

# Comparative studies of cytokine and histological profiles among two HPV vaccines : HPV vaccine adjuvants were safe and irrelevant to clinical signs or brain pathology



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PDF poster



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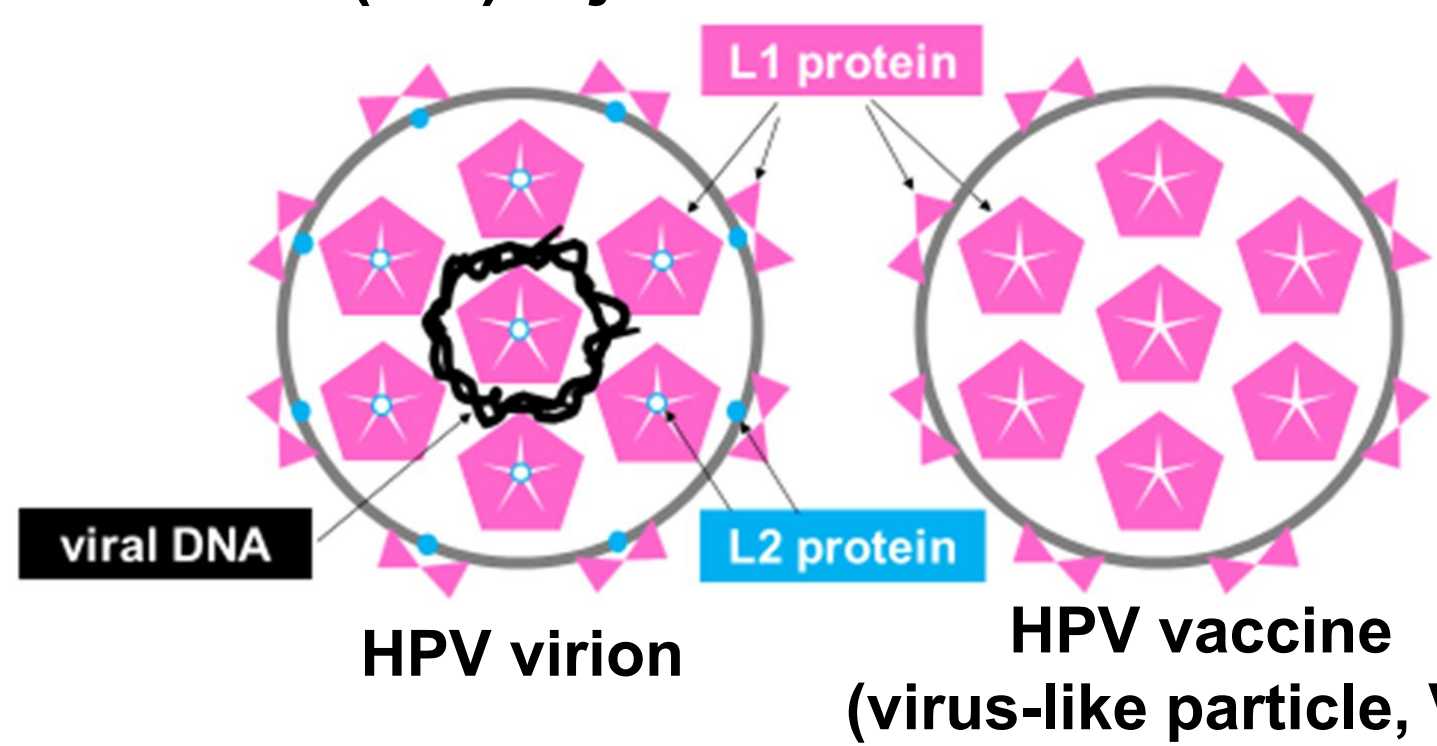
## Abstract

