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ORIGINAL ARTICLE

Morin Stain Detects Aluminum-Containing Macrophages in Macrophagic Myofasciitis and Vaccination Granuloma With High Sensitivity and Specificity

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Abstract

Macrophagic myofasciitis (MMF) is an inflammatory condition associated with the intramuscular (i.m.) injection of aluminum adjuvant-containing vaccines. It is clinically characterized by myalgia, weakness, and chronic fatigue and histologically by aggregates of cohesive macrophages with abundant basophilic, periodic acid-Schiff (PAS)-positive, diastase-resistant granules that percolate through the peri- and endomysium without eliciting substantial myofiber damage. The definitive diagnosis of MMF requires demonstration of aluminum within these macrophages. We evaluated the Morin stain, a simple, 2-step histochemical stain for aluminum, as a confirmatory diagnostic tool for MMF. Among 2270 muscle biopsies processed at UTSW between 2010 and 2015, a total of 12 MMF cases and 1 subcutaneous vaccination granuloma case were identified (11 pediatric, 2 adults). With the Morin stain, all 13 cases showed strong granular reactivity within the cytoplasm of macrophages but not in myofibers or connective tissue. Three cases of inflammatory myopathy with abundant macrophages (IMAM), 8 cases of granulomatous inflammation and 23 other deltoid muscle biopsies used as controls were all negative. Morin stain could be used in both formalin-fixed paraffin-embedded and cryostat sections. Thus, Morin stain detects aluminum with high sensitivity and specificity in human muscle and soft tissue and may improve the diagnostic yield of MMF and vaccination granuloma.

Key Words: Aluminum adjuvant, Inflammatory myopathy with abundant macrophages, Macrophagic myofasciitis, Vaccination granuloma.

INTRODUCTION

Macrophagic myofasciitis (MMF) is an inflammatory condition of skeletal muscle and fascia with unique macrophage morphology first described by Gherardi et al in 1998 in a case series of 14 adult patients from France (1). The dominant presenting symptoms were myalgia, arthralgia, and chronic fatigue. Muscle biopsies from all of those patients demonstrated muscle infiltration by large macrophages with abundant basophilic, periodic acid-Schiff (PAS)-positive, diastase resistant granules within the cytoplasm. These macrophages were cohesive but did not form multinucleated giant cells. Electron microscopy demonstrated lysosome-bound, spiculated inclusions in these macrophages that resembled mineral crystalloid. The lesions were restricted to the deltoid muscle, a common vaccination site in adults. Patients showed good responses to steroid treatment. Later in 2001, the same French group documented the association between MMF and the use of aluminum hydroxide adjuvant-containing vaccines and confirmed the presence of aluminum within the macrophage granules by atomic absorption spectroscopy and X-ray microanalysis (2). By 2014, this group had diagnosed over 600 cases of MMF in French adults (3). Outside of France, MMF has been reported more frequently in children (4–11) than in adults (12, 13). MMF in children was found exclusively in the quadriceps muscle, the site of pediatric vaccine injection; those patients had identical pathological findings but less distinctive clinical presentations compared to adults.

A close histological mimicker of MMF lesions is inflammatory myopathy with abundant macrophages (IMAM), a condition initially described in a cohort of patients with "dermatomyositis (DM)-like disease", whose muscle contained exceptionally large numbers of macrophages (14). Hemophagocytosis, a feature never reported in MMF, was noted in some of those patients. The pathogenesis of IMAM was thought to be T cell-triggered hyperactivation of macrophages. Subsequent studies from other groups showed that multiple hereditary and acquired conditions may lead to uncontrolled activation of macrophages in IMAM but found no consistent relationship between DM and IMAM (15–18).

The definitive diagnosis of MMF relies on the demonstration of aluminum within the macrophages. In previous studies, energy dispersive spectroscopy (7) or X-ray microanalysis (2) combined with scanning electron microscopy was used to confirm the presence of aluminum in the lesions.

However, these technologies are not readily available in most clinical laboratories in the United States.

Morin (2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one) is a flavonoid isolated from the leaves of *Psidium guajava* (19) that forms a green-blue fluorescent complex with aluminum (20). The complex can be visualized under the green channel under fluorescence microscopy. In addition to aluminum, Morin compound also binds boron, beryllium, zinc, gallium, indium, and scandium. However, none of those elements are used as vaccine adjuvants or are present in human or animal cells in detectable amounts. The utility of Morin stain to detect aluminum in human and animal cells has been previously validated in multiple *in vivo* and *in vitro* studies (19–24). It has also been used to detect aluminum in vaccination granulomas (25).

In this study, we report 12 additional pediatric and adult MMF cases, and test the validity of Morin stain, a simple, 2-step histochemical stain, as a confirmative diagnostic test for the detection of aluminum in MMF.

