

Narcolepsy and influenza vaccination-induced autoimmunity

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We read with great interest the editorial from Nellore and Randall (1) regarding narcolepsy and influenza vaccination and our recent publication (2). Their conclusions become even more important in light of a recent publication demonstrating an increased risk of narcolepsy in adults in England after receipt of Pandemrix vaccination (3). The authors' conclusions raise very important points regarding the production of future influenza vaccines. Because of the importance of other functional antibodies against nucleoprotein (NP) in promoting both CD4 and CD8 T cells responses against the influenza virus, formulating influenza vaccines exclusively with recombinant hemagglutinin (HA) may not be a viable solution to addressing vaccine-associated narcolepsy. As mentioned in their editorial, "*NP-specific CD8 T cell require NP-specific antibodies to maintain cross-priming during the primary response and to generate fully functional memory cells. Since these T cells are an important component of protection, having good NP-specific antibody responses is beneficial in controlling infection.*"

We agree with the authors' conclusions that "*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*". Furthermore, "*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*". Fortunately, with the current advances in vaccine manufacturing technologies, multiple options exist for achieving the abovementioned solutions (4).

The authors raised some important issues that merit

further clarification. Some of these have been discussed in a recent review and commentary published by us (5,6), but will be briefly mentioned here to provide necessary context to the reader.

Topic 1: "In fact, one study suggested that narcoleptic patients have T cells that react with both hypocretin (HCRT) and with HA expressed by the pandemic H1N1 virus. This paper was ultimately retracted due to an inability to reproduce the findings"

The reader is invited to visit the "Retraction Watch" website (<http://retractionwatch.com/2014/07/30/authors-retract-paper-confirming-that-narcolepsy-is-an-autoimmune-disease/>) and read the "Comments" section about the similarities between the ELISPOT images (image for control 58 and patient 44 are identical). The problem with that paper was more than just reproducibility of the data.

For our publication, we started with a bioinformatics analysis that did not focus primarily on HA or on HCRT but analyzed both the HCRT ligand and receptors and all the accessible influenza proteins. From this analysis, we observed the association between influenza NP and HCRT receptor 2 (HCRT_{r2}). Our analyses did not demonstrate a strong association between HCRT and HA which may support why the ELISPOT data trying to confirm the hypothesis in the retracted paper could not be reproduced by those authors.

Topic 2: “...about 25% of healthy Finnish subjects who had been infected with influenza pH1N1 also had antibodies against HCRT-R2 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”

Indeed we detected the presence of autoantibodies from some Finnish adults recovering from A(H1N1)pdm09 infection and some Finnish children who were not known to have narcolepsy in 2004/2005 (and whose HLA haplotype was unknown). One should note that the epitope identified in our publication (2) and contained in the A(H1N1)pdm09 virus causing infection was also contained in the seasonal vaccines administered in 2004/2005. As indicated in the Methods section of our publication (2), only one serum sample in either 2004 or 2005 was available for testing in these children. Since follow-up sera were not available on these subjects and their records were anonymous, it was not possible to determine whether these subjects were subclinical at the time of sample donation and subsequently went on to develop narcolepsy. The detection of HCRT₂ antibodies could be reflecting the first steps to disease development (7) in some individuals likely to be carrying the narcolepsy susceptibility allele. This later statement is supported by the observation that the projected coverage for the HLA-DQB1*0602 allele in Finland is 31.3% of the population (8).

Topic 3: “Surprisingly, the single amino acid polymorphism that distinguishes the NP used in Pandemrix and Focetria was irrelevant for cross-reactivity, as both peptides equivalently blocked antibody binding to HCRT-R2”

As indicated in our publication (2), this one residue difference in these variants may not be contributing to any difference in antibody cross-reactivity in vaccine-associated narcolepsy. However, these NP peptide variants differentially bound [table S3 (2)] to the major histocompatibility complex (MHC) allele product known to be tightly associated with narcolepsy development (HLA-DQB1*06:02). This differential binding could contribute to differences in T helper activity in antibody generation (9). Thus, the confirmed differences in total amount of influenza NP contained in AS03-adjuvanted A(H1N1)pdm09 vaccines compared to the MF59-adjuvanted A(H1N1)pdm09 vaccine

would translate into differences in amount of NP peptide available for HLA-binding and subsequent immune response generation in the setting of adjuvant.

