Vaccine Antigen Counts Do Not Provide An Accurate Measure of Immune System Stimulation

Fewer Antigens argument by CDC and media misleads parents

SafeMinds, May 11, 2016

SUMMARY

A common talking point to reassure parents who are concerned about the increasing number of immunizations recommended for infants is that today's vaccines contain *fewer antigens* and thus that infants' immune systems actually are taxed less than in the past. This argument is based on the same flawed reasoning that led Offit et al. (2002) to conclude that infants could safely receive as many as 10,000 vaccines at once. More recently it was used by DeStefano et al. (2013) to show that the increasing number of vaccines given to infants is not associated with risk of autism.

Here is a sampling of media headlines after publication of DeStefano et al.'s paper:

> NBC News: "New study finds no link between 'too many vaccines' and autism."
> Forbes: "Vaccines not linked to autism. Again."
> ACSH: "Yet another study disproves 'autism-vaccine' link. Yawn."
> NPR: "Number of early childhood vaccines not linked to autism."

What all these media reports fail to note is that DeStefano did not actually investigate the relationship between the number of *vaccines* received and autism. Instead, he investigated the relationship between the number of *antigens* received and autism.

The problem is that the Fewer Antigens argument is scientifically misleading, on many levels:

1) the argument is driven entirely by the large number of antigens (3002) assigned to the DTwP vaccine, which was replaced with DTaP in 1997 in the United States.

2) the DTwP antigen count of 3002 is a highball number that was estimated using a very different method than the DTaP antigen count of 5.

3) the available evidence suggests a strongly nonlinear relationship between Destefano's antigen type counts and documented adverse reactions to vaccines and clinical measures of immune system stimulation.

4) the *amount and kind* of antigen may be more important for stimulating the immune system than the total *types* of antigens.

5) last and most important, the narrow focus on antigens ignores the critical role of inflammation in immune system response and the associated increasing use of aluminum adjuvants.

INTRODUCTION: Children Receiving Increasing Numbers of Vaccines

Young children today receive more vaccines against more diseases than they did in the past. From 1983 to present, the cumulative number of vaccines received by 12 month-olds following the recommended U.S. childhood immunization schedule tripled from 11 to 32, and increased by at least a factor of 2 for all other well-baby visit ages (Figure 1). The expansion of the schedule has protected children against a wider variety of infectious diseases, including deadly Haemophilus influenzae type b (Hib) disease, but also has led to concerns that babies are receiving "too many vaccines too soon." Parents have been reassured that the increasingly full childhood schedule does not

overwhelm their babies' immune systems because vaccines today contain fewer antigens and thus are more targeted than in the past (Offit et al. 2002) (DeStefano et al. 2013).

In this analysis, the "fewer antigens" argument is examined critically against empirical evidence and immunological science. Are antigen counts a valid metric of immunologic stimulation and the potential for adverse reactions to vaccines?

