HUMAN VACCINES & IMMUNOTHERAPEUTICS
2016, VOL. 12, NO. 9, 2391–2402
http://dx.doi.org/10.1080/21645515.2016.1183077

Taylor & Francis
Taylor & Francis Group

REVIEW

# Effect of antipyretic analgesics on immune responses to vaccination

Ezzeldin Saleh[a], M. Anthony Moody[b], and Emmanuel B. Walter[c]

[a]Department of Pediatrics, Division of Pediatric Infectious Diseases, Duke Clinical Vaccine Unit, Duke University School of Medicine, Durham, NC, USA; [b]Duke Human Vaccine Institute, Department of Pediatrics, Division of Pediatric Infectious Diseases, Duke University School of Medicine, Durham, NC, USA; [c]Duke Clinical Vaccine Unit, Department of Pediatrics, Divisions of Primary Care and Pediatric Infectious Diseases, Duke University School of Medicine, Durham, NC, USA

**ABSTRACT**

While antipyretic analgesics are widely used to ameliorate vaccine adverse reactions, their use has been associated with blunted vaccine immune responses. Our objective was to review literature evaluating the effect of antipyretic analgesics on vaccine immune responses and to highlight potential underlying mechanisms. Observational studies reporting on antipyretic use around the time of immunization concluded that their use did not affect antibody responses. Only few randomized clinical trials demonstrated blunted antibody response of unknown clinical significance. This effect has only been noted following primary vaccination with novel antigens and disappears following booster immunization. The mechanism by which antipyretic analgesics reduce antibody response remains unclear and not fully explained by COX enzyme inhibition. Recent work has focused on the involvement of nuclear and subcellular signaling pathways. More detailed immunological investigations and a systems biology approach are needed to precisely define the impact and mechanism of antipyretic effects on vaccine immune responses.

**ARTICLE HISTORY**
Received 18 February 2016
Revised 10 April 2016
Accepted 22 April 2016

**KEYWORDS**
antibody responses; antipyretic analgesics; immune responses; NSAIDs; prophylaxis; vaccination

## Introduction

Antipyretic analgesics are widely used around the time of vaccination to ameliorate fever and pain.[1,2] They have been shown to decrease vaccine reactogenicity,[3-5] and until recently have not been associated with decreased vaccine immunogenicity.[6-8] However, an open label, randomized study by Prymula et al. demonstrated that while acetaminophen (paracetamol) prophylaxis significantly reduced fever following routine childhood immunization, it simultaneously blunted the immune response to several vaccine antigens.[9] In this study, infants receiving primary immunization were divided into two groups, a prophylaxis group who received acetaminophen and a control group. The same allocation was maintained during the booster "secondary" immunizations. The primary purpose of the study was to assess the effect of antipyretics in reducing fever and other vaccine related reactogenicity, but the preliminary immunogenicity report showed significantly reduced antibody levels in the prophylaxis group. This finding resulted in the rejection of the prevailing notion that prophylactic antipyretic use around the time of vaccination is harmless. Furthermore, this prompted discontinuation of enrollment in a placebo-controlled randomized trial of acetaminophen given for prevention of post-vaccine fever in infants.[10] To date, routine administration of antipyretics around the time of vaccination is discouraged by many.[11] Despite this, the current CDC Vaccine Information Statement (VIS) for DTaP instructs caregivers to use antipyretics at time of vaccination and for the next 24 hours to reduce fever and pain; however, this has not been updated since its publication in 2007.[12] The American Academy of Pediatrics in 2010 stated that more studies are needed to explore the clinical impact of antipyretics on vaccination and recommended discussing risks and benefits of prophylactic or therapeutic antipyretics with parents.[13] In a recent policy statement WHO advised against administration of prophylactic oral analgesics due to lack of evidence of effectiveness and/or the potential for affecting vaccine response.[14]

The focus of this review is to evaluate previous work exploring the effects of antipyretic analgesics on the immune responses following vaccination. A recent review by Das et al. examined the clinical studies that investigated the effect of prophylactic antipyretic analgesics on post-vaccination adverse reactions and antibody response to vaccination.[15] However, their analysis was restricted to children 6 years or less and did not discuss in vitro or laboratory studies.[15] Due to the paucity of clinical trials and studies examining this question, we expanded our review of the literature to cover clinical studies of all age groups, including pediatric and adult populations; in addition we reviewed in vivo and in vitro laboratory studies to explore potential mechanisms that could explain blunting of the immune response by antipyretic analgesics.

## Historical perspective

The role of non-steroidal anti-inflammatory drugs (NSAIDs) in modulating immune responses was first investigated decades ago. In 1922, Homer Swift tested the hypothesis that salicylates

**CONTACT** Ezzeldin Saleh, MD ezzeldin.saleh@duke.edu Department of Pediatrics, Division of Pediatric Infectious Diseases, Duke Clinical Vaccine Unit, Duke University School of Medicine, 2608 Erwin Road, Suite 210, Durham, NC 27705, USA.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/khvi.

may induce an antibody response, termed "immune bodies," following exposure to live and killed bacterial antigens.[16] He used strains of viridans streptococci and *Streptococcus pneumoniae* type 1 antigens as these bacteria were thought to be the etiologic agents for rheumatic fever at that time. He administered the antigens intravenously to rabbits treated with salicylates (given via gastric tube) and untreated controls. In these experiments, salicylates adversely affected antibody formation. Direct action of the salicylates on the antigen was implicated as rabbits that received antigen pre-incubated with salicylate had the lowest antibody response when compared to both rabbits that received antigen and oral salicylate without pretreatment or untreated controls.

Following Swift's report, no further studies appeared in the literature until after the discovery of prostaglandins and demonstration of their significant role in the regulation of inflammatory and immune responses. Numerous studies were done exploring the effects and mechanisms by which prostaglandins and their inhibitors affect the immune system.[17,18] Most of this pioneering work was done in animal models and focused on antibody production, although the results were often contradictory.[19-22]

Ambrose in 1966 studied the effect of salicylate on secondary antibody response in rabbits injected with BSA (bovine serum albumin) and diphtheria toxoid. Salicylate suppressed immunoglobulin production in a dose-dependent manner.[23] Similarly, salicylate inhibited thymidine incorporation and decreased the number of antibody forming cells found in spleen cells cultured from chickens immunized with sheep red blood cells (SRBCs).[24] However, other investigators reported differing results. Webb et al. injected mice with indomethacin 24 and 2 hours before injecting SRBCs, observing a block in splenic prostaglandin (PG F2$\alpha$) production associated with an increase in the number of antibody forming cells. Acetaminophen added to human peripheral blood lymphocytes in culture at concentrations of 2.5 to 300 mcg/mL resulted in increased responses to mitogen-induced blastogenesis; however these responses were inhibited by increasing the drug level to higher concentrations ($>$ 400 mcg/mL). Exposure of lymphocytes to the drug before mitogen stimulation did not result in increased responses.[25]

In 1978, Goodwin and colleagues published the first randomized, open-label controlled study to examine the effect of antipyretics on immune response following vaccination in healthy human subjects.[26] In this study, 15 healthy males and females were given indomethacin 25 mg orally for 12 days, starting one day before immunization with bivalent influenza vaccine (A/New Jersey and A/Victoria). A control group of 15 individuals matched by age and sex were similarly vaccinated without receiving indomethacin. Antibody titers were measured by hemagglutination inhibition (HAI) in both groups. In the indomethacin group, antibody titers to A/Victoria were increased compared to the controls (mean increase in tube dilution of $1.5 \pm 0.4$ versus $0.7 \pm 0.2$, $p < 0.025$), whereas titers to A/New Jersey were slightly lower ($2.2 \pm 0.6$ vs. $2.5 \pm 0.5$, not statistically significant). Baseline titers before vaccination indicated that about 90% of the subjects already had antibody titers to A/Victoria (mean titer between 1:20 and 1:40), whereas none had detectable titers to A/New Jersey ($\geq$ 1:10). In this study, indomethacin enhanced the antibody response to the A/Victoria strain that the participants had previously been exposed to but not to the novel A/New Jersey strain. Original antigenic sin might explain this result whereby increased antibody production to the older strain is produced at the expense to that of novel strains.[27,28] However, it is important to note that this phenomenon is controversial with some contradictory reports available.[29]

## Methods

This review is not a meta-analysis in that data were not combined between studies and subjected to additional statistical analysis. The literature search was performed by one reviewer and analyzed by all authors. Our review of the literature was not limited by year and included only English language reports. The search included the use of two electronic bibliographic databases, PubMed/MEDLINE and Embase. Search key words were used in a MeSH Terms and truncation strategy and included: (immunization OR vaccine), AND (antipyretics OR acetaminophen OR paracetamol OR ibuprofen OR aspirin OR anti-inflammatory agents, non-steroidal). For both databases keywords were mapped to appropriate subject headings. Only empirical studies were reviewed; case reports, letters to the editor, policy statements were excluded. A total of 1395 papers were screened based on title and abstract, of which 73 were examined for eligibility and 20 papers, representing clinical trials, were identified for review (including two abstracts identified through other sources). Additionally, we performed a manual review of historical and current in vitro and in vivo laboratory studies to explore the mechanistic effect of antipyretic analgesics on postvaccination immune response.