**[Introduction]** In Japan, neuropsychological symptoms following human papillomavirus (HPV) vaccinations were publicized as “adverse effects,” leading to vaccine hesitancy. Anti-vaccine activists claimed that aluminum (Al) adjuvants in HPV vaccines could cause immune abnormalities, such as cytokine changes and neuropathology; adjuvants in bivalent HPV vaccine (2vHPV) was AS04 [composed of **Al hydroxide (AH)** and **MPL**] and quadrivalent HPV vaccine (4vHPV) was **Al hydroxyphosphate sulfate (AHS)**. We aimed to determine whether HPV vaccinations in mice could reproduce alleged immunopathology. **[Methods]** We injected mice intramuscularly with 2vHPV, 4vHPV, hepatitis B virus (HBV) vaccines containing **AH** or **AHS**, or varicella-zoster vaccine (vZVZ) containing AS01 (**MPL** and **QS-21**), three times every 4 weeks. We monitored body weight, neurological signs, and serum cytokines for 12 weeks, and harvested the muscles, brain, and general organs. **[Results]** Histologically, all four Al-containing vaccine groups had Al-laden macrophage accumulation only at the injected muscle; no groups had damages in any other organs. Immunologically, four Al-containing vaccine groups, particularly 4vHPV, had continuously increased levels of several cytokines, including interferon (IFN)- $\beta$ . Principal component analysis (PCA) of 13 cytokine data showed overall cytokine profiles differed among the groups; no associations between cytokine profiles and muscle pathology or neurological signs which no group of mice had. Only vZVZ injection temporarily increased several cytokines, associated with body weight loss. Injections of AS01 alone or AS01-containing RS virus vaccine also caused weight loss. **[Conclusions]** Neither 2vHPV nor 4vHPV induced neurological deficits or damage in any organs, irrelevant to cytokine profiles. Continuously high levels of IFN- $\beta$  in vHPV groups may protect against other viral infections by trained immunity. Lastly, 2vHPV containing adjuvant AS04 (**AH** and **MPL**) did not induce weight loss; QS-21, but not **MPL**, in adjuvant AS01 seemed responsible for weight loss, demonstrating the safety of **MPL**.

## Background

### HPV vaccines

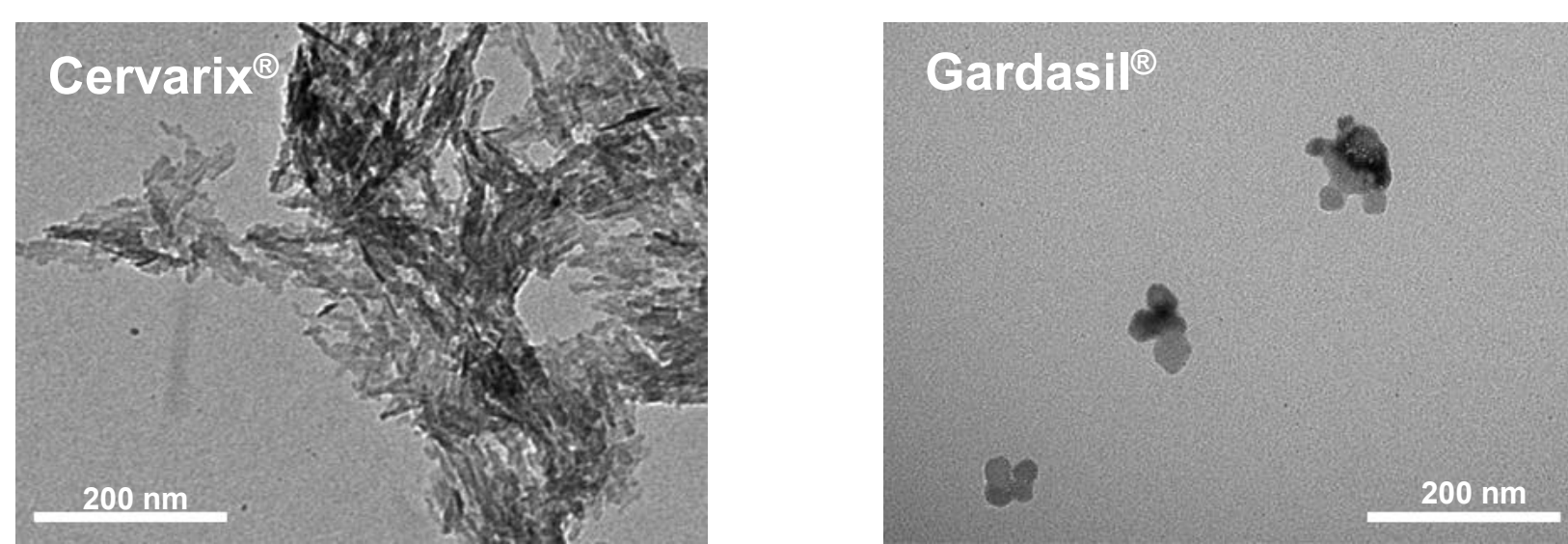
- Subunit vaccine composed of the HPV L1 protein
- Includes aluminum (Al) adjuvants to enhance immune responses
- Intramuscular (i.m) injection



Three HPV vaccines available in Japan

Trade name	Cervarix®	Gardasil®	Gardasil9®/Silgard9®
Generic name	Bivalent (2 ×) HPV VLP vaccine	Quadrivalent (4 ×) HPV VLP vaccine	9-valent 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	<b>Al hydroxide (AH) &amp; MPL</b> (Cervarix®) / <b>Al hydroxyphosphate sulfate (AHS)</b> (Gardasil®)		