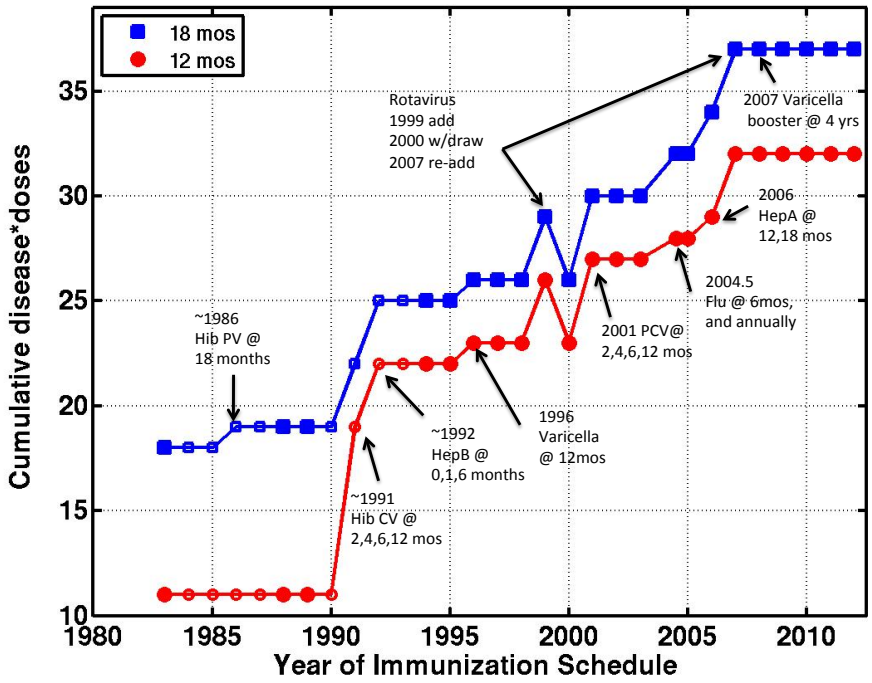

Figure 1. Change over time in cumulative diseases*doses received by 12 month-olds (red curve) and 18 month-olds (blue curve) following the CDC childhood immunization schedule. Annual schedules were obtained from http://www.cdc.gov/vaccines/schedules/past.html#prior-childhood, and are available for 1983, 1988-1989, and 1994-2012. These years are shown as filled large symbols. Educated guesses for the missing years 1984-1987 and 1990-1993 (shown as open small symbols) were made, as indicated by the annotations to the curve. In the case where a range of recommended ages was listed for a vaccine dose, the earliest recommended age is consistently used, e.g., 12-15 months is counted as 12 months. For the disease*dose calculation, DTwP, DTaP and MMR are counted as 3 diseases each and all other vaccines (including PCV7 and PCV13) are counted as a single disease.

INVESTIGATION

Fewer Antigens Argument Driven Entirely by DTwP to DTaP Transition

While the fewer antigens argument is commonly used to imply that *all* vaccines today are designed in a more targeted manner, the argument is driven entirely by the phaseout of a single trivalent vaccine, DTwP (Figure 2, blue curve). In 1997, the diptheria/tetanus/whole cell pertussis (DTwP) vaccine, which contained an estimated 3002 antigens, was replaced with the acellular pertussis DTaP vaccine, which contains only 4-6 antigens (DeStefano et al. 2013). The change was instigated to reduce the number of adverse reactions attributed to the whole cell pertussis component (Decker et al. 1995). The switch from 3 doses of DTwP to 3 doses of DTaP by age 12 months dramatically reduced infants' antigen exposure from ~9000 to ~15. All other vaccines on the recommended schedule contain 24 antigens or less (exception: varicella with 69 antigens) (DeStefano et al. 2013, Table 1). **Thus, Offit and DeStefano's antigen-based reasoning allows vast scope for adding new vaccines to the infant schedule and still staying below the pre-1997 antigen count.** It is notable (although not necessarily particularly

important, as discussed below) that when DTwP and DTaP are excluded from the tally, the cumulative total antigen exposure for 12 month-olds from all other vaccines has increased substantially since 1983, by a factor of nearly 10 (Figure 2, red curve).

