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Determination of influenza A virus titer from mouse lung tissues using the gentleMACS™ Dissociator

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Background

Influenza A virus is a negative-strand segmented RNA virus with eight segments in its genome. Antigenically distinct viral subtypes are defined by the hemagglutinin (HA) and neuraminidase (NA) major viral surface proteins which are the most important targets for antibody-mediated protection from infection. These HA and NA viral glycoproteins are classic T cell–dependent antigens for which antibody responses depend on influenza virus-specific CD4 T cell help in the form of surface expression of CD154 and secretion of cytokines.
An ideal inactivated vaccine for influenza A virus would induce not only highly robust strain-specific humoral and T cell immune responses but also cross-protective immunity in which an immune response to antigens from a particular viral subtype would protect against other viral subtypes. Cross-protective immunity would help limit outbreaks from newly emerging antigenically novel strains.
In this study, we directly compared the immunization of mice with whole inactivated influenza A virus adjuvanted with either cationic lipid/noncoding DNA complexes (CLDC) or aluminum hydroxide (alum) for their ability to induce both influenza-specific humoral and T cell immunity. We also evaluated the ability of each vaccine to confer cross-protection against a mismatched influenza virus challenge *in vivo*.

This protocol describes our standard procedure to determine influenza A virus titer from mouse lung using the gentleMACS™ Dissociator.

Materials and methods

Materials

- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS M Tubes
- Incubator (37 °C)
- Centrifuge
- Ice-cold phosphate-buffered saline (PBS)
- Minimal Essential Medium (MEM) with and without 2 µg/mL of TPCK-treated trypsin
- 96-well U-bottom plates
- Madin-Darby canine kidney (MDCK) cells
- Chicken red blood cells (CRBC)

Methods

C57BL/6J mice immunized with whole, inactivated influenza virus (H1N1) adjuvanted with either cationic-lipid DNA complexes (CLDC) or Alum (aluminum hydroxide) were challenged with a mismatched virus (H3N2). Lungs were collected at 4 days post-infection, the time of peak lung virus titers.

Dissociation

1. Collect both mouse lungs in 2 mL of ice-cold 1× PBS.
2. Transfer the sample into a gentleMACS M Tube.
3. Tightly close the M Tube and attach it upside down onto the sleeve of the gentleMACS Dissociator.
4. Run gentleMACS Program **RNA_01**.
5. Centrifuge the sample at 600×g for 10 minutes.
6. Collect supernatant and store at –80 °C or proceed with titer determination.

Influenza A virus titer determination

1. Prepare 10-fold dilutions of lung homogenate supernatants with MEM including trypsin in 100 µL.
2. Plate dilutions on 96-well U-bottom plates with 100 µL of MDCK cells at 2.5×10^6 cells/mL.
3. Incubate overnight at 37 °C.

4. Replace medium with fresh MEM without trypsin.
5. After 72 hours add 50 µL of 0.5% CRBCs in PBS.
6. Incubate for 1 hour at room temperature (19–25 °C) and record hemagglutination afterwards to determine 50% tissue culture infective dose (TCID50) (see figure 1). Hazy wells show CRBC agglutination while non-agglutinated wells show a distinct CRBC "button" at the bottom of the well.
7. TCID50 was then calculated by the Reed-Muench formula.

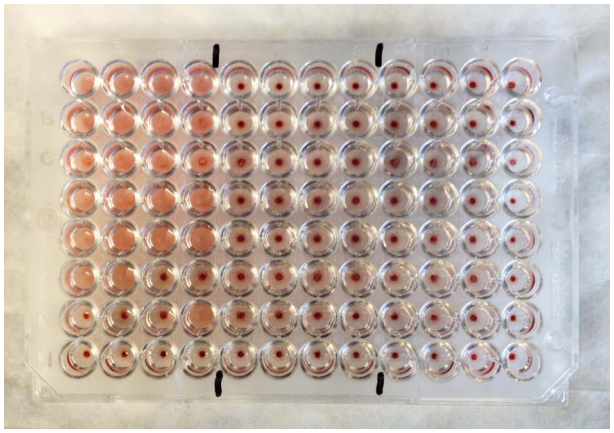

Figure 1: 96-well U-bottom plate used for influenza titering from lung homogenates. Serial dilutions of lung homogenate from influenza infected mice are plated with MDCK cells. CRBCs are then added to each well to demonstrate the presence of influenza virus. Hemagglutination results in hazy wells (upper left wells) while non-agglutinated RBCs fall to the bottom of the well and form a distinct button.

Results

This study demonstrates that the addition of CLDC as adjuvant to whole inactivated influenza A virus vaccine in mice induces greater protection against a mismatched influenza A challenge than alum, the most commonly used adjuvant in currently licenses vaccines (see figure 2). CLDC is a promising adjuvant for influenza virus vaccines that can enhance cross-protection against antigenic drift or novel emerging subtypes.

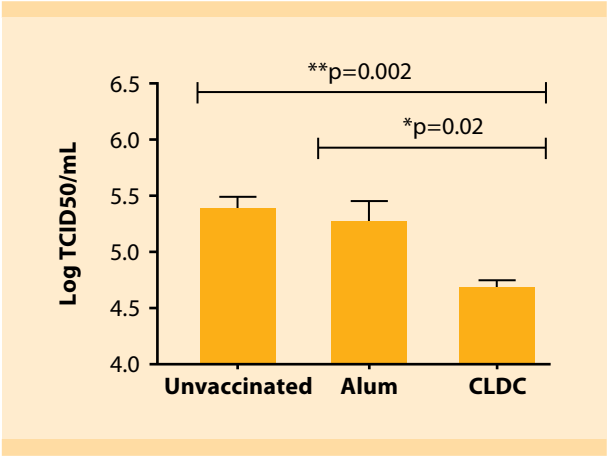

Figure 2: Influenza titers from influenza-infected mice. C57BL/6J mice were vaccinated with whole, inactivated influenza adjuvanted with either CLDC or Alum. These mice were challenged with a mismatched influenza strain and lung virus titers were determined 4 days post-infection. CLDC-adjuvanted influenza vaccination conferred greater protection against a mismatched influenza challenge.

Conclusion

Determination of influenza A virus titer can be accomplished with ease using the gentleMACS Dissociator.

Reference

1. Hong, D. K. *et al.* (2010) Cationic lipid/DNA complex-adjuvanted influenza A virus vaccination induces robust cross-protective immunity. J. Virol. 84: 12691–12702.

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