

The Safety of Adjuvanted Vaccines Revisited: Vaccine-Induced Narcolepsy

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ABSTRACT: Despite the very high benefit-to-risk ratio of vaccines, the fear of negative side effects has discouraged many people from getting vaccinated, resulting in the reemergence of previously controlled diseases such as measles, pertussis and diphtheria. This fear has been amplified more recently by multiple epidemiologic studies that confirmed the link of an AS03-adjuvanted pandemic influenza vaccine (Pandemrix®, GlaxoSmithKline Biologicals, Germany) used in Europe during the 2009 H1N1 influenza pandemic [A(H1N1) pdm09] with the development of narcolepsy, a chronic sleep disorder, in children and adolescents. However, public misperceptions of what adjuvants are and why they are used in vaccines has created in some individuals a closed “black box” attitude towards all vaccines. The focus of this review article is to revisit this “black box” using the example of narcolepsy associated with the European AS03-adjuvanted pandemic influenza vaccine.

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Vaccines are a key component of preventive medicine, and delays in vaccine uptake because of rare adverse events need to be balanced with the larger infection-associated morbidity and mortality in the general population not receiving the vaccine. Infrequent and delayed adverse events can arise in genetically susceptible subjects after adjuvanted (and non-adjuvanted) vaccine administration [1] as well as in individuals acquiring natural infection. Public misperceptions of what adjuvants are and why they are used in vaccines has created in some individuals a closed “black box” attitude towards all vaccines [2]. Using carefully designed studies that decipher the mechanism of action of rare adverse events as well as large-scale epidemiologic studies in human populations, it should be possible to develop vaccines (adjuvanted and non-adjuvanted) that minimize, if not eliminate, rare or delayed adverse events

including immune-mediated diseases. It should also be appreciated that associations will occur with the additional contribution of factors unrelated to vaccination. These factors include environment, diet, and age-related changes in the immune system, and conditions associated with triggering autoimmune disease in susceptible subjects (such as pregnancy or exposure to natural infections).

More than 1300 cases of vaccine-associated narcolepsy have been spontaneously reported to the EMA Eudravigilance database [3] as of January 2015 associated with an AS03-adjuvanted influenza vaccine manufactured in Europe that was distributed to more than 30.5 million people in European Union/European Economic Area countries (EU/EEA) during A(H1N1)pdm09 [4]. Subsequent epidemiologic studies and scientific investigations exploring the immunological mechanism that may have contributed to the development of narcolepsy with the European AS03-adjuvanted influenza vaccine have yielded valuable insights. Both approaches have helped vaccine manufacturers and regulatory agencies to (i) focus on the factors that most likely could contribute to the risk of immune-mediated diseases with vaccines, and (ii) identify the path forward for the development of even safer vaccines (with and without adjuvants). These same factors should also serve as a reminder to the public that avoiding vaccination is not a solution because of the risk of developing immune-mediated diseases by acquiring natural infection in addition to the morbidity and mortality associated with infection. Indeed, Toll-like receptors (TLRs) involved in the recognition and defense of the host from invading microorganisms are also suggested to play a critical role in the pathogenesis of autoimmune diseases [5]. Inappropriate stimulation or defective regulation of these pattern-recognition receptors has been evidenced in inherited inflammatory disorders [6].

EPIDEMIOLOGIC STUDIES OF VACCINE-ASSOCIATED NARCOLEPSY

Older epidemiologic studies with a follow-up of 16 to 18 years in 18,000 subjects receiving the influenza virus vaccine (adjuvanted with light mineral oil) demonstrated no increased risk of allergic and autoimmune diseases [7]. However, in 2012, observational

studies in Finland and the combined counties of Sweden reported an association between narcolepsy and vaccination with a European A(H1N1)pdm09 vaccine adjuvanted with AS03, an oil-in-water based emulsion [8]. At that time, the lack of increased reporting of narcolepsy in Norway, UK, Germany and Canada, where 3.5 million children/adolescents were vaccinated with Pandemrix® (GlaxoSmithKline Biologicals, Germany), and an analysis showing no cases of narcolepsy in recipients of an A(H1N1)pdm09 vaccine (Focetria®, Novartis Vaccines and Diagnostics, Italy) containing a different oil-in-water adjuvant, MF59 [9], most likely contributed to the caution applied by regulatory agencies in interpreting these single observational studies as having defined a causal relationship between vaccination and the identified safety signal of narcolepsy.

