HAL
open science

Macrophagic myofasciitis: characterization and pathophysiology.

Romain K. Gherardi, François-Jérôme Authier

To cite this version:

Romain K. Gherardi, François-Jérôme Authier. Macrophagic myofasciitis: characterization and pathophysiology.. Lupus, 2012, 21 (2), pp.184-9. 10.1177/0961203311429557 . inserm-00665883

HAL Id: inserm-00665883

https://inserm.hal.science/inserm-00665883

Submitted on 12 Jan 2013

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Macrophagic myofasciitis: characterization and pathophysiology

Romain K. Gherardi * **, François-Jérôme Authier**

Institut Mondor de Recherche Biomédicale INSERM : U955 , Université Paris-Est Créteil Val-de-Marne (UPEC) , IFR10 , 8 rue du Général Sarrail, 94010 Créteil,FR

* Correspondence should be addressed to: Romain Gherardi <romain.gherardi@hmn.aphp.fr >

Abstract
Summary

Aluminium oxyhydroxide (alum), a nano-crystaline compound forming agglomerates, has been introduced in vaccine for its immunologic adjuvant effect in 1927. Alum is the most commonly used adjuvant in human and veterinary vaccines but mechanisms by which it stimulates immune responses remains incompletely understood. Although generally well tolerated, alum may occasionally cause disabling health problems in presumably susceptible individuals. A small proportion of vaccinated people present with delayed onset of diffuse myalgia, chronic fatigue and cognitive dysfunction, and exhibit very long-term persistence of alum-loaded macrophages at site of previous intra-muscular (i.m.) immunization, forming a granulomatous lesion called macrophagic myofasciitis (MMF). Clinical symptoms associated with MMF are paradigmatic of the recently delineated "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA). The stereotyped cognitive dysfunction is reminiscent of cognitive deficits described in foundry workers exposed to inhaled Al particles. Alum safety concerns will largely depend on whether the compound remains localized at site of injection or may diffuse and accumulate in distant organs. Animal experiments indicate that biopersistent nanomaterials taken-up by monocytes-lineage cells in tissues, e.g. fluorescent alum surrogates, can first translocate to draining lymph nodes, and thereafter circulate in blood within phagocytes and reach the spleen, and, eventually, slowly accumulate in brain.

MESH Keywords Adjuvants, Immunologic ; adverse effects ; Alum Compounds ; adverse effects ; Animals ; Fasciitis ; chemically induced ; immunology ; pathology ; physiopathology ; Humans ; Myositis ; chemically induced ; immunology ; pathology ; physiopathology ; Nanostructures ; Phagocytes ; metabolism ; Syndrome

Introduction

In 1998, a consortium of French myopathologists described an emerging condition of unknown cause characterized by a pathognomonic lesion at muscle biopsy we called macrophagic myofasciitis (MMF).[1] MMF was detected in middle-aged adult patients with diffuse myalgias and fatigue.[1] Macrophages was the major cell type in the lesion, and enclosed agglomerates of nanocrystals in their cytoplasm.[1] Subsequently, these inclusions proved to be a key finding as they were constantly present at electron microscopy, and conspicuously contained aluminium as shown by ionic or X-ray microanalysis.[2] MMF was typically detected in the deltoid muscle, and could be differentiated both clinically and pathologically from Whipple's disease and other infectious histiocytoses, and from diffuse dysimmune fasciitis and panniculitis. [3] The crystalline rather than amorphous ultrastructural appearance of the inclusions was suggestive of aluminium hydroxide. Patients had normal renal function and had no peculiar exposure to aluminium other than previous immunization against hepatitis B (HBV), hepatitis A (HAV) or tetanus toxoid (TT) vaccines (100%), thus strongly suggesting that MMF inclusions correspond to aluminium oxyhydroxide (alum), an adjuvant incorporated in these vaccines to boost immunologic responses.[2] It is now clear that rapid emergence of MMF in France resulted from the specific combination of 3 factors : (1) replacement of the subcutaneous route by the i.m. route of vaccination in the early 1990s; (2) widespread extension of HBV primovaccination to the French adult population in the same time; and (3) the choice of the deltoid muscle (also used for i.m. vaccination) for routine muscle biopsy in France whereas biceps brachialis and quadriceps femoris muscles are preferred in most other countries. MMF lesion is now universally recognized to assess long-term persistence of alum at site of previous intramuscular (i.m.) immunization.[4] However, alum has been generally considered as safe on the basis of short-term surveys, and exact significance of longstanding MMF detection in a given patient remains uncertain because of (i) apparently "poorly specific" clinical manifestations, which of course does not mean non-disabling ones, and (ii) lack of self-evident link between persistence of alum agglomerates into macrophages at site of immunization and delayed onset of systemic and neurologic manifestations. Formal delineation of "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA),[5] and novel insights into the biodistribution of slowly biodegradable particles taken-up by monocyte-lineage cells in peripheral tissues provide settlement for a better understanding of this rare adverse effect of alum.