MATERIALS AND METHODS

Case Selection

The study was approved by UT Southwestern Medical Center institutional review board. We retrospectively reviewed the UTSW neuropathology database for cases diagnosed as MMF, IMAM, macrophagic inflammation, NOS, or granulomatous inflammation between 2010 and 2015. A total of 14 MMF, 3 IMAM and 18 cases with granulomatous inflammation were identified. All slides, electron microscopy images, and available electronic medical records were reviewed (by R.C. and C.C.). MMF was diagnosed when the characteristic infiltrate of densely packed macrophages with finely granular PAS-positive, diastase-resistant content was identified on muscle biopsy. In one case, the macrophages were confined to the epimysial connective tissue without involving the muscle and formed a necrotizing granuloma; this case was classified as vaccination granuloma. Another case had only a small focus of characteristic macrophages, which was lost on levels; that case was excluded.

Routine Muscle Processing

Fresh muscle specimens were oriented, snap-frozen in isopentane-cooled liquid nitrogen and submitted for frozen section histology and routine enzyme histochemical staining, including acid phosphatase and alkaline phosphatase. Immunohistochemical staining for major histocompatibility complex class I (anti-MHC1, US Biological, Salem, MA, M3886-10) and anti-CD68 (Ventana, Tucson, AZ) were performed on frozen sections in some inflammatory cases. Additional segments of skeletal muscle were received isometrically fixed in 10% formalin and processed for paraffin embedding, hematoxylin and eosin (H&E) histology, Masson's trichrome and Congo red (Sigma–Aldrich, St Louis, MO) stains; an average of 4 1–2-mm-blocks of fixed muscle were post-fixed in buffered glutaraldehyde and embedded in Epon-araldite for resin histology and electron microscopy.

Morin Staining Protocol

Morin stain was performed on formalin-fixed, paraffin-embedded (FFPE) and/or frozen sections on all 12 MMF, 1 vaccination granuloma, and 3 IMAM cases. An additional 8 cases of granulomatous inflammation in muscle and 23 consecutive adult deltoid muscle biopsies for other causes were used as controls. Morin staining solution was prepared from 0.2% Morin hydrate (MW 302.24, Sigma–Aldrich, St Louis, MO) dissolved in 85% alcohol and 0.5% glacial acetic acid solution. Prior to staining, FFPE slides were deparaffinized and rehydrated; frozen section slides were pre-fixed in 10% formalin for 10 minutes and then washed in water. Morin staining was performed as follows: formalin-fixed frozen and deparaffinized FFPE sections were placed in 1% hydrogen chloride for 10 minutes, followed by a rinse in distilled water. The slides were subsequently placed in 0.2% Morin solution for 10 minutes, followed by a few dips in 95% alcohol, and then transferred to xylene for Permount coverslipping.

Dealuminization

To examine the effect of a dealuminization step before Morin staining, both frozen and FFPE sections were placed in 10 mM ethylenediaminetetraacetic acid (EDTA), microwaved at the lowest setting (to avoid boiling of the solution) for 10 minutes; they were then incubated in the same solution for another 90 minutes and stained according to the protocol above, along with the non-EDTA treated slides as positive controls. Positively charged glass slides were used for every case to prevent detachment of the tissue from the slide during EDTA incubation.

RESULTS

Table summarizes the demographics and clinical characteristics for the MMF and IMAM patients. Among a total of 2270 muscle biopsies processed at UTSW between 2010 and 2015, 450 were from children (age 0–19), 1820 were from adults (20 and above). Of the 12 MMF cases identified, 10 were children (2%) and 2 were adults (0.1%). The biopsy sites were quadriceps muscle in all children and deltoid muscle in both adults. Four patients, including 2 adults (patients 12 and 13) and 2 of the older children (patients 9 and 10), presented mainly with myalgia (Table). Three patients (patients 10, 12, and 13) also had weakness. The pain was usually symmetric, starting in the lower extremities and spreading to involve trunk and upper extremities. CPK values were normal or high initially but had usually normalized by the time of muscle biopsy. The muscle pain was induced or intensified by physical activity, which led to avoidance of exercise and subsequent muscle wasting in some patients. In one child (patient 10), the pain was so severe that he refused to walk and was completely wheelchair-dependent several months after the onset of symptoms. He became tearful whenever his right leg was touched or when he was asked to stand. This patient had a history of Autism spectrum disorder and was initially suspected to have conversion disorder. Brain MRI, electromyography and nerve conduction studies were normal. The first muscle biopsy from