However, with larger epidemiologic studies, Ireland in 2012, England in 2013, Norway in 2013 and France in 2013 confirmed the association of narcolepsy in children and adolescents receiving the European AS03-adjuvanted pandemic influenza vaccine [8]. The vaccine-attributable risk estimates from the UK and Finnish studies ranged between one in 16,000 doses to one in 50,000 doses [10]. These epidemiologic studies in influenza demonstrated that rare immune-mediated adverse events such as narcolepsy associated with the European AS03-adjuvanted A(H1N1)pdm09 vaccine can be identified and require an understanding of the background occurrence of those events and the time needed for the clinical symptoms to manifest in order to enable signal detection despite biases (e.g., increased reporting that could be introduced later through increased media attention). Some of these recent epidemiologic studies additionally identified cases of narcolepsy developing in non-vaccinated subjects who acquired influenza illness and also in subjects receiving non-adjuvanted seasonal influenza vaccines (to be discussed in greater detail), which should serve as a hint that, at least for narcolepsy, the adjuvant AS03 by itself was not likely responsible for disease development.

ROLE OF THE ADJUVANT?

To date, no increased risk has been reported for the MF59-adjuvanted A(H1N1)pdm09 vaccine, of which an estimated 6.5 million doses were distributed in the EU/EEA and 25 million doses were used in Europe and Latin America [11], leading to the initial speculation that the AS03 adjuvant was the only factor responsible for triggering narcolepsy and has contributed to the closed “black box” attitude towards adjuvants in general. However, accumulating observations suggest that this adjuvant-only speculation is a misperception. First, in China, where vaccine uptake is less than 2% of the population, cyclical occurrences of narcolepsy cases was observed from 1996 to 2008 following seasonal influenza infections. Also, a threefold increase in narcolepsy was observed in children 6 months following infection with A(H1N1)pdm09 virus [12]. Second, a report from England in 2014 that examined 75 sub-

jects developing narcolepsy between 2008 and 2011 showed disease development after seasonal influenza vaccine (without adjuvant), after AS03-adjuvanted pandemic influenza vaccination, as well as with influenza illness [13].

In Canada, an estimated 12 million doses of another AS03-adjuvanted pandemic vaccine (Arepanrix®, GlaxoSmithKline Biologicals, Canada) were administered [14] and a lower association with narcolepsy was reported [15] compared to the AS03-adjuvanted A(H1N1)pdm09 vaccine (Pandemrix) used in Europe. In Canada, different manufacturing steps were followed in preparing the vaccine, which may have resulted in several differences between the vaccine antigen preparations despite using the same adjuvant AS03. This association with antigen-induced complications, rather than the presence or absence of adjuvant or composition of adjuvant, is echoed in a recent publication [16]. Investigators identified a mechanism by which molecular mimicry to a vaccine influenza antigen present in higher amounts in the European AS03-adjuvanted A(H1N1)pdm09 vaccine may have triggered a cross-reactive immune response to receptors in the brain known to be involved in sleep regulation and narcolepsy [16]. These findings are consistent with the previously mentioned observations and support the concept that vaccine-associated narcolepsy is not due solely to the characteristics of the adjuvant. The following section will briefly review narcolepsy; subsequent sections will discuss in greater detail the significance of vaccine-associated narcolepsy seen with the European AS03-adjuvanted A(H1N1)pdm09 vaccine [16].

NARCOLEPSY

Narcolepsy is a chronic disorder presenting with excessive daytime sleepiness, and its variant with cataplexy (a transient loss of muscle tone triggered by strong emotional stimuli) is tightly associated with HLA-DQB1*0602 [17]. Investigations in patients with narcolepsy-cataplexy revealed that in a majority of patients the neuropeptide hypocretin [18] is deficient in the cerebrospinal fluid. In the few postmortem brain studies conducted, investigators identified a loss of hypothalamic cells [19,20]. This neuropeptide hypocretin (HCRT) is also known by the term orexin [21]. To date, the exact mechanism culminating in the loss of HCRT-producing cell bodies of the perifornical lateral hypothalamus in narcoleptic patients remains unclear.

Investigations in animals demonstrated that dogs with hereditary narcolepsy have a mutation in the HCRT receptor 2 gene [22]. Murine models recapitulating the human narcolepsy phenotype can be observed with a loss of function of the HCRT receptor 2 [23] or the preprohypocretin gene [24] as well as with genetic ablation of HCRT neuron [25]. These findings related to the HCRT receptor 2 in canine and murine narcolepsy may be important clues to understanding human narcolepsy. In humans, another idiopathic sleep disorder termed

excessive daytime sleepiness has been linked by single-stranded conformational polymorphism analysis to an HCRT receptor 2 coding variant resulting in a Pro11Thr change that impaired HCRT ligand binding-related signaling [26]. These findings suggest that alterations in HCRT ligand or its receptors can disrupt neurotransmission and may play a key role in sleep-related disorders, including narcolepsy.