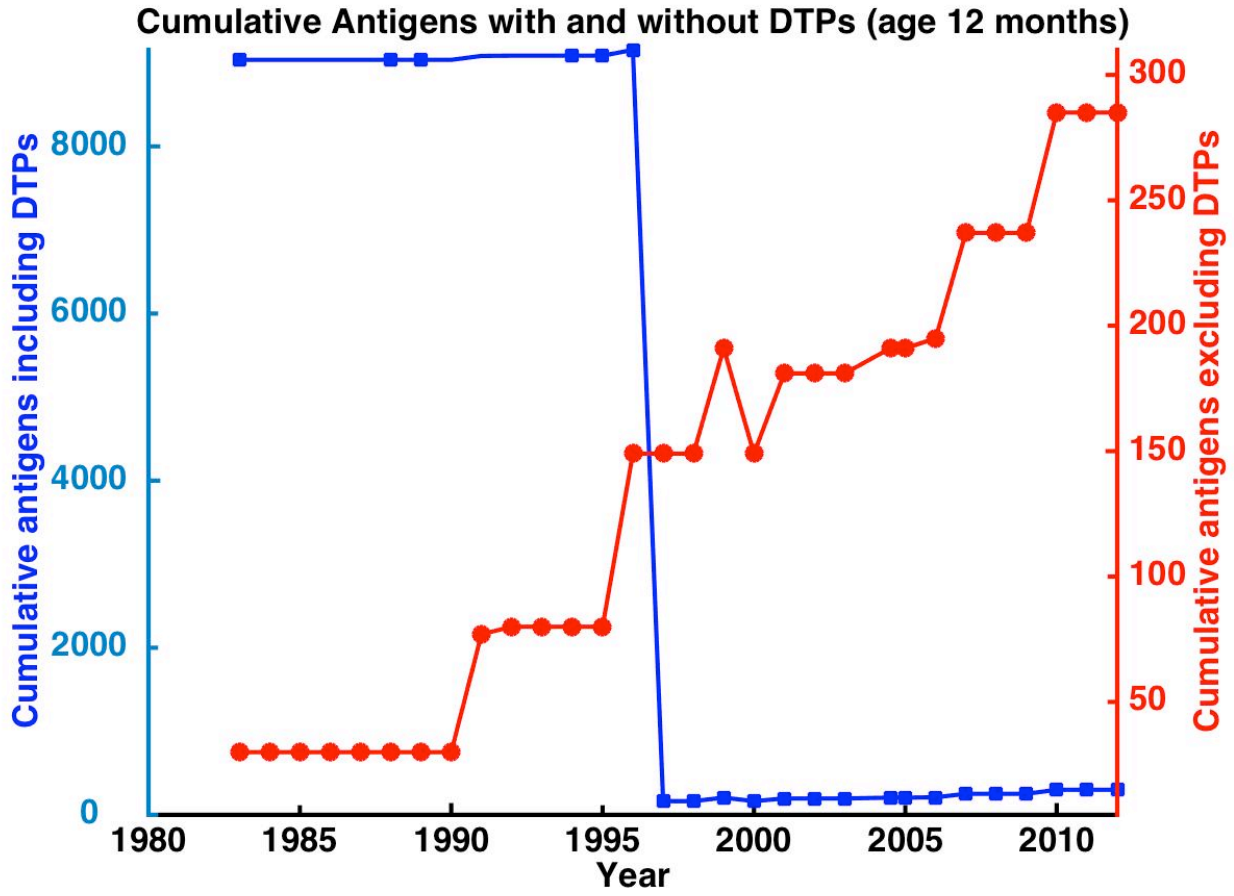

Figure 2. Change over time in cumulative antigens received by 12 month-olds following the CDC recommended childhood immunization schedule. Blue curve shows the change with DTwP and DTaP included. Red curve shows the changes for all vaccines except DTwP and DTaP. The calculations are based on the antigen counts in Table 1 of DeStefeno et al. (2013) applied to the yearly CDC immunization schedules starting in 1983 (http://www.cdc.gov/vaccines/schedules/past.html#prior-childhood).

Different Methods Were Used to Count Antigens in DTwP and DTaP

The antigen counts in the DTwP and DTaP vaccines were determined by very different methods. The DTwP antigen count was estimated by counting the total number of proteins and polysaccharides coded in the pertussis bacteria genome and assuming that each gene produces one antigen. In contrast, the 4-6 antigens assigned to the DTaP vaccine were based on the different types of proteins that were prepared by salt precipitation, centrifugation, and filtration. DTwP clearly did contain many more antigens than DTaP. Still, the method for the DTwP vaccine is almost certainly a highball estimate, since it's not reasonable to assume that every single gene coded in the genome will be expressed or that the human immune system will respond to every single protein or polysaccharide in the pertussis cell.