TABLE. Clinical and Pathological Data

ID	Age	Sex	Biopsy site	Clinical presentation	Dx	EM	Morin stain results	Genetic defects	Other Dx	Last vaccine[a] (m)
1	6 m	F	Quad	Hypotonia, DD	MMF		+	Inversion 1p		6
2	10 m	F	Quad	Hypotonia, DD	Vaccination granuloma		+			
3	12 m	M	Quad	Seizures	MMF		+			6
4	21 m	F	Quad	Hypotonia, DD	MMF		+	Gain 1q44	Mito. Dis.	8
5	21 m	M	Quad	Hypotonia, DD	MMF	Spiculated inclusion	+			<12
6	2 y	M	Quad	Myalgia, weakness	MMF	eTRI	+		DM	<12
7	3 y	M	Quad	Weakness	MMF		+			
8	4 y	M	Quad	Weakness, seizures	MMF		+	Deletion 11q12.3	Niemann–Pick type C	15
9	6 y	F	Quad	Myalgia, fatigue	MMF	Spiculated inclusion	+			34
10	6 y	M	Quad	Myalgia, weakness	MMF		+			23
11	7 y	M	Quad	Bilateral toe walking	MMF		+			
12	45 y	M	Deltoid	Myalgia and weakness	MMF		+			
13	55 y	F	Arm	Myalgia and weakness	MMF		+			
14	18 y	F	Quad	Sweet's syndrome	IMAM		−		Sweet's syndrome	
15	34 y	F	Quad	Myalgia and weakness	IMAM	eTRI	−		DM	
16	74 y	F	Quad	Proximal weakness	IMAM		−			

Quad, quadriceps; DD, developmental delay; DM, dermatomyositis; Dx, diagnosis; EM, electron microscopy findings; eTRI, endothelial tubuloreticular inclusions; F, female; IMAM, inflammatory myopathy with abundant macrophages; m, months; M, male; Mito dis; mitochondrial disease; MMF, macrophagic myofasciitis; y, years; +, positive; −, negative.

[a]Time between last aluminum-based vaccination and muscle biopsy.

left quadriceps showed mild type II atrophy only. A second muscle biopsy was obtained 3 months later from the right quadriceps and showed MMF as well as severe type II atrophy. He received oral steroid and analgesic treatments, as well as aggressive physical therapy several times per week, and was pain-free and ambulatory 1 month after the second muscle biopsy. Muscle pain waxed and waned in the other child (patient 9), without treatment. The muscle pain was controlled by oral opioids in the remaining adult (patient 12).

In younger children, the presenting symptoms were much more variable; hypotonia and developmental delay were the most common presentations. Nearly all had other comorbidities that led to the initial muscle biopsy. Three patients (patients 1, 4, and 8) had cytogenetic abnormalities including gain of 1q44, deletion of 11q12.3, and inversion of chromosome 1p, respectively; patient 4 was diagnosed with a mitochondrial abnormality and patient 8 with Niemann–Pick type C disease. Patient 3 had seizures and a history of perinatal sepsis. Patient 6 had the clinical diagnosis of juvenile DM and was found to have an inflammatory myopathy with multifocal endothelial tubuloreticular inclusions by electron microscopy in the biopsy. Four pediatric patients (patients 1, 3, 4, and 8) had vaccination records available; all had diphtheria–tetanus–pertussis (DTaP), hepatitis B (HepB), hemophilus influenza type b (HIB), and pneumococcal conjugate vaccines (PCV) at 5–12 months prior to the muscle biopsy. Clinic notes indicated that immunizations were "up to date" for patients 5 and 6. Patients 9 and 10 had the last set of vaccines 34 and 23 months prior to presentation, respectively. Vaccine histories for the remaining patients, including both adults, were unavailable (Table).

The patients with IMAM were juvenile (patient 14) or adults (patients 15 and 16). All had muscle pain, weakness, and elevated CK at presentation. Two patients had skin rashes that were felt to be atypical for DM; one had a clinical diagnosis of Sweet syndrome (patient 14); patient 15 was confirmed to have DM on a separate muscle biopsy, which demonstrated perifascicular atrophy and endothelial tubuloreticular inclusions.

Morphologically, biopsies from MMF cases typically contained aggregates of basophilic, cohesive macrophages with abundant granular cytoplasm centered in the perimysial connective tissue (Fig. 1A). A lymphocytic component was often present within the macrophage aggregate (Fig. 1A, asterisk). In cases where the macrophages infiltrated the endomysial compartment, they percolated through the interstitial connective tissue without causing obvious myofiber damage (Fig. 1B and C). The granular cytoplasm of the macrophages was highlighted by PAS with diastase (Fig. 1D) and anti-CD68 (Fig. 1E) stains. In frozen sections, the macrophages were basophilic (Fig. 1G), intensely positive for acid phosphatase (Fig. 1H) and esterase, and did not elicit alkaline phosphatase reactivity in connective tissue (Fig. 1I). MHC1 immunostaining was strongly positive in the macrophages but showed minimal sarcolemmal upregulation in myofibers (Fig. 1J). The background muscle often showed type II atrophy. Electron microscopy was performed on all cases but only 2 cases captured macrophages containing spiculated electron-dense inclusions characteristic of aluminum salt (Fig. 1K and L).