Epidemiological observations suggest that half of all adult narcoleptic patients report disease onset before the age of 15 years [27]. A report from China demonstrated that 70% of narcoleptic patients had disease onset before age 10, while in 15% onset occurred before 6 years of age [27]. The population prevalence in China is 0.034% and is similar to the reported prevalence rates in North America and Europe [27]. The delay between onset and diagnosis can be more than 10 years [28] but is reduced to only a few years when disease onset occurs in childhood [29].

HYPOTHESIZED MECHANISM FOR VACCINE-ASSOCIATED NARCOLEPSY

A recent publication [16] has demonstrated an increased frequency of antibodies to HCRT receptor 2 in sera obtained from narcoleptic patients with a history of vaccination with the European AS03-adjuvanted A(H1N1)pdm09 vaccine, Pandemrix. These antibodies were cross-reactive with a particular fragment in influenza nucleoprotein (NP), one of the influenza proteins contained in the virus used to make vaccines. Mass spectrometry of seasonal influenza vaccines detected the lowest amounts of nucleoprotein in the seasonal influenza subunit vaccine Agrippal[®] (Novartis Vaccines and Diagnostics, Italy) and its MF-59 adjuvanted formulation, Chiomas[®] (Novartis, Italy). Comparing A(H1N1)pdm09 vaccines, mass spectrometry demonstrated greater quantities of NP in Pandemrix compared to Arepanrix, in agreement with two other studies [30,31], and only trace quantities of NP in Focetria (similar to the trace amounts detected in Agrippal and Chiomas that are manufactured using similar subunit vaccine purification steps). Detailed time-point analyses of the immune response in three non-influenza-exposed subjects immunized with Focetria in 2009 demonstrated that the trace amounts of NP in Focetria would not elicit durable NP antibody responses necessary for subsequent cross-reactivity to HCRT receptor 2.

Thus, the differences between the European AS03-adjuvanted A(H1N1)pdm09 vaccine and the MF59-adjuvanted A(H1N1)pdm09 vaccine related to influenza nucleoprotein content and the respective immune response that would be triggered by increased amounts of nucleoprotein may explain the association of narcolepsy with Pandemrix-vaccinated subjects. In the presence of optimum amounts of total influenza nucleoprotein (containing the cross-reactive fragment), the adjuvant would most likely have boosted the immune response, generating higher amounts of antibodies cross-reacting with the

hypocretin receptor 2 fragment located in the external domain of this receptor in the human brain. This highly conserved NP peptide could explain the cyclical associations of narcolepsy with seasonal influenza infection in China [12] in the past decade. All of these earlier influenza strains contained the same nucleoprotein fragment.

The proposed mechanism for influenza infection/pandemic vaccine-associated narcolepsy is as follows: In subjects with genetic susceptibility to narcolepsy (carriers of the HLA DQB1*0602 haplotype), the presentation of NP antigen during infection or after immunization with adjuvanted influenza vaccines containing optimum amounts of NP generate high titers of NP antibodies that can persist in the systemic circulation. Either the high titers of NP antibodies or inflammation related to an unrelated infection (e.g., *Streptococcus*) may alter the blood-brain barrier [32], allowing NP antibodies to cross-react with neural tissue expressing HCRT receptors. Binding of the HCRT receptor with cross-reacting NP antibody could inhibit HCRT ligand-receptor interaction and additionally modulate signaling to the cells responsible for producing HCRT. This modulation in signaling may contribute to the disappearance of these HCRT-producing cells, as has been reported to occur in pathologic investigations of narcoleptic brains [20].