## Antipyretic effects on post-vaccination immune response

### *Studies reporting antipyretics used as a primary intervention*

#### *Studies done before the prymula 2009 publication*

Eight interventional clinical studies, published before the 2009 Prymula paper, investigated the effect of prophylactic antipyretic analgesics on vaccine immune response. While the majority of these studies used acetaminophen as the antipyretic,[5,6,30,31] the use of indomethacin,[26,32] piroxicam[33] and acetylsalicylic acid[34] were also evaluated [Table 1]. Influenza vaccines were used in five of the seven studies involving adult participants,[5,30,31] with pneumococcal and hepatitis B vaccines being the other two; whereas the only pediatric study evaluated diphtheria, tetanus and whole cell pertussis (DTP) vaccine[6] [Table 1].

We found only one study that replicated the findings of Goodwin et al., where an increase in measurable antibody production was observed after influenza vaccination (A/Taiwan, A/Beijing and B/Panama strains) of healthy adults $\geq$ 65 years, who were randomized to receive acetylsalicylic acid 300 mg or placebo on days 1, 2, 3, 5, and 7.[34] Influenza specific antibodies for the 3 strains were measured by ELISA at 3 weeks postvaccination, showing that a 4-fold or greater rise in antibodies to the

**Table 1.** Randomized Clinical Studies investigating effect of prophylactic antipyretic analgesics on postvaccination immune response.

| Author Year (ref) | Design, Setting, Subjects age, N | Reported Antipyretic, schedule | Vaccine/s | Measured outcomes, immune correlates or seroprotection cut-off | Reported significant difference in antibody response |
|---|---|---|---|---|---|
| Walter 2015 (41) [Abstract] | RCT, double blind, placebo, (USA), 12–35 mo, N = 40 | Acetaminophen 0, q 4–6 hrs for 24 hrs | Influenza (IIV3) | HAI ≥ 40 | |
| Wysocki 2014 (39) [Abstract] | RCT, Open label, placebo (Poland), infants, N = 800 | Paracetamol[a], Ibuprofen 0, 6–8 hrs, then q 6–8 hrs [with each vaccination] | Prevnar-13 DTaP/IPV/Hib/HBV [Primary series and booster] | Pneumococcal anticapsular IgG | |
| | | | | Pertussis FHA and tetanus IgG | Decreased pneumococcal antibody GMCs (for 5 of 13 serotypes) with Paracetamol prophylaxis. Decreased pertussis and tetanus toxins antibody GMCs with Ibuprofen prophylaxis. |
| Doedeé 2014 (38) | RCT, Open label, placebo, (Netherlands), Young adults ≥18 yr, N = 496 | Paracetamol Prophylaxis:0, 8, 16 hrs; Treatment: 6, 14, 22 hrs[ with first 2 vaccine doses] | Hepatitis B vaccine [3-dose series] | Anti-HBs (≥10 IU/L) | Decreased anti-HBs levels after 3rd dose in prophylaxis group. Antibody levels were protective in all groups. No baseline antibody levels. |
| Prymula 2014 (36) | RCT, Open-label (Czech Republic, Italy, Hungary, Chile, Argentina), 2 mo, N = 558 | Paracetamol Prophylaxis: 0, 4–6 hrs, 8–12 hrs [with each vaccination] | 4 CMenB DTaP-HBV-IPV/HiB PCV7 MenC [Primary series and booster] | hSBA, Ab to fHbp, NadA, NZ OMV (titer ≥ 5) DT & TT Ab ≥0.1 IU/mL. HepB = 10 mIU/mL. Polio virus type1, 2, 3: 1:8 dilution, Pneumococcal Abs serotypes 4, 6B, 9V, 14, 18C, 19F, 23F ≥ 0.35 ug/mL, Pertussis Abs. | |
| Prymula 2013 (35) | RCT, Open label, (Czech Republic), 31–44 mo and 40–48 mo, N = 443 | None in this study (Follow up to Prymula 2009) | 10-valent Pneumococcal non- typable *H. influenzae* protein D conjugate (PHiD-CV) | Anti-pneumococcal serotype-specific total IgG. Pneumococcal opsonophagocytic titers (opsonic titer = 8)[b], Anti-protein D GMC Nasopharyngeal swab cultures | |
| Prymula 2009 (9) | RCT, Open label,(Czech Republic)16 wks at enrolment, 12–15 mo at booster, N = 459 | Paracetamol0, q 6–8 hr for 24 hrs [with each vaccination] | 10-valent Pneumococcal non-typable *H. influenzae* protein D conjugate (PHiD-CV) DTaP-HBV-IPV/Hib Rotavirus [Primary series and booster] | Anti-pneumococcal IgG ≥ 0.2 μg/mL, Pneumococcal opsonophagocytic titers = 8, Anti-PRP ≥ 0.15 μg/mL, Antidiphtheria ≥ 0.1 IU/mL, Antitetanus ≥ 0.1 IU/mL, Anti-PT ≥ 5ELU/mL, Anti-FHA ≥ 5 ELU/mL, Anti-pertactin ≥ 5 ELU/mL, Anti-HBs ≥10 mIU/mL, Anti-polio ≥ 1:8, Anti-rotavirus IgA ≥ 20 U/mL[c] | Primary series: Decreased antibody responses to all vaccines except for Polio; decreased antipneumococcal GMCs (all 10 serotypes), protein D, anti-PRP, anti-DT, anti-TT and anti-pertactin in prophylaxis group. Booster series: Decreased GMCs for anti-pneumococcal (all except 19F), anti-tetanus, anti-protein D in prophylaxis group. |
| Gross 1994 (31) | RCT, Placebo(USA), 73–88 yrs, N= 80 | Acetaminophen 0, q 6 hrs × 2 days | Influenza IIV3 | Influenza HAI ≥ 40 | |
| Hsia 1994 (34) | RCT double-blind-placebo (USA), ≥ 65 yrs, N=281 | Acetylsalicylic acid day 1,2,3,5 and 7 | Influenza IIV3 | Serum specific antibody for 3 Influenza strains by ELISA-4 fold riseBlastogenic and interleukin-2 response | Increased A/Beijing influenza antibody titers (4-fold rise) among acetylsalicylic acid group compared to placebo -more marked in >75 yrs. |

(*Continued on next page*)

**Table 1.** (*Continued*)

| Author Year (ref) | Design, Setting, Subjects age, N | Reported Antipyretic, schedule | Vaccine/s | Measured outcomes, immune correlates or seroprotection cut-off | Reported significant difference in antibody response |
|---|---|---|---|---|---|
| Chernesky 1993 (30) | RCT double-blind placebo (Canada), ≥ 65 yrs, N = 185 | Acetaminophen 0, 6 hrs | Influenza IIV3 | Influenza HAI ≥40 | |
| Aoki 1993 (5) | RCT, double blind, placebo, (Canada), 27–48 yrs, N = 262 | Acetaminophen 0, 4, 8, 12 hours | Influenza IIV3 | Influenza HAI ≥ 40 ≥, 4-fold change in serum HAI | |
| Uhari 1988 (6) | RCT, double blind, placebo, (Finland) 5 mo, N = 233 | Acetaminophen 4 hrs after vaccination | DTPDTP-IPV | Diphtheria, Pertussis, Tetanus IgG by enzyme immunoassay. | |
| Ceuppens 1987 (33) | Double blind, placebo (Belgium), 22–26 yrs, N = 50 | Piroxicam Daily, 3 days before each vaccination and 7 days after [with each vaccination] | Hepatitis B vaccine [3-dose series] | Anti-HBs >10 U/mL by RIA, Immune complexes by RIA, Lymphocyte subpopulations/activation markers/functional studies | |
| Lafferty 1981 (32) | Open label, controlled (USA), ≥ 65 yrs, N = 40 | Indomethacin Day of vaccination for 5 days | 14-valent Pneumococcal polysaccharide vaccine | Antibodies to pneumococcal polysaccharides by RIA | |
| Goodwin 1978 (26) | Open label, controlled (USA), Healthy adults N = 30 | Indomethacin - 48 hrs., 0, then q 4 hrs for 12 days | Influenza IIV2 | Influenza HAI | Increased antibody titers to A-Victoria (strain with high prevaccination titers). No similar increase to novel A-New Jersey strain. |

[(a)]Paracetamol: international nonproprietary name for acetaminophen.
[(b)]Pneumococcal Opsonophagocytic titers measured by killing assay using HL60 cell line.
[(c)]Anti-polio (1,2 or 3) measured by microneutralization; all other antibodies levels were measured by ELISA.
***Abbreviations by cited order***: N: number of study participants; RCT: Randomized Controlled Trial; IIV3: Trivalent Inactivated Influenza vaccine; HAI: Hemagglutination inhibition; DTaP: Diphtheria, tetanus and acellular pertussis vaccine; IPV: Inactivated polio vaccine; Hib: *Haemophilus influenza*e type B; HBV: Hepatitis B virus vaccine; FHA: filamentous hemaglutnin; GMC: geometric mean concentration; AntiHBs: anti-hepatitis B surface antigen; 4CMenB: Meningococcal type B vaccine; PCV7: Pneumococcal pentavalent vaccine ; MenC; Meningococcal vaccine type C; hSBA: Serum complement bactericidal activity; fHbp: factor H binding protein; NadA: Neisserial adhesion A; NZOMV: New Zealand strain outer membrane vesicles; DT: Diphtheria toxoid; TT: Tetanus toxoid; PRP: polyribose ribitol phosphate, a Hib antigen; ELISA: Enzyme -linked Immunosorbent Assay; IIV2: Bivalent Inactivated Influenza vaccine. RIA: Radioimmunoassay.

