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Review

# Is narcolepsy a classical autoimmune disease?

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ABSTRACT

Narcolepsy is a neurological disorder characterized by excessive daytime sleepiness. It is caused by the loss of orexin producing neurons in the lateral hypothalamus. Current evidences suggest an autoimmune mediated process causing the specific loss of orexin neurons. The high association of the disease with the HLA DQB1*06:02, as well as the link with environmental factors and are important clues supporting this theory. Recently, the association between the occurrence of the disease and vaccination campaign after the 2009 H1N1 pandemic highlighted the importance to increase the knowledge in the Pandora box of the vaccines. This review discusses the last finding regarding the pathogenesis of the disease and its relationship with the H1N1 vaccines.



**Contents**



*Abbreviations:* ASIA, autoimmune/autoinflammatory syndrome induced by adjuvants; REM, rapid eye movement; ICSD-3, International Classification of Sleep Disorders version 3; MSLT, Multiple Sleep Latency Test; CSF, cerebrospinal fluid; Trib2, tribbles homolog 2 protein; NEI, neuropeptide glutamic acid-isoleucine; αMSH, α-melanocyte-stimulating hormone; MCH, melanin-concentrating hormone neurons; SGA, streptococcal group A; HLA, human leukocyte antigen; PANDAS, pediatric autoimmune disorders associated with streptococcal infections; SOREMPs, sleep onset REM periods.

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## Introduction

Autoimmune diseases are chronic inflammatory conditions which can be organ specific or systemic. They are initiated by the loss of immunological tolerance to self-antigens. Their pathogenesis involved both intrinsic (e.g. genetic, epigenetic) and environmental factors (e.g. infections, vaccines, adjuvants). In 2011, Shoenfeld and Agmon-Levin described the Autoimmune/autoinflammatory syndrome induced by adjuvants (ASIA) to summarize the spectrum of immune-mediated diseases and symptoms triggered by an adjuvant stimulus [1,2]. In addition, they detailed a collection of data demonstrating the association between different vaccines and autoimmune diseases [1,2]. In particular, influenza vaccine has been related with systemic lupus erythematosus, rheumatoid arthritis, vasculitis, reactive arthritis and Guillain Barré syndrome [3]. Interestingly, after the declaration of a global H1N1 influenza pandemic an impressive amount of evidence linked the vaccination campaign with an increment in the number of cases of narcolepsy in Europe [4].

Narcolepsy is a sleep disorder characterized by excessive sleepiness daytime. It is considered a rare disease with world prevalence between 25 and 50 per 100,000 people [5]. However, its prevalence varies from one country to another highly depending on the genetic background of the population. For instance, the lowest has been reported in the middle east, being 0.23/100,000 in Israeli Jewish population [6] and in Saudi Arabia 4/100.000 [7] while Japan presents the highest reported up to 160/100,000 [8]. According to the International Classification of Sleep Disorders version 3 (ICSD-3), this disease is characterized by uncontrollable rapid eye movement (REM) attacks in which the previous non-REM stage is absent. In addition, patients may present other symptoms such as cataplexy (loss of muscle tone), disrupted nocturnal sleep, sleep paralysis, hallucinations and obesity [9,10]. In 2014, the ICSD-3 criteria established that narcoleptic type 1 patients (orexin deficiency) must present daily periods of irrepressible need to sleep or daytime lapses into sleep. Furthermore, they must meet at least one of the following characteristics. First, presence of cataplexy and the sleep tests should show mean sleep latency up to 8 min or to have two or more sleep onset REM periods (SOREMPs) on a Multiple Sleep Latency Test (MSLT) under standard conditions. Second, the levels of orexin 1 cerebrospinal fluid (CSF) should be less than 110 pg/mL or 1/3 of mean values lower than the mean obtained in normal subjects with the same immuno-reactive standardized assay [11].

