Narcolepsy and influenza vaccination—the inappropriate awakening of immunity

Anoma Nellore[1], Troy D. Randall[2,3]

[1]Department of Medicine, Division of Infectious Diseases; [2]Division of Clinical Immunology, [3]Division of Rheumatology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Correspondence to: Troy D. Randall. Department of Medicine, Division of Clinical Immunology and Rheumatology, University of Birmingham, 1720 2nd AVE S. SHEL 507, Birmingham, AL 35294, USA. Email: randallt@uab.edu.

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Introduction

Narcolepsy is a chronic neurological disorder characterized by an inability to regulate sleep/wake cycles leading to abruptly occurring periods of daytime sleepiness, cataplexy, hypnogogic hallucinations and disrupted nocturnal sleep (1). The symptoms of narcolepsy often emerge over time and a definitive diagnosis requires a combination of behavioral and biochemical tests. As a result, the identification of narcolepsy in any particular patient is difficult and is often delayed by up to a decade following the initial onset of symptoms.

The symptoms of narcolepsy are due to impaired signaling by the neuropeptide, hypocretin (HCRT—also known as orexin) (2-4). For example, narcoleptic patients often have greatly reduced numbers of neurons in the hypothalamus that produce HCRT (4), resulting in abnormally low levels of HCRT. Narcolepsy can also be caused by naturally-occurring or experimentally-induced mutations in HCRT itself, its precursor protein or in its receptors, such as HCRT-R2 (2,3).

The incidence of narcolepsy is strongly associated with the HLA DQB1*0602 haplotype (5) and is weakly associated with other immune-related genes, such as the T cell receptor (6), suggesting that in some cases, narcolepsy can be an autoimmune disease mediated by CD4 T cells. Genetic polymorphisms are only part of the mechanism, as there is a high rate of discordance between monozygotic twins for the development of narcolepsy (7). Thus, environmental factors also play an important role in triggering the disease process. Consistent with this idea, infections with streptococcus (8) or influenza H1N1 virus (9) are associated with the onset of narcolepsy symptoms. However, the way these infections promote the onset of narcolepsy is not entirely clear.

Link with influenza vaccination

The appearance of the H1N1 pandemic strain of influenza in 2009 prompted the rapid development and distribution of vaccines containing antigens from the new virus. These vaccines included (among others), Pandemrix, which was administered to approximately 30M patients in Europe, Focetria, which was administered to approximately 25M patients globally, including Europe, and Arepanrix, which was administered to approximately 12M patients, mostly in Canada. Unexpectedly, some cases of narcolepsy were associated with Pandemrix vaccination in Sweden and Finland (10,11). Following public health alerts, many more cases were reported, mostly from northern Europe, leading to intensive investigations about the potential mechanism.

Given that the initial cases of narcolepsy (and many follow-ups) were reported following vaccination with Pandemrix, but not with Focetria, the investigation initially focused on the different adjuvants used in each vaccine (10). Pandemrix was formulated with the relatively new adjuvant, AS03, whereas Focitriea was formulated with MF59. AS03 and MF59 are both squalene-based emulsion adjuvants, but AS03 also contains the immune-potentiator, DL-α-tocopherol. The idea that AS03 might be responsible for the association with narcolepsy was abandoned following the realization that a third vaccine, Arepanrix, which was also formulated with AS03, was not associated with the

onset of narcolepsy (12), at least in the populations in which it was administered.

Despite the many cases of narcolepsy reported following Pandemrix vaccination, it is important to note that the epidemiology is not straightforward and has many confounding factors [detailed in (13)]. In particular, the rapid and widespread media exposure of the potential link between vaccination and narcolepsy likely introduced a strong bias into the detection and reporting. For example, both physicians and patients were likely to be hyper-vigilant to potential signs of narcolepsy (awareness bias), particularly in vaccinated patients (selection bias), leading to a skewing of the data (13). Moreover, it is difficult to separate the effects of influenza infection from the effects of vaccination. In fact, clinical studies suggested that infection was already widespread in Northern Europe at the time that overlapped with vaccination (14). Given that a seasonal increase in narcolepsy was reported in China during the 2009-2010 pandemic (9), despite the lack of a vaccination campaign, influenza infection may pose an equal or higher risk of developing narcolepsy in susceptible subjects. Unfortunately, serum samples taken at the appropriate times from affected and control subjects in Northern Europe are not necessarily available. Thus, the actual risk of developing narcolepsy following Pandemrix vaccination is difficult to assess.

