Vaccine 30 (2012) 3897–3907

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Characterization of Th17 responses to *Streptococcus pneumoniae* in humans: Comparisons between adults and children in a developed and a developing country

Anna Lundgren[a,*], Taufiqur R. Bhuiyan[b], Daniel Novak[a,c], Joanna Kaim[a], Adi Reske[d], Ying-Jie Lu[d], Firdausi Qadri[b], Richard Malley[d]

[a] *Gothenburg University Vaccine Research Institute (GUVAX), Dept. of Microbiology and Immunology, University of Gothenburg, Box 435, 405 30, Gothenburg, Sweden*
[b] *International centre for diarrhoeal disease research, Bangladesh (icddr,b), GPO Box 128, Dhaka 1000, Bangladesh*
[c] *Queen Silvia's Children's Hospital, Sahlgrenska University Hospital, SU/Östra, 416 85 Gothenburg, Sweden*
[d] *Division of Infectious Diseases, Dept. of Medicine, Children's Hospital, Harvard Medical School, Boston, 300 Longwood Avenue, Boston MA, 02115 USA*

ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form 25 February 2012
Accepted 29 March 2012
Available online 12 April 2012

Keywords:
T cell
IL-17A
Streptococcus pneumoniae
Children
Developing country

ABSTRACT

Intranasal exposure to *Streptococcus pneumoniae* as well as mucosal or parenteral immunization with a recently developed killed pneumococcal whole cell vaccine, confer Th17-mediated protection against subsequent *S. pneumoniae* colonization in mice. Given our interest in the function of Th17 cells and the ongoing efforts to develop this vaccine for use in infants and children in developing countries, we analyzed Th17 responses to the whole cell antigen (WCA) and individual pneumococcal antigens in healthy individuals and patients with pneumococcal disease and compared responses in children and adults from Sweden and Bangladesh. Peripheral blood mononuclear cells (PBMCs) isolated from Swedish adults produced IL-17A after stimulation with WCA, with the pneumolysoid PdT and with the protein required for cell separation in group B streptococci (PcsB). IL-22 and IFN-γ responses were also detected, but these cytokines originated from separate CD4+ T cell subsets. PBMCs from Swedish children produced lower levels of IL-17A in response to WCA compared to adults, whereas no such difference was noted from the samples from Bangladesh, where responses by children and adults were both significantly higher than those in Sweden. High IL-17A responses to stimulation with WCA were also observed in children with proven or probable pneumococcal pneumonia. Our results thus demonstrate the presence of Th17-type T cells that are specific for pneumococcus in both children and adults. The different levels of Th17 responses to pneumococci in children and adults in developing and developed countries, which may at least partly be due to differences in exposure to pneumococci, are important factors to consider in the evaluation of candidate pneumococcal protein-based vaccines in human trials.

1. Introduction

Streptococcus pneumoniae colonizes the nasopharynx as part of the normal flora, but is also an important cause of diseases, including otitis media, pneumonia, sepsis and meningitis [1,2]. Over 800,000 children under 5 years of age are estimated to die from pneumococcal diseases worldwide each year, with a majority of cases in developing countries [3]. *S. pneumoniae* colonization precedes development of disease [2]. Children in developing countries have higher colonization rates and earlier acquisition of disease than children in the developed world [4,5]. Although the introduction of pneumococcal capsular polysaccharide conjugate vaccines has been accompanied by impressive reductions in invasive disease attributable to pneumococcal strains covered by the vaccine, issues of serotype coverage, serotype replacement and cost may limit the applicability of this strategy for the developing world [6,7]. Therefore, there is a continued search for new pneumococcal vaccines

Abbreviations: BCG, Bacillus Calmette-Guérin vaccine; COPD, chronic obstructive pulmonary disease; GMP, good manufacturing practice; PBMCs, peripheral blood mononuclear cells; PcsB, protein required for cell separation in group B streptococci; PdT, pneumolysoid; PHA, phytohemagglutinin; PPD, *Mycobacterium tuberculosis* purified protein derivative; PsaA, pneumococcal surface adhesin A; PspC, pneumococcal surface protein C; SEB, staphylococcus enterotoxin B; TLR, toll like receptor; WCA, whole cell antigen.

* Corresponding author. GUVAX, Dept. of Microbiology and Immunology, University of Gothenburg, Box 435, 405 30, Gothenburg, Sweden. Tel.: +46 31 786 6213; fax: +46 31 786 6205.

E-mail addresses: anna.lundgren@microbio.gu.se (A. Lundgren), taufiqur@icddrb.org (T.R. Bhuiyan), daniel.novak@vgregion.se (D. Novak), joanna.kaim@microbio.gu.se (J. Kaim), adi.reske@childrens.harvard.edu (A. Reske), yingjie.lu@childrens.harvard.edu (Y.-J. Lu), fqadri@icddrb.org (F. Qadri), richard.malley@childrens.harvard.edu (R. Malley).

0264-410X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2012.03.082

Table 1
Subjects included in the study.