MMF histopathology

Deltoid muscle biopsy findings are stereotyped,[1 –4] consisting of focal infiltration of the epimysium, perimysium and perifascicular endomysium by well-circumscribed and cohesive sheets of large mononuleated cells of the monocyte and macrophage lineage, usually intermingled with a minor lymphocytic population. The maximum observed section size of the lesion is 1cm. Aluminium salts are positively stained by hematoxylin and, consistently, the cytoplasm of macrophages is basophilic (dark blue) on hematoxylin-eosin stained cryostat sections. Probably due to specific chemical reactions, this is not observed on formalin fixed material in which macrophages

exhibit a finely granular grey/beige content. In both cryostat and paraffin sections, macrophages are strongly periodic Acid Schiff (PAS)$^+$. They express CD68 and major histocompatibility complex (MHC) class 1 and MHC class 2 antigens. CD3$^+$ T-cells, mainly CD8$^+$, forming perivascular cuffs are constantly found. Occasional CD19$^+$ B cells, rarely forming lymphoid follicles, and CD138$^+$ plasma cells may be detected. Giant multinucleated cells are not detected except when another foreign material, e.g. coton wool, is present. In rare instances (about 1%) the granuloma may be encircled by thick fibrotic tissue and centered by a large necrotic area, forming a lesion reminiscent of a rheumatoid nodule. Myofibers remote from the infiltrate are typically intact, but MMF may be occasionnaly associated with typical dermatomyositis or autoimmine necrotizing myopathy. At electron microscopy, macrophages appear heavily loaded with submicron/micron-sized agglomerates of spiculated osmiophilic structures surrounded by discontinuous lysosomal membranes. In routine, inclusions can be visualized by the Morin stain for aluminium. Micro-organisms are not detected by appropriate stainings or electron microscopy.

Similar MMF lesions can be detected in the quadriceps muscle in babies and children because this muscle is used for i.m. vaccine administration in young individuals. MMF can be experimentally reproduced by i.m. vaccination in mice, rats and monkeys,[2 ,6 ,7] progressively shrinking with time.[6] It is, therefore, important to determine if the MMF lesion is unusually persistent in biopsied patients by precisely recapitulating history of previous vaccinations. In practice we consider MMF to be so when the time elapsed from last vaccine shot to MMF detection is >18 months. This point is particularly important in small children who receive numerous alum-containing vaccine shots in the first year of life, increasing risk of chance associations between MMF lesions and unrelated conditions, e.g. congenital myopathies and muscular dystrophies.[8] The risk also exists in adults but accounts for no more than 5–10% of MMF$^+$ biopsies, including fully asymptomatic patients and patients investigated for hereditary disorders.