In narcolepsy, the specific group of neurons in charge of orexin production is loss, which is a neurotransmitter involved in different metabolic process. This resembles the specific destruction of insulin-producing β-cells in the pancreas of patients with diabetes mellitus type 1 [12,13]. Therefore, narcolepsy has been considered an autoimmune disease. Orexin absence inhibits the ability to regulate the sleep-arouse balance, resulting in sleep disturbances [14,15]. The orexin levels in the CSF are low or undetectable compared with healthy individuals as consequence of the absence of these neurons [9,15]. According to Rose revision of Witebsky's postulates, five criteria are necessary to recognize an autoimmune disease [16]. Initially, the identification of the auto-antigen(s) involved in the disease is necessary; followed by the identification and isolation of auto-antibodies or auto-reactive T cells. This will allow the inoculation of the antigen with adjuvant in naïve animals inducing the progression of the disease and the appearance of T reactive cells or auto-antibodies. Consequently, the passive transfer of T cell or antibodies from the sick in to naïve animals should replicate the disease. Finally, additional circumstantial evidence such as genetic association and environmental triggers of autoimmune mediated process among others should be considered [2,16,17]. In this review, we will examine evidences supporting an autoimmune origin of narcolepsy, as well as the emerging concepts regarding the association between narcolepsy and environmental factors.

## Immune response to the unknown auto-antigen in narcolepsy

The identification of specific auto-antigen(s) is a key factor to unravel the autoimmune process of a particular disease. It is expected that the discovery of the auto-antigen(s) will allow the understanding of the mechanisms which are altered in the disease. In narcolepsy the auto-antigen has not yet been identified, rendering impossible an active immunization to induce an animal model. Moreover, some authors reported higher levels of inflammatory cytokines (i.e. G-CSF and IL-8) in the plasma of narcoleptic patients [18] and altered cytokine profile in patients with narcolepsy-cataplexy compared to controls, particularly involving IL-4 and significant Th1/Th2 imbalance [19]. However, no inflammatory process, including lymphocytic infiltration has been observed in the hypothalamus during post-mortem brain analysis of long-term disease patients [20,21]. It is noteworthy that the impossibility to analyze the brain of patients in early stages is a major obstacle [4,22,23].

## Exploration for auto-antibodies: clues for auto-antigens

The presence of auto-antibodies is a main characteristic of autoimmune diseases. Therefore, in order to find evidence for the autoimmune etiology of narcolepsy some studies have searched the presence of specific auto-antibodies. Passive transfer of antibodies from narcoleptic patients to murine models has demonstrated either sleep behavioral disturbances or brain histological changes [24]. Moreover sera from narcoleptic patients can bind brain or muscle structures [4,25]. However, the specific mechanisms by which the antibodies are inducing these changes are still unknown. So far, some attempts to identify a possible auto-antigen have been done base on the analysis of the specificity of antibodies from narcoleptic patients.

In 2010, Cvetkovic-Lopes et al., described the presence of antibodies against Tribbles 2 (Trib2) in narcoleptic patients, which bound orexin producing neurons in mouse brains [26]. These autoantibodies were described originally in uveitis (11) and they were found to have higher titers in a small group of narcoleptic patients (14%) when they were compared with healthy controls [26–28]. Total IgG from narcoleptic patients (confirmed to be anti-Trib2 positive) was intra-ventricular brain injected in naïve mice. This induced narcoleptic-like attacks similar to those observed in narcoleptic patients as well as loss of orexin producing neurons in the lateral hypothalamus of the injected mice, as well as behavioral and cognitive changes [24]. Moreover, the animals injected with IgG from narcoleptic patients had also loss of NeuN (neuronal marker), and synaptophysin (synaptic marker) which did not necessarily correspond to the same spots where the orexin neurons are located. This may indicate that the antibodies can have also effects on other groups of neurons contributing to the changes observed in the animals. This study reported for the first time the importance of humoral response in narcolepsy [24]. Despite this association there is no clue about the involvement of anti-Trib2 in the disease, since Trib2 is not reported to be involved in a process specifically associated with orexin or sleep [29,30]. Indeed, just one report indicates that this Trib-2 may be associated with brain processes as it is highly express in female mice with anxiety, yet a specific mechanism linking it with narcolepsy has not been described [31].

