The Chinese University of Hong Kong, Faculty of Medicine, Department of Microbiology
Joint Graduate Student Seminar

How Does Measles Give You "Immune Amnesia"?

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Date: 11th December 2019

Outline

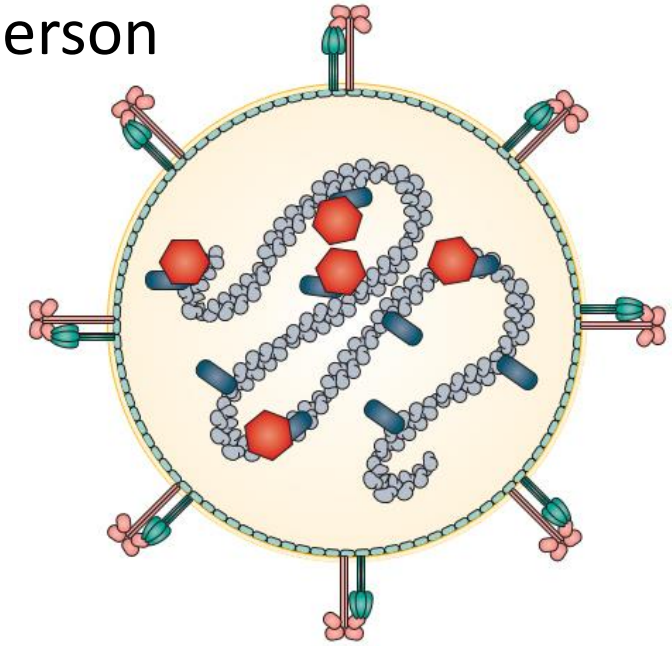
Introduction to measles

3 studies of evidences to “Immune Amnesia” hypothesis

Summary

Introduction – What is measles?

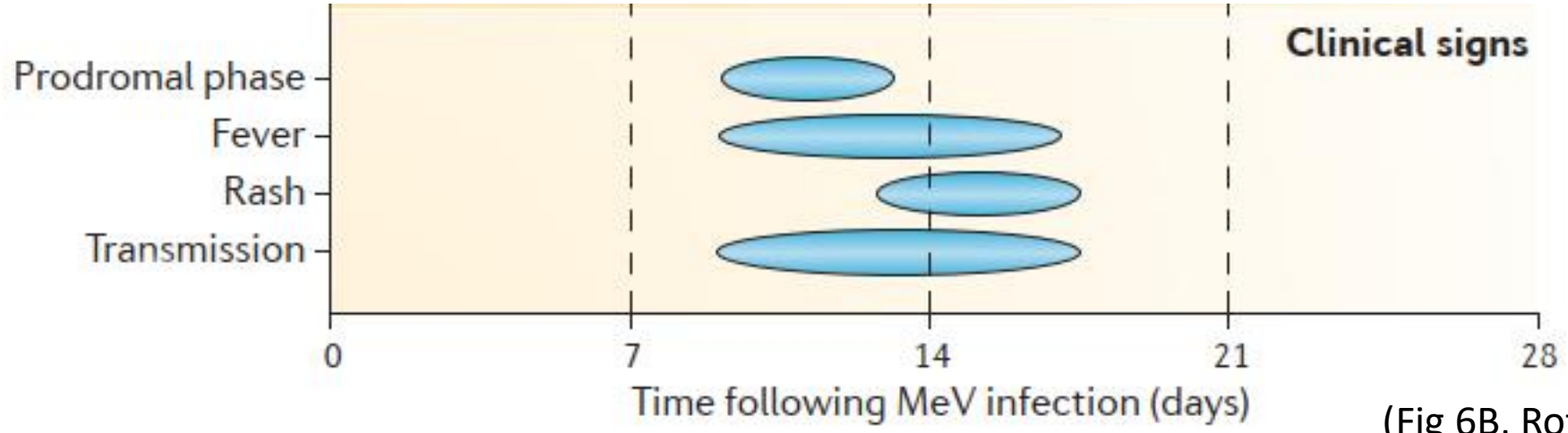
- Pathogen: Measles virus (MeV)
  - Single-Stranded, negative sense RNA virus in genus *Morbillivirus*
- Airborne disease
  - Spread through coughs and sneezes of infected person
  - Direct contact with infected secretions
- Clinical signs include
  - Fever
  - Skin rash
  - Cough, coryza and conjunctivitis



(Fig 4A, Rota et al., 2016)