In contrast to i.m. injections, alum-containing vaccines administered by the s.c. route may elicit chronic lesions that are somewhat different from MMF, so-called cutaneous pseudo-lymphoma, associated with a rim of alum-containing macrophages.[9]

From MMF-associated syndrome to ASIA

According to the patient association, about 1000 patients with documented MMF have been identified in France. Occasional cases have been reported in many other countries.[8 ,10 –15] The structure of symptoms was strikingly similar in independent cohorts of French adult patients.[4] We recently reviewed the files of 457 adult MMF patients collected from 1994 to 2011 in our centre. Patients were either investigated and biopsied (n=270) at the Neuromuscular Centre of Créteil (Neuromuscular Reference Centre Garches-Necker-Mondor-Hendaye), or were referred for follow-up or complementary investigation after MMF detection in other French hospitals by one of the myopathologists that had described the lesion (n=187). Most patients were females (70%) and at the middle age at time of biopsy (median 45 years, range 12–83). They had received 1 to 17 i.m. alum-containing vaccine administrations (mean 5.3) in the 10 years before MMF detection, and these included HBV vaccination in 85%. Patients mainly complained of chronic diffuse myalgias >6mois (89%) with or without arthralgias, disabling chronique fatigue >6 months (77%), overt cognitive alterations affecting memory and attention (51%), and dyspnea 50%. As previously reported, onset of these clinical symptoms was always posterior to, and delayed from, immunization, median time elapsed from last vaccine administration being 7 months (range 0.5–84) for initial systemic symptoms, and 11 months (range 0–72) for first myalgia.[4] Time elapsed from last vaccine administration to biopsy was 65 months (range 3–219). Compared to our previous reports, this delay has progressively increased (36 months in the initial series of 2001, 53 months in series of 2003), [4] indicating that MMF patients are chronically diseased and, though mainly vaccinated in the late nineties or early 2000', frequently looked for diagnosis long after onset of symptoms.

Myalgias and fatigue may not be synchronous. Myalgia may follow an exercise of unusual intensity and often begin in lower limbs,[4] and almost never at site of previous vaccine injection. Myalgia progressively extend upward to affect paravertebral muscles and become diffuse at time of biopsy.[4] Muscle weakness is rare. Myopathic electromyogram and CK elevation are found in less than one half of patients. Some fibromyalgic tender points are detected in a minority of patients, but the 1990 ACR criteria for fibromyalgia are rarely fulfilled.[4] Interestingly, ^{67} Gallium scintigrams has shown the presence of subtle radionuclide uptake predominating in the painful areas along the lower limb muscle fascias and in para-articular tissues in all tested patients.[17] This was not found in fibromyalgic controls.

Fatigue, sleep disturbances with unrereshing sleep, and sometimes headaches may be very disabling and often deeply impacts professional and personal life. A case-control study conducted by AFSSAPS pointed out chronic fatigue as more frequent and more pronounced in patients with than without MMF in deltoid muscle (http://afssaps.sante.fr/htm/10/myofasci/etude.pdf). In fact, a majority of patients fulfil international criteria of chronic fatigue syndrome.[18] Consequently, history of exposure to alum-containing vaccines should be checked carefully in patients with CFS, and muscle biopsy searching persistent MMF at site of injection should be considered when chronology is consistent, even many years after onset of symptoms.

CNS involvement is assessed by cognitive dysfunction. Patients complain of subjective memory impairment, difficulties in sustaining attention, and mood disturbancies. Although often disabling, cognitive dysfunction is often underestimated or remains undetected by routine examination. A comprehensive battery of neuropsychological tests in unselected MMF patients without MS showed alterations in

all individuals, consistent with mild cognitive impairment (MCI) but including at least one test reaching the dementia threshold in 96%.[19] Compared to arthritis controls matched for pain severity and duration, depression and educational level, MMF patients displayed distinctive impairment of visual memory, working memory and dichotic listening, a pattern suggestive of cortico-subcortical organic damage involving fronto-parieto-thalamo-striatal areas, with deep white matter alterations.[19] Very similar cognitive alterations have been documented workers exposed to inhaled Al fumes or powder.[20 –22] These alterations are also reminiscent of those described in HIV- or HCV-infected individuals.[19]