Study group	*N*	Gender (females/males)	Age (median, range)
Healthy Swedish adults	45	29/16	34 years (21–58)
Swedish control children	11	5/6	24 months (6–65)
Swedish children with pneumonia	4	1/3	17 months (8–54)
Healthy Bangladeshi adults	10	6/4	27 years (19–32)
Healthy Bangladeshi children	23	10/13	12 months (6–60)

that can give rise to broader protection against pneumococcal colonization and disease, by inducing other arms of immunity than the anticapsular antibody responses elicited by the conjugate vaccines [8].

Intranasal exposure to live *S. pneumoniae* [9] as well as mucosal [9–11] and parenteral vaccination [12] with killed whole cell antigen (WCA) in combination with adjuvants can protect mice against pneumococcal colonization in the absence of antibodies. This protection is critically dependent on CD4+ IL-17A producing Th17 cells and levels of IL-17A produced by blood cells stimulated with WCA correlate with protection in this vaccination model [13]. Th17-dependent protection can also be induced in mice by mucosal immunization with a combination of purified pneumococcal proteins (a non-toxic derivative of pneumolysin, PdT, pneumococcal surface protein C, PspC, and pneumococcal surface adhesin A, PsaA), administered together with cholera toxin [14].

The broad protection afforded by the whole cell vaccine as well as its low production costs may make it particularly suitable for use in developing countries. This vaccine has been produced under good manufacturing practice (GMP) conditions for use in human trials [12] and a Phase I clinical study of healthy adult volunteers has been initiated. However, little is known about Th17 responses to pneumococci in humans. In particular, it has been suggested, although not proven, that these T cell responses contribute to the age-dependent decrease in pneumococcal colonization and disease in children. In support of this possibility, a recent epidemiological study in Bangladesh suggested serotype-independent protection against *S. pneumoniae* in infants, indicating that factors other than anticapsular antibodies may be important for early pneumococcal protection [15]. IL-17A responses to WCA have previously been described in studies using whole blood from healthy American adults as well as from mononuclear cells derived from tonsils collected from 2 to 12-year-old British children [13]. Preliminary data also suggest that production of the Th17-associated cytokine IL-22 in response to WCA stimulation may be inversely correlated to the risk of subsequent pneumococcal colonization in patients with chronic obstructive pulmonary disease (COPD) [16]. However, the cellular source of the IL-17A and IL-22 measured in these studies has not been investigated. Furthermore, it is unclear whether responses differ in children and adults and whether Th17 responses to pneumococci are influenced by the different levels of pneumococcal colonization and disease present in various parts of the world.

In this study, we analyzed Th17 and antibody responses to pneumococcal antigens in adults and children in Sweden and Bangladesh. Pneumococcal colonization is established very early in life in Bangladesh, with 50% of infants colonized at least once by 8 weeks of age, 90% by the age of 21 weeks and 100% at 1 year [5]. The colonization rate also remains high in older children and young adults in this population. In contrast, a later onset of pneumococcal acquisition and lower carriage rates have been observed in Swedish infants [17], a pattern that is consistent with several other reports comparing pneumococcal epidemiology in developing and developed countries [18–22].

Here we demonstrate the presence of Th17 type T cells that are specific for pneumococcus in both children and adults. We also show that, in contrast to Swedish children who produce low levels of IL-17A in response to WCA, children from Bangladesh have robust IL-17A responses to these antigens that are comparable to that of adults from the same country.

2. Materials and methods

2.1. Subjects and sample collection

Heparinized venous blood was collected from Swedish and Bangladeshi adults and children (Table 1). Healthy Swedish adults were recruited among students and staff at the Sahlgrenska Academy, University of Gothenburg. A majority of the adults were previously immunized with the Bacillus–Calmette–Guérin (BCG) vaccine. Control Swedish children were recruited from patients at Queen Silvia's Children's hospital, Gothenburg (Table 2). These healthy children came to the hospital for control visits ($n = 8$) or

Table 2
Swedish children included in the study.

Subject	Gender	Age (months)	Diagnosis	IgG titer (fold rise)	Pneumococcal infection
Swedish control children					
1	F	6	Healthy, control visit	–	–
2	M	6	Healthy, control visit	–	–
3	F	12	Healthy, control visit	–	–
4	M	13	Healthy, control visit	–	–
5	F	15	Healthy, control visit	–	–
6	M	24	Healthy, control visit	–	–
7	M	28	Healthy, control visit	–	–
8	F	35	Healthy, control visit	–	–
9	M	62	Broken elbow	–	–
10	M	65	Testicular torsion	–	–
11	F	71	Dog bite	–	–
Swedish children with pneumonia					
12	M	15	Pneumonia with hemolytic uremic syndrome, blood culture pos.	37	Definite
13	M	18	Pneumonia with pulmonary abscess, urine test pos.	3	Likely
14	F	51	Pneumonia with pleural effusion, urine test pos.	2	Likely
15	M	8	Otitis followed by pneumonia, urine test neg.	1	Unlikely