Aluminium from adjuvanted subcutaneous allergen immunotherapeutics in rats is mainly detected in bone

To the Editor,

Aluminium (Al) hydroxide (AH) is frequently used as adjuvant in subcutaneous allergen immunotherapy (SCIT) in Europe. With regard to the cumulative Al amount administered per year, SCIT constitutes an important source of Al in humans, and its safety is constantly questioned in the public. Toxicokinetics of Al after SC injection of those poorly soluble Al particles is unknown. We conducted a study in rats receiving a single SC injection (1 mL) of either plain AH adjuvant (pAH: Alhydrogel®, 1.25 mg Al/mL; n = 6 rats), each of two marketed AH-adjuvanted SCIT products at highest marketed strength with high Al content but different in adjuvant manufacture (P1: Alhydrogel®-adjuvanted mixed grass pollen allergen, 1 mg Al/mL; P2: in situ co-precipitated AH-adjuvanted mixed grass pollen allergen, 1.13 mg Al/mL; n = 6 rats each), or vehicle (saline; n = 3 rats). Al in plasma (during 80 days) and tissues (at day 80; whole humerus bone and right brain hemisphere) was determined by atomic absorption spectrometric (AAS) procedures (full Method section in Appendix S1).

Al plasma time courses after treatment did not exhibit profiles distinctive from that of the vehicle group (Figure 1); however, for P2, but not for P1 and pAH, a significant increase in Al plasma AUC(0–80 d) vs vehicle (mean [SD]: 1431 [314] vs 909 [48] µg/L*d) was found.

Al concentration in bone was significantly increased in all groups (Figure 2; Table S2), again strongest after P2 (mean [SD]: 1.28 [0.26], 0.64 [0.12], 0.72 [0.10] and 0.40 [0.11] µg/g wet weight (ww) for P2, P1, pAH and vehicle, respectively). However, concentrations were far below the levels of toxicological concern (>10–15 µg/g in both rats and humans1,2). A more visible increase in bone rather than plasma exposure is not surprising as in contrast to fast renal Al plasma clearance, elimination of Al from bone is very slow and skeleton is the major storage compartment for Al in the body of both rats and humans.3

No significant difference in brain Al concentration between any treatment and vehicle was observed (means: 0.07–0.29 vs 0.08 µg/g ww; Figure 2; full data see Table S2), which is consistent with observations of very small fractions of dose (<0.01%) retaining in brain.3 Since we measured the Al amount in a whole hemisphere, clusters due to focal accumulation as well as any undissolved Al particles potentially transported into the brain4 are fully captured. We conclude from our results that contribution of such particulate Al amounts in brain, if any, is negligible.

The findings in plasma and bone were corroborated by determinations at the injection site: a high Al release (65.9% of injected dose) was found for P2 in contrast to small dose fractions for plain Alhydrogel® and Alhydrogel®-adjuvanted product P1 (1.7 and 11.2%, resp.; Figure S3 and Table S2). The highest absolute Al amount released after 80 days was 659.1 µg for P2, being about fivefold compared with that for P1 (126.6 µg). Frequency of persistent nodules at day 80 was higher in the groups receiving pAH (6/6 rats) and P1 (5/6) compared with P2 (3/6), showing a plausible inverse relation to the Al release from the injection site.

A positive linear relationship was found between Al amount released from the injection site until day 80 and Al plasma AUC(0–80 d) (y = 0.73x + 927; r = .79) or bone Al concentration (y = 0.00079x + 0.63; r = .70), respectively (Figure S4). These correlations confirm that Al release was an indirect measure of the systemically available amount. However, we cannot exclude some overestimation of this amount as a fraction of insoluble AH might have been transported to the draining lymph node but not yet entered the system.

To our knowledge, this is the first time that Al from marketed SCIT products has been systemically detected. Increase in Al levels was mainly visible in bone, less in plasma, but not in brain. Furthermore, our data indicate that Al-adjuvanted SCIT products do not behave uniformly: we observed a remarkable difference in the degree of systemic Al availability at day 80 between two marketed AH-adjuvanted SCIT products, suggesting a higher rate of Al release from the co-precipitated product. This is most probably due to the physicochemical differences (ie chemical composition...
and amorphous state) of co-precipitated AH adjuvants compared with preformed commercial AH adjuvants such as Alhydrogel®. However, once absorption is completed, dose-normalized cumulative extent of bone Al increase is expected to be comparable between products. Crude linear extrapolation of our injection site release data over time (including literature data) suggests that complete absorption from Alhydrogel®-adjuvanted allergen preparations takes at least 350 days, whereas absorption from co-precipitated products might be completed much earlier (after ca. 120 days).

Our results cannot one-to-one be translated to humans. In relation to body weight, the doses applied to our rats (350 g) were 170 times higher compared with a 60 kg human adult. Considering an allometric scaling factor of 6.2 usually applied for dose conversion on mg/kg basis between rats and humans in pharmacology, this ratio is still 27. Thus, increase in bone Al concentration in a human adult after absorption of a SCIT Al dose of 659 µg (estimated Al release after P2 on day 80) is assumed 1/27 of that observed in our rats. Based on this calculation, we expect that after a single SCIT dose in humans, Al levels in bone, and even more valid in plasma and brain, will be indistinguishable from baseline levels. However, assuming full accumulation during a 3-year perennial SCIT applying 36 main-tenance doses each containing up to 1250 µg Al in adults a cumulative bone Al increase of 1-2 µg/g wet weight is roughly extrapolated. Compared with an upper limit of normal bone Al content in healthy humans of <10 µg/g dry weight, this increase is considered substantial but without clinical relevance. For more precise predictions of Al exposure according to various treatment schedules, particularly in children, a physiology-based toxicokinetic (PBTK) model is desirable. For its establishment, the results of this study are considered essential.