In all 12 cases, the Morin stain demonstrated strong, blue-green, granular, fluorescence staining in the macrophage cytoplasm (Fig. 1F). Morin reactivity was restricted
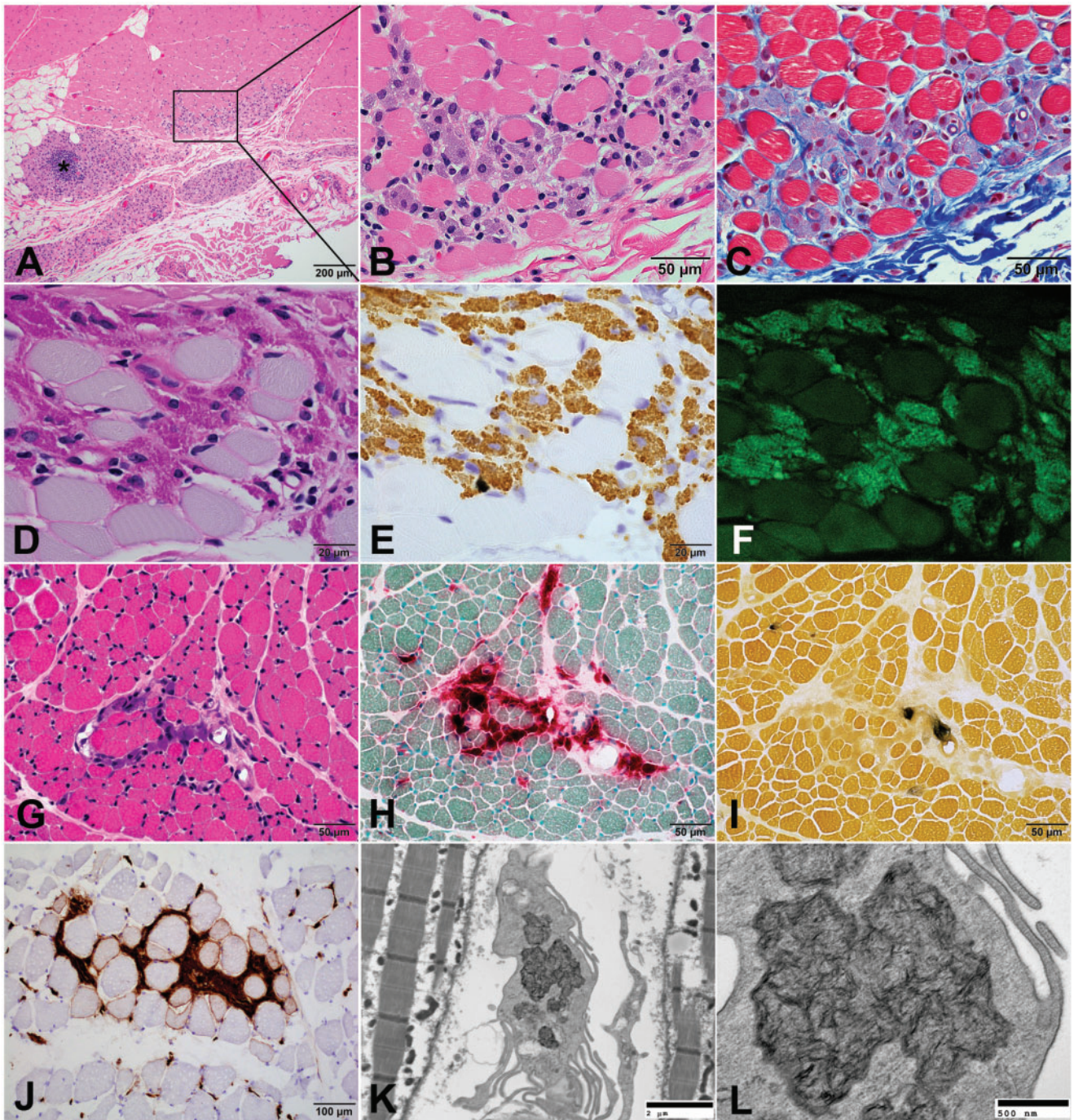
FIGURE 1. Macrophagic myofasciitis morphology. **(A–F)** FFPE tissue. Low-power H&E stain shows perimysial and endomysial aggregates of macrophages with a lymphocytic component (**A**, asterisk). High-power H&E **(B)** and trichrome **(C)** stains on consecutive sections show macrophages percolating through the endomysial connective tissue without inducing apparent myofiber damage. The granular cytoplasm of the macrophages is highlighted by PAS with diastase **(D)**, anti-CD68 **(E)** and Morin **(F)** stains on consecutive sections. **(G–J)** Frozen sections. The macrophages are intensely basophilic by H&E **(G)**, strongly positive for acid phosphatase **(H)**, but do not elicit alkaline phosphatase reactivity in connective tissue **(I)**. Immunostain for MHC1 highlights the macrophages but shows minimal sarcolemmal upregulation in myofibers **(J)**. **(K, L)** Electron microscopy shows macrophages containing spiculated electron dense inclusions characteristic of aluminum crystalloid.

to macrophages and was never detected alone in connective tissue or myofibers. The stain was effective in both FFPE and frozen sections. Removing aluminum by pre-stain chelation with EDTA significantly reduced the fluorescence intensity in MMF specimens compared to non-treated controls (Fig. 2). After several days, however, the de-aluminized slides typically gained fluorescence signal similar to the positive control, likely because of a continued low rate reaction from residual aluminum within the mounted slides.

