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$Al(OH)_3$-adjuvanted vaccine-induced macrophagic myofasciitis in rats is influenced by the genetic background

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Abstract

Macrophagic myofasciitis (MMF) is a specific histopathologic lesion involved in the persistence for years of aluminum hydroxide [$Al(OH)_3$] at the site of previous intramuscular (i.m.) injection. In order to study mechanisms involved persistence of MMF lesions, we set up an experimental model of MMF-lesion in Sprague–Dawley and Lewis rat, by i.m. injections of 10 μL of an $Al(OH)_3$-adjuvanted vaccine. An evaluation carried out over a 12-month period disclosed significant shrinkage of MMF lesions with time. A radioisotopic study did not show significant aluminium uptake by $Al(OH)_3$-loaded macrophages. A morphometric approach showed that Lewis rats with Th1-biased immunity had significantly smaller lesions than Sprague–Dawley rats with balanced Th1/Th2 immunity. Concluding, our results indicate that genetic determinatives of cytotoxic T-cell responses could interfere with the clearance process and condition the persistence of vaccine-induced MMF-lesions.

Keywords: Inflammatory myopathies; Macrophagic myofasciitis; Experimental model; Aluminium hydroxide; Vaccine; Adverse effects; Histopathology; Morphometry; Th1; Th2; Radioisotopic study; Sprague–Dawley rat; Lewis rat

1. Introduction

Macrophagic myofasciitis (MMF) is a recently described histopathologic lesion, mainly detected in adult patients with chronic fatigue and diffuse myalgias [1,2]. MMF consists of a pathognomonic focal epi-, peri- and endomysial infiltration of large PAS-positive and major histocompatibility complex class 1 antigen (MHC-1)-positive macrophages, intermingled with $CD8^+$T-cells, in the absence of conspicuous muscle fibre damage [1,2]. At electron microscopy, macrophages constantly enclose crystal material in their cytoplasm [1,3], representing aluminium hydroxide [$Al(OH)_3$], an immunologic adjuvant incorporated in vaccines to stimulate Th2 immune responses [2]. Analysis of patients history established that MMF assesses long-term persistence of $Al(OH)_3$ at site of previous intramuscular (i.m.) injection [2,4], time elapsed from last immunization with an $Al(OH)_3$-containing vaccine to muscle biopsy ranging from 3 months to 8 years in our series (median: 53 months) [5].

The low detection rate of MMF among vaccine receivers undergoing deltoid muscle biopsy prompted WHO to propose the working hypothesis that MMF could occur in a predisposed subset of individuals with impaired ability to clear aluminium from muscle [4]. In fact, the main difficulty relies on the lack of firm data about normal residence time of $Al(OH)_3$ in muscle tissue after i.m. injection in normal individuals [2]. Long-term follow-up of vaccinated monkeys indicates that MMF lesions are destinated to vanish, only 2/4 monkeys still displaying macrophagic infiltration 12 months after injection [6]. This experimental result notably differs from that observed in patients, in which the median persistence time of macrophagic infiltrates was

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53 months. This led us to study factors that could interfere with the clearance process.

For this purpose, we induced in rats MMF lesions by i.m. administration of a human-equivalent dose of an $Al(OH)_3$-adjuvanted vaccine. Once phagocytozed by macrophages [7–9], $Al(OH)_3$ promotes cell survival [10], and antigen presenting cell functions [11]. Such vaccine-loaded macrophages strongly express the ferritin receptor (CD71) through which extracellular aluminium can enter cells [12]. Therefore, we first investigated whether exogenous soluble aluminium from another source than vaccine could be captured by $Al(OH)_3$-loaded macrophages and eventually impede solubilization of aluminium from the crystal. Genetic determinatives of cytotoxic T-cell responses could also represent factors interfering with the clearance process. Indeed, while a large number of macrophages remain accumulated at site of $Al(OH)_3$ injection [2], an unknown proportion of aluminium-loaded antigen presenting cells migrate to the regional lymph node [7] where they likely initiate the primary immune response to the vaccine antigen. It is widely accepted that effector $CD8^+$T-cells generated in the lymph node are in charge of clearing out cells bearing the cognate antigen on MHC-1 molecules in peripheral tissues [13]. Consistently, CD8 + T-cells in MMF closely contact MHC-1-expressing macrophages accumulated in muscle and fascia [2] suggesting an effector-target interplay. To test this hypothesis, we compared the size of vaccine-induced lesions in inbred rat strains Sprague–Dawley and Lewis that differ by the intensity of cell-mediated immune responses [14].