Available Evidence Does Not Support a Linear Relationship Between Immune Activation and Antigen Count

Using DeStefano's DTwP-dominated antigen counts, young children clearly were exposed to more *types* of vaccine antigens in the past than they are today. However, as shown below, tallying the total number of types of antigens does not provide an accurate measure of a vaccine's ability to stimulate the immune system and cause adverse reactions.

One way to test the linearity assumption is to review the literature on adverse reactions to DTwP vs. DTaP. If adverse reactions scale linearly with antigen count, one would expect a ~600-fold difference in adverse reactions (i.e., 3002/5). An analysis of serious adverse reactions reported to the U.S. Vaccine Adverse Events Reporting System (VAERS) database between 1991 and 2000 found a DTwP/DTaP adverse event ratio of 3.8 for seizures, 2.3 for disabilities, 2.0 for chronic brain damage, and 1.6 for life-threatening reactions (Geier & Geier 2002). The VAERS analysis thus suggests a ratio of 1.6 to 3.8 for serious reactions, which is very different from the ratio of 600 required by the assumptions of the DeStefano study.

Similar adverse reaction ratios were obtained from a controlled clinical study of 2200 infants enrolled in a 3-shot clinical trial of DTwP or DTaP series for 2,4, and 6 month year-olds (Decker et al. 1995). That study examined milder reactions including redness and swelling at the injection site, fussiness, drowsiness and fever. Adverse reaction ratios for DTwP/DTaP generally ranged from < 2.0 to 5. Again, the DTwP/DTaP adverse reaction ratio was nowhere near the factor of 600 required by DeStefano.

The above studies show that DTaP is safer than DTwP, and this likely *is* related to the removal of certain antigens that were in whole cell pertussis. However, it is notable that DTaP is less *efficacious* than DTwP in conferring immunity to pertussis (Klein et al. 2013), again likely due to the removal of some of the whole cell antigens. In fact, the switch to DTaP has led to an unfortunate resurgence of pertussis in highly vaccinated populations (which sometimes is blamed unfairly on parents who decline to vaccinate). The breakthrough cases of pertussis illustrate that some vaccine issues do not necessarily have easy, one-size-fits-all answers and highlight the need for new vaccines that are at once both safer and more efficacious.

Objective clinical measures of immune stimulation, such as C-reactive protein (CRP) or proinflammatory cytokines, provide an additional way to test the antigen linearity assumption. Pourcyrous et al. (2007) measured serum CRP in premature infants following immunization with the following 5 vaccines: DTaP, PVC7, Hib, IPV, and HepB. According to Table 1 of Destefano et al. (2013), the antigen counts for these vaccines are ~5, 8, 2, 15, and 1, respectively. Among these, the Hib vaccine (with only 2 antigens) had the greatest immune-stimulating potential, provoking an abnormal CRP response in 70% of infants tested. In contrast, the IPV vaccine, which at 15 had the largest number of antigens, provoked an abnormal CRP response in 0% of infants tested. Thus Pourcyrous' results argue against a linear relationship, if indeed any relationship, between antigen type counts and immune activation.

Antigen Amount vs. Type

A final problem with the antigen linearity theory is that it is based exclusively on different *types* of antigens. However, the overall *amount* of a given type of antigen may be the more relevant quantity, since white blood cells in the human immune system react strongly when a single type of antigen is expressed repeatedly across a pathogen cell surface (Parham 2009).

A relevant issue is that the immune system may respond more weakly to incremental numbers of antigen types. The Gardasil and Prevnar vaccines provide evidence for this. New antigen types were added to both these vaccines relative to their original formulations, with an apparent need for an increase in the *amount* of the original antigen types to offset the effect of the new additions (Figure 3, Merck 2011, Merck 2015). For example, the change from Prevnar 7 to Prevnar 13 involved a 10% increase in the amounts of each of the original 7 antigen types, while the change from Gardasil 4 to Gardasil 9 involved a 50-100% increase in the amounts of 3 out of 4 of the original antigen types. Also notable is that the amount of aluminum adjuvant was more than doubled from 225 to 500 mcg Al as AAHS in the change from Gardasil 4 to Gardasil 9. The importance of aluminum adjuvants to immune system activation is discussed further below.