The Morin stain was also positive in a case of vaccination granuloma from a 10-month-old infant (patient 2). Biopsy of the left thigh demonstrated a superficial necrotizing granuloma confined to the epimysial connective tissue (Fig. 3A). PAS, FITE, and GMS special stains showed no mycobacterial or fungal organisms. The macrophages contained characteristic blue, finely granular cytoplasm as those seen in MMF, and were strongly positive in the Morin stain. Necrotic debris was also reactive in the CD68 and Morin stains, albeit less intensely than in viable macrophages (Fig. 3B–D).

One of the MMF biopsies demonstrated an inflammatory myopathy with multiple tubuloreticular endothelial inclusions (patient 6); staining of this biopsy with the Morin stain demonstrated unequivocal cytoplasmic reactivity in aggregates of macrophages in the perimysial compartment. Seven cases had concomitant diffuse type II atrophy (patients 4, 5, 7, 9, 10, 12, and 13) and one patient (patient 8) had mild neurogenic changes. The muscles were otherwise unremarkable.

Thirty-four other cases were stained with Morin as controls, including 3 patients with IMAM, 5 patients with granulomatous myositis, 2 patients with sarcoidosis, one lipogranuloma, and 23 consecutive adult deltoid muscle biopsies that had been performed for other conditions. None contained Morin-positive macrophages. The IMAM cases were characterized by diffuse macrophage infiltrates within the perimysium and endomysial connective tissue (Fig. 4A). The macrophages were positive for acid phosphatase (Fig. 4B) and

esterase. Unlike MMF, however, the macrophages in IMAM were more dispersed, lacked basophilic, PAS-positive granules (Fig. 4C), and elicited strong alkaline phosphatase reactivity in connective tissue (Fig. 4D). Myopathic changes including necrotic and regenerating fibers were apparent in all 3 IMAM cases, although there were no significant MHC1 upregulation in myofibers (Fig. 4E). Endothelial tubuloreticular inclusions were identified in one case. As noted, the Morin stain was negative in those macrophages for all 3 IMAM cases (Fig. 4F).

DISCUSSION

Aluminum adjuvants have been widely used in many vaccines worldwide for decades, including for DTaP, hepatitis A (HepA), HepB, human papilloma virus (HPV), HIB, and PCV. It functions as an immunostimulant to prolong and augment the immune response to the antigen. Subcutaneous granuloma induced by aluminum adsorbed vaccine was reported as early as the 1950s (26); this was followed by multiple case series under the names "aluminum granuloma", "aluminum hydroxide granuloma", "pruritic granuloma" or "vaccine granuloma" (27–39). Surgically biopsied or resected cases usually demonstrated necrotizing granuloma containing aluminum crystalloid, as seen in our patient 2. Aluminum granuloma may occasionally present as breast mass (40), away from the injection site, presumably through lymphatic spread of aluminum-containing macrophages. Morphologically, aluminum granuloma may resemble granuloma annulare, rheumatoid nodule or caseating granuloma. Morin stain can serve as a valuable diagnostic tool in these situations.

The onset of MMF detection coincides with introduction of the i.m. route of vaccination (41) when it was found that i.m. injection of DPT vaccine caused significantly less local adverse effects such as redness, swelling, abscess or granuloma formation than the subcutaneous route (42–44). One study found more systemic symptoms such as fussiness, severe
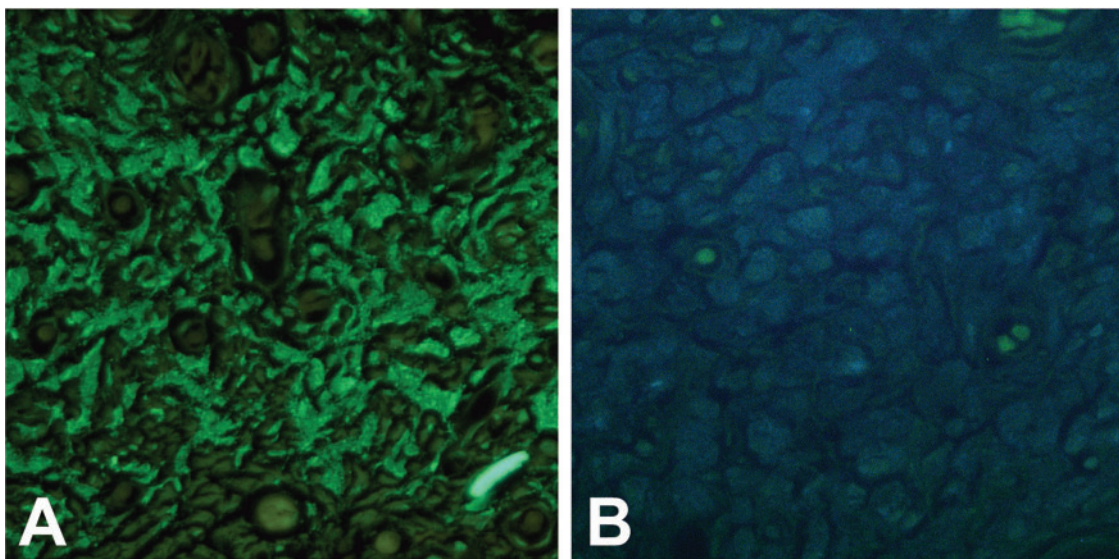