A recent study also found that passive transfer of IgG from narcoleptic patients in rat brains induced changes in different

sleep parameters. Three different patterns in which sera of narcoleptic patients can bind to brain rat tissue were identified. In the first pattern, antibodies bound mainly hypothalamic melanin-concentrating hormone and pro-opiomelanocortin but not orexin neurons. In the second pattern, GABAergic cortical interneurons were recognized by the antibodies. In the last pattern antibodies mainly bound globus pallidus neurons. Interestingly, a more detailed analysis of the first pattern showed that antibodies recognized a common C-terminal epitope in the neuropeptide glutamic acid-isoleucine (NEI) and the α-melanocyte-stimulating hormone (αMSH) peptides. These two molecules are present in the hypothalamic in the melanin-concentrating hormone neurons (MCH) but not in orexin producing neurons [32]. These results contrast Cvetkovic-Lopes report who showed that sera from patients can bind orexin producing neurons [26]. Unlike anti-Trib2 the presecence of antibodies to MSH expresing neurons can potentially interfer in the process associated with narcolepsy, since MSH neurons can moduluate orexin neurons functions [33,34].

## T cell involvement

In 2013, an exciting report suggested orexin to be an auto-antigen. Functional analyzes of $CD4^+$ lymphocytes from narcoleptic patients showed that these cells were able to recognize orexin peptides presented by dendritic cells (homozygotic for DQA1*01:02/DQB1*06:02 haplotype). Moreover, those cells could also recognize peptides from H1N1 indicating a possible molecular mimicry between orexin and similar peptides from H1N1. This may partially explain the relationship described between the H1N1 vaccine and narcolepsy (See Sections "AH1N1 vaccine and influenza infections" and "The vaccine dilemma") [35]. However, the authors were not able to replicate the results leading to the retraction of the paper [36,37]. It is to note that the original paper still demonstrated structural similarities between orexin peptides and the haemaglutinin 1 employed for the elaboration of the vaccine which can be further explored.

## Genetic associations

One of the common characteristics of autoimmune diseases is to be frequently associated with specific HLA. The strongest evidence that narcolepsy has an autoimmune etiology comes from genetic studies. Several studies demonstrated that narcolepsy is highly associated with HLA risk polymorphisms, in particular DQB1*06:02 and DQA1*01:02. The higher risk is reported for DQB1*06:02 carriers as it is present in 82–99% of narcoleptic patients [38,39], while only 12–38% of healthy individuals have this allele. Indeed, homozygote individuals for DQB1*06:02 have an increased risk to develop narcolepsy [38–40]. Other polymorphic associations have been described with other genes such as tumor necrosis factor alpha [9], tumor necrosis factor (ligand) superfamily member 4, T cell receptor alpha chain [41], Cathepsin H, DNA methyltransferase I [42,43], among others [44]. Of note, many of the polymorphisms are in immune related genes supporting the hypothesis of an immune-mediated mechanism which may involve antigen presentation, including lymphocyte sub-populations as well as antigen presenting cells [23].

## Additional autoimmune related aspects

Due to the common mechanisms in autoimmune diseases [17], the presence of more than one autoimmune disease in one patient is common [45]. A study in a Spanish cohort showed that approximately 16% of the narcoleptic patients had one or more immunopathological disorders including allergies and autoimmune diseases, such as systemic lupus erythematosus and multiple sclerosis. In addition, familial autoimmunity was recently demonstrated to be a frequent condition [46]. In the case of narcolepsy, first degree relatives of patients have a higher risk to develop the disease [47,48], supporting the importance of genetic background. However, twin studies demonstrated that the concordance rate of narcolepsy in monozygotic twins is 20–35% suggesting that the development of the disease does not depend only on the genetic background but also on environmental factors [48].

## Environment, infections and vaccines

In genetically susceptible individuals autoimmune diseases can be triggered by exposure to external molecules or factors. As mentioned above, the concordance rate of narcolepsy in twins indicates the importance of environmental factors. Interestingly, the age of onset in narcolepsy is frequently at teenage suggesting that hormonal changes in puberty might trigger the disease [8,49–52]. Few reports regarding the exposure to toxic substances have been done. For example, one study comparing narcoleptic patients with a group of match controls showed that exposure to heavy metals, woodwork, fertilizers and pesticides are a risk for narcolepsy in a particular population [53]. Passive smoking has also been related with the onset of the disease in HLA DQB1*06:02 carriers [54]. In addition, other external stressors such as major changes in sleeping habits or changes in living style, carried out an additional risk [52]. Unfortunately, all this evidence is limited to small cohorts and specific populations making necessary to clarify the role of these factors in the development of the disease. Nevertheless, the strongest and most discussed evidence regarding environmental factors and narcolepsy is the association with infections and vaccination.