In addition to CFS, 15–20% of patients with MMF concurrently develop an autoimmune disease, the most frequent of which being multiple sclerosis (MS)-like demyelinating disorders [12 –23 , 23 ,24] Hashimoto's thyroiditis, and diffuse dysimmune neuromuscular diseases, such as dermatomyositis, necrotizing autoimmune myopathy, myasthenia gravis, and inclusion body myositis. Even in the absence of overt autoimmune disease, low titers of various autoantibodies, increased inflammatory biomarkers, and abnormal iron status are commonly detected.[4]

Taken individually, none of the clinical manifestations commonly associated with persistent MMF is specific of a given cause. Combination of chronic myalgias, fatigue, and cognitive dysfunction is consistent with CFS,[18] a poorly understood condition also known as myalgic encephalomyelitis,[25] which may be triggered by various infectious and non-infectious agents. We previously noted the closely similar structure of symptoms in individuals with MMF and with the so-called Gulf war syndrome[4] which is increasingly recognized as linked to multiple vaccinations,[26 ,27] with special emphasis put on anthrax vaccine, an alum-adjuvanted vaccine administered in 6 shots, that was recently shown to also induce MMF.[13] On these grounds, we proposed to consider MMF-associated symptoms as an adjuvant-induced syndrome.[28] Therefore, we fully support the term ASIA (autoimmune/inflammatory syndrome induced by adjuvants) coined by Pr Shoenfeld to designate these symptoms, regardless of the nature of the involved immunologic adjuvant (alum, silicone gel, viral components, etc).[5]

Handling and transport of poorly soluble nanomaterials by phagocytes : a possible clue for understanding MMF and ASIA

For decades, aluminium oxyhydroxide, is the most commonly used adjuvant in human and veterinary vaccines. The mechanism by which it stimulates the immune response remains incompletely understood. [29]

Imbalance between the huge number of alum-vaccine receivers and the small number of biopsy-proven MMF cases strongly suggest that individual susceptibility factors play a crucial role in intolerance to alum. In rats, the genetic background strongly influences the size of lesions induced by i.m. injection of alum.[6] Adverse response to alum injection may also depend on susceptibility genes, such as HLA-DRB1*01, that may favour the development of autoimmune diseases.[30] Thus, aluminium likely represents one environmental factor able to trigger adverse effects in individuals with as yet largely unknown susceptibility genes. In keeping with this view, several closely related conditions have been shown to be associated with Al overload, including MMF,[14] idiopathic CFS,[31] and MS.[32] Moreover, strong suspicion of a possible link between Gulf war syndrome and alum administration has been experimentally supported.[33] Quite logically, questions are currently burgeoning about the exact safety level of aluminium adjuvants.[34]

However, if biopersistence of the adjuvant in the body is a priori undesirable, the exact significance of MMF remains uncertain since a conceptual link is still a missing between the observed persistence of particle-loaded MPs at site of previous immunization and the systemic, especially neurologic, clinical manifestations. Alum is potentially highly neurotoxic,[33] but it is used at concentrations viewed as an acceptable compromise between adjuvanticity and toxicity by industry and regulatory agencies. In fact, the potential toxicity of alum will be influenced by whether the bioactive nanomaterial remains localized at injection points or rather scatters and accumulates in distant organs and tissues. Characterization of the fate of i.m. injected particles is therefore crucial for understanding pathophysiology of MMF and related disorders.

A reference study based on isotopic ^{26}Al showed poor ^{26}Al clearance in the urine after i.m. injection of isotopic alum to rabbits (6% at d28 endpoint), and detected ^{26}Al, in an unknown form, in lymph nodes, spleen, liver, and brain.[35] However, as for other slowly biodegradable nanomaterials, the biodistribution of alum particles following injection into muscle is currently unknown.