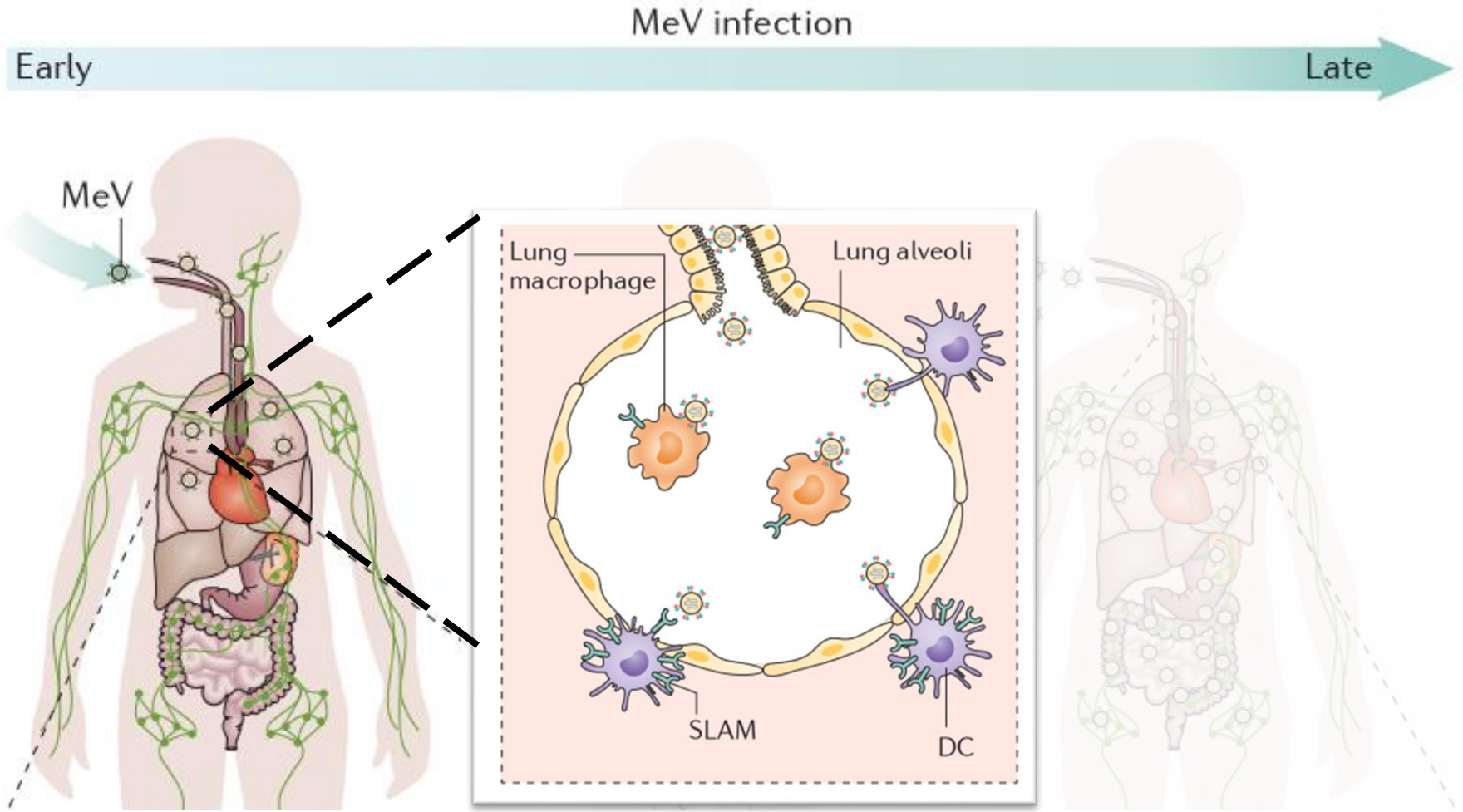
Introduction – What is measles?

- Incubation period
  - 10 days to onset of fever, 14 days to onset of rash
- Contagious period
  - 4 days before to 4 days after the onset of rash
- Recovery
  - Resolves spontaneously after 1 to 3 weeks
  - Lifelong immunity



(Fig 6B, Rota et al., 2016)