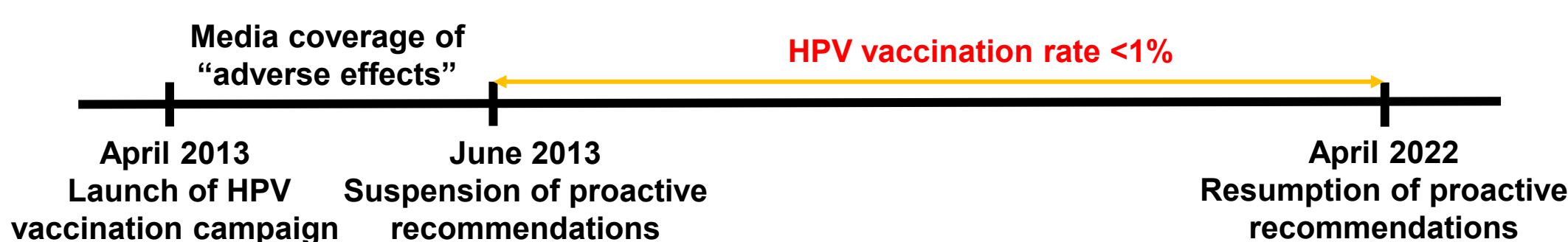
VLP: virus-like particle, Al: aluminum, MPL: monophosphoryl lipid A



The images of HPV vaccines by transmission electron microscopy

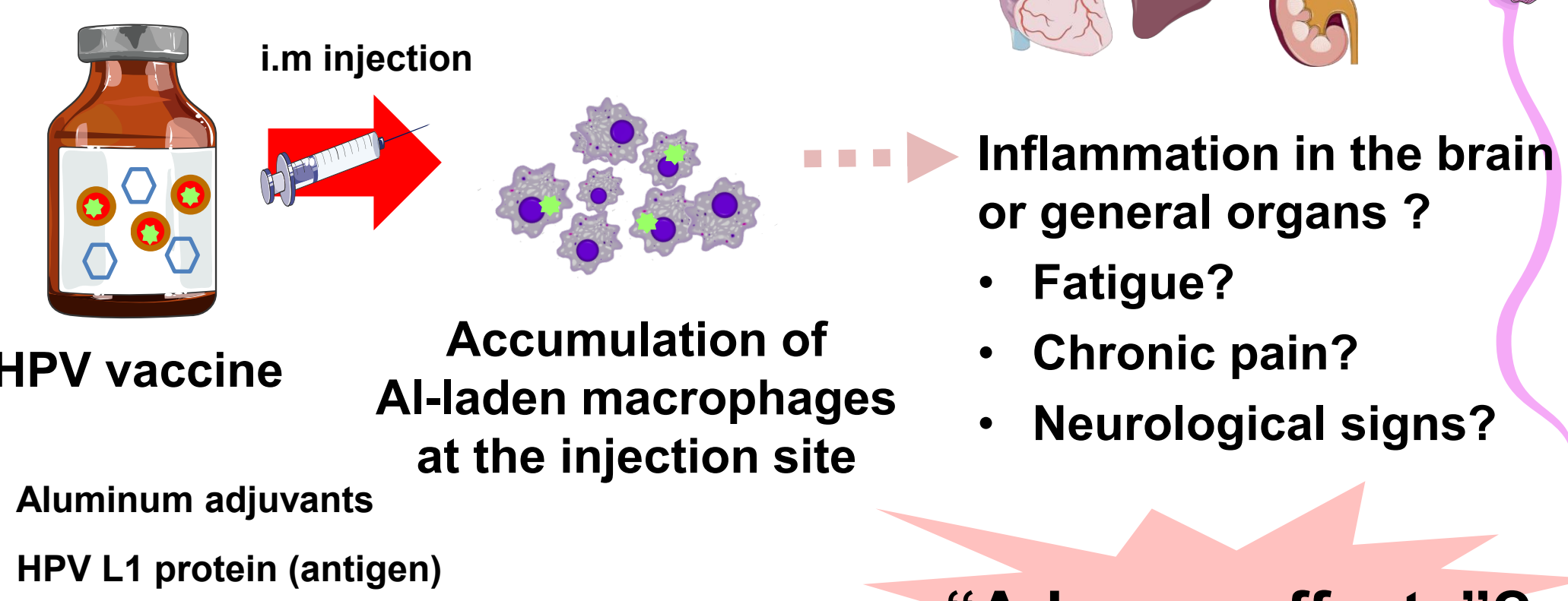
## HPV vaccine hesitancy in Japan

- Neuropsychological symptoms following HPV vaccinations were publicized as “adverse effects” by mass media
- Vaccination rate <1% due to the suspension of proactive recommendations for HPV vaccination
- Continuous concerns for the “adverse effects” after resuming the proactive recommendations