FIGURE 2. Dealuminization prior to staining diminishes Morin reactivity. Consecutive sections from an MMF case stained with Morin without **(A)** and with **(B)** EDTA pre-treatment.
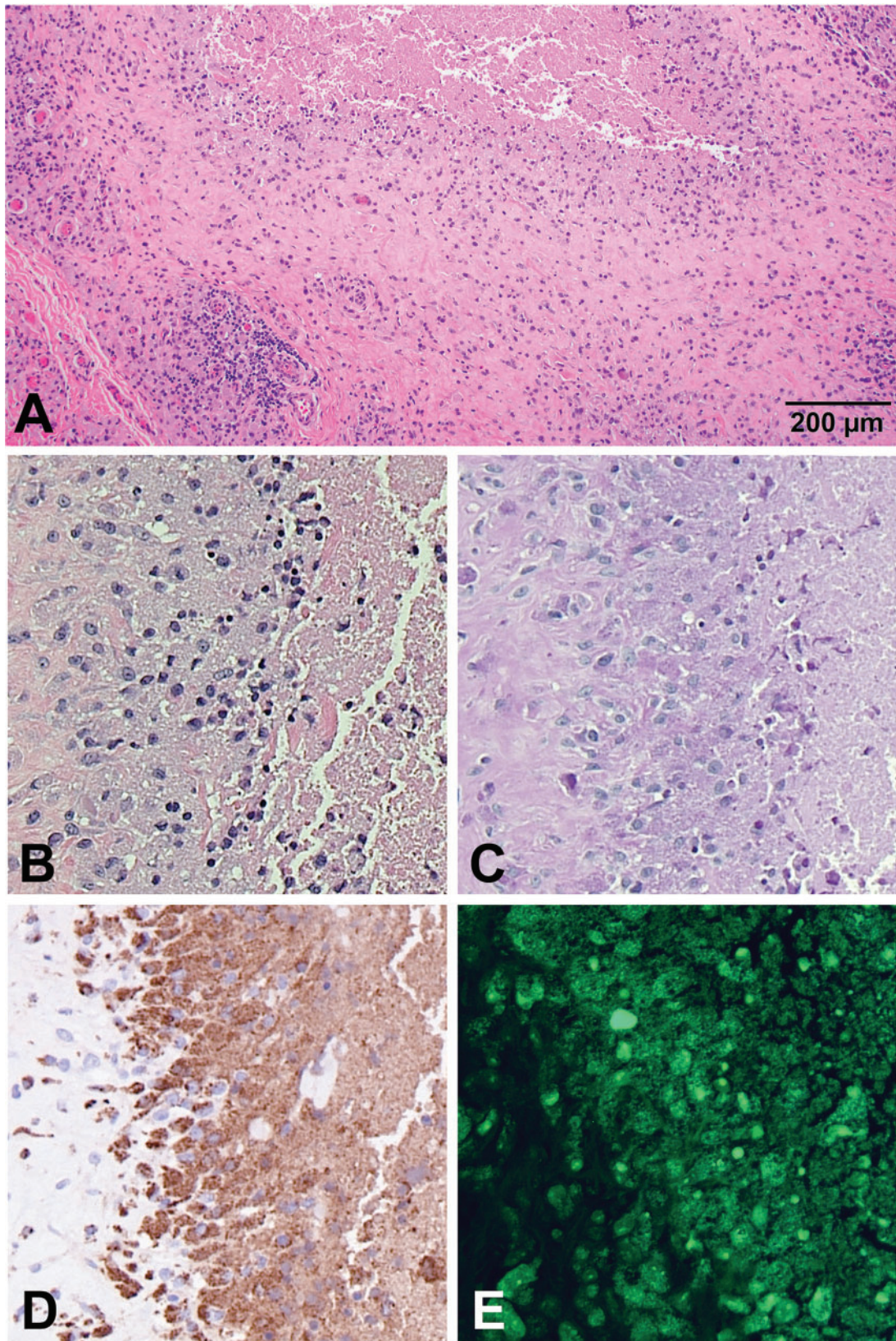
FIGURE 3. Vaccination granuloma. (**A**) Low-power H&E stain shows a necrotizing granuloma in epimysial connective tissue. (**B–E**) High-power images of H&E (**B**), PAS-D (**C**), anti-CD68 (**D**) and Morin (**E**) stains show similar macrophage morphology as seen in MMF cases.