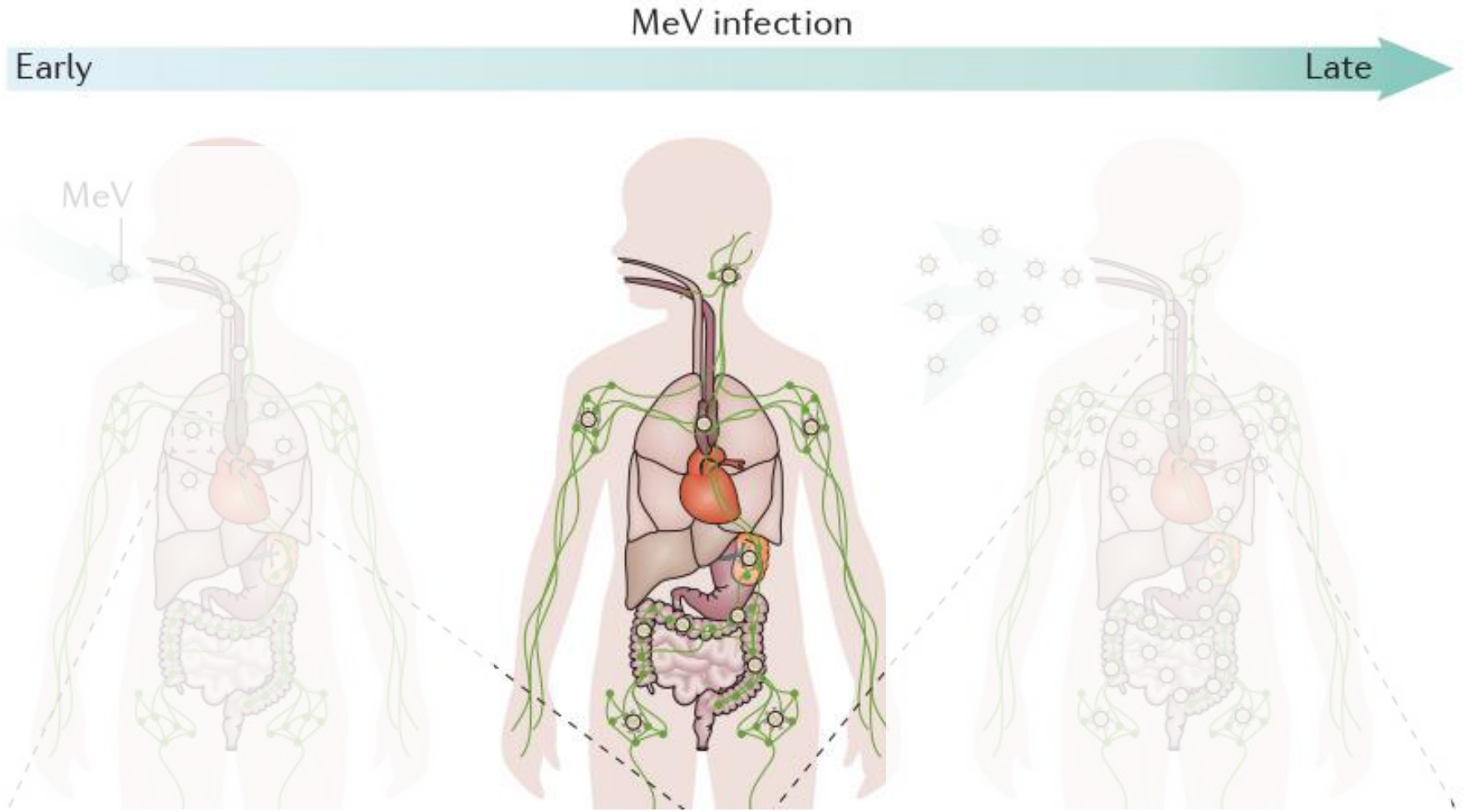
Introduction – MeV infection



Initial targets: Respiratory tract-resident dendritic cells (DCs) and alveolar macrophages

