## Macrophagic myofasciitis (MMF) is likely irrelevant to adverse effects of HPV vaccinations in Japan: experimental evidence that MMF is a "vaccine tattoo"



reonashiro@med.kindai.ac.jp

<u>Reona Shiro<sup>1</sup>, Fumitaka Sato<sup>2</sup>, Seiichi Omura<sup>2</sup>, Ah-Mee Park<sup>3</sup>, Cong Thanh Nguyen<sup>2</sup>, Ijaz Ahmad<sup>2</sup>, Ikuo Tsunoda<sup>2</sup></u>

**PDF** poster







#### Abstract

[Introduction] In Japan, neuropsychological symptoms following human papillomavirus (HPV) vaccinations were widely reported as "adverse effects," leading to vaccine hesitancy with less than 1% of the vaccination rate. As one of the rationales for the "adverse effects," vaccine opponents proposed that HPV vaccinations can cause macrophagic myofasciitis (MMF). MMF is a disease entity hypothesized by one French group that the aluminum (AI) adjuvants in HPV vaccines cause the accumulation of Al-laden macrophages at the injected muscles locally, leading to inflammation in the brain and general organs. We aimed to determine whether experimental HPV vaccinations in mice can reproduce MMF-like changes locally and alleged "adverse effects" in the brain and general organs. [Methods] We injected mice intramuscularly with five vaccines or phosphate-buffered saline (PBS) three times every 4 weeks and monitored body weight changes and neurological signs. We harvested the muscles, general organs, and brain tissues for histological analyses and sera for 13-cytokine quantifications. [Results] We found MMF-like histological changes with Al-laden F4/80+ macrophage accumulation in the injected muscles in all four Al(+) vaccine groups, but not in the PBS or Shingrix group. No groups had inflammation in the brain or general organs. We did not observe neurological signs in any groups, except for body weight loss in the Shingrix group, which were associated with temporal increases in IL-6, MCP-1, and IFN-y. [Conclusions] We observed MMF-like changes at the injection sites in not only two HPV-vaccine groups, but also the other two Al(+) vaccine groups. We observed neither neurological nor systemic signs in any Al(+) vaccine groups. Thus, the MMF muscle pathology reported in humans is likely a "vaccine tattoo," a physiological reaction as the result of Al-adjuvant injection, but not the cause of brain or systemic inflammation.

#### Aim

**Can experimental HPV vaccinations reproduce MMF**like changes locally and the "adverse effects" in the brain and general organs?

#### Materials and methods

Group	Vaccine	Type of vaccines	Viral antigen	Adjuvant	AI
1	Cervarix®	2× HPV VLP vaccine	HPV L1 (16, 18)	AS04 (Al hydroxide & MPL)	+
2	Gardasil®	4× HPV VLP vaccine	HPV L1 (6, 11, 16, 18)	Al hydroxyphosphate sulfate	+
3	Bimmugen®	Hepatitis B	HBs antigen	Al hydroxide	+
4	HEPTAVAX®	vaccine		Al hydroxyphosphate sulfate	+
5	Shingrix®	Varicella-zoster vaccine	Varicella-zoster virus gE	AS01B (QS-21 & MPL)	_
6	PBS	injection control		_	_

#### No inflammation in the brain or general organs in any groups



#### Background

#### **HPV** vaccines

- Subunit vaccine composed of the HPV L1 protein
- Includes aluminum (AI) adjuvants to enhance the immune responses
- Intramuscular (i.m) injection



HBs antigen; hepatitis B virus surface antigen, gE; glycoprotein E

#### 1st i.m injection Day 0

2nd i.m injection **Day 28** 

Day 56 3rd i.m injection

**Day 84** Collection of sera, muscles, general organs, and brains

#### Results

No groups have clinical signs except for weight losses in the Shingrix group

Luxol fast blue stain and immunohistochemistry against lba1+ microglia were conducted to examine neuropathology. General organs including the liver, heart, kidney, and gut had no inflammation.

#### Efficient induction of anti-HPV L1 antibody in HPV-vaccinated groups



Using the serum samples on day 84, we determined the levels of anti-HPV16 L1 antibody by enzyme-linked immunosorbent assays (ELISA). \* P < 0.05, ANOVA. N.D., not detectable.

# Quadriceps

Female 5-weeks-old C57BL/6

mice (n=5/group) were

injected 50 µL of vaccines or

PBS into the right quadriceps.

viral DNA L2 protein							
	HPV virio	HPV vaccine (virus-like particle, VLP)					
Trade name	Cervarix®	Gardasil®	Silgard9®				
Generic name	Bivalent (2 × ) HPV VLP vaccine	Quadrivalent (4 × ) HPV VLP vaccine	9-valent (9 × ) HPV VLP vaccine				
Antigen		HPV L1 protein					
HPV type	16, 18	6, 11, 16, 18	6, 11, 16, 18, 31, 33, 45, 52, 58				
Adjuvant	Al hydroxide & MPL	AI hydroxyphosphate sulfate					
AI amount	0.5 mg / 0.5 mL	0.225 mg / 0.5 mL					

VLP: virus-like particle, AI; aluminum, MPL; monophosphoryl lipid A

#### HPV vaccine hesitancy in Japan

- Reports of "adverse effects" including neurological and systemic symptoms by mass media
- Vaccination rate <1% due to the suspension of proactive recommendations for HPV vaccination
- Continuous concerns for the "adverse effects"



Following administration of vaccines or PBS into mice, we monitored body weight changes and clinical signs for 3 months.

MMF-like muscle pathology with Al-laden macrophages only in Al(+) vaccine groups



#### Serum IL-6, MCP-1, and IFN-y associate only with Shingrix group's weight losses



We harvested the sera at several time points and quantified serum interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and interferon-γ (IFN- γ) concentrations by the Legendplex<sup>™</sup> Mouse **Inflammation Panel.** 

### Conclusions

#### after resuming the proactive recommendations

![](_page_0_Figure_48.jpeg)

On day 84, we harvested the quadriceps and conducted hematoxylin and eosin (H & E) and aluminum (Al, lumogallion) stains. We also visualized F4/80+ macrophages by immunohistochemistry. Shown are histology of injected quadriceps.

- MMF-like changes at the injection sites were induced in not only the HPV-vaccinated groups, but also the other two Al(+) vaccine groups.
- Neither neurological nor systemic signs were seen in any Al(+) vaccine groups.

The MMF muscle pathology reported in humans is likely a "vaccine tattoo," a physiological reaction as the result of Al-adjuvant injection, but not the cause of brain or systemic inflammation.

## References, grant, and COL

• References:

1) Matsumura N, Tsunoda I. Scientific evaluation of alleged findings in HPV vaccines: Molecular mimicry and mouse models of vaccineinduced disease. Cancer Sci. 2022;113(10):3313-3320.

2) Matsumura N, Shiro R, Tsunoda I. Critical evaluation on roles of macrophagic myofasciitis and aluminum adjuvants in HPV vaccineinduced adverse events. *Cancer Sci.* 2023;114(4):1218-1228.

• Grant: KAKENHI-JSPS (JP23K08901, Reona Shiro)

• COI: The authors have no financial relationships to disclose.

![](_page_0_Picture_59.jpeg)