A/Beijing strain occurred more frequently among the acetylsalicylic acid recipients ($p < 0.05$), and was conspicuously evident in those ≥ 75 years of age ($p < 0.01$). However, in vitro studies showed a different effect with a ≥ 3-fold increase in influenza-antigen-stimulated [$^3$H]-thymidine incorporation (following incubation with A/Beijing strain) occurring more commonly in the placebo group compared to the acetylsalicylic group (25% vs. 16%), and there was no observed difference in antigen-stimulated IL2 production[34] Another study done during the same influenza season (1991–1992) in elderly outpatients and nursing home residents, with a mean age of 80 years, who were randomized to receive either acetaminophen or placebo, showed no effect of acetaminophen on influenza HAI.[31]

Two randomized, double blind placebo controlled studies were done in Canada and examined immune responses following vaccination with trivalent inactivated influenza vaccine (A/Taiwan/1/86 [H1N1], A/Shanghai/16/89 [H3N2] & B/Yamagata/16/88) during the 1990–1991season.[5,30] Both studies evaluated the use of prophylactic acetaminophen with the first dose given at the time of vaccination. One study enrolled 474 healthcare workers who were randomized to three groups: 1) half dose acetaminophen (162.5 mg), 2) full dose acetaminophen (325 mg), or 3) placebo. Study medications were given in four consecutive doses at 4-hour intervals.[5] The other study randomized 100 healthy adults ≥ 65 years old attending outpatient clinics into two groups, receiving either acetaminophen 975 mg every 6 hours for two doses or placebo.[30] HAI titers in both studies were judged to be protective and there was no difference in antibody response detected between the treatment and control groups.

Two of the early studies, separately evaluated antipyretic analgesics use in adults receiving antigens other than influenza vaccine.[32,33] Firstly, 14-valent pneumococcal polysaccharide vaccine was given to 40 healthy adults ≥ 65 years old, who were divided into indomethacin prophylaxis (25 mg four times daily for five days after vaccination) and control group.[32] Baseline and postvaccination antibody levels were similar, and did not correlate with absolute lymphocyte count, delayed hypersensitivity testing or response to phytohemagglutinin.[32] Secondly, in a double blinded placebo controlled study, the hepatitis B vaccine series were given to 50 healthy subjects 22–26 years old previously unimmunized and who had no evidence of hepatitis B infection pre and postvaccination.[33] Prophylaxis with daily piroxicam was given daily for 10 days starting three days before each vaccine dose. Similarly, antibody levels obtained after each vaccination were comparable between the two groups, as well as peripheral lymphocyte subpopulations, activation markers and functional studies.[33]

The single randomized controlled pediatric study was reported by Uhari and colleagues in 1988.[6] It included healthy, five-month-old children who received either a single acetaminophen dose or a placebo 4 hours after vaccination with DTP or DTP-inactivated polio vaccine. Antibody titers to diphtheria and tetanus toxoids and pertussis antigens measured 6 weeks

later did not differ significantly between the groups; however, there were no pre-immunization baseline levels measured. Our search algorithm identified no subsequent randomized controlled pediatric studies addressing the question of antipyretic effects on vaccine immune response until the Prymula study in 2009.

### Prymula 2009 publication and later studies

Prymula and colleagues in 2009 reported a randomized open-label parallel group study that included healthy infants 9–16 weeks old at enrollment; the infants received their first set of primary immunizations (at 3, 4, and 5 months of age) and then booster doses at 12 to 15 months of age.[9] Children were randomized to an acetaminophen (paracetamol) prophylaxis group or a control group (no placebo or drug). Prophylactic acetaminophen total daily dosage of 40–50 mg/kg was administered in the first 24 hours. Study staff administered the initial dose immediately after each vaccination and parents gave second and third doses at home every 6 to 8 hours. All doses were given rectally. The vaccine antigens evaluated included the following: 10 pneumococcal capsular polysaccharides (serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F) included in the pneumococcal non-typeable *H. influenzae* protein D-conjugate vaccine (PHiD-CV); diphtheria toxoid; tetanus toxoid; pertussis antigens including pertussis toxoid, filamentous haemagglutinin, and pertactin; hepatitis B surface antigen; polio virus type 1, 2, and 3 antigens; and *Haemophilus influenzae* type b polysaccharide (polyribosylribitol phosphate, PRP) contained in the hexavalent diphtheria-tetanus-3 component acellular pertussis (DTaP), inactivated hepatitis B (HBV), inactivated poliovirus (IPV) types 1, 2 and 3, *H. influenzae* type b (Hib) vaccine. In addition, anti-rotavirus IgA levels were used as the immune correlate for oral rotavirus vaccination.

Baseline antibody levels were similar between the prophylaxis and no prophylaxis groups. One month following the primary vaccination series, blunting of antibody responses was noted among children receiving acetaminophen prophylaxis. Antibody geometric mean concentrations (GMCs) to all 10 pneumococcal serotypes were significantly lower in the prophylaxis group. However, the correlate of protection level of $\geq$ 0.2 mcg/mL was similar for all pneumococcal serotypes in the two groups, except for serotype 6B, which was significantly lower in the prophylaxis group. Protective opsonophagocytic titers (> 8 dilutions) for pneumococcal serotypes 1, 5 and 6B were significantly lower in the prophylactic group. Seroprotection rates against *Haemophilus influenzae* type b and GMCs of antibodies to diphtheria, tetanus, and pertactin were also significantly lower in the prophylaxis group.

Antibody levels were again measured before and one month after booster vaccination. Pneumococcal antibody GMCs, opsonophagocytic activity geometric mean titers (GMTs) and seropositivity rates were lower in the acetaminophen prophylaxis group for most serotypes except 9V. Antibody concentrations for all other antigens were similar after vaccine boost, except for lower levels found against tetanus in the prophylaxis group. A post hoc analysis indicated the reduced antibody levels in the prophylaxis group occurred regardless of the presence or absence of fever. Additionally, upon review of previous vaccine trials, the authors confirmed a similar reduction in responses to all pneumococcal serotypes (except serotype 14) in children group who received prophylaxis on the day of vaccination.

In 2013, Prymula et al. reported a follow up study evaluating the effect of acetaminophen on the long term persistence and boosting of antibody as well as the rate of nasopharyngeal carriage of *S. pneumoniae and H. influenzae*. Children in the acetaminophen group in the 2009 study were compared to controls after the groups received a booster dose of 10-valent pneumococcal capsular polysaccharide non-typeable *Hemophilus influenzae* conjugate vaccine at 40 to 48 months of age.[35] The group who received acetaminophen prophylaxis had lower titers prior to the boost, but both groups had similar robust increase in titers following the boost. The blunted response observed with the primary immunization was not persistent, suggesting that there was no adverse effect on memory B cells. Also, there was no difference in nasopharyngeal carriage rates for non-typeable *H. influenzae* or other tested bacteria, suggesting that the observed differences in antibody levels may not be clinically significant.

In a separate randomized open label controlled phase 2 study reported in 2014, Prymula et al. evaluated the immunogenicity and reactogenicity of multicomponent meningococcal serogroup B vaccine (MenB-4C) given together with routine childhood vaccinations (DTaP-HBV-IPV/Hib and PHiD-CV). Healthy children enrolled at 2 months were randomized to receive acetaminophen or no analgesic at each vaccination, and the children received 3 doses of primary immunizations at 2, 3, and 4 months of age and a fourth dose of PHiD-CV at 12 months. In contrast to the findings for other studies noted above, prophylactic acetaminophen did not impact the antibody response to any of the tested antigens post-vaccination.[36] This study reported higher rate of fever ($\geq$ 38°C) when MenB-4C was co-administered with combination vaccines, and that prophylactic acetaminophen use effectively decreased fever. Accordingly, guidelines adopted in 2015 by UK Joint Committee on Vaccination and Immunisation (JCVI) recommended prophylaxis with acetaminophen to infants under 12 months of age when MenB-4C vaccine is co-administered with other routine vaccines at 2 and 4 months.[37]

The 2009 Prymula study found that antibodies against hepatitis B surface antigen (anti-HBs) were comparable in the prophylaxis and no prophylaxis groups. In contrast, a controlled, open label study in adults reported blunting of the antibody response to HBV with acetaminophen prophylaxis.[38] Healthy young adults ($\geq$ 18 years) were randomly assigned to receive no drug or acetaminophen for 48 hours, either as prophylaxis (first dose at vaccination) or as treatment (first dose 8 hours after vaccination). HBV was administered in a three dose series, at 0, 1 and 6 months. None of the participants used acetaminophen around the third dose. Anti-HBs levels were measured immediately before and one month after the third dose. No baseline antibody levels were measured; however, to reduce the chance that a participant had a prior vaccine series, those individuals who had anti-HBs > 10,000 mIU/mL prior to the third dose had their vaccination records reviewed and individuals were excluded if they had previously received HBV. Anti-HBs after the third dose were significantly lower in the prophylactic acetaminophen group compared with the no drug group (4257 mIU/mL vs. 5768 mIU/mL, respectively; $p = 0.048$), while no

difference between the therapeutic acetaminophen and control groups was observed ($p = 0.34$). While the result showed blunting of vaccine response, all groups had seroprotective antibody levels at series completion, and this study was not able to examine the response following the first dose since anti-HBs were not measured.