Figure 3. Antigen, adjuvant and other components of Gardasil 4 & 9 and Prevnar 7 & 13 vaccines. Source: Merck 2011, Merck 2015

Gardasil 4 (Merck, 2011) Each 0.5 ml vaccine contains: 20 mcg of HPV- 6 L1 protein 40 mcg of HPV-11 L1 protein 40 mcg of HPV-16 L1 protein 20 mcg of HPV-18 L1 protein 225 mcg of aluminum as AAHS 780 mcg of L-histidine 35 mcg of sodium borate 50 mcg of polysorbate 80 <7 mcg of yeast impurities Gardasil 9 (Merck, 2015) Each 0.5 ml vaccine contains: 30 mcg of HPV- 6 L1 protein (increased from 20 mcg) 40 mcg of HPV-11 L1 protein 60 mcg of HPV-16 L1 protein (up from 40 mcg) 40 mcg of HPV-18 L1 protein (up from 20 mcg) 20 mcg of HPV-31 L1 protein 20 mcg of HPV-31 L1 protein 20 mcg of HPV-33 L1 protein 20 mcg of HPV-45 L1 protein 20 mcg of HPV-52 L1 protein 20 mcg of HPV-58 L1 protein 500 mcg of aluminum as AAHS 780 mcg of L-histidine 35 mcg of sodium borate 50 mcg of polysorbate 80 <7 mcg of yeast impurities **Summary**: From Gardasil 4 to Gardasil 9, the total **antigen amount increased from 120 mcg to 270 mcg** and the total **aluminum adjuvant increased from 225 mcg to 500 mcg** of aluminum as AAHS.	Prevnar7 Each 0.5 mL dose is formulated to contain: 2 µg of each saccharide for serotypes 4, 9V, 14, 18C, 19F, and 23F, and 4 µg of serotype 6B per dose (16 µg total saccharide); approximately 20 µg of CRM197 carrier protein; and 125 µg of aluminum as aluminum phosphate adjuvant. http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM137038.pdf Prevnar13 Each 0.5 mL dose of the vaccine is formulated to contain approximately 2.2 µg of each of Streptococcus pneumoniae serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 23F saccharides, 4.4 µg of 6B saccharides, 34 µg CRM197 carrier protein, 100 µg polysorbate 80, 295 µg succinate buffer and 125 µg aluminum as aluminum phosphate adjuvant. http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM201669.pdf **Summary**: From Prevnar 7 to 13, the total **antigen amount increased from 16 mcg to ~30.8 mcg total saccharide** and the total **aluminum adjuvant held steady at 125 mcg** of aluminum as aluminum phosphate.

The Critical Role of Vaccine Adjuvants in Immune System Response

The fewer antigens argument assumes that pathogenic proteins and polysaccharides (i.e., antigens) are the only immune-stimulating components of vaccines. This argument ignores harmful ingredients such as mercury preservative in the flu vaccine and aluminum adjuvants present in many vaccines. The cumulative amount of aluminum, a known neurotoxin (Vera-Lastra et al. 2013), received by children following today's recommended vaccine schedule has increased by about a factor of 3 compared to the schedule of the early 1980s (Tomljenovic & Shaw 2011).

The use of aluminum and other adjuvants, particularly in vaccines that lack whole cell components and contain only isolated toxoids, is needed to activate T cells, which are the primary drivers of the human adaptive immune system. T cell activation requires that the antigen-presenting cell (APC) be in a state of real or perceived inflammation upon detecting the presence of infection. A T cell receptor (TCR)-antigen bond alone is not sufficient to activate a T cell. Rather, T-cell activation requires a co-stimulatory signal from the binding of CD28 and B7 cell surface proteins, in which B7 is expressed on the APC only when the latter detects the presence of a stereotypical pathogen associated molecular pattern (PAMP) or is "tricked" into expressing B7 through the use of a vaccine adjuvant (Parham 2009). Thus, the narrow focus on antigens as the only immune-activating components of vaccines ignores the critical role of inflammation and neurotoxic aluminum adjuvant in immune system response.