Aluminium oxyhydroxide is composed of micron/submicron-sized aggregates of nano-sized (ca 13 nm) particles and these aggregates were initially believed to remain extracellular until their complete solubilisation in interstitial fluids.[35] We now know that quite the reverse is the case and that APCs avidly take up alum particles,[36] and, in so-doing, become long-lived cells,[37] and impede alum solubilization.[2] Inflammatory monocytes (MOs) are attracted into muscle by danger signals, becoming macrophages and MO-derived dendritic cells (DCs), before migrating to the draining lymph nodes (DLNs).[38] Since one function of migratory DCs is to transfer antigenic material to a large network of distant resident APCs, we examined if fluorescent nanomaterials injected into muscle could translocate to distant organs as part of a general mechanism linked to phagocytosis.

Preliminary results have substantiated this view.[39 ,40] We observed that fluorescent surrogates of alum particles injected into mouse muscle were rapidly taken up by macrophages to form a MMF-like granuloma. An important proportion of particles escaped the injected muscle, mainly within immune cells, gaining access to the regional lymph nodes. Then particle-loaded cells exited the lymphatic system to reach the blood stream (presumably through the thoracic duct, a terminal lymphatic vessel plugged to the subclavian vein), allowing them to gain access to distant organs such as spleen, liver and, eventually, the brain. Using lymph node ablation and genetically manipulated animals, we documented that systemic biodistribution of particles injected into muscle necessitates early cell loading in muscle or lymph nodes, and crucially depend on the presence of attracting signals for monocytes (namely the MCP-1/CCL2 chemokine) in tissues. Thus, immune cells loaded with alum-like particles circulate after the i.m. injection and can reach distant tissues such as brain, especially if they produce attracting signals for inflammatory cells or exhibit weak blood brain barrier (BBB).[39 ,40] This may also apply to other poorly degradable nanomaterials such as silicone, another compound suspected to cause ASIA.[5] Of course, lot remains to be done to determine if, in what conditions, and to what extent alum and other mineral particles gaining access to the brain by a Trojan horse mechanism, as HIV and HCV particles do, can cause significant inflammatory and neurotoxic damage.

In conclusion, MMF revealed an almost complete lack of knowledge on the fate, systemic diffusion, and long-term safety of alum particles. On the grounds of our clinical and experimental data, we believe that increased attention should be paid to possible long-term neurologic effects of continuously escalating doses of alum-containing vaccines administered to the general population. Special emphasis should be put on individuals with immature/altered BBB or inflammatory states.

References:

Références

- 1 . Gherardi RK , Coquet M , Chérin P . Macrophagic myofasciitis: an emerging entity . Lancet . 1998 ; 352 : 347 - 52
- 2 . Gherardi RK , Coquet M , Chérin P . Macrophagic myofasciitis lesions assess long-term persistence of vaccine-derived aluminum hydroxide in muscle . Brain . 2001 ; 124 : 1821 - 31
- 3 . Bassez G , Authier FJ , Lechapt-Zalcman E . Inflammatory myopathy with abundant macrophages (IMAM): a condition distinct from macrophagic myofasciitis, sharing similarities with cytophagic histiocytic panniculitis . J Neuropathol Exp Neurol . 2003 ; 62 : 464 - 74
- 4 . Gherardi RK , Authier FJ . Aluminum inclusion macrophagic myofasciitis: a recently identified condition . Immunol Allergy Clin North Am . 2003 ; 23 : 699 - 712
- 5 . Shoenfeld Y , Agmon-Levin N . 'ASIA' Autoimmune/inflammatory syndrome induced by adjuvants . J Autoimmun . 2011 ; 36 : 4 - 8
- 6 . Authier FJ , Sauvat S , Christov C . AlOH3-adjuvanted vaccine-induced macrophagic myofasciitis in rats is influenced by the genetic background . Neuromuscul Disord . 2006 ; 16 : 347 - 52
- 7 . Verdier F , Burnett R , Michelet-Habchi C , Moretto P , Fievet-Groyne F , Sauzeat E . Aluminium assay and evaluation of the local reaction at several time points after intramuscular administration of aluminium containing vaccines in the Cynomolgus monkey . Vaccine . 2005 ; 23 : 1359 - 1367
- 8 . Müller HD , Landeghem FK , Schmidt PF , Sommer C , Goebel HH . Macrophagic myofasciitis plus (distinct types of muscular dystrophy) . Neuropediatrics . 200 ; 40 : 174 - 178
- 9 . Maubec E , Pinquier L , Viguier M . Vaccination-induced cutaneous pseudolymphoma . J Am Acad Dermatol . 2005 ; 52 : 623 - 629
- 10 . Gruis KL , Teener JW , Blaivas M . Pediatric macrophagic myofasciitis associated with motor delay . Clin Neuropathol . 2006 ; Jul–Aug 25 : (4) 172 - 9
- 11 . Ryan AM , Bermingham N , Harrington HJ , Keohane C . Atypical presentation of macrophagic myofasciitis 10 years post vaccination . Neuromuscul Disord . 2006 ; 16 : 867 - 869
- 12 . Kalil RK , Monteiro A , Lima MI . Macrophagic myofasciitis in childhood: the role of scanning electron microscopy/energy-dispersive spectroscopy for diagnosis . Ultrastruct Pathol . 2007 ; 31 : 45 - 50
- 13 . Theeler BJ , Simper NB , Ney JP . Polyglandular autoimmunity with macrophagic myofasciitis . Clin Rheumatol . 2008 ; 27 : 667 - 669
- 14 . Exley C , Swarbrick L , Gherardi RK , Authier FJ . A role for the body burden of aluminium in vaccine-associated macrophagic myofasciitis and chronic fatigue syndrome . Med Hypotheses . 2009 ; 72 : 135 - 139
- 15 . Israeli E , Agmon-Levin N , Blank M , Shoenfeld Y . Macrophagic Myofaciitis a Vaccine (alum) Autoimmune-Related Disease . Clin Rev Allergy Immunol . 2010 ; Sep 30 (http://www.springerlink.com/content/720l4415255625x6/fulltext.pdf
- 16 . Pereira JP , Barroso C , Evangelista T , Fonseca JE , Pereira da Silva JA . Macrophagic myofasciitis: a case report of autoimmune/inflammatory syndrome induced by adjuvants (ASIA) . Acta Reumatol Port . 2011 ; 1 : 75 - 76
- 17 . Chérin P , Authier FJ , Gherardi RK . Gallium-67 scintigraphy in macrophagic myofasciitis . Arthritis Rheum . 2000 ; 43 : 1520 - 1526
- 18 . Authier FJ , Sauvat S , Champey J , Drogou I , Coquet M , Gherardi RK . Chronic fatigue syndrome in patients with macrophagic myofasciitis . Arthritis Rheum . 2003 ; 48 : 569 - 70
- 19 . Couette M , Boisse MF , Maison P . Long-term persistence of vaccine-derived aluminum hydroxide is associated with chronic cognitive dysfunction . J Inorg Biochem . 2009 ; 103 : 1571 - 1578
- 20 . Rifat SL , Eastwood MR , McLachlan DR , Corey PN . Effect of exposure of miners to aluminium powder . Lancet . 1990 ; 336 : 1162 - 1165
- 21 . Akila R , Stollery BT , Riihimaki V . Decrements in cognitive performance in metal inert gas welders exposed to aluminum . Occup Environ Med . 1999 ; 56 : 632 - 639
- 22 . Polizzi S , Pira E , Ferrara M . Neurotoxic effects of aluminium among foundry workers and Alzheimer's disease . NeuroToxicol . 2002 ; 23 : 761 - 774
- 23 . Authier FJ , Cherin P , Creange A . Central nervous system disease in patients with macrophagic myofasciitis . Brain . 2001 ; 124 : 974 - 83
- 24 . Simpson DA . Systemic histiocytosis presenting as macrophagic myofasciitis . J Clin Neuromusc Dis . 2002 ; 4 : 19 - 22
- 25 . Hooper M . Myalgic encephalomyelitis : a review with emphasis on key findings in biomedical research . J Clin Pathol . 2007 ; 60 : 466 - 471
- 26 . Hotopf M , David A , Hull L , Khalida I , Unwin C , Wessely S . Role of vaccinations as risk factors for ill health in veterans of the Gulf war: cross sectional study . BMJ . 2000 ; 320 : 1363 - 1367
- 27 . Cherry N , Creed F , Sliman A . Health and exposures of United Kingdom Gulf war veterans. Part II: The relation of health to exposure . Occup Environ Med . 2001 ; 58 : 299 - 306
- 28 . Gherardi RK . Lessons from macrophagic myofasciitis: towards definition of a vaccine adjuvant-related syndrome . Rev Neurol (Paris) . 2003 ; 159 : 162 - 164
- 29 . Exley C , Siesjö P , Eriksson H . The immunobiology of aluminium adjuvants: how do they really work? . Trends Immunol . 2010 ; 31 : 103 - 109
- 30 . Guis S , Pellissier JF , Nicoli F . HLA-DRB1*01 and macrophagic myofasciitis . Arthritis Rheum . 2002 ; 46 : 2535 - 2537
- 31 . van Rensburg SJ , Potocnik FCV , Kiss T . 2001 ; Serum concentrations of some metals and steroids in patients with chronic fatigue syndrome with reference to neurological and cognitive abnormalities . Brain Res Bull . 55 : 319 - 25
- 32 . Exley C , Mamutse G , Korchazhkina O . 2006c ; Elevated urinary excretion of aluminium and iron in multiple sclerosis . Multiple Sclerosis . 12 : 533 - 540
- 33 . Shaw CA , Petrik MSJ . Aluminum hydroxide injections lead to motor deficits and motor neuron degeneration . J Inorg Biochem . 2009 ; 103 : 1555 - 1562
- 34 . Tomljenovic L , Shaw CA . Aluminum Vaccine Adjuvants: Are they Safe? . Curr Med Chem . 2011 ; 18 : 2630 - 2637