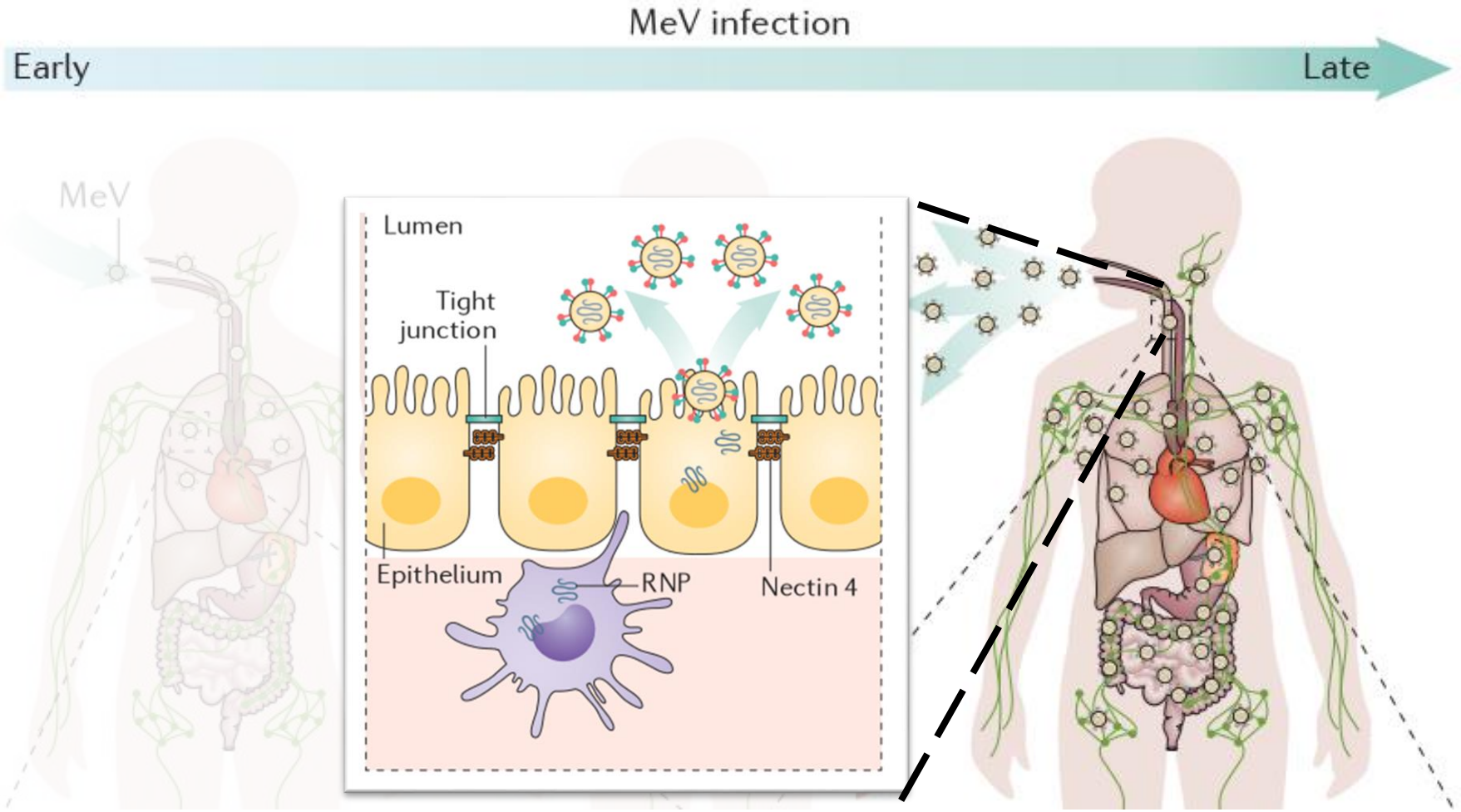
(Fig 5, Rota et al., 2016)

Introduction – MeV infection



Amplification: In regional lymphoid tissues followed by systemic infection

Introduction – MeV infection



Transmission: MeV is transmitted to epithelial cells by infected lymphocytes and DCs. As a result, large amount of progeny viruses are released into respiratory tract.

Introduction – MeV infection

- Immune suppression caused by MeV infection
  - Leads to secondary infections, which is causes majority of measles death
  - Lasts for weeks to months after acute stage of infection
- Proposed mechanisms of MeV-induced immunosuppression
  - Lymphopenia during acute phase
  - Suppression of lymphocyte proliferation
  - Long-term changes in cytokine secretion
  - “Immune Amnesia”

(Permar et al., 2006; de Vries et al., 2012)

Introduction – MeV infection

- Hypothesis “Immune Amnesia”
  - During the lymphopenia during acute phase, pre-existing memory lymphocytes depletes.
    Immunosuppression is the result of impaired previously acquired immunological memory.
  - Proposed recently in 2012
  - Provides explanation to
    - Prolonged immunosuppression after recovery from lymphopenia
    - Greater reduction of all-cause child mortality than proportion of measles death prevented after mass measles vaccination campaigns (Aaby et al., 1995)

1st Study

PLOS | PATHOGENS

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RESEARCH ARTICLE

Measles Immune Suppression: Lessons from the Macaque Model

Rory D. de Vries, Stephen McQuaid, Geert van Amerongen, Selma Yüksel, R. Joyce Verburgh, Albert D. M. E. Osterhaus, W. Paul Duprex, Rik L. de Swart