Evidence for an autoimmune mechanism

The known link between narcolepsy and the HLA DQB1*0602 haplotype suggests that narcolepsy can have an immune component—even in the absence of vaccination. Thus, one can envision that some component of the Pandemrix vaccine may stimulate T or B cells that cross-react with HCRT, its receptors or with cells that express these proteins. In fact, one study suggested that narcoleptic patients have T cells that react with both HCRT and with hemagglutinin expressed by the pandemic H1N1 virus (15). This paper was ultimately retracted due to an inability to reproduce the findings. However, subsequent studies suggested a link between vaccination and the formation of antibodies against the HCRT receptor, HCRT-R2 (16).

Following up on potential differences between Pandemrix and Focetria, this study compared the sequences of the influenza proteins used in the two viruses and found three amino acid differences, one in the hemagglutinin and two in the nucleoprotein (16). Interestingly, one of these differences was in a region of NP that has sequence homology to the human receptors for HCRT—the HCRT-R1 and HCRT-R2, suggesting that this difference may elicit a cross-reactive immune response. Consistent with this idea, they showed that Finnish patients with narcolepsy and with the HLA-DQB1*0602 haplotype had circulating antibodies against HCRT-R2 following vaccination with Pandemrix (16), whereas healthy Italian subjects vaccinated with Focetria did not. Although this was a striking finding, about 25% of healthy Finnish subjects who had been infected with influenza pH1N1 also had antibodies against HCRT2 and more than half of healthy Finnish children had antibodies against HCRT-R2 in the 2004/2005 season - prior to any possible exposure to the antigens in the pandemic H1N1 virus (16). Thus, the link between antibodies against HCRT-R2 and Pandemrix vaccination is not clear-cut.

Despite the caveats in the epidemiology, antibodies against HCRT-R2 did cross-react with the homologous region of NP, since peptides from this region of NP could block antibody binding to HCRT-R2 (16). Surprisingly, the single amino acid polymorphism that distinguishes the NP used in Pandemrix and Focitriea was irrelevant for cross-reactivity, as both peptides equivalently blocked antibody binding to HCRT-R2 (16). These data clearly demonstrated that antibodies against HCRT-R2 also bind the homologous region of NP and suggest the converse—that NP-specific antibodies elicited following vaccination with Pandemrix could cross-react with HCRT-R2 and promote narcolepsy.

Why might Pandemrix more efficiently elicit cross-reactive antibodies than Focitriea? To address this point, the authors compared the amounts of NP protein in a wide array of vaccines and showed using multiple methods that the Pandemrix vaccine contained much higher amounts of NP than almost any other vaccine (16), particularly Focitriea, which was on the low end of the spectrum. Thus, they concluded that antibodies elicited by the larger amounts of influenza NP in the Pandemrix vaccine cross-react with human HCRT-R2 and promote narcolepsy. However, the serological data did not necessarily support this idea, as they found roughly similar titers of NP-specific antibodies in serum from a small cohort of patients vaccinated with either Pandemrix or Focitriea (16). In contrast, they found much higher titers of NP-specific antibodies in subjects who had recovered from H1N1 infection.

Interestingly, another vaccine, Arepanrix, contains a similar amount of NP as Pandemrix and is also formulated with the adjuvant, AS03, but was not associated with the

development of narcolepsy. Thus, it seems that the amount of NP in a particular vaccine was not the most important factor in triggering narcolepsy. Importantly, Pandemrix and Arepanrix were manufactured using slightly different processes (17). The antigens used in the Pandemrix vaccine were generated using the Dresden protocol, which involves treating the virus with detergent prior to diafiltration and inactivation using deoxycholate and formaldehyde. In contrast, the antigens used in the Arepanrix vaccine were prepared using the Quebec protocol, which involves inactivating virus by ultraviolet irradiation followed by formaldehyde, then purification by centrifugation, and disruption with deoxycholate.

The differences in these protocols almost certainly led to differences in antigen cross-linking and the exposure of internal epitopes. By treating the virus with detergent first, the protocol used to make the Pandemrix vaccine likely released the NP proteins from inside the virus, thereby exposing their epitopes to the immune system. In contrast, by first fixing the virus with formaldehyde, the protocol used to make the Arepanrix vaccine likely cross-linked the NP proteins inside intact virions, where they would not be exposed to the immune system. Thus, despite having similar amounts of NP, Pandemrix and Arepanrix would likely trigger different repertoires of B cells, with Pandemrix triggering more NP-specific B cells.

