Pollen Grains as ‘Trojan Horses’ for Oral Vaccination

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ABSTRACT SUMMARY
This is the first report that describes development and in vivo evaluation of pollen grains (PGs) as a novel oral vaccination system. PGs were found to help induce serum anti-ovalbumin (OVA) antibodies significantly higher than those induced by use of cholera toxin (CT) as an oral adjuvant.

INTRODUCTION
Oral vaccine delivery has been a longstanding goal in the field of vaccination because it is needle-free, can be self-administered and can produce both systemic and mucosal immune responses. The major roadblocks that continue to obstruct successful oral vaccination are the degradation of vaccines in the stomach and their poor uptake across the intestinal epithelial cell lining. PGs are a natural engineering marvel with potential to address these roadblocks. PGs have very tough exterior shells, which can withstand the acidic and enzymatic environment of the stomach and they can pass in to the body as intact particles across the tight epithelial cells of the gastrointestinal mucosa¹. We propose to exploit these fantastic natural particles and transform them into ‘Trojan horses’ to safely ferry vaccine antigens across the harsh environment of the stomach and across the tight epithelial barrier, into the body (Fig 1). Accordingly, we present the first study demonstrating the potential of PGs for oral vaccination.

EXPERIMENTAL METHODS
Lycopodium clavatum spores (LSs) (Fig 2A) were used as a representative of plant PGs. LSs were defatted and deproteinated using a series of chemical treatment steps². Residual protein content was measured using elemental analysis. Cleaned LSs were filled with OVA as a model antigen. To fill spores with OVA, dry LSs were added to an aqueous OVA solution and vacuum was applied. To visually confirm localization of OVA inside spores, OVA labeled with Texas Red was used and LSs were subsequently examined using confocal microscopy. To evaluate the potential of LSs for oral vaccination, Balb/c mice (n=5 per group) were orally immunized with OVA filled into spores. A dose of 100 µg OVA with either 1 mg (LS1 group) or 5 mg spores (LS5 group) was fed to mice using a feeding needle. In addition, we used CT (5 µg, CT1 group) and the safer but less immunogenic B subunit of CT (CTB - 50 µg, CTB group) as positive control adjuvants while keeping OVA at 100 µg. It has been shown that CT is more effective if a higher dose of OVA is used³. Accordingly we also included a group of mice fed with 5 mg OVA and 5 µg CT (CT2 group). Mice were orally immunized at days 0 and 28. Blood was collected at days 0, 28 and 56 to determine immunoglobulin G (IgG) levels using enzyme-linked immunosorbent assay (ELISA).

RESULTS AND DISCUSSION
Chemical treatment of LSs was effective in removal of native plant material. The final protein content in processed spores was 0.5% compared to 7% in natural (unprocessed) spores. Scanning electron micrographs of LSs

Fig 1. Concept of pollen grains as a delivery system.
before (Fig 2B) and after treatment (Fig 2C) provide a visual indication that processed LSs are devoid of plant biomolecules. OVA was successfully loaded into the spore interior as demonstrated by the confocal micrograph of LSs (Fig 2D). ELISA for serum at a 1:200 dilution (Fig 2E) shows that by using CT as an adjuvant if the OVA dose is increased from 100 µg (CT1 group) to 5mg (CT2 group) the IgG production increases by almost three fold. In comparison, mice immunized with 100 µg OVA+5mg LSs (LS5 group) had IgG levels even higher (about 4-fold higher) than the CT2 group. This is exciting data because CT is the current gold standard in oral mucosal vaccination and our study shows that LSs are even superior to CT.

Processed PGs are expected to be safe when consumed orally because native plant proteins, lipids and other biomolecules are removed during pollen-processing. The pollen shells by themselves are made of an inert biomaterial that does not cause allergic reactions. Two previous human studies, one using unprocessed LSs and the second using processed LSs have shown that no adverse effects are seen upon oral LS consumption by humans.

CONCLUSION
We present the first study demonstrating the adjuvant effect of LSs for oral vaccination. Chemical treatment of spores successfully removed native plant biomolecules and OVA was successfully loaded into the cleaned spores. When fed to mice, OVA+spores induced a significantly higher serum anti-OVA IgG response compared to even CT, which is considered a gold standard for oral adjuvants. Overall, this study provides exciting data that LSs (perhaps all PGs in general) can act as oral mucosal adjuvants, and may provide a safe and effective vehicle for oral vaccination.

REFERENCES

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