In 2007, the medical records analyses of narcoleptic DQB1*06:02 carriers and matched controls as well as the evaluation of detailed questionnaires demonstrated the importance of infectious agents. Thus, measles infection and the presence of unexplained fever in the past history were reported to be associated with higher risk of developing subsequently narcolepsy [52]. It is widely recognized that infections can induce autoimmunity through different mechanisms, such as molecular mimicry, epitope spreading, bystander activation and superantigens [55–59]. In the narcolepsy *scenario*, it has been suspected that streptococcal and influenza A infections as well as the H1N1 vaccine play a role in the pathogenesis of the disease [60–62].

### *Streptococcal infections*

The importance of streptococcal group A (SGA) infection in autoimmunity has been extensively studied. The molecular mimicry between SGA and human proteins helps to the progression of immune-mediate damage which may involve heart, joints, skin and brain as a consequence of antigen mimicry with M – protein (40). Moreover, the production of superantigens by SGA can also stimulate auto-reactive B and T cells leading to the production of autoantibodies [63,64]. High anti-streptococcal antibodies were detected in the sera of newly diagnosed narcoleptic patients close to the onset of the disease [60]. Consequently, the analysis in narcolepsy patients and matched control carriers of HLA DQB1*06:02 concluded that childhood streptococcal throat infection was a risk factor for narcolepsy when it compared childhood infectious diseases, such as mononucleosis, pneumonia, or hepatitis [65]. An interesting example was described in 2013, when an eight year old child positive for DR2 (DR 15) and HLA DQB1*0602 was diagnosed with Sydenham Chorea and narcolepsy. Interestingly, besides the HLA this patient had CSF orexin deficiency and elevated titers of anti-streptococcal antibodies (i.e. anti-streptolysin O) [65]. It is important to highlight that SGA infections have also been related

with neurological conditions other than narcolepsy. Indeed, Sydenham chorea syndrom, pediatric autoimmune disorders associated with streptococcal infections (PANDAS) and different dystonias have been described following this infection [63,66–68]. Of note, the association is not only with the infection itself but also with the presence of auto-antibodies against neuronal proteins including neuronal receptors which can alter signaling pathways, such as CaMKII, tyrosine hydroxylase with eventual dopamine release or the direct stimulation of dopamine receptors [66–71].

*AH1N1 vaccine and influenza infections*

A recent report summarized the history of narcolepsy research in Stanford University. It describes the first observation regarding a temporal association between sleep related disturbances and H1N1 pandemic infection. In 1918, during the influenza pandemia, sleep disturbances and movement disorders as well as extreme sleepiness were noted in flu patients (for more details see [22]). These findings were also supported in the Chinese population after the last pandemic H1N1 influenza. The pandemic infection in China was followed by a parallel increment in the incidence of narcolepsy. The study found 3–4 fold rises in the incidence of narcolepsy in Beijing-China after H1N1 infections [72]. It is noteworthy that in 2011 the incidence of narcolepsy in the Chinese population decreased notoriously returning to the usual incidence [72,73].

The outbreak of the 2009 pandemic influenza led to the fast development and approval of different vaccines resulting in massive immunization campaigns all over the world [4]. In Europe, the use of 8 different commercial vaccines was approved (all designed from the A/California/7/2009 (H1N1) v-like strain). The most widely used was the ASO3-adjuvanted vaccine [4,74,75]. Following the 2009 H1N1 vaccination campaing, in 2010 an increment in the diagnosis of narcolepsy in Finish children was noticed [75,76]. This observation led to through deeper epidemiological analysis of the association. In late 2011 the National Institute for Health and Welfare created a task force to determine whether there was a causal relationship between the rise in narcolepsy cases in children from Finland and the 2009 vaccination campaign. The analysis was done comparing the narcolepsy incidence before and after 2009 showing an increased risk of narcolepsy in the 4–19 age group (9-fold) among those who received the ASO3-adjuvanted vaccine, Pandemrix™ [77]. Later, the analysis of the individuals infected with the virus show no increased risk of narcolepsy in the overall population [78]. In consequence, the risk of narcolepsy associated with the H1N1 vaccination was evaluated in other countries by retrospective studies based on health care databases and the annual incidence of the disease [23,76,79–86]. It is important to mention that in other countries such as Italy and the United Kingdom there was no evidence of increased risk of narcolepsy with Pandemrix™ [79].