In fact, other studies show that the antigens in Pandemrix and Arepanrix are functionally different (12). The antigens in Arepanrix poorly block antibody responses to the antigens in Pandemrix—a result that is true in both narcoleptic and healthy control patients (12), suggesting that different epitopes and perhaps different proteins are available for binding. A Western blot analysis of NP proteins in Pandemrix and Arepanrix showed generally more NP in Pandemrix than in Arepanrix and that much of the NP protein was a higher molecular weight (12), consistent with cross-linked multimers of NP. Again, this would suggest that the proteins and epitopes in the Pandemrix vaccine are different than those in the Arepanrix vaccine.

The epitope of NP that has homology to HCRT-R2 can be mapped on the NP crystal structure (18), to an exposed region of the protein that should be available for antibody binding. Thus, the NP protein does not have to be denatured, which might occur during vaccine production, in order to expose the homologous epitope. This result also suggests that the native NP protein generated during viral infection should have a similarly exposed epitope that is homologous with HCRT-R2. Given that NP is one of the most abundant proteins in influenza virus and that influenza infection triggers a robust inflammatory response and releases a large amount of NP protein from dying epithelial cells (19), it is no surprise that the antibody response against NP following infection is very high. However, this result would also suggest that influenza infection should more robustly elicit a cross-reactive response against HCRT-R2 and more potently trigger the onset of narcolepsy than any vaccine. In fact, a seasonal incidence of narcolepsy was associated with influenza infection in China during the last pandemic (9) and may have been associated with previous pandemics as well (20). Therefore, influenza infection as well as vaccination may be an important trigger of narcolepsy in susceptible individuals.

Potential models

The production of autoantibodies is normally circumvented, in part, by the deletion of autoreactive B cells as they emerge from the bone marrow (21). Thus one could hypothesize that, in some individuals, HCRT-R2-specific B cells are not deleted during in the bone marrow, but are normally held in check in the periphery – perhaps because HCRT-R2 is expressed in an immune privileged site or perhaps by the absence of HCRT-R2-specific CD4 helper T cells (22). However, if HCRT-R2-specific B cells can also be stimulated by a homologous region on NP, they can be helped by NP-specific T cells, even ones that are not restricted to the HLA DQB1*0602 allele. This model would imply that vaccination with NP-containing vaccines or natural influenza infection should trigger the activation and differentiation of HCRT-R2-specific B cells in any individual that does not delete them. Given that influenza infection triggers much stronger T cell responses than does vaccination, one would expect that the development of HCRT-R2-specific autoantibodies would be more efficient following infection than after vaccination.

An alternative model suggests that individuals with HLA DQB1*0602 are likely to strongly bind peptides of HCRT-R2, but for some reason, CD4 T cells responding to these peptides are not efficiently eliminated during thymic selection. Instead, these T cells are kept in check through peripheral tolerance mechanisms or perhaps because HCRT-R2 is expressed in an immune privileged site. However, following influenza infection, these T cells are strongly stimulated by homologous peptides from NP, leading to a robust T cell response that provides help to B cells responding to the cross-reactive epitope of NP

and HCRT-R2. This model would explain the linkage of narcolepsy with HLA DQB1*0602. Moreover, the region of NP that is homologous to HCRT-R2 contains peptides that bind HLA DQB1*0602 (16), particularly from the NP protein used in the Pandemrix vaccine. If this is the case, CD4 T cells may be reacting with HCRT-R2-specific B cells or with NP-specific B cells. They may also be reacting with HCRT-R2-expressing cells in the brain and directly causing neurological damage—perhaps in combination with HCRT-R2-specific antibodies.

Regardless of whether vaccine-induced narcolepsy is triggered by autoreactive B cells or T cells (or both), it is important to consider how these cells or antibodies cross the blood-brain barrier. Clearly this process can occur, as it does in patients with multiple sclerosis (23), but the mechanisms responsible are not entirely clear. Antibodies are normally prevented from crossing the blood-brain barrier unless there is local inflammation or they are engineered to be transported (24). It seems unlikely that parenteral vaccination with Pandemrix would trigger an acute inflammatory response in the brain, but evidence for or against this possibility is not available. However, given that HCRT-R2-specific antibodies appear to be commonly produced (16), perhaps a subset of those individuals coincidentally develop an inflammatory response in the brain, which would then allow antibody translocation and lead to symptoms of narcolepsy.