Topic 4: “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... (but)...they found roughly similar titers of NP-specific antibodies in serum from a small cohort of patients vaccinated with either Pandemrix or Focetria”

As the authors correctly point out, we compared the amounts of NP protein in a wide array of seasonal and pandemic influenza vaccines. Using multiple methods, these studies demonstrated high quantities of NP in the Pandemrix vaccine but trace amounts in Focetria. Regarding the titers of NP-specific antibodies in the cohort of patients vaccinated with either Pandemrix or Focetria, one should note that the Pandemrix titers are reflecting day 500 post-vaccination while those from Focetria are from day 22 or day 202 post-vaccination. The titers of Focetria at day 22 being similar to day 500 with Pandemrix further reinforce that Focetria contained suboptimal amounts of NP to induce a durable immune response to NP and would be much lower than what one would expect to find with Pandemrix vaccine at day 22 (we did not have access to blood samples taken from subjects 22 days after Pandemrix).

Topic 5: “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”

As indicated in table 2 of our publication (2), the amount of NP contained in Arepanrix is not similar to Pandemrix as these vaccines products are normalized for HA quantity (as indicated on their package inserts). This is why the NP to HA ratio becomes important. The NP to HA ratio for Pandemrix is 0.96, for Arepanrix is 0.69, and for Focetria is 0.12–0.13 (two different lots were available for testing for Focetria). One should note the NP:HA ratios for the seasonal vaccines, and that the lowest amounts are found for Agrippal and Chiromas (i.e., Fluad) which use the same subunit purification procedure as Focetria.

The low projected population coverage for HLA-

DQB1*06:02 in Canada (5.60% based on linkage disequilibrium for DR-DQ) may explain the lower incidence of narcolepsy in Canada with Arepanrix. In figure 1 of a recent review paper (6), we indicated in a world heat map the projected population coverage for HLA-DQB1*06:02 in the various countries. It is worth noting that the countries reporting vaccine-associated narcolepsy with Pandemrix also have a greater percentage of the population carrying the narcolepsy risk-allele, HLA-DQB1*06:02, compared to countries not reporting A(H1N1)pdm09 vaccine-associated narcolepsy (including Canada).

Topic 6: “In fact, other studies show that the antigens in Pandemrix are Arepanrix are functionally different...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, consistent with cross-linked multimers of NP...suggest that the proteins and epitopes in the Pandemrix vaccine are different than those in the Arepanrix vaccine”

One should note that the Arepanrix and Pandemrix vaccines were only manufactured in 2009–2010. Therefore, the publication from 2014 (10) was studying the antigen composition of expired vaccines. Antigen modification of the NP protein (e.g., NP protein multimers) in the MES-PAGE analysis images could also be consistent with protein alterations occurring in expired vaccines. An image of the full-length vertical MES-PAGE analysis “lane” was not provided which would have been helpful in confirming the extent of protein alterations in protein bands lower down the “lane”. Furthermore, as correctly stated by the authors of that publication, they received only ten vials of Arepanrix and were therefore unable to perform all immunological experiments in parallel with both Arepanrix and Pandemrix (no parallel data were available for Arepanrix). Therefore, the authors’ conclusions should be interpreted with caution, and they correctly echo this in their discussion of the “limitations” of their study.

Topic 7: “Vaccination of HLA-DQB1*06:02 transgenic mice with Pandemrix does not trigger narcolepsy...”

As mentioned in our publication (2), back translation from

clinical studies will likely require additional engineering to ensure that the appropriate human T cell repertoire is present in humanized mice. Although a recent report (10) indicated that Pandemrix immunization in mice transgenic for HLA-DQB1*06:02 did not elicit narcolepsy, the investigators suggested that the T cell receptor repertoire (not humanized) in such mice may have been inadequate for elicitation of narcolepsy after immunization with Pandemrix.

We hope that the abovementioned clarifications provide context to the reader and help to reinforce the importance of the conclusions drawn by Nellore and Randall. Our studies suggest that vaccine-associated narcolepsy contributes to the spectrum of immune-mediated disease development involving modulation of HCRT₂ signaling and then culminating in loss of HCRT-producing neurons. Indeed, modulation of HCRT₂ signaling has been demonstrated in five seminal studies of the HCRT ligand and receptor (11-16). The complexity of this disease process (17-19) is why our original investigation (2) brought together twenty experts spanning vaccine discovery and development, informatics, formulations, *in vitro* cell biology, biologics (antibody) profiling, quantitative sciences, assay development, epidemiology, neurosciences, immunology, and mass spectrometry. As indicated in the title of the editorial, vaccine-associated narcolepsy may indeed reflect the “inappropriate awakening of immunity” which hopefully we are getting closer to putting back to sleep by ongoing investigations into this complex immune-mediated disease.

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None.

Footnote

Conflicts of Interest: SS Ahmed and L Steinman are two of three co-authors from the original publication (the third being W Volkmuth) who are inventors on a patent (“Avoiding narcolepsy risk in influenza vaccines”) with application number PCT/EP/2014/059672 with priority date May 10, 2013, and publication number WO/2014/180999 on November 13, 2014.

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