Wysocki et al. recently investigated effect of acetaminophen and ibuprofen prophylaxis.[39] In their placebo-controlled study, 908 healthy infants receiving primary vaccines (PCV-13, DTaP/IPV/Hib/HBV) were randomized to five groups—two groups received either acetaminophen or ibuprofen at vaccination, two groups received one or the other drug 6–8 hours after each vaccination, and the fifth control group received no medications. The study was limited as no baseline serologies were obtained. Following the primary series, pneumococcal anticapsular IgG GMCs were significantly lower in the acetaminophen prophylaxis group compared to the control group for 5 of 13 serotypes. Similarly, pertussis filamentous hemagglutinin and tetanus IgG GMCs were significantly lower among the ibuprofen prophylaxis group compared to the control group after the primary series. Notably, there were no differences observed for antibody responses to any antigen after the toddler vaccine dose.

Sponsored by the Centers for Disease Control and Prevention Clinical Immunization Safety Assessment Project, Duke University is currently conducting a double blind, placebo-controlled study to assess the effect of prophylactic antipyretics on immune responses and rates of fever after inactivated influenza vaccine (IIV) in children 6 through 47 months of age.[40] The study groups include blinded therapy with prophylactic acetaminophen or placebo immediately following and every 4 to 6 hours in the 24 hours after receipt of IIV, or open-label ibuprofen immediately following and every 6 to 8 hours in the 24 hours after IIV receipt. The preliminary pilot data from 40 children, randomized to receive either acetaminophen or placebo, did not show a difference in antibody responses to three influenza antigens as measured by HAI.[41]

### *Observational studies reporting antipyretic use*

In addition to the controlled studies summarized above, there have been several observational studies describing the effect of antipyretic prophylaxis or treatment around vaccination time on vaccine immune responses [Table 2]. These studies primarily assessed the immunogenicity and reactogenicity of different vaccines and did not include antipyretic analgesics as a prespecified intervention. Antipyretic analgesic use was either included in post hoc analysis or derived from reported use by caregivers.

In a longitudinal study of safety and immunogenicity of a 3- versus 4-dose immunization schedule for DTP, parents were instructed to record temperature, adverse reactions and use of acetaminophen during four intervals (0–6 hours, 7–12 hours, 13–24 hours, 25–48 hours). Acetaminophen was not given prophylactically; however parents were instructed to use as needed for fever, pain, or local reactions. Results showed 73.5% of children received acetaminophen at least once within 48 hours of vaccination, and 2.1% were given it prophylactically in a time period when no adverse reactions were recorded.[7]

In a trial comparing acellular versus whole cell pertussis vaccine in children 15–24 months and 4–6 years of age, antipyretics were not given prophylactically but parents were called 3–7 days after vaccination to obtain information about reactions and medication use.[42] Children who received whole cell vaccine had significantly higher rate of reactions and also reported a higher rate of use of acetaminophen compared to the acellular pertussis group (53% vs. 12%, $p < 0.00001$)[42]; another study comparing the two vaccines among 15–20 month old children reported rates of 63% versus 31% for use of acetaminophen when parents were contacted by phone at 1, 3, and 14 days after vaccination.[43]

Similarly, in another study, prophylaxis with acetaminophen for fever was recommended in study centers in Alberta and British Columbia but not in Quebec. As expected the use of prophylactic acetaminophen was significantly higher in the study centers where recommended. Prophylactic use was 93.4% and 80.6% in Alberta and British Columbia, respectively, and 53.8% in Quebec. The use of acetaminophen in the first 24 hours following vaccination increased by 3.6%, 12.4% and 25.2%, respectively, among the centers.[44] Interestingly, despite the differences in acetaminophen usage, the frequency of adverse reactions was not different among the three centers.

A study in the UK evaluated reactogenicity and immunogenicity of adjuvanted split virion and non-adjuvanted whole virion H1N1 (2009) pandemic influenza vaccine among healthy children 6 to 12 years of age. Antipyretic use was 36.5% and 28.4% for the first and second doses of adjuvanted vaccine, respectively; compared to 22.1% and 16.6% for the first and second doses of the non-adjuvanted whole virion vaccine. Acetaminophen or ibuprofen use on day 0 or 1 after the first or second dose of either vaccine did not affect antibody titer regardless of whether fever was included in the regression analysis.[8] Similarly, recent results of a meta-analysis of four randomized trials of monovalent H1N1 (2009) pandemic influenza vaccine in adults, found no significant difference in hemagglutinin inhibition titers in low-dose aspirin users (43% of the study subjects) compared to non-users.[45] This study evaluated multiple vaccine formulations and medications use was self-reported by subjects.

Reported use of prophylactic antipyretics in these studies varied significantly, ranging from as little as 2% to more than 90%. All the studies concluded that antipyretic use had no effect on antibody responses following immunization.

## The relationship between novel antigen exposure, timing of antipyretic use and vaccine response

The timing of antipyretic analgesic administration, and vaccination with novel antigens appear to be important determinants of humoral immune response following vaccination. In all studies that reported decreased immunogenicity with antipyretic prophylaxis, the significant negative impact on immune response was evident only when antipyretics were given at time of vaccination and not when they were given as a treatment thereafter.[9,38,39] Results from in vitro studies support this observation. In a rabbit spleen culture system, antibody suppression by salicylates was noted mainly during the inductive phase of the culture system (first 9 days), with very little effect

**Table 2.** Observational studies reporting antipyretics analgesics use around vaccination time.

| Author Year (ref.) | Study vaccine/s, Setting, Subjects age, N | Antipyretic analgesic reported use | Measured outcomes, immune correlates or seroprotection | Results/Antibody response |
|---|---|---|---|---|
| Jackson 2016 (45) | Influenza (monovalent 2009 pandemic H1N1), (USA) ≥ 18 and ≤ 50 yr N= 1597 | Low-dose Aspirin, self-reported chronic use by 43% | Hemagglutination-inhibition titers | Low-dose Aspirin use was not significantly associated with hemagglutination-inhibition titers. |
| Andrews 2011 (8) | Influenza (split virion, AS03B-adjuvanted AND non-adjuvanted whole virion H1N1 (2009), (UK) 6 mo −12 yr N= 943 | Antipyretic analgesics therapeutic use: Adjuvanated 1st dose: 36.5%, 2nd dose: 28.4% Whole virion: 1st dose: 22.1%, 2nd dose: 16.6% | Hemagglutination-inhibition titers (≥ 1:32) Microneutralisation titers (≥1:40) | Paracetamol[(a)] or ibuprofen use on day 0 or day 1 did not affect antibody titer after 1st or 2nd vaccine dose |
| Mills 1998 (44) | WC-DTP-IPV/RPR-T, Acellualar Pertussis, DTP-IPV/RPR-T, (Canada) ≥ 2 and <3 mo infants N=560 | Acetaminophen prophylaxis use among 3 sites (93.4%, 80.6% and 53.8% and increased to 97.0%, 93.0% and79.8%, respectively after therapeutic use in first 24 hrs | Anti-PRP 20.15 and 2 1.0 ug/ml. Polio types 1, 2 and 3 neutralizing antibody titers ≥ 8. DT Ab levels ≥ 0.01, ≥ 0.10 and 1.0 IU/mL. TT Ab levels ≥ 0.01, ≥ 0.10 and ≥ 1.0 EU/ml. Pertussis agglutination titer ≥ 64. Pertussis Ag responses ≥ 25 and ≥ 100 EU/mL. | Acetaminophen prophylaxis did not affect immunogenicity. Differences in antibody responses clinically insignificant (tetanus seroprotection is 100%, diphtheria 99%). |
| Marcinak 1993 (43) | DTaP, WC-DTP, (USA) 15 – 20 mo N=246 | Acetaminophen therapeutic use 63% in WC-DTP, 31% in DTaP | PT Abs using CHO cell assay FHA and PT Abs by ELISA, TT by ELISADT by VERO cell assay | FHA, PT and functional PT were higher in acellular DTP compared to whole cell even in subgroup analysis by race, gender, and practice setting. Antipyretics use reported but not analyzed for correlation with antibody response. |
| Auerbach 1992 (42) | WC-DTP, Acellular DTP, (USA) 15–24 mo, 4–6 yr N=111 | Acetaminophen use 53% in WC-DTP, 12% acellular DTP | Pertussis agglutinin (1:2 lower limit of detection). PT Ab by toxin neutralization. DT & TT ( ≥ 0.01 IU/mL considered protective). | No differences in antibody responses between the 2 doses of acellular DTP. All 3 groups had significant increases in pertussis agglutinins, but higher GMT for acellular DTP |
| Long 1990 (7) | WC-DTP, (USA)2 mo,N=538 | Acetaminophen therapeutic use 73.5% within 48 hr in DTP, 21% in placebo. DTP prophylactic use 2.1%. | DT Ab assay by toxin neutralization. Tetanus toxoid Ab by hemagglutination. Pertussis Ab by direct cell agglutination and by toxin neutralization. | Acetaminophen use did not correlate with Pertussis antibody response. |

[(a)]Paracetamol: international nonproprietary name for acetaminophen.

