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A novel oral mucosal immune enhancer *Bacillus subtilis* spores which can instruct efficiently program the generation of memory T cells

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Avian influenza virus (AIV) mainly results in severe respiratory syndrome or even death. *Bacillus subtilis* spores were a mucosal immune enhancer promising mucosal immunity. Here, we firstly established DC/EC co-culture system and spores provided assistance in recruiting DCs to the intestinal epithelial cells (IECs) and forming trans epithelial dendrites (TEDs). In addition, we showed that spores plus H9N2 WIV was tested in a murine oral immunization model, and the levels of local respiratory tract and systemic immune responses were measured. We showed that H9N2 WIV plus spores could enhance antigen-specific IgG, IgG1 and IgG2a in the serum and IgA titers in intestinal and lung wash fluid respectively compare to H9N2 WIV alone. Similarly, spores assisted with H9N2 WIV immunization generate superior memory T-cell proliferation, such as CD3+CD45RA-CD62L+CCR7- central memory T cell (TCM) at 7d and CD3+CD44+CD69+ intestinal resident memory T cells (TRM) at 45d. Taken together, our data showed that spores lead to the activation of TRM including recruitment of sub mucosal DCs, expression of chemokines and adhesion molecules. Taken together, *Bacillus subtilis* spores, a mucosal immune enhancer, was superior to promising immune memory in this study by up regulating TRM which has major implications for vaccine development.

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