetically, the WCV preparation should preserve these noncapsular components in native configuration as well as many other as of yet uncharacterized antigens that can serve as targets for protective antibody production. Empirically WCV: a) elicits antibodies to the known well studied proteins PspA, PsaA, and Ply; and to the CWPS, which perhaps has protective potential [17, 18] b) elicits antibodies to a large number of other proteins, not identified, as seen with Western blotting, and c) expresses protective antigens redundantly, e.g. is still protective even if the choline-binding class of proteins [19] or the pneumolysin protein (unpublished data) are eliminated. Therefore it is reasonable to expect that the humoral response elicited by WCV could contribute to protection regardless of serotype and other genetic variations.

As is shown by the titers in Table 2 and the flow cytometric binding in Figure 1, antibodies to WCV bound to pneumococcal cells across all tested MLST sequences and serotypes. The magnitude of the fold-rise varied, especially in the titers to clinical strains, for structural reasons unknown. This variation appears not due to the effect of capsule on antigen accessibility to antibody: the fold rises in the isogenic capsule switch variants in the TIGR4 background were less variable, and the noncapsulated variant reacted similarly to the capsulated strains. This may suggest that the role of capsule *per se* on IgG antibody access to noncapsular antigens is minimal, but the inherent differences across sequence types in representation of accessible antibody targets contribute to greater variability. The CWPS, which has only slight antigenic variation, is common to all pneumococci examined, but the protective capacity of CWPS antibody in invasive infection is controversial [17, 18, 20–23]. Although our ELISA showed a reduction in titer in all the strains following adsorption with CWPS, the majority of WCV-generated antibody appears to be directed at non-CWPS targets in most of the strains.

Interpretation of protective potential of these antibody titers and binding capacities is not clear; unlike anticapsular antibody for which threshold levels have been determined that correlate with protection from disease with encapsulated organisms such as *S. pneumoniae* and *Haemophilus influenzae* type b [24], such correlates for noncapsular pneumococcal antibodies do not exist and may be difficult to establish. In active immunization studies thus far, both 2- and 3-dose schedules of subcutaneous WCV administered at two-week intervals provided dose-dependent protection from sepsis via aspiration of WU2 serotype 3 to 100% of animals compared with 0–30% survival in animals injected with Al(OH)3 alone [5]. The challenge strain in this study is the same serotype 3 strain to which the titer rise (10-fold, Table 2) was lowest indicating that, at least for this strain in this model, a modest rise in titer is sufficient for protection from invasive disease. It may be possible to further evaluate the function of the WCV-elicited antibody in an opsonin-dependent killing assay using various strains *in vitro* with a human neutrophil source and WCV-immunized sera. Such an assay is in development and may further inform us about the serotype coverage of WCV immunization. It may also identify a correlate of protection against invasive disease by which WCV-generated immunity can be measured.

While the effect of PCV immunization programs on reduction of carriage of vaccine- types demonstrates that anticapsular antibody is *sufficient* to prevent colonization [25], it is not *necessary.* We have demonstrated antibody-independent mechanisms of WCV- elicited clearance of colonization [6, 7], and others have similarly shown that reduction in the duration of carriage following exposure to live pneumococcus is not antibody- mediated [26, 27]. Furthermore, the critical role of the CD4+ T cell effector cytokine IL- 17A in clearance of carriage has been confirmed in models of primary colonization [28] as well as in WCV-elicited mechanisms of immunity to colonization [7]. The capacity of T cells (in pre-challenge blood samples) of WCV-immunized animals to produce IL-17A in culture correlates inversely with the burden of pneumococci recovered from nasal washes following intranasal challenge with serotype 6B [5, 7, 8]: IL-17A levels of greater than 300 pg/ml predict a low to non-detectable pneumococcal count in post-challenge nasal washes. In these IL-17A assays, the WCA used to stimulate the T cells is noncapsulated. To assess the effect of capsule on the accessibility of antigens able to stimulate IL-17A secretion in WCV-immunized T cells, we used the isogenic capsule- switch panel of TIGR4 strains. As shown in Figure 2, all 20 strains elicited IL-17A responses well above the 300 pg/ml correlate; most elicited medians (among 10 mice) of 1000–4000 pg/ml. While it is difficult to directly apply the cited protective threshold (determined following intranasal immunization and produced in whole blood samples) to that determined here with subcutaneous vaccination and produced in splenocyte samples, the magnitude of the response is encouraging and does not suggest that capsule alone significantly impacts the accessibility of T cell antigens that trigger the IL- 17A responses generated by WCV immunization.