***Abbreviations by cited order*** N: number of study participants; WC: Whole Cell; DTP: Diphtheria, tetanus, pertussis vaccine; IPV: Inactivated polio vaccine; RPR polyribose ribitol phosphate; T: tetanus vaccine; DT: Diphtheria toxoid; TT: tetanus toxoid; DTaP: Diphtheria, tetanus, acellular pertussis vaccine; FHA: filamentous hemaglutnin.

afterwards; although most of the antibody production was noticed after the inductive period.[23] In similar studies using human peripheral blood mononuclear cells (PBMCs), indomethacin maximally inhibited immunoglobulin secretion when the drug was added early on or in the first 24 hours of culture.[46-48] Ibuprofen added during the first days of PBMC culture (days 1, 2 and/or 3) produced greater IgM suppression versus adding the drug at later time points (day 5 and/or day 6).[49] Indomethacin administered to mice immunized with human serum albumin showed a reduction in antibody affinity and production, and this effect was greater when the drug was given one week before or one week immediately after immunization ($p < 0.05$ and $p < 0.02$, respectively), whereas no significant reduction was observed when indomethacin was given during the second week (day 7 to 14).[50]

These laboratory data correlate with human studies, such as antibody blunting in the 2009 Prymula study that occurred only in the primary vaccine series "novel antigens" and not when booster vaccines were given, and in the study of Wysocki et al. that noted similar results. Additionally, in the 1978 Goodwin study, indomethacin prophylaxis resulted in lower, but not statistically significant, antibody response to the novel influenza A/New Jersey strain but not against the A/Victoria strain that the participants had already been exposed to. Taken together, these studies suggest that antipyretic prophylaxis primarily affects vaccine response to novel antigens, meaning that this phenomenon will have greater impact on children, as they are the group who receives most of the novel vaccine antigens and are commonly given antipyretic analgesics around the time of vaccination.[1,51]

## Mechanisms of antipyretic analgesics action on the immune system

The mechanisms by which antipyretic analgesics affect the antibody response following immunization are not clear. An early study demonstrated that salicylate inhibits complement, but antibody binding was not affected.[52] Antipyretic analgesics

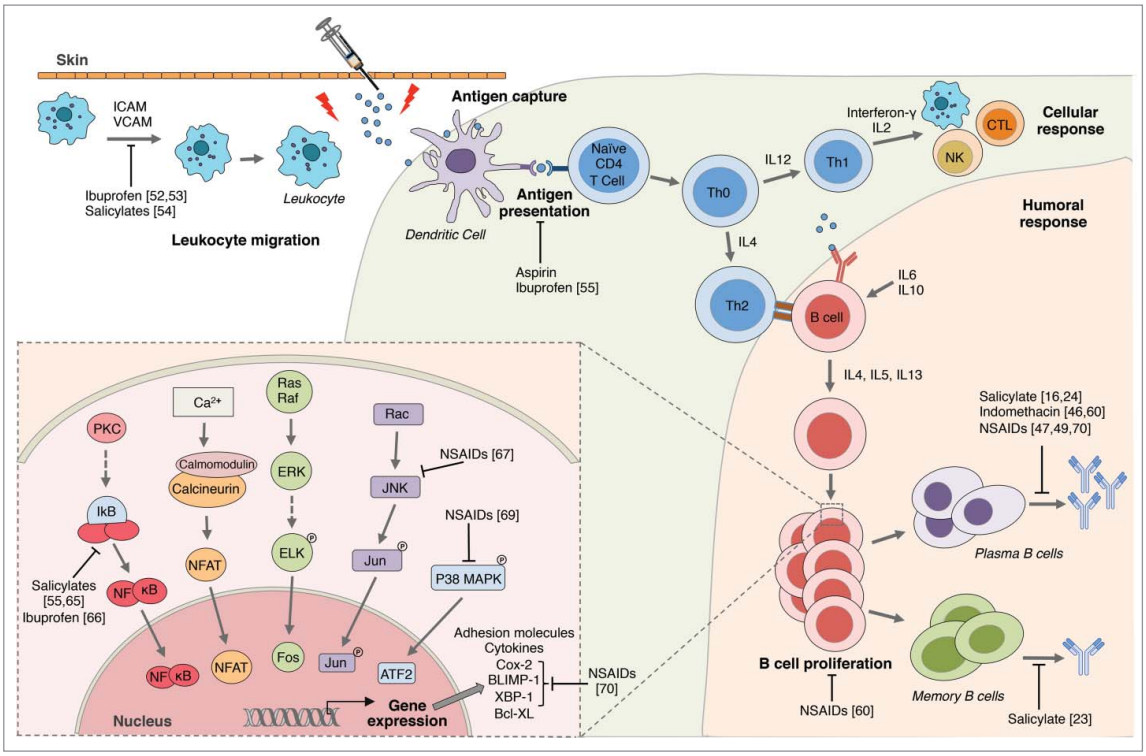


**Figure 1.** Antipyretics analgesics inhibition of post-vaccination immune response. This figure illustrates the different mechanisms by which antipyretic analgesics might inhibit post-vaccine adaptive immune response as suggested by the referenced studies. Vaccine antigen delivered at injection site induces immune and inflammatory mediators which triggers leukocyte migration and activates dendritic cells (DC) [upper left]. DCs capture, process and present antigen to naive CD4 T cells and induce their proliferation and differentiation into T-helper cells (Th0). Th0 influenced by cytokines and other stimuli differentiate into T-helper subsets Th1 (associated with cellular responses) and Th2 (associated with humoral responses). Th2 cells interact with B cell and secrete cytokines (IL4, IL5, IL13) leading to B cell proliferation and differentiation into antibody-secreting plasma cells and memory B cells. Insert: Major intracellular signaling pathways that lead to activation of nuclear factors and expression of cellular end products. PKC: Protein Kinase C; NF-kB: Nuclear factor $\kappa$ B; NFAT: Nuclear factor of activated T-cells; ERK: extracellular signal regulated kinases; JNK: Jun N terminal kinase; MAPK: mitogen-activated protein kinase; ATF2: Activating transcription factor-2; Cox-2: Cyclo-oxygenase 2; Bcl-XL: B lymphocyte; BLIMP-1:B lymphocyte induced maturation protein-1; XBP-I: X-box-binding protein1.

have been shown to affect the adaptive arm of the immune response at different points along the pathway from initial cellular response at the injection site to the final step of antibody production. This is shown in Figure 1 together with corresponding reference study. Ibuprofen and salicylates have been shown to inhibit leukocyte migration[53] and reduce PBMC adhesion through reduced surface expression of VCAM-1 and ICAM-1;[54,55] furthermore, both inhibited antigen presentation in dendritic cells.[56] These antipyretic analgesics inhibit cyclooxygenase enzymes (COX-1 and COX-2) leading to a suppression of prostaglandin release.[57-59] Inhibition of COX-2 leads to reduced interferon-$\gamma$ producing T cells and reduced antibody production by B cells in mice infected with vaccinia virus.[60] COX-1 and COX-2 inhibitors have been shown to markedly reduce antibody production.[61-63] Human B cells strongly express COX mRNA and protein and produce prostaglandins upon activation[61] suggesting a potential target for antipyretic analgesics in antibody producing cells. However, this reduction was also observed in COX-deficient mouse models,[49,61] indicating that the mechanism is not solely dependent on that pathway. Furthermore, studies on the effect of prostaglandins on antibody response have reported opposing results. PGE2 inhibited antibody production in cultured peripheral human B cells;[20,22] on the contrary, other studies reported that PGE2 enhanced antibody production.[46,48]

Taken together, these data suggest that other COX-independent mechanisms are involved in the blunting of the antibody response by antipyretic analgesics. A major mechanism is through the inhibition of nuclear signaling and transcription pathways. In a recent review, Purssell proposed that COX inhibitors decrease antibody response through inhibition of the mitogen activated protein kinase (MAP) and extracellular regulated protein kinase pathways (ERK).[64] Aspirin and sodium salicylate inhibit IkB kinase (IKK-b) through binding of these agents to IKK-b to reduce ATP binding.[65] Ibuprofen and other NSAIDs suppressed in vitro production of IL-1$\beta$ and TNF-$\alpha$ by blocking nuclear factor- kB (NF-kB) translocation after stabilization of the NF-kB/IkB complex in cytoplasm.[66,67] NSAIDs suppressed T-cell activation by inhibiting p38 MAP induction, an effect that was reversed by PGE2.[68,69] COX-2 selective inhibition reduced BLIMP-1, an essential transcription factor for plasma cell differentiation.[70] Several additional transcriptional factors are involved in the early initiation phase of the germinal center that leads to the development of antibody-secreting plasma and memory B cells.[71] These include: B cell lymphoma 6 (BCL6),[72] Interferon-regulatory factor 4 (IRF4),[73] Myocyte-specific enhancer factor 2C (MEF2C),[74] B cell-specific transcription coactivator (OCA-B/OBF/Bob-1)[75] and cell-cycle regulator *c-Myc* (MYC).[76] Considering that the antipyretics effect on antibody response is present only at vaccination time, inhibition of these transcriptional factors could theoretically be implicated; however at this time there is no literature evidence to support this.