Published: August 30, 2012 • https://doi.org/10.1371/journal.ppat.1002885

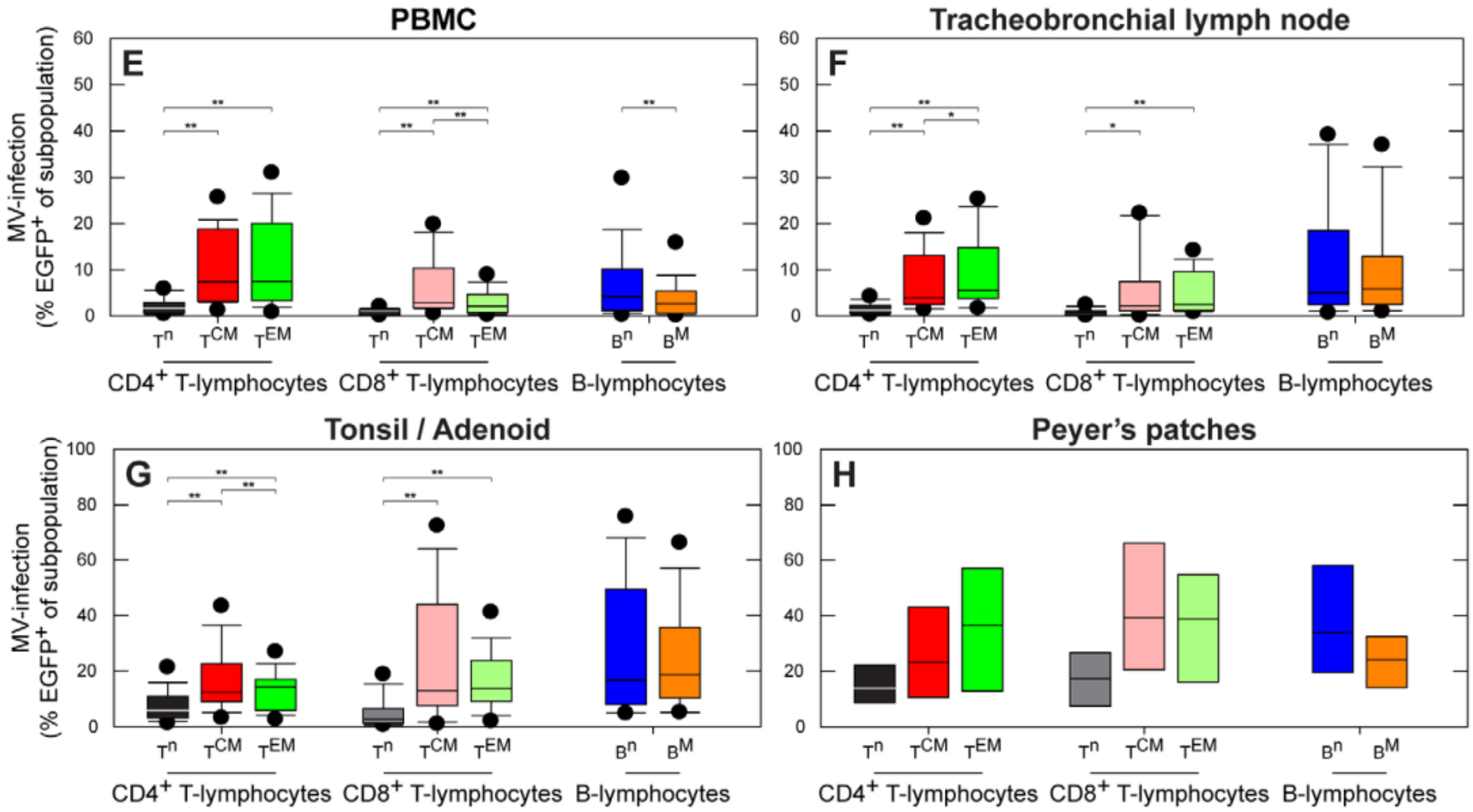
Methodology

- Macaques infection model
  - Rhesus (n=5) and cynomolgus macaques (n=35)
  - Infected with
    - Recombinant MeV strains (rMV^{IC323} or rMV^{KS}) expressing EGFP (EGFP, enhanced green fluorescent protein)
  - Blood collected daily from 0 to 13 days post infection (d.p.i)
    - Total white blood cell counts
    - Peripheral blood mononuclear cell (PBMC) isolation
  - Necropsy
    - Macaques were euthanized at different time points (2 to 15 d.p.i.)
    - Lymphoid tissues were collected for immunohistochemistry and flow cytometry

Methodology

- Cell sorting by flow cytometry
  - T-lymphocytes
    - naïve ($CD45RA^{+}$, T^{n}), central memory($CD45RA^{-}CCR7^{+}$, T^{CM}), effector memory ($CD45RA^{-}CCR7^{-}$, T^{EM})
  - B-lymphocytes
    - naive (IgD+CD272, B^{n}) & memory ($IgD^{-}CD27^{+}$, $CD20^{+}HLA^{-}DR^{+}$, B^{M})
  - Detection of MeV infection by EGFP