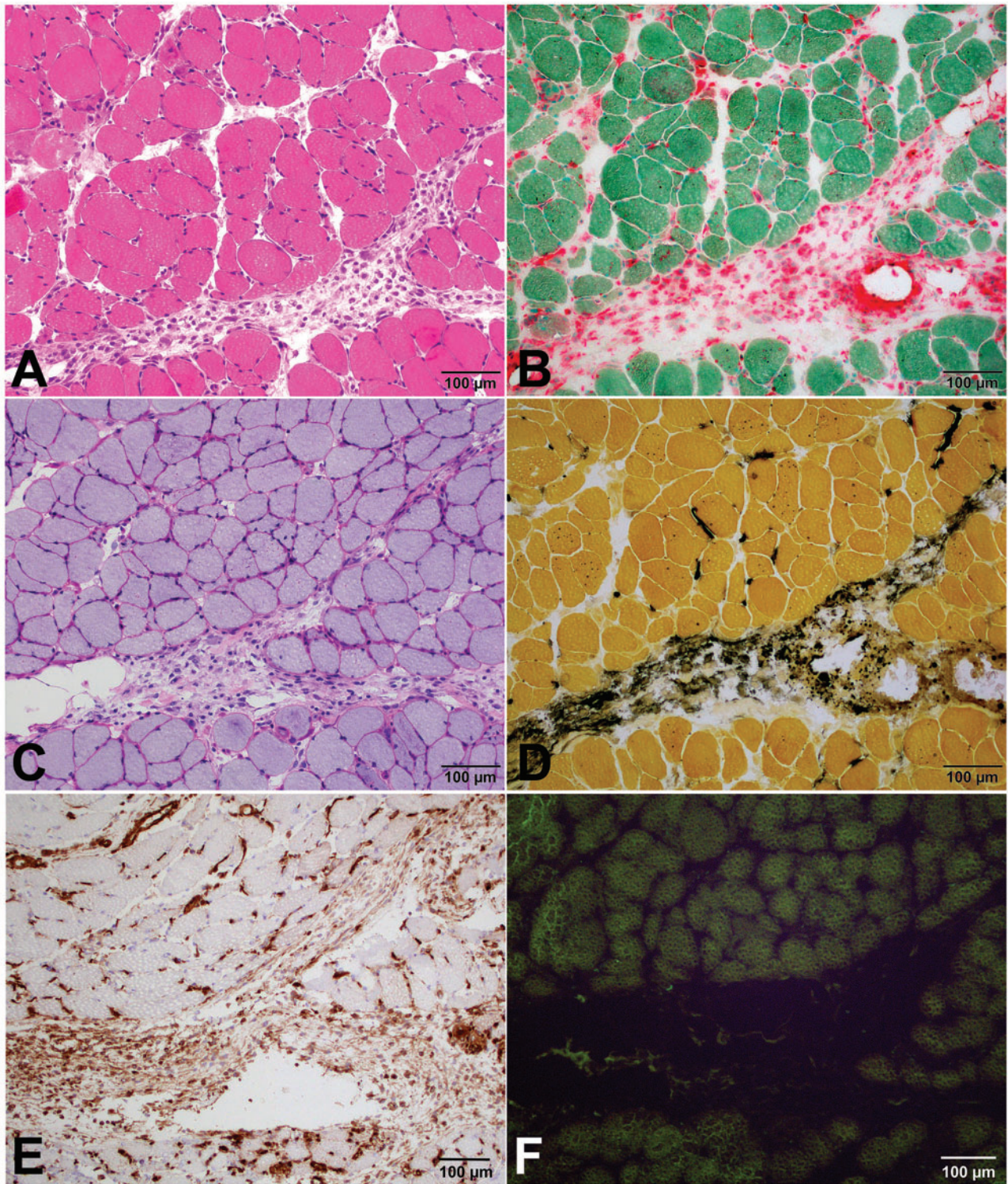
FIGURE 4. Inflammatory myopathy with abundant macrophages (IMAM). **(A–F)** H&E **(A)** and PAS-D **(C)** stains show diffuse perimysial and endomysial infiltrations of macrophages without basophilic, granular cytoplasm. The macrophages are strongly positivity for acid phosphatase **(B)** and elicit intense alkaline phosphatase relativity in the connective tissue **(D)**. No significant MHC1 upregulation was observed in myofibers **(E)**. Morin stain is negative in macrophages **(F).**