## Macrophagic myofasciitis (MMF)

A disease entity proposed by a French research group



## Aim

To determine whether experimental HPV vaccinations in mice reproduce alleged immunopathology

## Materials and methods

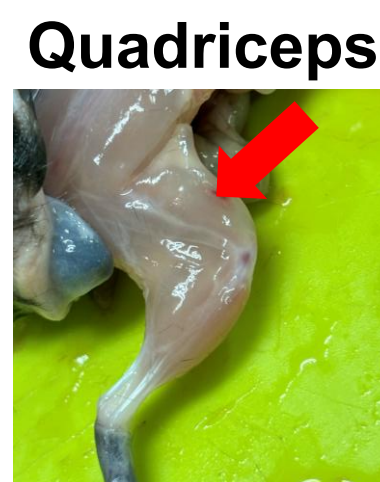
Group	Vaccine	Vaccine	Viral antigen	Adjuvant	Al
1	2vHPV	2× HPV VLP vaccine	HPV L1 (16, 18)	AS04 ( <b>AH</b> & <b>MPL</b> )	+
2	4vHPV	4× HPV VLP vaccine	HPV L1 (6, 11, 16, 18)	<b>AHS</b>	+
3	vHBV-AH	Hepatitis B vaccine	HBs antigen	<b>AH</b>	+
4	vHBV-AHS			<b>AHS</b>	+
5	vZVZ	Varicella-zoster vaccine	Varicella-zoster virus gE	AS01 ( <b>QS-21</b> & <b>MPL</b> )	—
6	PBS	injection control		—	—

Day 0 1st i.m. injection

Day 28 2nd i.m. injection

Day 56 3rd i.m. injection

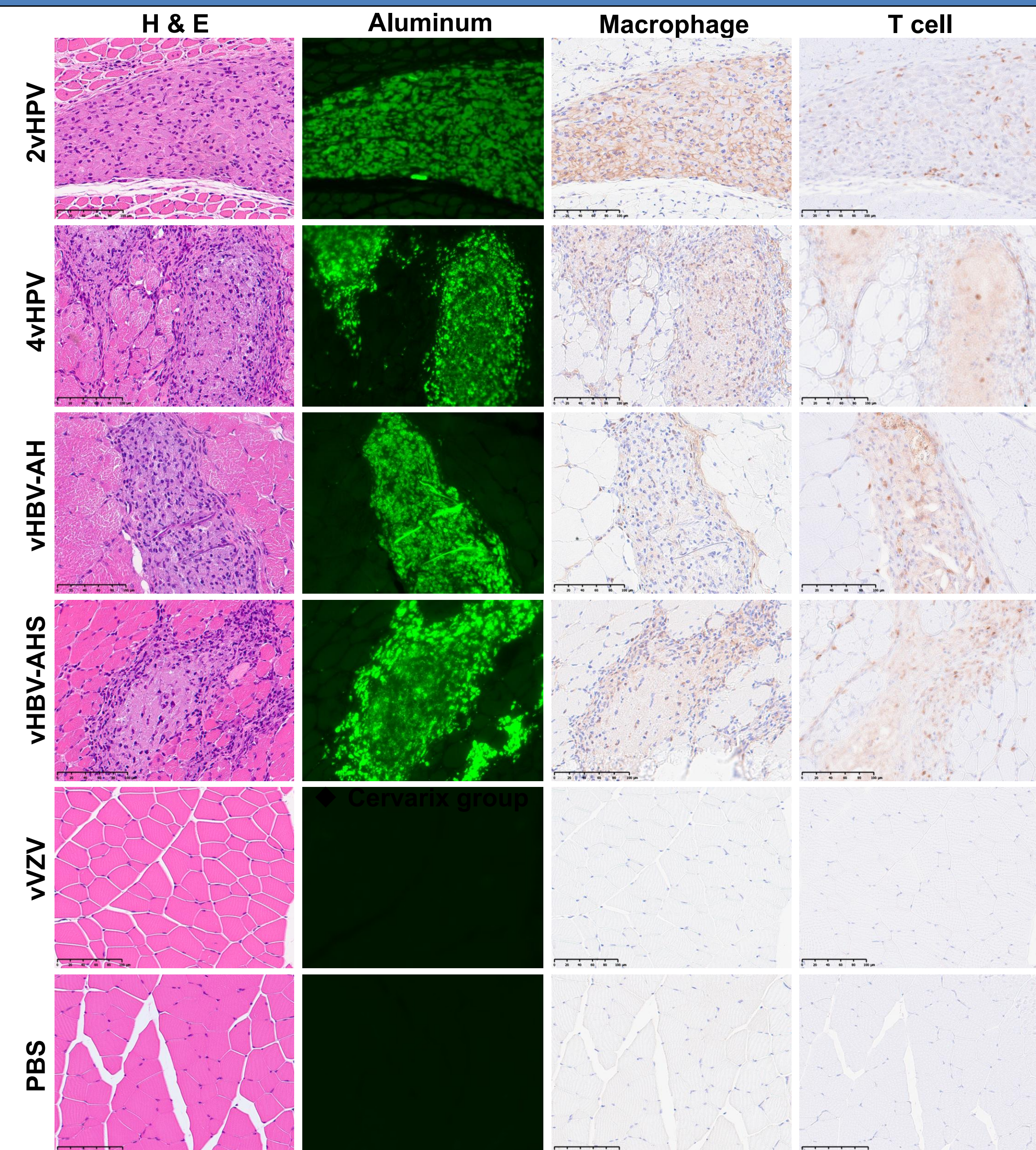
Day 84 Collection of sera, muscles, general organs, and brains