Studies have demonstrated that IL-17A facilitates clearance of pneumococci from mucosal surfaces by recruiting neutrophils that effectively kill the bacteria [7, 28]. Furthermore, using the same isogenic capsule-switch library as that used here to stimulate splenocytes, it had been shown that survival of killing by neutrophils *in vitro* correlated with the size of the capsule expressed in the TIGR4 background [9]. Here we found that the magnitude of the IL-17A response from WCV-immunized splenocytes correlated significantly and inversely with the resistance to killing by neutrophils that had been determined for that capsular type (Figure 3). For example, variants expressing capsular serotypes such as 23F and 19F that elicited the lowest IL-17A responses demonstrated higher mean relative survival rates in the neutrophil assay than serotypes such as 4 and 5 that elicited the highest IL-17A responses. This suggests the hypothesis that a capsular type that is both more susceptible to neutrophils and that primes for more robust IL-17A responses would be less fit for survival in carriage. As demonstrated in the cited study, the capsule size of these isogenic variants correlates with

prevalence in carriage [9]. The effect of capsule on eliciting IL-17A responses may also be reflected in these differences in the ability of pneumococci of varying serotype to colonize at mucosal sites, differences represented by differing carriage prevalence rates.

In summary, the data presented here demonstrate robust WCV-elicited humoral and cellular immunogenicity to a comprehensive panel of pneumococci of varying MLST and serotypes. In light of this potential for omni-strain coverage from WCV immunization, as well as the economy of manufacture, plans for Phase I clinical trials of the WCV in the USA are underway.

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Abbreviations

WCV	whole cell vaccine
Al(OH)3	aluminum hydroxide
CWPS	cell wall polysaccharide
MLST	multilocus sequence typing
PCV	pneumococcal conjugate vaccine
MFI	mean fluorescence intensity

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Highlights

- We show the immunologic responses to a species-specific pneumococcal whole cell vaccine administered with aluminum hydroxide
- Antibody elicited by the vaccine cross-reacts to a panel of clinical pneumococcal isolates of diverse MLST and serotypes
- Immunization with this vaccine primes T cells to produce robust IL-17A responses to pneumococci regardless of serotype
- This data suggest broad responses and provide further support for the clinical development of this vaccine

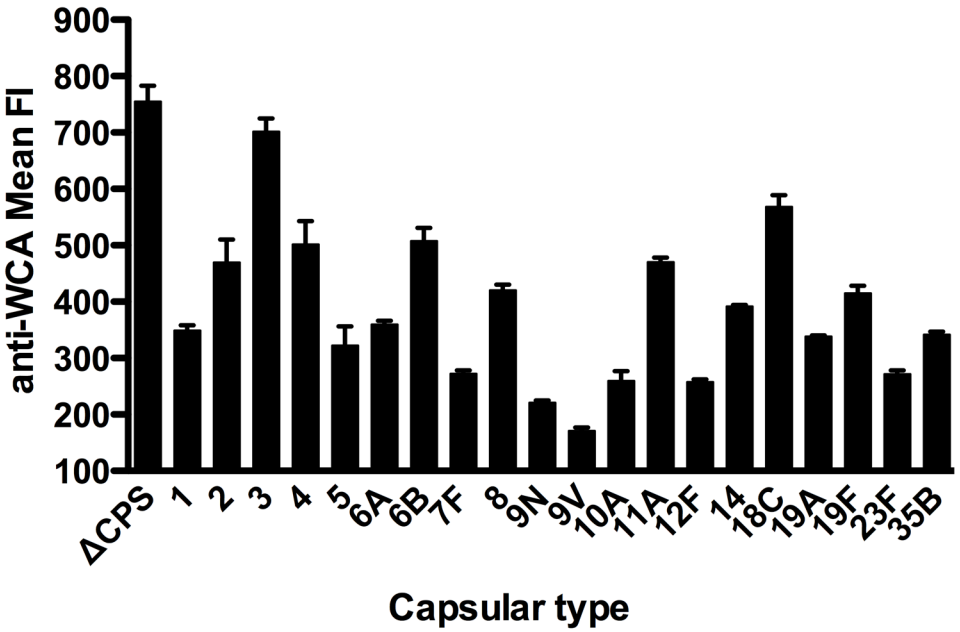

Figure 1. WCV-elicited antibody reactivity against isogenic strains bearing different capsular types

Heat-killed TIGR4 isogenic strains of varying capsular type were detected with sera from rabbits immunized with WCV then stained with an anti-rabbit IgG fluorophore and measured by flow cytometry. The same strains were detected with pre-immune sera from the same animal with range of mean fluorescence intensity 2.9 – 4.9. The data represent the median and interquartile range from at least three separately prepared samples.