2. Material and methods

2.1. Animals and experimental procedures

This study was conducted in accordance with the EC guidelines for animal care [Journal officiel des communautés européennes, L358, December 18, 1986]. We used 5-week-old female rats, including 50 Sprague–Dawley (SD) rats that have a naturally well-balanced immunity, and 40 Lewis (Lw) rats that predominantly develop Th1 immune responses [14]. The animals were kept at constant temperature (22 °C) and pressure (15 Pa) under a 12:12 h day/night cycle with food and water ad libitum. All experiments were carried out under general anesthesia by i.p. injection of 0.1 mL/100 g of 6% phenobarbital.

Since most cases of human MMF are caused by anti-HBV vaccines immunization was performed using a commercially available aluminium hydroxide-adjuvanted vaccine. According to Smil [15], 10 μL was considered as a human equivalent dose of vaccine since average weight of rats was about 120 g at time of injection. Rats received i.m. injection of 10 μL of either Engerix® B20 (vaccinated groups, V) or NaCl 0.9% (controls) into one *tibialis anterior* muscle. Skin was incised then i.m. injection was performed longitudinally with a needle inserted 10 mm deep into muscle.

2.2. Myopathological study

Muscle samples were conventionally processed for light microscopy, and studied as 10 μm cryosections stained by hematoxylin–eosin. Vaccine-induced MMF-like lesions, defining experimental myofasciitis (EMF), were assessed by the presence of at least one infiltrate of basophilic large cohesive macrophages [2]. Macrophages were assessed by immunohistochemical expression of ED-1 antigen [16]. Lymphoid component of EMF lesions was semi-quantitatively evaluated and expressed as lymphocyte score (LS): 0, absent; 1+, mild, 2+, moderate; 3+, marked (more lymphocytes than macrophages). In addition, a quantitative approach was performed at 2 and 12 months by counting the total lymphocyte number observed within MMF lesions. Detection of T-cells in EMF lesion was performed by immunofluorescence using monoclonal antibody to rat CD5 (MCA52R, Serotec, UK) 1/200.

2.3. Radio isotopic study

To assess whether circulating aluminium could be trapped in the MMF lesion, a single dose of 0.4 ng ^{26}Al was injected i.v. in 6 V-SD at day 30 post-injection (fully constituted EMF lesion) and 4 SD controls. Animals were sacrificed 24 and 72 h post-i.v. injection and both *tibialis anterior* muscles were removed. Quantification of the radioisotope was done in muscle tissue by accelerator mass spectroscopy (AMS), as previously described [17]. The $^{26}Al/^{27}Al$ ratio and ^{26}Al concentration were determined in each sample. The ratio of trapped ^{26}Al fractions in right and left *tibialis anterior* muscles ($AbsFr_{r/l}$) was calculated, allowing evaluating aluminium uptake in EMF lesions.

2.4. Comparative clearance of MMF lesions in two different rat strains

Rats were sacrificed at 1, 2, 3, 6 and 12 months post-injection. At each time point, 10 vaccinated (5 V-SD; 5 V-Lw) and 6 controls (3 SD; 3 Lw) had removal of both *tibialis anterior* muscles. Muscles were serially sectioned for pathologic evaluation. EMF lesions were irregular and consisted of central vaguely fusiform bulk, often accompanied by small satellite lesions. Evaluation of EMF lesions was based on size and lymphoid component of lesions. The lymphoid component was evaluated by LS determination (see supra). The size of lesions was assessed using the Cavalieri estimator procedure that allows accurate estimation of an irregularly shaped lesion volume [18]. Briefly, 10–15 equally spaced cross-sections were systematically sampled from an array of serial sections representing 1 mm of muscle, section count starting from the first one hitting the lesion. The volume of lesion (V_L) was calculated