Female 5-weeks-old C57BL/6 mice (n=5/group) were injected 50  $\mu$ L of vaccines or PBS into the right quadriceps.

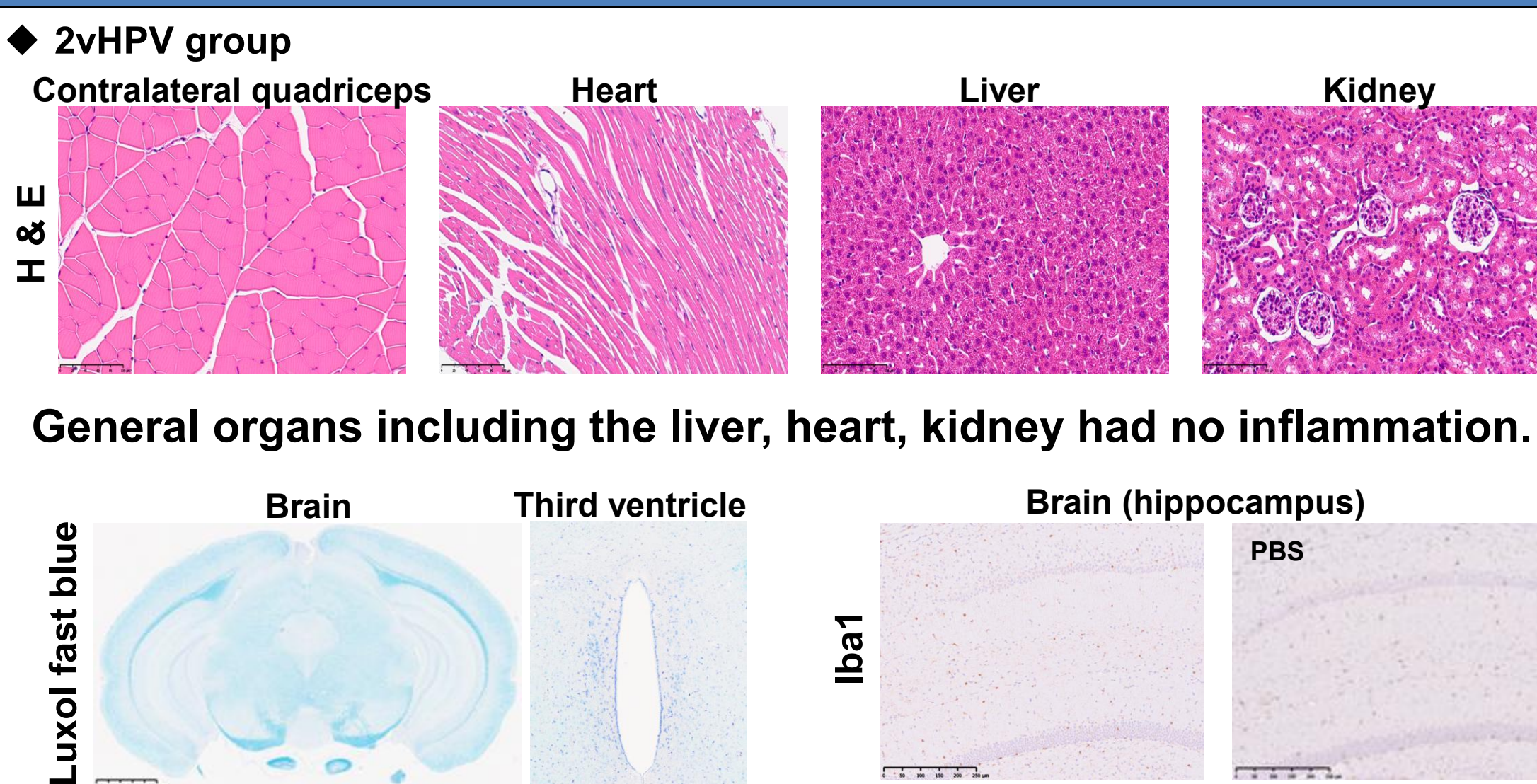
## Results

### MMF-like histology in muscle injected with Al-containing vaccines



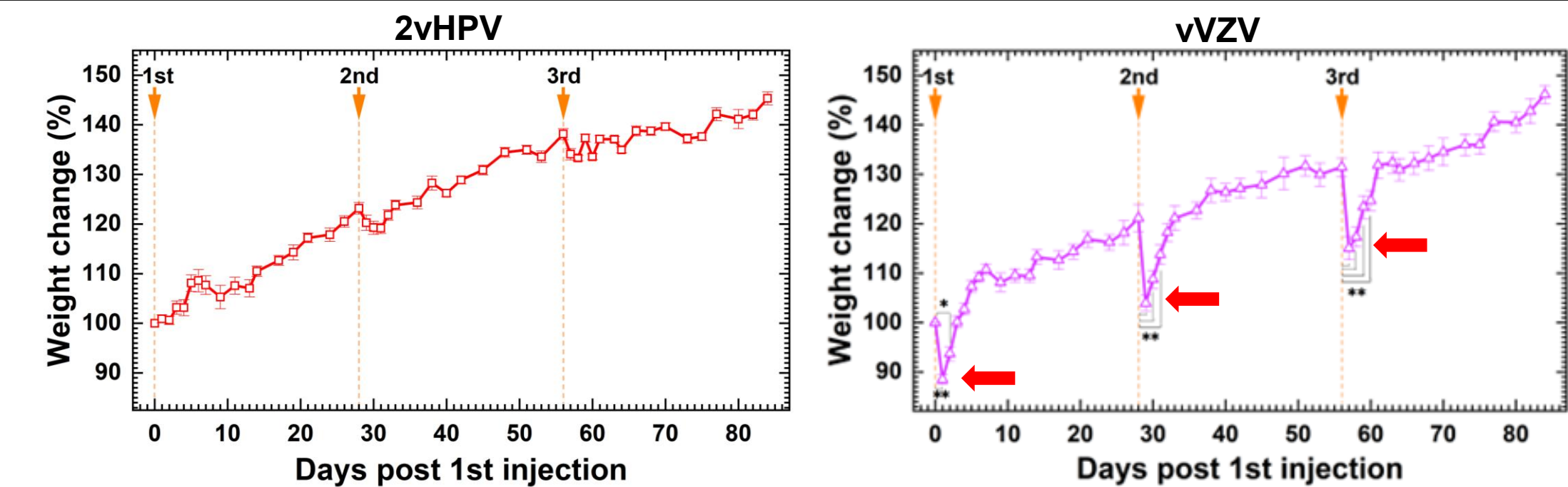
On day 84, we harvested the quadriceps and conducted hematoxylin and eosin (H & E) and aluminum (Al, lumogallion) stains. We visualized F4/80<sup>+</sup> macrophages and CD3<sup>+</sup> T cells by immunohistochemistry. Shown are histology of injected quadriceps.