DOWNSTREAM EVENTS TRIGGERED BY ANTIBODIES TO HCRT RECEPTOR 2

HCRT receptor 2 dysregulation through binding of cross-reactive autoantibodies finds similarities in another anti-receptor-mediated neurological autoimmune disease in which autoantibodies target the acetylcholine receptor, namely myasthenia gravis [33]. These cross-reactive antibodies in myasthenia gravis lead to functional consequences resulting in clinical disease. What is interesting is that the published influenza NP mimic peptide aligns with residues 34–45 of HCRT receptor 2. This region corresponds to the N-terminal region of the canine HCRT receptor 2 in which a single amino acid substitution of glutamic acid with lysine at residue 54 abolishes HCRT binding in Dachshunds afflicted with narcolepsy [34]. Since both HCRT receptors 1 and 2 are highly conserved across mammalian species [21], the binding of a cross-reactive antibody to this region in humans could similarly modulate HCRT signaling through HCRT receptor 2. However, the effects of cross-reactive antibodies may not be limited only to the HCRT receptor 2-expressing targets that receive neuronal projections from hypothalamic HCRT-producing cells. In transgenic mice studies published in 2010, investigators demonstrated direct and indirect activation of HCRT-producing neurons by HCRT via the HCRT receptor 2 [35]. Detailed immunoelectron microscopic analyses along with three-dimensional reconstruction of the HCRT-producing neurons demonstrated direct contacts among these neurons, suggesting that connections between HCRT-producing neurons form a positive-feedback circuit. The recently published

findings of antibodies against the HCRT receptor 2 do not preclude a direct modulation of HCRT-producing neurons via an autoreceptor or an indirect modulation of these neurons via different neurons (e.g., histaminergic) important in sleep arousal that express HCRT receptor 2 and send neural projections back to regulate HCRT-producing neurons [36]. Antibodies binding to a receptor could have different effects, including (i) inhibition of receptor signaling, (ii) stimulation of receptor signaling, (iii) triggering of programmed cell death, (iv) cellular cytotoxicity, (v) cell clearance by complement-mediated pathways, and (vi) receptor internalization [36].

POTENTIAL IMPLICATIONS FOR INFLUENZA VACCINES

So is vaccine-associated narcolepsy only linked with an AS03-adjuvanted pandemic influenza vaccine, or could it also be occurring in an occult fashion with seasonal influenza vaccines (both inactivated and live-attenuated) containing significant amount of nucleoprotein? It is possible that the use of an adjuvant in a vaccine may have amplified and accelerated the time to development of narcolepsy, allowing detection with current epidemiologic tools [8]. It is not surprising that antibodies to HCRT receptor 2 were also reported to occur in some Finnish individuals with A(H1N1)pdm09 infection or Finnish children not known to have narcolepsy at the one time-point that was available for testing in 2004/2005 [16]. There is a known observed time lag between disease onset and disease diagnosis in both narcolepsy and vaccine-associated narcolepsy [28,37]. The presence of autoantibodies in some of these normal-appearing individuals could be reflecting the first pathogenic steps to disease development [13] in individuals who may be carrying the narcolepsy susceptibility allele (the projected coverage for the HLA-DQB1*0602 allele in Finland is more than one-third of the population [16]).

PATH FORWARD

With the continued development of sophisticated technologies and informatics tools, our ability to identify cross-reactive epitopes in vaccines leading to rare adverse events in genetically susceptible individuals will undoubtedly improve. This will increase our opportunity to potentially refine these vaccines to avoid potential risks like narcolepsy and to maximize the clear benefits afforded by vaccination. In the case of influenza vaccine (where a cross-reactive epitope has been identified from influenza nucleoprotein with high similarity to the human hypocretin receptor implicated in narcolepsy), one could remove this cross-reactive epitope by applying subunit purification steps known to effectively minimize the total amount of influenza nucleoprotein transferring into the final vaccine product [16]. Alternatively, one could use molecular biological techniques to engineer influenza virus reassortant strains (similar to the approach used for some live-attenuated HSV vaccines [38]) such that the cross-reactive epitope is deleted or

sufficiently mutated so that its similarity to the human HCRT receptor is lost. This would enable vaccine manufacturers to continue using their preferred technology for producing their specific influenza vaccine (i.e., live-attenuated, inactivated split-virion, or inactivated subunit).

While the recombinant influenza vaccine produced in a continuous insect cell line and licensed in 2013 only contains influenza hemagglutinin (HA) [39], it is not approved for use in people under the age of 18 years. When such HA-only vaccines receive approval for younger ages, yet another possible solution to the problem of vaccine-associated narcolepsy (mediated by cross-reactivity to a fragment of influenza nucleoprotein) will be within reach. However, any advantages (i.e., generation of cross-clade protection/development of a “universal” influenza vaccine) afforded by other epitopes contained in the highly conserved influenza nucleoprotein or other non-HA influenza proteins would not be retained in HA-only vaccines.

The lessons from the narcolepsy cases related to A(H1N1)pdm09 are instructive. We trust that the ongoing collaborative efforts of academic researchers, vaccine manufacturers and regulatory agencies [40] will rebuild the public trust in the safety of both adjuvanted and non-adjuvanted influenza vaccines.

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