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Animal studies are mandatory to investigate the poorly understood fate and effects of aluminum adjuvants administered to billions of humans and animals worldwide

In a recent paper Ameratunga; Languth, and Hawkes [1] raised "scientific and ethical concerns" pertaining to animal models of autoimmunity/autoinflammatory syndrome induced by adjuvants (ASIA) [1]. The authors have previously questioned the existence of ASIA using arguments that were dismissed [2]. Now, they try to convince the scientific community to forbid animal studies evaluating safety of aluminum adjuvants.

This is a shocking recommendation (i) because there has been only one reference experimental study on aluminum adjuvants toxicokinetics [3] and it suffers major conceptual and methodological limitations [4]; (ii) because aluminum adjuvants safety has never been epidemiologically evaluated on the long term, the Centers for Disease Control and Prevention stating "there have been no population-based studies specifically designed to evaluate associations between clinically meaningful outcomes and non-antigen vaccine ingredients, other than thimerosal" [5]; and (iii) because these poorly understood compounds used in 60% of current vaccines are intended to be administered to billions of individuals over the next years in the setting of a massive expansion of vaccine prevention strategies announced worldwide [6].

Ameratunga et al. [1] reviewed a list of animal studies said to have been conducted to demonstrate ASIA [7–17]. This selection is inadequate at least for the first study [7] which included no clinical evaluation because it was designed to explore and understand systemic translocation of aluminum and other biopersitent particles injected in muscle. In contrast, Ameratunga et al. omitted a number of mouse studies documenting neurologic effects of aluminum adjuvant administration [18–20].

Ameratunga et al. [1] listed several areas of concern in the evaluated studies.

1- Inappropriate dose and/or delivery of adjuvant used

Three studies, including the irrelevant one mentioned above, were criticized because they used doses considered "far in excess" of those used in humans [7,9,10]. Another one, that appropriately used a conversion factor to adjust the dose, was pointed out because it administered Gardasil® on 3 consecutive days instead of an over 6 months period used in humans [14]. Extrapolating mouse to human dosage is a challenging issue since a firm scientific basis for allometric conversion is still lacking. To approximate human to mouse dosage for aluminum studies, a conversion factor of ×30 is used by the Agency for Toxic Substances and Disease Registry [21] and by the US Food and Drug Administration [22]. Since ASIA usually occurs after multiple aluminum-containing vaccine administrations (up to 12 injections-not restricted to a single vaccine- in our experience [23]) administration of important doses may not be so unrealistic. It is also inevitable that the administration schedules differ in mouse and humans, since mice live no more than 2 years. To avoid endless debate on these points, it could be useful to follow recommendations made by the experts of the national drug agencies as we did in a recent study [20].

2- Inappropriate adjuvant used

Freund's Complete adjuvant (FCA) is one of the first adjuvants developed and has been widely used in research. However FCA causes local inflammatory lesions which can be quite severe and its use can be only accepted by ethic committees if their use is scientifically justified for the induction of autoimmune disease models for which currently no comparable alternatives are known to exist. Less problematic alternatives to FCA exist, mainly including Aluminum hydroxide which is recommended as a "non-inflammatory adsorptive adjuvant "by Institutional Animal Care and Use Committees [24]. It is therefore dishonest to consider a few papers that conventionally used FCA to elicit given autoimmune reactions (8,9,11,12), including one comparative study that unambiguously recommended the use aluminum adjuvant instead of FCA (12), and take pretext of these papers to dismiss aluminum adjuvants-based studies on ASIA.

We would like to stress that there are 3 commonly used aluminum adjuvants. If Aluminum hydroxide (Alhydrogel®) and aluminum phosphate (Adjuphos®) are commercially available, the new generation adjuvant amorphous aluminum hydroxyphosphate sulfate (AAHS) used in Gardasil® is a Merck proprietary adjuvant [25]. To our knowledge, this adjuvant is not available for independent experimental safety studies, which may raise ethical problems since it has been placed in both vaccine and placebo in Gardasil premarket trials [26].

3- Use of genetically susceptible mice

Ameratunga et al. [1] pointed out two studies that used SLE prone mice (9,13). Their criticism is difficult to understand. These studies evidently aimed at evaluating if adjuvanted vaccines could represent an environmental factor that may trigger and perpetuate an autoimmune disorder in an individual genetically susceptible to this autoimmune disease. The mouse model used was specifically susceptible to SLE, and not to other autoimmune diseases. Whether or not the experimental results could be translated to the human situation is another question. Of note, the vast majority of aluminum adjuvants-based experimental studies did not use genetically susceptible animals.