### No abnormalities in the brain or general organs by any vaccines



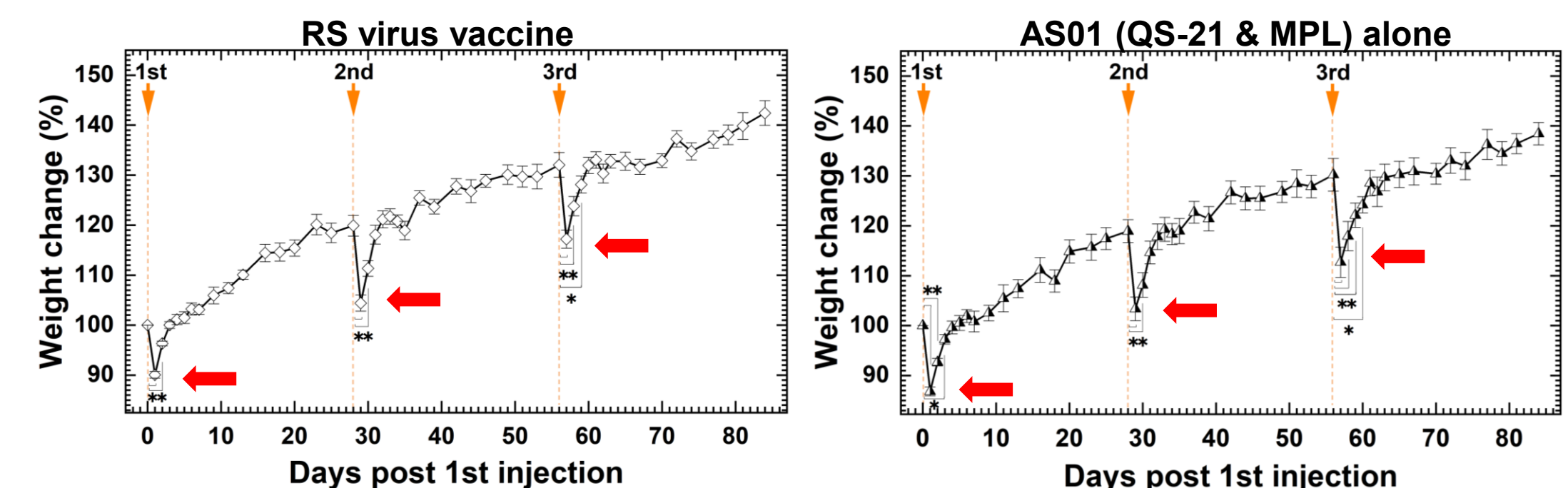
The brain, including the hippocampus and ventricles, was normal. No inflammatory demyelination or microglial activation was observed by Luxol fast blue stain or Iba1 immunohistochemistry.

### No neurological signs by any vaccines, but weight loss by AS01-containing vZVZ



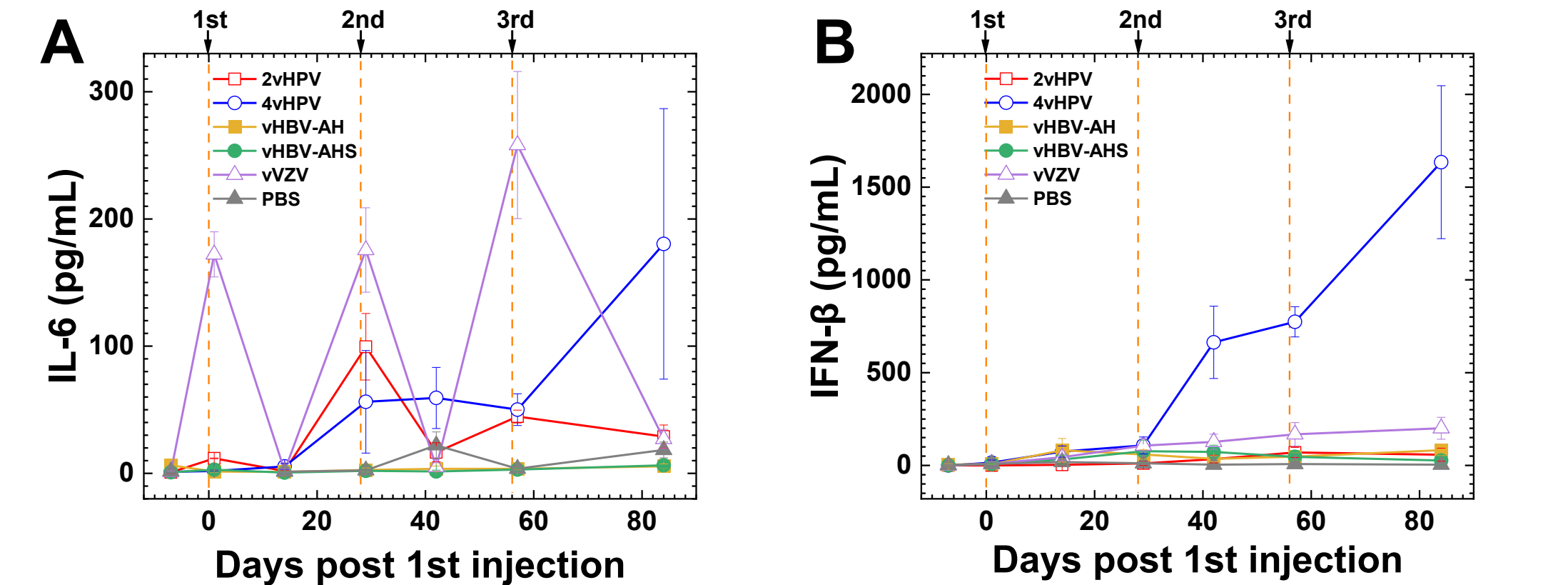
Following administration of vaccines or PBS into mice, we monitored body weight changes and clinical signs for 84 days.