Results



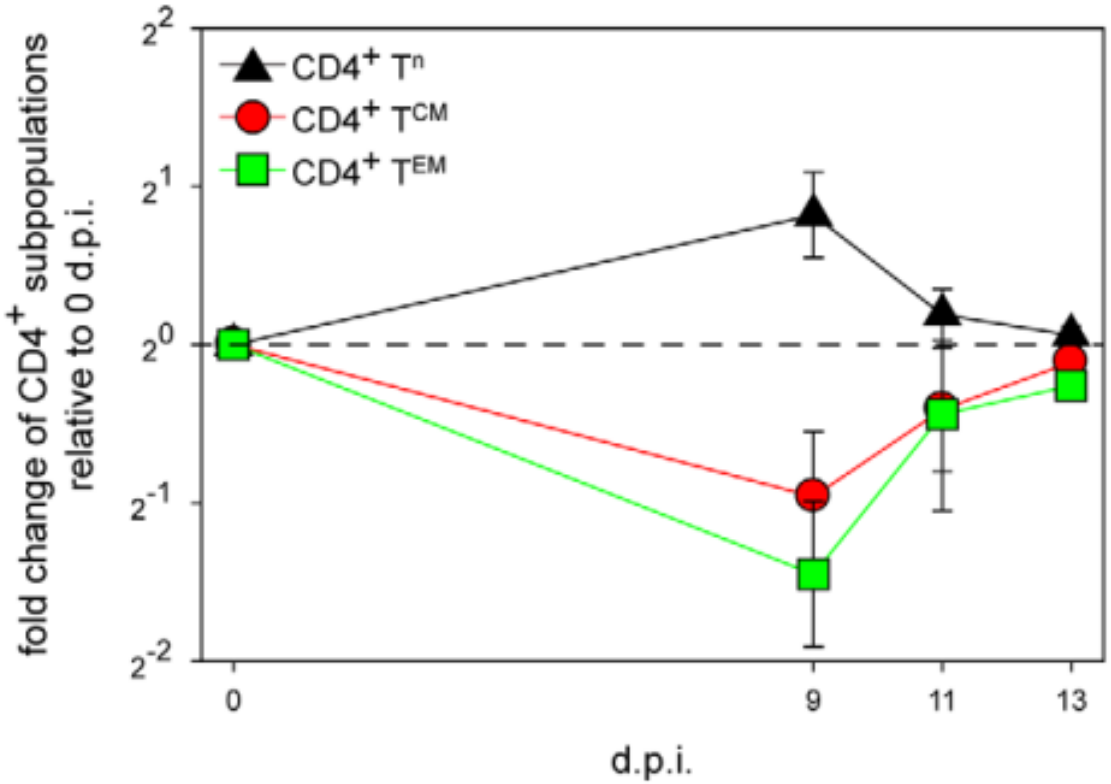
% of MeV-infection of different cell types at different locations during the approximate peak viremia

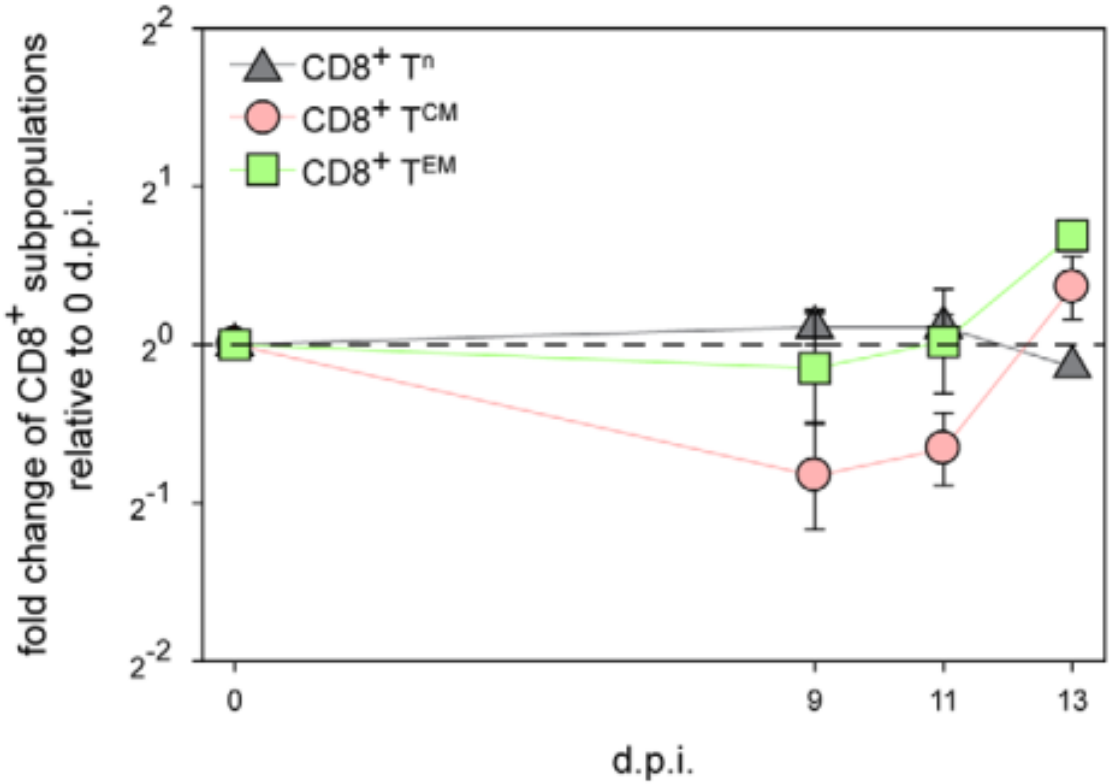
(Panel E to G, n=14; Panel H, n=3)