In addition, there have been concerns that antipyretic analgesics may reduce the ability of cells to proliferate or induce cell death. To date, results have been mixed, with one study showing that indomethacin inhibited antibody production without loss of cell viability. However, other studies showed that NSAIDs decreased antibody secreting cells[61] or resulted in a modest reduction in B cell proliferation without inducing apoptosis.[49]

Overall, antipyretic analgesics actions on cells and on signaling pathways appear to be diverse and studies to date have shown opposing effects. This highlights our lack of understanding of the mechanisms behind antipyretic blunting of vaccine-elicited antibody response and the need for further work in this field.

## Conclusions

The answer to the question of whether antipyretic analgesics have a clinically significant impact on vaccine response has significant public health implications. Although generating a great deal of interest in the topic, the 2009 Prymula study did not answer the question because the acetaminophen-associated antibody blunting that was observed following vaccination still resulted in protective antibody levels. Additionally their follow up study showed a robust antibody response following booster vaccine doses. The studies included in our review reported no significant blunting of the immune response in papers published prior to the 2009 Prymula study, but since that report there have been several studies that have suggested immune blunting. One study showed lower response to a novel influenza strain following vaccination; however the difference was not statistically significant.[26] Thus, at this time, there is no clear answer as to whether antipyretic analgesic administration blunts the immune response to a degree that could result in vaccine failure.

The timing of administration of antipyretic analgesics appears to be paramount. In all studies that reported a negative effect on antibody response, the medications were given prophylactically. Interestingly, this effect was not seen when acetaminophen was given only four hours after immunization.[6] Additionally, all reported decreases in antibody response occurred only with novel antigen vaccination, with little to no impact observed following booster immunizations. These findings underscore the notion that relationship between antigen exposure and the timing of the medication dosage plays a vital role in modifying the immune response, and this set of observations can direct the focus of future research to explore the underlying mechanism.

The array of vaccine antigens in use today has evolved over the last several decades, and this evolution continues as new vaccines are being developed, and as new technologies and advanced manufacturing techniques become available. Modern vaccines employ more purified proteins as well as novel adjuvant formulations,[77] and many older vaccines are being mixed into single dose combination vaccines. However, the increased availability of vaccines means that simultaneous multiple vaccines may be given during the same visit. Do any of these factors come into play to shape the immune response when antipyretics are given? Further work will be needed to elucidate the effects of these changes on vaccine response.

Another intriguing and unanswered question is whether antipyretics exert a negative effect by suppressing a beneficial increase in temperature that could augment vaccine responses. A recent review by Evans et al. illustrated how thermal stress stimulates and augments the innate and adaptive immune responses.[78] Given the mixed antipyretic and anti-inflammatory effects of clinically available NSAIDs and other analgesics, studies to examine this question may be difficult to perform in humans, and animal studies using novel compounds and/or in vitro studies may be needed. However, one limitation of in vitro studies is that they may artificially simplify the immune response. For example, studies that examined B cell development in vitro used a limited biological environment and thus were unable to evaluate other immunologic, metabolic and physiologic factors that may contribute to the mechanism of antibody inhibition.[64]

It is clear that more clinical trials are needed to evaluate effect of antipyretic analgesics on immune responses to common vaccine antigens, different vaccine combinations and different vaccine schedules. Studies are also needed to assess the clinical impact of the different classes of antipyretic analgesics and to assess if the effect of antipyretic analgesics exists in a dose-dependent response. Increasing the scope of the clinical trials investigating antipyretic analgesics effects beyond immunogenicity to include vaccine efficacy will provide insight into the potential impact on public health.

In addition, data are lacking about this effect in other clinically important cohorts like immunocompromized populations, pregnant women, and chronic users of antipyretic analgesics. A systems biology approach, looking at the correlation of these responses with T and B cell phenotypes and responses, together with profiling of cytokines and intracellular signaling may provide insight into some of these questions and guide future research directions. Regardless, more work is needed to generate an evidence base to inform the development of recommendations for the use of antipyretics around vaccination time.

## Abbreviations

| | |
|---|---|
| COX | Cyclooxygenase |
| NSAIDs | non-steroidal anti-inflammatory drugs |
| HAI | hemagglutination inhibition |
| DTP | diphtheria, tetanus and whole cell pertussis vaccine |
| PHiD-CV | pneumococcal non-typeable H. influenzae protein D-conjugate vaccine |
| HBV | inactivated hepatitis B vaccine |
| GMC | geometric mean concentration |
| anti-HBs | antibodies against hepatitis B surface antigen |

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Acknowledgments

We thank Eric Monson, Ph.D. and Angela Zoss (Duke University Libraries, Data and Visualization Services Department) for their consultation and reviewing of the figure design; Emily Mazure, MSI, AHIP (Duke

University Medical Center Library & Archives) for assisting with biomedical literature search.

## Funding

This work was supported by National Institute of Child Health and Human Development of the National Institutes of Health under award number 5T32HD060558. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

## References

[1] Taddio A, Manley J, Potash L, Ipp M, Sgro M, Shah V. Routine immunization practices: use of topical anesthetics and oral analgesics. Pediatrics 2007; 120:e637-43; PMID:17766503; http://dx.doi.org/10.1542/peds.2006-3351

[2] Manley J, Taddio A. Acetaminophen and ibuprofen for prevention of adverse reactions associated with childhood immunization. Ann Pharmacother 2007; 41:1227-32; PMID:17519301; http://dx.doi.org/10.1345/aph.1H647

[3] Ipp MM, Gold R, Greenberg S, Goldbach M, Kupfert BB, Lloyd DD, Maresky DC, Saunders N, Wise SA. Acetaminophen prophylaxis of adverse reactions following vaccination of infants with diphtheria-pertussis-tetanus toxoids-polio vaccine. Pediatr Infect Dis J 1987; 6:721-5; PMID:3313232; http://dx.doi.org/10.1097/00006454-198708000-00005

[4] Lewis K, Cherry JD, Sachs MH, Woo DB, Hamilton RC, Tarle JM, Overturf GD. The effect of prophylactic acetaminophen administration on reactions to DTP vaccination. Am J Dis Child 1988; 142:62-5; PMID:3277388

[5] Aoki FY, Yassi A, Cheang M, Murdzak C, Hammond GW, Sekla LH, Wright B. Effects of acetaminophen on adverse effects of influenza vaccination in health care workers. CMAJ 1993; 149:1425-30; PMID:8221426

[6] Uhari M, Hietala J, Viljanen MK. Effect of prophylactic acetaminophen administration on reaction to DTP vaccination. Acta Paediatr Scand 1988; 77:747-51; PMID:3059756; http://dx.doi.org/10.1111/j.1651-2227.1988.tb10741.x

[7] Long SS, Deforest A, Smith DG, Lazaro C, Wassilak GF. Longitudinal study of adverse reactions following diphtheria-tetanus-pertussis vaccine in infancy. Pediatrics 1990; 85:294-302; PMID:2304782

[8] Andrews NJ, Walker WT, Finn A, Heath PT, Collinson AC, Pollard AJ, Snape MD, Faust SN, Waight PA, Hoschler K, et al. Predictors of immune response and reactogenicity to AS03B-adjuvanted split virion and non-adjuvanted whole virion H1N1 (2009) pandemic influenza vaccines. Vaccine 2011; 29:7913-9; PMID:21875635; http://dx.doi.org/10.1016/j.vaccine.2011.08.076

[9] Prymula R, Siegrist CA, Chlibek R, Zemlickova H, Vackova M, Smetana J, Lommel P, Kaliskova E, Borys D, Schuerman L. Effect of prophylactic paracetamol administration at time of vaccination on febrile reactions and antibody responses in children: two open-label, randomised controlled trials. Lancet 2009; 374:1339-50; PMID:19837254; http://dx.doi.org/10.1016/S0140-6736(09)61208-3

[10] Jackson LA, Peterson D, Dunn J, Hambidge SJ, Dunstan M, Starkovich P, Yu O, Benoit J, Dominguez-Islas CP, Carste B, et al. A randomized placebo-controlled trial of acetaminophen for prevention of post-vaccination fever in infants. PLoS One 2011; 6:e20102; PMID:21698100; http://dx.doi.org/10.1371/journal.pone.0020102

[11] Homme JH, Fischer PR. Prophylactic paracetamol at the time of infant vaccination reduces the risk of fever but also reduces antibody response. Evidence-Based Medicine 2010; 15:50-1; PMID:20436123; http://dx.doi.org/10.1136/ebm1049

[12] CDC. Vaccine Information Safety, DTap 05/17/2007 http://www.cdc.gov/vaccines/hcp/vis/vis-statements/dtap.html. Accessed online 04/06/2016

[13] Brady MT, Swanson JT. More study needed on antipyretics' effect on vaccine responses. AAP News 2010; 31-1

[14] WHO Weekly Epidemiological Record 25 September 2015; 90(39): 505-510

[15] Das RR, Panigrahi I, Naik SS. The effect of prophylactic antipyretic administration on post-vaccination adverse reactions and antibody response in children: a systematic review. PLoS One 2014; 9:e106629; PMID:25180516; http://dx.doi.org/10.1371/journal.pone.0106629

[16] Swift HF. The action of sodium salicylate upon the formation of immune bodies. J Exp Med 1922; 36:735-60; PMID:19868706; http://dx.doi.org/10.1084/jem.36.6.735