Many of the chronic conditions that afflict children today, e.g., autism, asthma, and severe food allergies, are associated with hyperinflammatory and hyperimmune response (Bilbo et al. 2012). It is biologically plausible that increased adjuvant exposure and early immune stimulation may be contributing to the increasing prevalence of these conditions. The CDC should be seriously considering and investigating this hypothesis rather than misleading the public with false and oversimplified arguments about antigens.

CONCLUSION

The available evidence does not support the assumption that immune activation scales linearly with vaccine antigen type count. The fewer antigens argument also ignores the critical role of inflammation in human immune system response and the increased use of neurotoxic adjuvants like aluminum to achieve that state of inflammation. A valuable research goal would be to define a more scientifically defensible measure of immune stimulation that could be quantified objectively. One possible such measure might be the release of pro-inflammatory cytokines such as TNF-α and Il-6, or Il-1β following vaccination (Li et al., 2015).

References

Bilbo, S.D., Jones, J.P. & Parker, W., 2012. Is autism a member of a family of diseases resulting from genetic/cultural mismatches? Implications for treatment and prevention. *Autism Res Treat*, vol. 2012, article ID 910946. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22928103.

Decker, M.D. et al., 1995. Comparison of 13 Acellular Pertussis Vaccines: Adverse Reactions. *Pediatrics*, Supplement, pp.557–565.

DeStefano, F., Price, C.S. & Weintraub, E.S., 2013. Increasing Exposure to Antibody-Stimulating Proteins and Polysaccharides in Vaccines Is Not Associated with Risk of Autism. *J. Pediatr.*, 163(2), pp. 561-7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23545349.

Geier, D.A. & Geier, M.R., 2002. Serious neurological conditions following pertussis immunization: an analysis of endotoxin levels, the vaccine adverse events reporting system (VAERS) database and literature review. *Pediatric Rehabilitation*, 5(3), pp.177–182.

Klein, N.P. et al., 2013. Comparative effectiveness of acellular versus whole-cell pertussis vaccines in teenagers. *Pediatrics*, 131(6), pp.e1716–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23690518.

Li, Q. et al., 2015. Neonatal vaccination with bacillus Calmette-Guerin and hepatitis B vaccines modulates hippocampal synaptic plasticity in rats. *J. Neuroimmunology,* 288, pp. 1-12.

Merck 2011. Gardasil, Highlights Of Prescribing Information: https://www.merck.com/product/usa/pi_circulars/g/gardasil/gardasil_pi.pdf

Merck 2015. Gardasil 9, Highlights Of Prescribing Information: https://www.merck.com/product/usa/pi_circulars/g/gardasil_9/gardasil_9_pi.pdf

Offit, P.A. et al., 2002. Addressing parents' concerns: Do multiple vaccines overwhelm or weaken the infant's immune system? *Pediatrics*, 109(1), pp.124–129.

Parham, P. (Stanford U.), 2009. *The Immune System* 3rd ed., New York, NY: Garland Science, Taylor & Francis Group, LLC.

Pourcyrous, M. et al., 2007. Primary immunization of premature infants with gestational age <35 weeks: cardiorespiratory complications and C-reactive protein responses associated with administration of single and multiple separate vaccines simultaneously. *J. Pediatr.*, 151(2), pp.167–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17643770.

Tomljenovic, L. & Shaw, C.A., 2011. Do aluminum vaccine adjuvants contribute to the rising prevalence of autism?

J Inorg Biochem, 105(11), pp.1489–1499. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22099159.

Vera-Lastra, O. et al., 2013. Autoimmune/inflammatory syndrome induced by adjuvants (Shoenfeld's syndrome): clinical and immunological spectrum. *Expert review of clinical immunology*, 9(4), pp.361–73. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23557271.