4- Small numbers of animals

Ameratunga et al. [1] criticized the same two studies for the limited number of animals used. This was not the case for the vast majority of aluminum adjuvants-based experimental studies. The exploratory study in sheep is a particular case since this study was conducted to

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confirm clinical observations made in the same species [15]. Confirmatory studies on larger number of animals are in progress.

5- Poorly defined experimental protocols and irreproducible data

According to Ameratunga et al. [1] the experimental protocols were not sufficiently detailed in two studies [8,10]. They also made vague statements about omission of the adjuvant type and route of administration, but they didn't quote precise references supporting their contention, which is unacceptable. They also raised a point about the method used to assess proteinuria in one paper (12), which could not be viewed as precluding reproducibility of the experiments.

Finally, the efforts of Ameratunga et al. [1] to pick up punctual limitations in a small number of papers to dismiss the whole appear obvious. On these weak grounds, instrumentalizing the Bradford Hill criteria for causality is vain.

6- Severe pain induced in animal

In this section, Ameratunga et al. [1] reiterated the same strategy. They pointed out a very limited number of studies using CFA, approved by local ethic committees, to which they added two clearly irrelevant studies not initially incorporated into their list [27,28]. This was done to raise the spectre of animal torture and call "for an immediate moratorium on animal experiments of ASIA". As stated above, aluminum adjuvants is recommended as an alternative to CFA by ethic committees and has been used in billions of humans and animals with an excellent local tolerance profile. Taking advantage of the rise of anti-animal experimentation movements to claim for a global rejection of animal studies on adjuvant safety is, in our opinion, an unethical way of raising a proper scientific discussion.

Confronting the attempt to block further experimental investigations on aluminum adjuvants made by Ameratunga et al. [1] to some advances made by our two groups using experimental approaches may indicate why further investigations in the field are strongly needed:

- In contrast to previous belief [3], Aluminum hydroxide injected in muscle is not solubilized in the interstitial fluid and vaccine-derived aluminum is not quickly eliminated in urine: instead, this nearly insoluble particulate compound is quickly captured by monocyte/macrophage lineage cells and may persist within these cells from many months after injection in animals [29] to many years in some human beings [30].
- In contrast to the classical depot formation hypothesis in which local deposition of the adjuvants was thought to play a crucial role [31], it is now clear that a substantial part of the injected adjuvant is transported within cells to distant organs [7,32] where they may persist as long as in the injected muscle [33]. These organs include the regional lymph nodes, spleen and liver, and particles can eventually enter in the brain using a CCL2-dependent Trojan horse mechanism [7,32,33]
- In contrast to previous belief that innocuity of aluminum adjuvants can be inferred from the low quantitities of Al3+ injected with vaccines ("the dose makes the poison"), neurotoxic effects of Alhydrogel® were shown to respond to a non-linear dose response curve with selective toxicity of the lowest dose [20]. It seems that toxicity of this nearly insoluble particulate adjuvant that selectively concentrates in immune cells may obey the specific rules of small particle toxicology rather than any simplistic dose-response relationship.
- If small animal studies showing toxic effects of aluminum adjuvants are often suspected to be irrelevant to the human situation, this is not the case of large animal models. In sheep, multiple aluminum-containing vaccine administrations were shown to be associated with a biphasic neurologic disease including initial meningoencephalomyelitis with behavioral alterations followed by progressive spinal neurodegenerative changes, offering an invaluable model to understand human ASIA [15,34].

If aluminum-containing vaccines seem globally safe and will probably continue to prove most useful in future years, we believe that the optimization of both vaccine products and practices is desirable to limit the occurrence of rare adverse events. Our two groups have long clinical experience of neurologic manifestations temporally associated with multiple injections of aluminum hydroxide adjuvant-containing vaccines, in humans [23,30,35,36] and sheep [15]. These observations are in line with a huge number of convergent medical observations described as ASIA cases in all parts of the world [37]. On the one hand, temporal association does not equal causation, of course. On the other hand, the existence of a camodel to understand human ASIusal link between multiple vaccine administrations and neuro/immunologic adverse effects is difficult to establish by epidemiology [38]. In 2012, the Institute of Medicine (IOM) indicated "the evidence was inadequate to accept or reject a causal relationship" for the vast majority of vaccine adverse effects they examined, and considered "the inadequate understanding of biologic mechanisms underlying vaccine adverse effects" as one major cause of uncertainty. The IOM committee declared that "the value of dialogue between both epidemiologic and mechanisms approaches cannot be overstated. These conversations between different types of research can be difficult, but the results are worth it"[38]. This is why animal studies are mandatory in the field of ASIA.

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