More detailed analyses of some narcolepsy cases linked to the AS03 adjuvanted vaccine have shown interesting characteristics. Slight differences between patients who received or not the vaccine were found in a French study. In particular, vaccinated patients had a shorter delay in diagnosis and the MSLT analysis showed higher number of sleep onset REM periods [86]. Moreover, genotypification of Narcolepsy/Cataplexy post vaccination cases from Switzerland, United States, the United Kingdom, France, and Brazil found that all the patients were carriers of DQB1*06:02 [61,76,87], and one particular case with an additional diagnosis of multiple sclerosis was also DRB1*15:01 positive [88].

## The vaccine dilemma

Vaccines can be classified within two groups according to their components. The first one is live attenuated vaccines that try to resemble the natural infection. The second group includes subunit, toxoid, carbohydrate, and conjugated vaccines which usually contains adjuvants to enhance and modulate the immune response [89]. In response to the global H1N1 influenza pandemic, both kind of vaccines were design and commercialized [4,74,79]. During the vaccination campaign the vaccines containing squalene based adjuvants were the most used (Table 1 – [80]). Interestingly, in Europe the association between narcolepsy H1N1 vaccinations was specifically related to the ASO3 (Pandemrix™) and not to the MF59 adjuvanted vaccine (Focetria®) [4,23].

*The manufacturing process hypothesis*

There is no evidence of narcolepsy in other adjuvanted or non-adjuvanted H1N1 vaccines [90–93]. Just one case has been reported

**Table 1**
Commercial H1N1 vaccines authorized for 2009 pandemic by the European Center for Disease Prevention and Control or Helath Canada discussed in this review.

| Name | Producer | Components | Haemagglutinin content (μg) | Adjuvant | Adjuvant Emulsion per dose | Manufacture inactivation protocol |
|---|---|---|---|---|---|---|
| Pandemrix | GSK | Split-virion, reassortant A/California/7/2009 (H1N1)v like strain, inactivated, Adjuvanted | 3.75 | AS03 | Squalene 10.69 mg<br>α-Tocopherol 11.86 mg<br>Polysorbate 80 4.86 mg | Influenza virus concentration and purification by zonal centrifugation using on a linear sucrose density gradient solution containing detergent to split the virus.<br>Additional purification by diafiltration<br>Inactivation by deoxycholeate and formaldehyde |
| Arepanrix | GSK | Split influenza virus, A/California/7/2009 (H1N1)v like strain (X-179A) inactivated, Adjuvanted | 3.75 | AS03 | Squalene 10.69 mg<br>α-Tocopherol 11.86 mg<br>Polysorbate 80 4.86 mg | Influenza virus inactivation by UV followed by formaldehyde<br>Purification by centrifugation and disruption by deoxycholate (Tween 80) |
| Focetria | Novartis | Surface-antigens (haemagglutinin and neuraminidase), reassortant A/California/7/2009 (H1N1)vlike strain, inactivated | 7.5 | MF59C.1 | Squalene 9.75 mg<br>Polysorbate 80 1.175 mg<br>Sorbitan trioleate 1.175 mg | |

Adapted from "Q&A for Heath Professionals on Vaccines and Vaccination in Relation to 2009 Influenza A(H1N1) Pandemic", "Arepanrix H1N1. AS03-Adjuvanted H1N1 Pandemic Influenza Vaccine" and "Association of receipt of Pandemrix™ and narcolepsy in children and adolescents in the UK (England) (ECDC 2009-2013, GSK, 2009)
GSK: GlaxoSmithKline

in Brazil, where a 19 year old women carrier of the DQB1*06:02 allele developed narcolepsy after she was vaccinated with Arepanrix (AS03 adjuvanted vaccine manufactured in Canada – Table 1) [4,87]. Recently, the hypothesis that the manufacturing process of the vaccines can be the culprit of this situation has been emerged, since they vary in composition as well as in the production process (Table 1) [4,23].