[17] Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. Immunol Today 1991; 12:349-52; PMID:1958288; http://dx.doi.org/10.1016/0167-5699(91)90064-Z

[18] Goodwin JS, Ceuppens J. Regulation of the immune response by prostaglandins. J Clin Immunol 1983; 3:295-315; PMID:6140268; http://dx.doi.org/10.1007/BF00915791

[19] Schleimer RP, Benjamini E. Effects of Prostaglandin Synthesis Inhibition on the Immune Response. Immunopharmacol 1981; 3:205-19

[20] Simkin NJ, Jelinek DF, Lipsky PE. Inhibition of human B cell responsiveness by prostaglandin E2. J Immuno 1987; 138(4):1074-1081

[21] Ishizuka M, Takeuchi T, Umezawa H. Promotion of antibody formation by prostaglandin. Experientia 1974 Oct; 30:1207-8; PMID:4611783; http://dx.doi.org/10.1007/BF01923694

[22] Jelinek DF, Thompson PA, Lipsky PE. Regulation of human B cell activation by prostaglandin E2. Suppression of the generation of immunoglobulin-secreting cells. J Clin Invest 1985; 75:1339-49; PMID:3872889; http://dx.doi.org/10.1172/JCI111835

[23] Ambrose CT. Inhibition of the secondary antibody response in vitro by salicylate and gentisate. J Exp Med 1966; 124:461-82; PMID:5922744; http://dx.doi.org/10.1084/jem.124.3.461

[24] Alm GV, Pachman LM. The effects of sodium salicylate on the anamnestic immune response in vitro. Experientia 1971; 27:924-5; PMID:4947882; http://dx.doi.org/10.1007/BF02135747

[25] Panush RS, Ossakow SJ. Effects of acetaminophen on normal human peripheral blood lymphocytes: enhancement of mitogen- and antigen-stimulated incorporation of tritiated thymidine. Clin Exp Immunol 1979; 38:539-48; PMID:535186

[26] Goodwin JS, Selinger DS, Messner RP, Reed WP. Effect of indomethacin in vivo on humoral and cellular immunity in humans. Infect Immun 1978; 19:430-3; PMID:631882

[27] Song JY, Cheong HJ, Seo YB, Kim IS, Noh JY, Heo JY, Choi WS, Lee J, Kim WJ. Comparison of the long-term immunogenicity of two pandemic influenza A/H1N1 2009 Vaccines, the MF59-adjuvanted and unadjuvanted vaccines, in Adults. Clin Vaccine Immunol 2012; 19:638-41; PMID:22379067; http://dx.doi.org/10.1128/CVI.00026-12

[28] Miller MS, Gardner TJ, Krammer F, Aguado LC, Tortorella D, Basler CF, Palese P. Neutralizing antibodies against previously encountered influenza virus strains increase over time: a longitudinal analysis. Sci Transl Med 2013; 5:198ra07; http://dx.doi.org/10.1126/scitranslmed.3006637

[29] Andrews SF, Kaur K, Pauli NT, Huang M, Huang Y, Wilson PC. High preexisting serological antibody levels correlate with diversification of the influenza vaccine response. J Virol 2015; 89:3308-17; PMID:25589639; http://dx.doi.org/10.1128/JVI.02871-14

[30] Chernesky M, O'Neill D, Pickard L, Castriciano S, Kraftcheck D, Sellors J, McLean D, Flett N, Mahony J. Immunogenicity and adverse reactions of influenza vaccination in elderly patients given acetaminophen or placebo. Clin Diagn Virol 1993; 1:129-36; PMID:15566726; http://dx.doi.org/10.1016/0928-0197(93)90021-V

[31] Gross PA, Levandowski RA, Russo C, Weksler M, Bonelli J, Dran S, Munk G, Deichmiller S, Hilsen R, Panush RF. Vaccine immune response and side effects with the use of acetaminophen with influenza vaccine. Clin Diagn Lab Immunol 1994; 1:134-8; PMID:7496933

[32] Lafferty WP, Selinger DS, Schiffman G, Goodwin JS. Response to pneumococcal vaccine in the elderly: no enhancement by indomethacin. J Immunopharmacol 1981; 3:241-50; PMID:7338631; http://dx.doi.org/10.3109/08923978109026429

[33] Ceuppens JL, Desmyter J. Effect of prostaglandin synthesis inhibition in vivo on the immune response following hepatitis B vaccination in normal subjects. Int J Immunopharmacol 1987; 9:803-10; PMID:2962954; http://dx.doi.org/10.1016/0192-0561(87)90076-2

[34] Hsia J, Tang T, Parrott M, Rogalla K. Augmentation of the immune response to influenza vaccine by acetylsalicylic acid: a clinical trial in a geriatric population. Methods Find Exp Clin Pharmacol 1994; 16:677-83; PMID:7746030

[35] Prymula R, Habib A, Francois N, Borys D, Schuerman L. Immunological memory and nasopharyngeal carriage in 4-year-old children previously primed and boosted with 10-valent pneumococcal nontypeable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) with or without concomitant prophylactic paracetamol. Vaccine 2013; 31:2080-8; PMID:23391599; http://dx.doi.org/10.1016/j.vaccine.2013.01.044

[36] Prymula R, Esposito S, Zuccotti GV, Xie F, Toneatto D, Kohl I, Dull PM. A phase 2 randomized controlled trial of a multicomponent meningococcal serogroup B vaccine (I). Hum Vaccin Immunother 2014; 10:1993-2004; PMID:25424809; http://dx.doi.org/10.4161/hv.28666

[37] David Salisbury MR, Karen N, editors. Immunisation against infectious disease [Internet]. Public Health England, Department of Health. 2013, updated edition. [Updated 2015 Sep 22; cited 2016 April 9]. Available from: https://www.gov.uk/government/uploads/system/uloads/attachment.data/file/462629/2904512 Green Book Chapter 22 v6 0W.PDF

[38] Doedee AM, Boland GJ, Pennings JL, De Klerk A, Berbers GA, Van Der Klis FR, de Melker HE, van Loveren H, Janssen R. Effects of prophylactic and therapeutic paracetamol treatment during vaccination on hepatitis B antibody levels in adults: Two open-label, randomized controlled trials. PLoS One 2014; 9:e98175; PMID:24897504; http://dx.doi.org/10.1371/journal.pone.0098175

[39] Wysocki J, Center KJ, Brzostek J, Majda-Stanislawska E, Giardina P, Sundaraiyer V, Patterson S, Gruber WC, Scott D, Gurtman A. Does Use of Prophylactic Antipyretics (PAP) Affect Immune Response To Vaccination In Infants? Pneumonia 2014; 3:197

[40] Duke University, Centers for Disease Control and Prevention. The Effect of Prophylactic Antipyretics on Immune Responses and Fever After 2014–2015 and 2015–2016 Inactivated Influenza Vaccine. In: ClinicalTrials.gov [Internet]. Besthesda, MD: National Library of Medicine (US). 2014 [Cited 2016 March 26]. Available from https://clinicaltrials.gov/ct2/show/NCT02212990 NLM Identifier: NCT02212990

[41] Walter EB HC, Harrington L, McGee CE, Grohskopf L, Museru O, Broder K. Pilot Study to Assess the Effect of Prophylactic Antipyretics on Immune Responses and Fever after Inactivated Influenza Vaccine (IIV) in Young Children [Abstract]. In: Proceedings of the 18th Annual Conference on Vaccine Research, National Foundation for Infectious Diseases, April 13–15, 2015; Bethesda, MD. Abstract number S32

[42] Auerbach BS, Wilson ME, Lake AM, Deforest A, Steinhoff M, Halsey NA. Dose-response to acellular pertussis vaccine and comparison with whole cell pertussis vaccine at 15–24 months and 4–6 years of age. Acellular Pertussis Vaccine Study Team. Vaccine 1992; 10:14-20; PMID:1539456; http://dx.doi.org/10.1016/0264-410X(92)90413-E

[43] Marcinak JF, Ward M, Frank AL, Boyer KM, Froeschle JE, Hosbach PH, 4th. Comparison of the safety and immunogenicity of acellular (BIKEN) and whole-cell pertussis vaccines in 15- to 20-month-old children. Am J Dis Child 1993; 147:290-4; PMID:8438810

[44] Mills E, Gold R, Thipphawong J, Barreto L, Guasparini R, Meekison W, Cunning L, Russell M, Harrison D, Boyd M, et al. Safety and immunogenicity of a combined five-component pertussis-diphtheria-tetanus-inactivated poliomyelitis-Haemophilus B conjugate vaccine administered to infants at two, four and six months of age. Vaccine 1998; 16:576-85; PMID:9569468; http://dx.doi.org/10.1016/S0264-410X(97)00241-7

[45] Jackson ML, Bellamy A, Wolff M, Hill H, Jackson LA. Low-dose aspirin use does not diminish the immune response to monovalent H1N1 influenza vaccine in older adults. Epidemiol Infect 2016; 144:768-71; PMID:26330204; http://dx.doi.org/10.1017/S0950268815002058

[46] Staite ND, Panayi GS. Regulation of human immunoglobulin production in vitro by prostaglandin E2. Clin Exp Immunol 1982; 49:115-22; PMID:6957277