- 35 . Flarend RE , Hem SL , White JL . In vivo absorption of aluminium-containing vaccine adjuvants using 26 Al . Vaccine . 1997 ; 15 : 1314 - 1318
- 36 . Morefield GL , Sokolovska A , Jiang D , HogenEsch H , Robinson JP , Hem SL . Role of aluminum-containing adjuvants in antigen internalization by dendritic cells in vitro . Vaccine . 2005 ; 23 : 1588 - 1595
- 37 . Hamilton JA , Byrne R , Whitty G . Particulate adjuvants can induce macrophage survival, DNA synthesis, and a synergistic proliferative response to GM-CSF and CSF-1 . J Leukoc Biol . 2000 ; 67 : 226 - 232
- 38 . Brigitte M , Schilte C , Plonquet A . Muscle resident macrophages control the immune cell reaction in a mouse model of notexin-induced myoinjury . Arthritis Rheum . 2010 ; 62 : 268 - 279
- 39 . Khan Z , Christov C , Lux F . Bio-distribution of nanosized aluminic particles after i.m. injection in the mouse . Communication to the Nanotoxicology Meeting Edinburgh, UK 2010 ;
- 40 . Khan Z , Tillement O , Cadusseau J , Gherardi RK . Nanoparticle neurodelivery depends on a MCP-1-driven Trojan horse mechanism . Communication to the American Academy of Neurology Hawaï April 2011