pain, and decreased movement associated with i.m. vaccination (42). The possible mechanism is that skeletal muscle contains much more vasculature than subcutaneous fat, which allows faster mobilization, antigen processing and absorption of injected material, thereby reducing the chance of granuloma formation and decreasing the likelihood of local signs and symptoms. Histopathologically, both vaccination granuloma and MMF are composed of aggregates of basophilic macrophages with granules in the cytoplasm that contain aluminum salt. Vaccination granulomas are more superficial, manifesting as a necrotizing granuloma within the subcutaneous fat, whereas in MMF the macrophages percolate through the perimysial and endomysial connective tissue without attacking the myofibers.

In the United States, aluminum adjuvants are used in DTaP, HepA, HepB, HPV, HIB, and PCV vaccines (http://www. cdc.gov/vaccinesafety/concerns/adjuvants.html). Last accessed 2/10/2017. Despite the large number of people undergoing vaccination each year, only 3 cases of MMF have been reported in the United States to date (8, 13). In our experience, MMF is not rare and we suspect that it is underdiagnosed. There are likely several reasons for this: (1) Symptoms of myalgia and fatigue are highly non-specific. (2) A history of prior vaccination is often difficult to elicit at the time of presentation because symptoms in adults are typically delayed for 4–5 years after the vaccination (3) and can be delayed for as long as 10 years (45); therefore, clinicians looking for a "recent" vaccination history can be misguided. (3) The lesions of MMF are localized to the site of vaccination, and the deltoid muscle, the most common site of vaccination in adults, is much less frequently chosen as a biopsy site in the United States. (4) Definitive pathologic diagnosis relies on demonstration of aluminum in macrophages.

Currently, there is no widely accepted and cost effective method to detect aluminum in tissue in routine clinical laboratory settings. Ultrastructural detection of diagnostic spiculated inclusions relies on the capture of these focal collections of basophilic macrophages in tissue samples submitted for electron microscopy that, by virtue of their small size, may not contain foci of macrophagic infiltration. We routinely examine 4 randomly sampled, 1–2-mm-sized resin blocks for all suspected MMF cases; only 2 of our 13 cases demonstrated spiculated inclusions. Energy dispersion spectroscopy or X-ray microanalysis are not available in most institutions, limiting the value of this specialized procedure. Our results indicate that the Morin stain can be a reliable tool to assess aluminum containing granular cytoplasmic material in macrophages with100% sensitivity and 100% specificity in our small cohort. Morin stain may show weak nuclear reactivity in lymphocytes and macrophages. This is likely due to the presence of zinc-containing transcription factors in the nucleus but this is easily distinguished from the strong granular cytoplasmic staining in MMF. The Morin stain is a simple procedure that can be set up easily in most clinical laboratories. The staining solution is fairly stable when kept at room temperature in the dark; in our hands, it maintained the same staining quality 3 months after preparation. The signal to noise ratio was high on both FFPE and frozen sections. Viewing the Morin stained slides does, however, require a fluorescence microscope and when Permount was used for cover-slipping, a fluorescence-quenching phenomenon was observed after few minutes under the green

fluorescent channel. Fluorescence was regained after several hours in dark. When kept in room temperature, fluorescence intensity gradually decreased over 2–3 months.

We found that ~2% of pediatric and 0.1% of adult muscle biopsies at our institution between 2010 and 2015 demonstrated macrophages characteristic of MMF. Taking into consideration that MMF is restricted to the vaccine injection site, it is likely that this series underestimates the frequency of MMF given that only 9% of adult muscle biopsies were from the deltoid muscle. The clinical significance of MMF remains unclear in some cases, particularly in the younger pediatric population. Many pediatric patients, particularly those under 4 years of age, presented with hypotonia and developmental delay that were possibly attributable to other genetic or metabolic disorders; the MMF lesions may well represent an incidental finding unrelated to the patients' clinical features in this group. Steroid treatment had little utility or efficacy in those patients. Other pediatric MMF series, totaling 39 cases in the English literature reported similar findings (2, 4–11). Studies in animal models have shown that aluminum containing macrophage could persist in normal animal muscle for up to 18 months after injection (3), and it is conceivable that a large percent of these MMF lesions found in children under 4 without myalgia may represent a "vaccination tattoo" rather than a true pathology. In contrast, in older children and adults with MMF, prolonged exercise induced pain that was responsive to steroid therapy; in those cases, an absence of other significant medical conditions suggest that MMF is the likely cause of symptoms.

IMAM is morphologically distinct from MMF. The macrophages in IMAM are diffuse, discohesive, lacking granular cytoplasm and elicit strong alkaline phosphatase reactivity in the connective tissue. Myofiber damage is usually apparent. Importantly, we found no direct association between IMAM and classical DM. Therefore, the presence of classic DM-associated changes such as perifascicular atrophy and endothelial tubuloreticular inclusions does not permit reliable distinction between IMAM and MMF.

In conclusion, each year, vaccination protects millions of people from severe infectious diseases. MMF is an adverse effect associated with i.m. injection of aluminum hydroxide adjuvant-containing vaccines. Although non-life threatening, MMF may cause myalgia, weakness, and chronic fatigue if left untreated. MMF is probably underdiagnosed in the United States. Increased awareness and appropriate tools are necessary for the correct diagnosis of this condition. We found that the Morin stain detects aluminum deposits in both FFPE and frozen muscle tissue with high sensitivity and specificity. The stain can be completed in regular histology laboratories in <30 minutes and may serve as a valuable diagnostic tool for MMF and vaccination granuloma.

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