This theory has been discussed since the risk of narcolepsy is associated with Pandemrix™ but not with Arepanrix™ or other H1N1 vaccines [90]. Lately, the first two epidemiological studies in Canada were published [94,95]. The vaccine used during the vaccination campaign after the 2009 H1N1 pandemic was Arepanrix® which differs from Pandemrix™ in the place and the protocol of manufactured (Table 1) [4,23]. In agreement with previous results the analysis done in Ontario did not find any risk associated with the vaccine. Briefly, 1604 adverse events were reported following the immunization, 5 cases went to clinical verification but none of them were consistent with narcolepsy [94]. Likewise, the results in Quebec reported a small risk mainly in persons less than 20 years of age. However, the authors claim that influenza infection could be a confounding factor [95]. All this together suggests that the different protocols employed during the inactivation and the splitting of the virion can influence the final conformation of the immunogenic viral proteins within the vaccine. Therefore, these changes may be reflected in slight modifications in the epitopes that are potentially recognized by T or B cells in genetically susceptible individuals [4,23,96].

*ASO3 adjuvant and the α-tocopherol hypothesis*

The analysis of clinical and epidemiological results of MF-59 did not find any risk of narcolepsy [97,98]. Worth mentioning, the major difference between the ASO3 and the MF59 adjuvants is the presence of the α-tocopherol (Table 1). The adjuvant MF59 induces the expression of cytokines and chemokines by muscle cells at the injection place in mice model resulting in the migration of immune cells including monocytes and granulocytes, especially neutrophils [99]. In consequence, the interaction between these cells amplifies the immune signal improving the antigen processing as well as the transportation to regional lymph nodes. This whole process results in the enhancement of cellular immune response [98–100]. In contrast, the ASO3 adjuvant contains α-tocopherol which is a form of vitamin E. The immunomodulator activity of this molecule promotes the innate immune system activation not solely at the injection site but also in non-regional lymph nodes, which makes it more potent in terms of immune system activation. Actually, it was demonstrated that α-tocopherol can achieve higher titers and more stable antibody response [101]. Moreover, AS03 modulates the expression of cytokines incrementing the antigen processing and transportation to the lymph nodes, thus leading to enhancement of the antigen-specific adaptive immune response [102,103]. Remarkably, a recent study demonstrated by *in vitro* analysis that α-tocopherol can increase the production of orexin as well as the proteosome activity in a murine hypothalamic cell line. Within the cell, the overproduction of a specific protein is controled, among other by ubiquitination and posterior degradation of the protein in excess. Therefore, this suggested that the overproduction of orexin fragments can conduce to a deregulation of internal cells mechanisms. This may conduce to antigen presentation via HLA, triggering an autoimmune process [104]. However, the authors did not report increment in the expression of HLA molecules leaving an unanswered important aspect. However, all these results may suggest that the differences between vaccine components as well as their manufactured protocol can have an important effect not only in its immunogenic efficiency, but also in the induction of non-specific immune responses and perhaps autoimmunity. Unfortunately, there are not enough data regarding the differences in the immune reaction to each adjuvant [103].

## Conclusions

Different hypothesis have been proposed to clarify the autoimmune etiology of narcolepsy. Based on these facts we believe that narcolepsy may be an autoimmune disease mainly driven by humoral response. However, the absence of evidence cannot exclude the role cellular response. Autoimmune diseases result from the interaction of 3 factors leading to the clinical manifestations: first inherited factors (i.e. genetics), second environmental factors, and third the breakdown of autoimmune tolerance in particular the appearance of auto-antibodies or auto-reactive T cells. In narcolepsy, the third factor is still unclear. Nevertheless, data are missing regarding the classical criteria for autoimmune diseases. For instance, it is imperative to identify the auto-antigen in order to develop experimental models with active immunization. Therefore, further analyzes on the role of Trib2 and of melanin-concentrating hormone as well as other neural networks are needed to clarify their role in the pathogenesis of the disease.

The association between the H1N1 vaccination or flu infection itself and narcolepsy onset in Europe is well recognized. The epidemiological results showed the importance of geographic location and genetic background. It also raised the concern regarding the effects of vaccines in susceptible individuals. Moreover, it is necessary to homogenize protocols for safety evaluation of vaccines and studies on the effects of adjuvants both on immune system and other physiological processes. This will allow the comparison between components as well as the proper evaluation of the safety. Further follow-up regarding the association with H1N1 vaccination should be done to avoid the induction of the disease in the scenario of future pandemic. Therefore, it is necessary to understand how the manufactured process can alter the H1N1 molecules and the immune system response, as well as if the component of the adjuvant can deregulate neuronal and immune cells.

## Competing interests

Y. Shoenfeld has acted as a consultant for the no-fault U.S. National Vaccine Injury Compensation Program. The other authors declare no competing interests.

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