### Weight loss by AS01-containing RS virus vaccines and AS01 alone

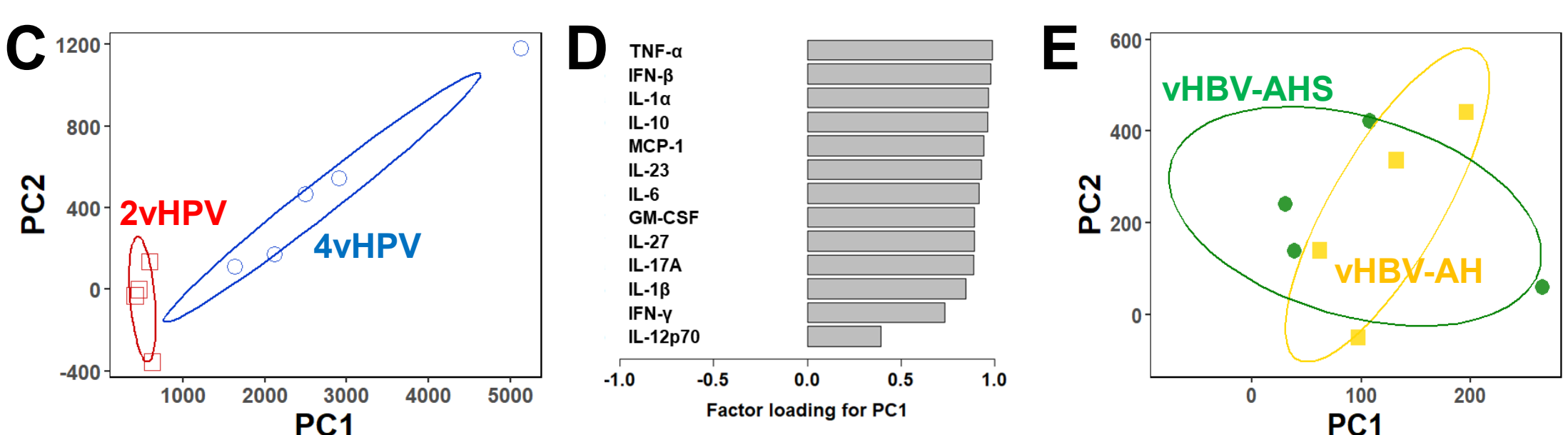


We injected RS virus vaccine containing adjuvant AS01<sub>E</sub>, which was composed of half the amount of AS01 in vZVZ, and AS01 alone three times and monitored body weight.

### Cytokine profiles irrelevant to muscle pathology or clinical signs



We quantified concentrations of 13 cytokines in sera by the flow cytometry-based multiplex immunoassays. (A) The vZVZ group had temporal increases in serum IFN- $\gamma$ , CCL2/MCP-1, and IL-6 after each injection, which was associated with body weight loss. (B) IFN- $\beta$ , IL-10, IL-27, and GM-CSF increased over the time course in all groups; the 4vHPV group had the highest cytokine levels.



We conducted principal component analysis (PCA) of 13 cytokine multiplex data on day 84. (C) PCA separated the two HPV vaccine groups by PC1 values. (D) Factor loading for PC1 showed that several cytokines contributed positively to the PC1 distribution. (E) On the other hand, PCA did not separate the two HBV vaccine groups.

## Conclusions

- Injections of all Al-containing vaccines, including two HPV vaccines, reproduced MMF-like pathology at the injected muscle, demonstrating that the MMF-like pathology is a physiological change, “vaccine tattoo,” by Al adjuvants.
- Neither 2vHPV nor 4vHPV induced neurological deficits or damage in any organs, irrelevant to cytokine profiles.
- 2vHPV containing adjuvant AS04 (AH and MPL) did not induce weight loss; QS-21, but not MPL, in adjuvant AS01 was responsible for weight loss, demonstrating the safety of MPL.
- Continuously high levels of IFN- $\beta$  in the 4vHPV group may provide protection against other viral infections by trained immunity.

HPV vaccines and their adjuvants are safe irrelevant to clinical signs of brain pathology.

## References, grant, and COI

- References:
  - 1) Matsumura N, Tsunoda I. Scientific evaluation of alleged findings in HPV vaccines: Molecular mimicry and mouse models of vaccine-induced 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 vaccine-induced adverse events. *Cancer Sci.* 2023;114(4):1218-1228.
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- COI: The authors have no financial relationships to disclose.