Depending on how CD4 T cells differentiate, they can express a wide variety of chemokine receptors, some of which are associated with their ability to migrate into the brain (25). Interestingly, the chemokine receptors expressed by influenza vaccine-specific CD4 T cells differ depending on their antigen-specificity as well as the adjuvant used (26,27). Moreover, CD4+ T follicular helper (Tfh) cells, the T cells that promote antibody responses (28), can also be divided based on their expression of CXCR5, CXCR3 and CCR6 (29), suggesting that they have different functional and homing properties. Importantly, these subsets are produced in different proportions, depending on the type of immune responses (30,31). Thus, Pandemrix may elicit different Tfh subsets than do other vaccines or infection, leading to a preferential targeting of the brain. Although we can infer the existence of Tfh cells that cross-react with HCRT-R2 and NP by the production of antibodies, these cells have not yet been characterized and we do not know what types of chemokine or homing receptors they might express.

Tissue-specific CNS immune responses may also play a role in the development of narcolepsy after Pandemrix vaccination. For example, local Tfh cells support the activity of ectopic B cell follicles in the meninges and the generation of intrathecal immunoglobulins, which are both associated with the pathogenesis of autoimmune encephalitis in mice and in patients with secondary progressive multiple sclerosis (32). If Pandemrix induces an acute CNS inflammatory response, NP antigen may cross the blood-brain barrier and promote a local cross-reactive immune response to HCRT that triggers the development of narcolepsy.

Should NP be excluded from vaccines?

Given the potential risk of developing HCRT-R2-specific antibodies and T cells following vaccination with NP-containing vaccines, one might conclude that the formulation of vaccines using split virus is inappropriate. Instead, one could formulate influenza vaccines exclusively with recombinant HA in order to focus the antibody response on neutralizing, or even broadly-reactive, epitopes on this molecule. Although this approach has some merit, antibodies against other proteins of influenza are functional and it might be rash to discount them altogether. For example, NP-specific antibodies play an important role in the immune response by forming immune complexes with NP protein and targeting it to antigen-presenting cells (33), which take up the antigen and promote both CD4 and CD8 T cell responses against the virus. In fact, NP-specific CD8 T cells require NP-specific antibodies to maintain cross-priming during the primary response (19,33) and to generate fully functional memory cells (33). Since these T cells are an important component of protection, having good NP-specific antibody responses is beneficial in controlling infection.

Even if influenza vaccines did not include NP, seasonal influenza infection, regardless of severity, will elicit a robust T cell and antibody-mediated response against NP. Therefore, unless we can completely prevent infection altogether (an unlikely possibility), almost everyone will have antibodies to NP. However, if a specific step in the vaccine manufacturing process creates or exposes a cross-reactive epitope of NP, then that step should be identified and eliminated. Similarly, if a specific peptide sequence of NP stimulates autoreactive T cells, then that peptide should be engineered out of the NP used in vaccine production. Unfortunately, we will likely not be able to control the sequence of NP in circulating viruses and will simply have

to be aware of how B and T cell responses to these viruses affect the physiology of their hosts.

Conclusions and future directions

The etiology of narcolepsy remains elusive, most likely because of multiple, probably independent, causal mechanisms. For example, Type 1 narcoleptic patients have impairments in HCRT production because of fewer neurons that produce HCRT (1), which are not the same neurons that express HCRT receptors. This difference suggests that vaccine-induced or infection-induced narcolepsy is mechanistically different than Type 1 narcolepsy—a possibility that can be tested by measuring HCRT levels in cerebrospinal fluid.

In the context of influenza vaccine-induced or infection-induced narcolepsy, despite the clear demonstration of antibodies that cross-react with both influenza NP and HCRT-R2 (16), there is no causal demonstration that either antibodies or T cells cross-reacting with HCRT-R2 induce narcolepsy. Vaccination of HLA DQB1*0602 transgenic mice with Pandemrix does not trigger narcolepsy (12) and many apparently healthy subjects have titers of HCRT-R2-specific antibodies that are equivalent to those in narcoleptic patients (16). Thus, identification and characterization of T and B cells that cross-react with NP and HCRT-R2 in narcoleptic patients will be key to understanding the potential link between vaccination, infection and narcolepsy.

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Footnote

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