CONFLICT OF INTEREST

Author Jennifer D. Oduro declares that she is employee at preclinics GmbH, a contract research organization that has received payment for the conductance of the animal study. All other authors declare that they have no conflict of interest.

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Allergenomics of the tick *Ixodes ricinus* reveals important α-Gal–carrying IgE-binding proteins in red meat allergy

To the Editor

Red meat allergy known as mammalian meat allergy, caused by IgE antibodies against galactose-α-1,3-galactose (α-Gal), is nowadays recognized worldwide, and strong associations with tick bites have been identified for different tick species and geographical locations.1 Time relationship between tick exposure and increased IgE levels to α-Gal has further supported the strong evidence that tick bites are the primary cause of the IgE antibodies.2 Ticks in all developmental stages can bite humans, and in the United States, high IgE levels to α-Gal following bites from larvae have been reported.3 While we have previously demonstrated the presence of α-Gal in the gut of the European tick *Ixodes ricinus*,4 α-Gal–containing proteins in tick saliva from the South American, Japanese and European ticks, *Amblyomma sculptum*,5 *Haemaphysalis longicornis*6 and *Hyalomma marginatum*,7 have recently been reported. However, the α-Gal content of the *I ricinus* proteome has not been investigated yet. Here, we used allergenomics8 and shotgun proteomics approaches to identify IgE-binding α-Gal–carrying proteins in adult and larval stages and in saliva of *I ricinus* ticks. Allergen-specific IgE antibody responses were assessed by ImmunoCAP (Thermo Fisher Scientific) among 32 Swedish and 18 US mammalian meat allergic patients to reveal differences in IgE reactivity between two geographical populations. IgE- and α-Gal–binding capacities, as well as allergenicity of *I ricinus* tick proteins, were evaluated (details on Methods and Patients’ characteristics are presented in the Appendix S1).

Our data revealed that nearly all Swedish and American meat allergic patients had IgE responses against both the larval and adult stages of *I ricinus*, and we noted that the four individuals IgE negative to *I ricinus* had comparably low IgE levels to α-Gal (Table S1). Similar to previous reports, moderate-to-high correlations between IgE to α-Gal and adult ticks were seen in the two patient groups (Figure S1).6 Furthermore, the strong correlations between IgE reactivity to adult and larvae in both patient groups suggest that growth stages of *I ricinus* ticks seem to be of less importance for the IgE recognition (Figure S1). In Western blot, IgE-reactive protein bands in the molecular range of 25-150 kDa in *I ricinus* ticks were noted with comparable results for both tick stages using Swedish and American serum pools (Figure 1A,B, respectively). To investigate whether the observed IgE reactivities were α-Gal-related, the pools were preincubated with the α-Gal–carrying glycoprotein bovine thyroglobulin prior to immunoblot analysis. The IgE-binding to proteins from both adult and larval ticks was strongly diminished in Swedish and in US serum pools, revealing α-Gal as the main IgE target (Figure 1A,B lanes with α-Gal+) in both populations. Interestingly, proteins at the similar size were identified in *I ricinus* saliva that bound red meat allergic patients’ IgE and were recognized by the anti-α-Gal antibody (Figure 1C). Similar results have been reported for the analysis of saliva from the Japanese tick *H longicornis*.6 In addition, we investigated the IgE-binding capacity with IgE-inhibition ELISA where protein extract from *I ricinus* was able to inhibit IgE binding to HSA-α-Gal by up to 77% (Figure S2). We also evaluated the allergenic potential of *I ricinus* using blood from four mammalian meat allergic patients and noted that protein extract from adult ticks induced basophil activation in 13 patients (Figure 1D and Figure S3). The allergenic activity towards ticks was higher compared with HSA-α-Gal; however, HSA-α-Gal showed to be more sensitive. This shows that *I ricinus* protein epitopes also are of importance in basophil activation, which is in line with the IgE-binding capacity results (immunoblots and ELISA). When blood samples from four patients were stimulated with extract from *I ricinus* larvae (Figure 1E), the allergenic activity was found to be similar to adult *I ricinus* protein extract in three patients. None of the antigens activated basophils in two nonallergic individuals (Figure S3), indicating that the observed reactions were IgE-dependent. Basophil activation with adult *I ricinus* protein extract was dose-dependent reaching 72.7% of CD63-positive cells (median, 34.4%; range, 7.5% to 72.7%, at concentration 50 µg/mL) giving a sensitivity of 93%. Furthermore, a strong correlation between %CD63-positive basophils for adult *I ricinus* protein extract and HSA-α-Gal (Figure S4) was noted, pointing out the dominant role of the α-Gal epitope in activating red meat allergic patients’ basophils.

We used an allergenomics approach with 2D PAGE and 2D immunoblots together with mass spectrometry to identify α-Gal–carrying IgE-binding proteins in adults and larve ticks (Figure S5 and Figure S6; for details, please see Appendix S1). Analysis of the obtained MS/MS spectra gave high identification scores to 43 protein accession numbers for adult and 37 for larve from the *Ixodida* order (Table S2), grouped into six protein groups: vitellogenins, SERPIN, actin, α-2-macroglobulin, chitinase-like lectins and transport or