[47] Martinez F, Coleman JW. The effects of selected drugs, including chlorpromazine and non-steroidal anti-inflammatory agents, on polyclonal IgG synthesis and interleukin 1 production by human peripheral blood mononuclear cells in vitro. Clin Exp Immunol 1989; 76:252-7; PMID:2788047

[48] Ceuppens JL, Goodwin JS. Endogenous prostaglandin E2 enhances polyclonal immunoglobulin production by tonically inhibiting T suppressor cell activity. Cell Immunol 1982; 70:41-54; PMID:6214316; http://dx.doi.org/10.1016/0008-8749(82)90131-9

[49] Bancos S, Bernard MP, Topham DJ, Phipps RP. Ibuprofen and other widely used non-steroidal anti-inflammatory drugs inhibit antibody production in human cells. Cell Immunol 2009; 258:18-28; PMID:19345936; http://dx.doi.org/10.1016/j.cellimm.2009.03.007

[50] Phillips C. Prostaglandin E2 production is enhanced in mice genetically selected to produce high affinity antibody responses. Cell Immunol 1989; 119:382-92; PMID:2522824; http://dx.doi.org/10.1016/0008-8749(89)90252-9

[51] Taddio A, Ipp M, Thivakaran S, Jamal A, Parikh C, Smart S, Sovran J, Stephens D, Katz J. Survey of the prevalence of immunization non-compliance due to needle fears in children and adults. Vaccine 2012; 30:4807-12; PMID:22617633; http://dx.doi.org/10.1016/j.vaccine.2012.05.011

[52] Whaley K, Sloane DJ. Studies of the action of some anti-inflammatory drugs on complement mediated immune haemolysis. Br J Clin Pharmacol 1975; 2(2):123-129; PMID:1234496; http://dx.doi.org/10.1111/j.1365-2125.1975.tb01567.x

[53] Hofbauer R, Speiser W, Kapiotis S. Ibuprofen inhibits leukocyte migration through endothelial cell monolayers. Life Sci 1998; 62:1775-81; PMID:9585108; http://dx.doi.org/10.1016/S0024-3205(98)00139-8

[54] Kapiotis S, Sengoelge G, Sperr WR, Baghestanian M, Quehenberger P, Bevec D, Li SR, Menzel EJ, Mühl A, Zapolska D, et al. Ibuprofen Inhibits Pyrogen-dependent Expression of VCAM-1 and ICAM-1 on human endothelial cells. Life Sci 1996; 58(23):2167-81; PMID:8649201; http://dx.doi.org/10.1016/0024-3205(96)00210-X

[55] Pierce JW, Read MA, Ding H, Luscinskas FW, Collins T. Salicylates inhibit I kappa B-$\alpha$ phosphorylation, endotheliaL-leukocyte adhesion molecule expression, and neutrophil transmigration. J Immunol 1996; 156:3961-9; PMID:8621937

[56] Kim HJ, Lee YH, Im SA, Kim K, Lee CK. Cyclooxygenase Inhibitors, Aspirin and Ibuprofen, Inhibit MHC-restricted Antigen Presentation in Dendritic Cells. Immune Netw 2010; 10:92-8; PMID:20631879; http://dx.doi.org/10.4110/in.2010.10.3.92

[57] Graham GG, Scott KF. Mechanism of action of paracetamol. Am J Ther 2005; 12:46-55; PMID:15662292; http://dx.doi.org/10.1097/00045391-200501000-00008

[58] Hinz B, Cheremina O, Brune K. Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. FASEB J 2008; 22:383-90; PMID:17884974; http://dx.doi.org/10.1096/fj.07-8506com

[59] Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 1971; 231:232-5; PMID:5284360; http://dx.doi.org/10.1038/newbio231232a0

[60] Bernard MP, Bancos S, Chapman TJ, Ryan EP, Treanor JJ, Rose RC, Topham DJ, Phipps RP. Chronic inhibition of cyclooxygenase-2 attenuates antibody responses against vaccinia infection. Vaccine 2010; 28:1363-72; PMID:19941994; http://dx.doi.org/10.1016/j.vaccine.2009.11.005

[61] Ryan EP, Pollock SJ, Murant TI, Bernstein SH, Felgar RE, Phipps RP. Activated Human B Lymphocytes Express Cyclooxygenase-2 and Cyclooxygenase Inhibitors Attenuate Antibody Production. J Immunol 2005; 174:2619-26; PMID:15728468; http://dx.doi.org/10.4049/jimmunol.174.5.2619

[62] Chang CL, Ma B, Pang X, Wu TC, Hung CF. Treatment with cyclooxygenase-2 inhibitors enables repeated administration of vaccinia virus for control of ovarian cancer. Mol Ther 2009; 17:1365-72; PMID:19471247; http://dx.doi.org/10.1038/mt.2009.118

[63] Ryan EP, Malboeuf CM, Bernard M, Rose RC, Phipps RP. Cyclooxygenase-2 Inhibition Attenuates Antibody Responses against Human Papillomavirus-Like Particles. J Immunol 2006; 177:7811-9; PMID:17114452; http://dx.doi.org/10.4049/jimmunol.177.11.7811

[64] Purssell E. Cyclooxygenase inhibitors inhibit antibody response through interference with MAPK/ERK pathways and BLIMP-1 inhibition. Med Hypotheses 2014; 83:372-7; PMID:25012778; http://dx.doi.org/10.1016/j.mehy.2014.06.015

[65] Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-$\beta$. Nature 1998; 396:77-80; PMID:9817203; http://dx.doi.org/10.1038/23948

[66] Stuhlmeier KM, Li H, Kao JJ. Ibuprofen: New Explanation for an Old Phenomenon. Biochem Pharmacol 1999; 57:313-20; PMID:9890559; http://dx.doi.org/10.1016/S0006-2952(98)00301-3

[67] Paccani SR, Patrussi L, Ulivieri C, Masferrer JL, D'Elios MM, Baldari CT. Nonsteroidal anti-inflammatory drugs inhibit a Fyn-dependent pathway coupled to Rac and stress kinase activation in TCR signaling. Blood 2005; 105:2042-8; PMID:15514010; http://dx.doi.org/10.1182/blood-2004-04-1299

[68] Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, Santoro MG. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. Nature 2000; 403:103-8; PMID:10638762; http://dx.doi.org/10.1038/47520

[69] Paccani SR, Boncristiano M, Ulivieri C, D'Elios MM, Del Prete G, Baldari CT. Nonsteroidal anti-inflammatory drugs suppress T-cell activation by inhibiting p38 MAPK induction. J Biol Chem 2002; 277:1509-13; PMID:11700329; http://dx.doi.org/10.1074/jbc.M110676200

[70] Bernard MP, Phipps RP. Inhibition of cyclooxygenase-2 impairs the expression of essential plasma cell transcription factors and human B-lymphocyte differentiation. Immunology 2010; 129:87-96; PMID:20050331; http://dx.doi.org/10.1111/j.1365-2567.2009.03152.x

[71] De Silva NS, Klein U. Dynamics of B cells in germinal centres. Nat Rev Immunol 2015; 15:137-48; PMID:25656706; http://dx.doi.org/10.1038/nri3804

[72] Fukuda T, Yoshida T, Okada S, Hatano M, Miki T, Ishibashi K, Okabe S, Koseki H, Hirosawa S, Taniguchi M, et al. Disruption of the Bcl6 gene results in an impaired germinal center formation. J Exp Med 1997; 186:439-48; PMID:9236196; http://dx.doi.org/10.1084/jem.186.3.439

[73] Willis SN, Good-Jacobson KL, Curtis J, Light A, Tellier J, Shi W, Smyth GK, Tarlinton DM, Belz GT, Corcoran LM, et al. Transcription factor IRF4 regulates germinal center cell formation through a B cell-intrinsic mechanism. J Immunol 2014; 192:3200-6; PMID:24591370; http://dx.doi.org/10.4049/jimmunol.1303216

[74] Wilker PR, Kohyama M, Sandau MM, Albring JC, Nakagawa O, Schwarz JJ, Murphy KM. Transcription factor Mef2c is required for B cell proliferation and survival after antigen receptor stimulation. Nat Immunol 2008; 9:603-12; PMID:18438409; http://dx.doi.org/10.1038/ni.1609

[75] Hess J, Nielsen PJ, Fischer KD, Bujard H, Wirth T. The B lymphocyte-specific coactivator BOB.1/OBF.1 is required at multiple stages of B-cell development. Mol Cell Biol 2001; 21:1531-9; PMID:11238890; http://dx.doi.org/10.1128/MCB.21.5.1531-1539.2001

[76] Calado DP, Sasaki Y, Godinho SA, Pellerin A, Kochert K, Sleckman BP, de Alborán IM, Janz M, Rodig S, Rajewsky K. The cell-cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. Nat Immunol 2012; 13:1092-100; PMID:23001146; http://dx.doi.org/10.1038/ni.2418

[77] Reed SG, Orr MT, Fox CB. Key roles of adjuvants in modern vaccines. Nat Med 2013; 19(12):1597-608; PMID:24309663; http://dx.doi.org/10.1038/nm.3409

[78] Evans SS, Repasky EA, Fisher DT. Fever and the thermal regulation of immunity: the immune system feels the heat. Nat Rev Immunol 2015; 15:335-49; PMID:25976513; http://dx.doi.org/10.1038/nri3843