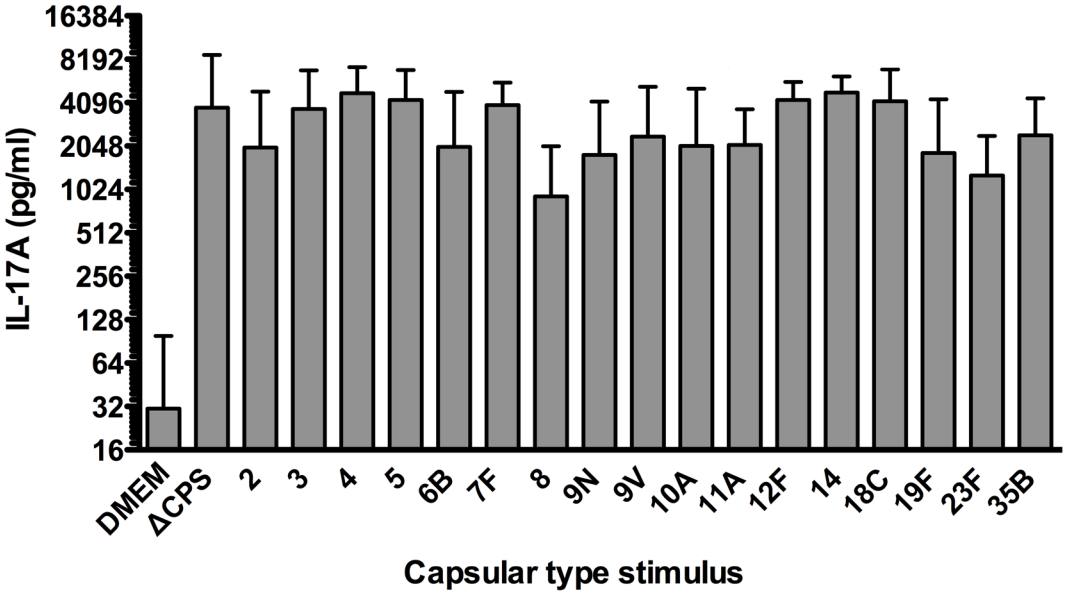

Figure 2. WCV-induced IL-17A response to isogenic pneumococcal strains bearing differing capsular types from splenocytes obtained from mice immunized with WCV

Splenocytes from WCB immunized C57Bl/6 mice (n=10) were stimulated with killed TIGR4 isogenic strains of the indicated capsular type. Stimulation was with 1×10^6 pneumococcal cells per 1×10^6 splenocytes. IL-17A was measured from the supernatants following a 72-hour incubation. Height of the columns represents median values with interquartile ranges indicated by bars.

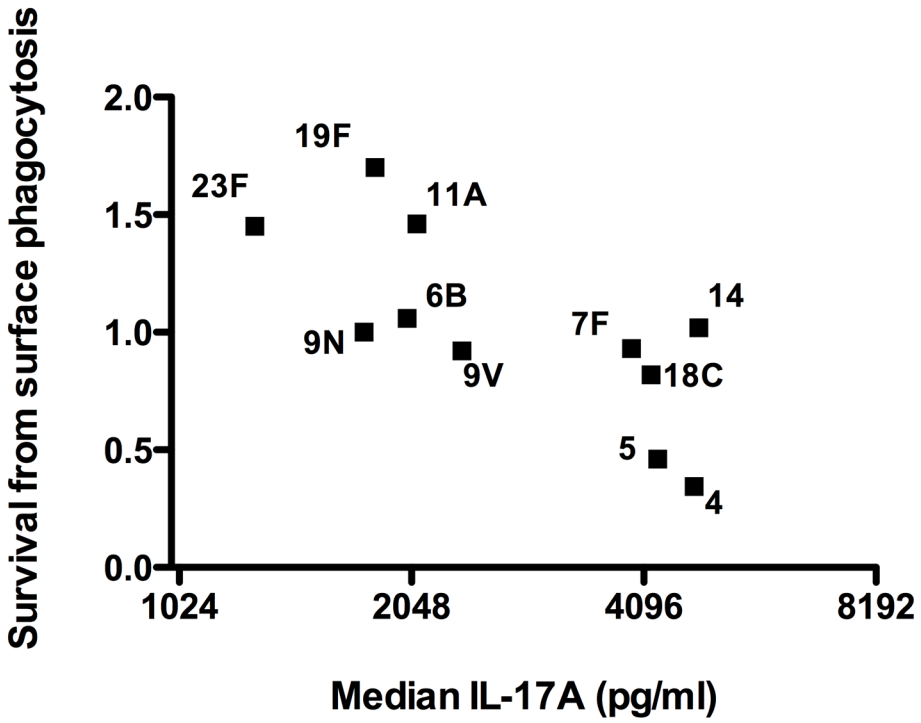

Figure 3. Correlation of IL-17A response and survival in a surface phagocytosis assay