(Fig 1, de Vries et al., 2012)

Results

Relative population sizes of T-lymphocytes in PBMC at different d.p.i. (n=9)



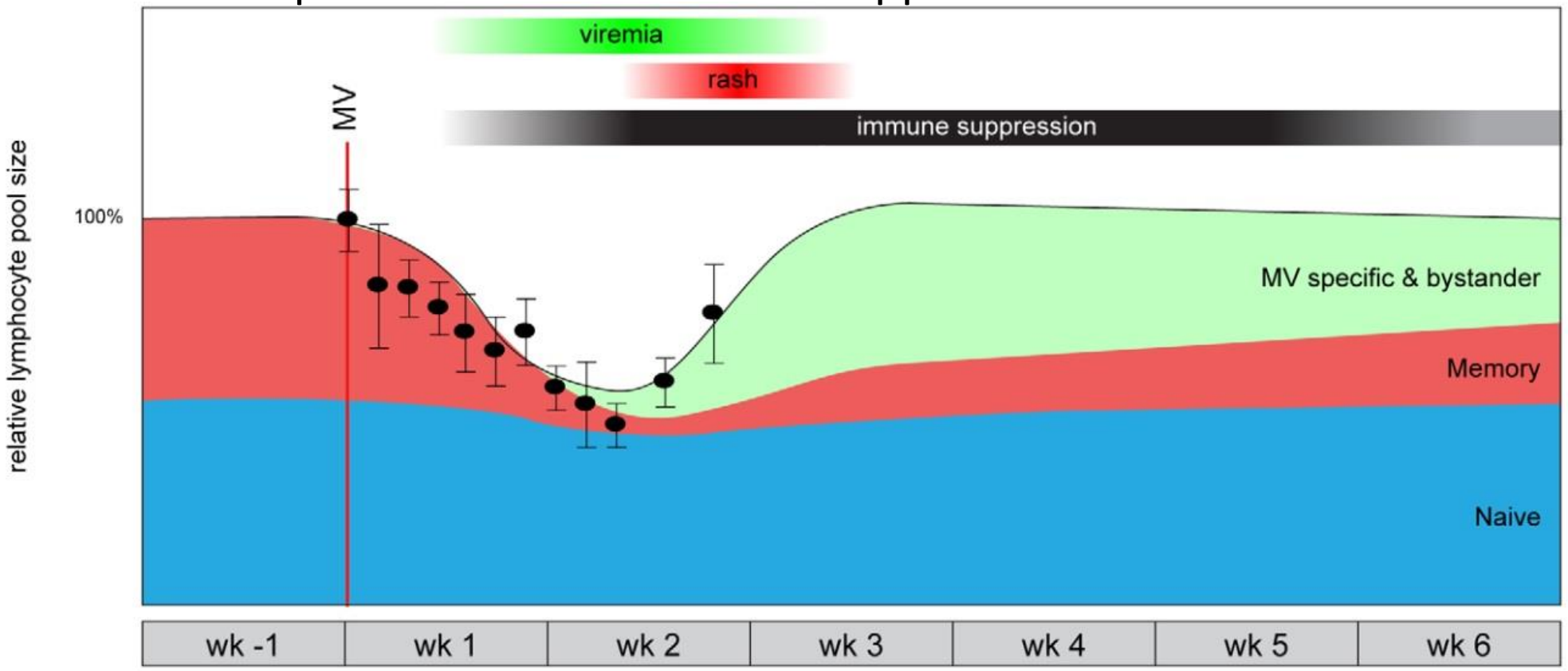


(Fig 5A, de Vries et al., 2012)

1st study: Conclusions

1. MeV preferentially infected CD45RA$^-$ memory T-lymphocytes more than naïve T cells
2. MeV infection caused transient leukopenia followed by massive lymphocyte expansion

Proposed model for immune suppression of MeV infection



(Fig 5B, de Vries et al., 2012)

2nd study: Epidemiological data analysis based on "immune amnesia" hypothesis

REPORT

Long-term measles-induced immunomodulation increases overall childhood infectious disease mortality

Michael J. Mina[1,2,*], C. Jessica E. Metcalf[1,3], Rik L. de Swart[4], A. D. M. E. Osterhaus[4], Bryan T. Grenfell[1,3]

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Science 08 May 2015:
Vol. 348, Issue 6235, pp. 694-699
DOI: 10.1126/science.aaa3662

Hypothesis

- If loss of immunological memory after measles exist, host with impaired resistance will be more susceptible to infectious diseases.
- Therefore, non-measles infectious disease mortality should correlate with measles incidence data.
- The association should be strengthened when measles incidence data are transformed to reflect the accumulated population with measles-induced immunomodulation