Correlation of median IL-17A responses stimulated by capsule-switch isogenic pneumococcal whole cell antigens in WCV-immunized splenocytes with relative survival from surface phagocytosis (normalized to survival by the serotype 14 strain, as measured in [9]). IL-17A responses correlate significantly and inversely with survival from neutrophil-mediated surface phagocytosis (Spearman $\rho = -0.6545$; $p = 0.03$). The isogenic capsule-switch variants eliciting the highest IL-17A demonstrate the lowest survival index in an *in vitro* assay of surface phagocytosis.

Table 1

Description of clinical strains used to determine IgG titer from WCV-immunized sera.

Serotype	Site of isolation	MLST
1	Nasopharynx (NP)	227
4	NP	205
7C	NP	1757
7F	Blood	191
9V	NP	156
12F	Blood	218
15B	NP	199
18C	NP	113
22F	NP	433
23F	NP	33
3	Blood	NA (not available)
4 (two different isolates)	Blood	NA
6B (four different isolates)	Blood	NA
9V (two different isolates)	Blood	NA
14 (three different isolates)	Blood	NA
23F	Blood	NA

Table 2

Titers of IgG antibody in alum control or WCV-immunized rabbit sera to live log- phase pneumococci of the indicated strain/serotype. Strains are described in Table 1 and represent a variety of clinical carriage and disease isolates as well as a library of capsule-switch variants constructed on the TIGR4 genetic background [9]. The assays were determined with and without adsorption with the cell wall polysaccharide to indicate presumed protein-specific and total antibodies, respectively. Titers are reported as reciprocal of the dilution at which OD_{450} 0.3 was reached.

Serotype	CWPS-adsorbed titer			Non-adsorbed titer		
	Al(OH)3 control	WCV-immunized	Fold-difference	Al(OH)3 control	WCV-immunized	Fold-difference
Clinical isolates						
3	65	670	10	55	1000	18
4	38	1700	45	45	2300	51
4	100	2000	20	120	2900	24
4	25	1500	60	88	3200	36
6B	110	2500	23	125	3800	30
6B	25	1500	60	90	2100	23
6B	25	1600	64	92	3500	38
9V	97	1800	19	120	3800	32
9V	67	1300	19	110	3100	28
9V	54	2200	41	46	3000	65
12F	35	2100	60	39	3800	97
14	25	1400	56	90	2000	22
14	85	1800	21	90	2900	32
15B	74	3600	49	110	6600	60
23F	25	3400	140	100	5000	50
1	17	2200	130	NA	NA	NA
6B	58	3000	52	NA	NA	NA
7C	32	3300	100	NA	NA	NA
7F	19	3000	160	NA	NA	NA
14	33	2300	70	NA	NA	NA
18C	6	1800	300	NA	NA	NA
22F	31	1600	52	NA	NA	NA

Serotype	CWPS-adsorbed titer			Non-adsorbed titer		
	Al(OH)3 control	WCV-immunized	Fold-difference	Al(OH)3 control	WCV-immunized	Fold-difference
23F	30	2300	77	NA	NA	NA
Isogenic TIGR4 strains TIGR4:Capsular Type						
TIGR4:1	70	2400	34	80	2900	36
TIGR4:2	88	2500	28	110	3300	30
TIGR4:3	78	3400	43	80	4000	50
TIGR4:4	85	3700	43	95	4200	44
TIGR4:5	80	2900	36	80	3400	43
TIGR4:6A	105	2900	27	110	3100	28
TIGR4:6B	100	3200	32	110	3700	34
TIGR4:7F	80	2400	30	85	2800	33
TIGR4:8	100	3000	30	120	3400	28
TIGR4:9N	100	3000	30	110	3400	31
TIGR4:9V	100	2800	28	120	3500	29
TIGR4:10A	100	2700	27	120	3800	32
TIGR4:11A	120	3100	26	130	3900	30
TIGR4:12F	70	2800	40	80	3200	40
TIGR4:14	78	3200	41	80	3400	43
TIGR4:18C	90	2800	31	95	3100	33
TIGR4:19A	90	3000	33	130	4400	34
TIGR4:19F	110	3100	28	120	4200	35
TIGR4:23F	100	2900	29	120	3400	28
TIGR4:35B	80	3000	38	80	3200	40
TIGR4: Δcapsule	100	4400	43	110	5000	45

NA – not assayed

Vaccine. Author manuscript; available in PMC 2013 June 19.