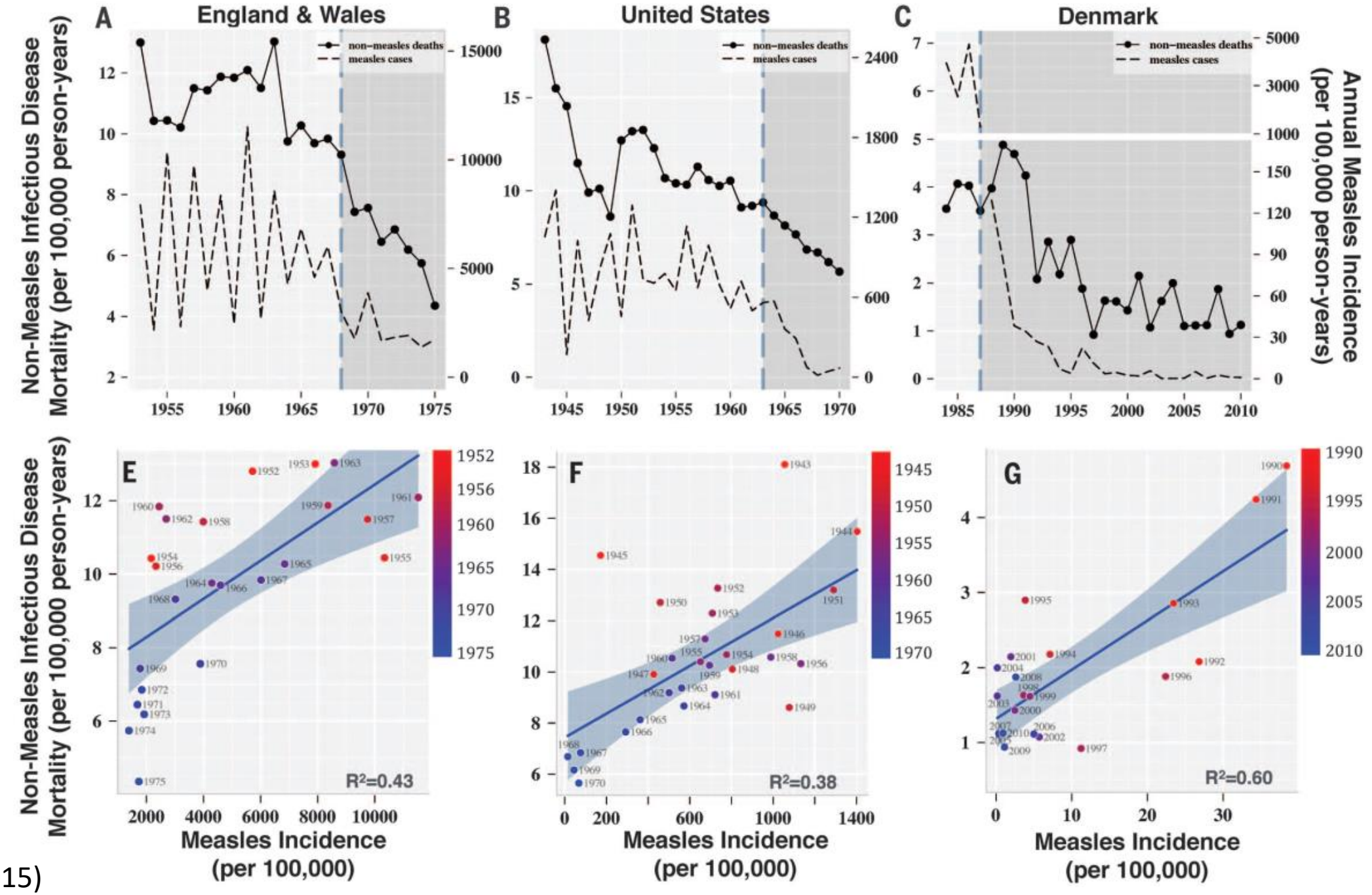
Methodology

- Data sets: National-level epidemiological data
    - From (i)England and Wales, (ii) the United States and (iii) Denmark
    - For children aged 1 to 9 years in Europe or 1 to 14 years in US
    - Period around the introduction of mass measles vaccination
- Data analysis
    - Regression analysis of non-measles infectious disease mortality against measles incidence or prevalence of measles-induced immunomodulation

Methodology

- Data analysis
  - Transformation of measles incidence to measles-induced immunomodulation
    - To reflect accumulated immunomodulated population size at a certain time
    - Simplified example: If immune memory loss last for 3 years,
      Total number of immunomodulated individuals (S) =
      Sum of measles cases of last 3 years
    - Prevalence of measles-induced immunomodulation = S / Total population
  - Best-fit duration of immunomodulation
    - Transformation were repeated with different duration of immunomodulation
    - Best-fit duration =
      Duration that gave highest R^2 in regression of transformed data against mortality

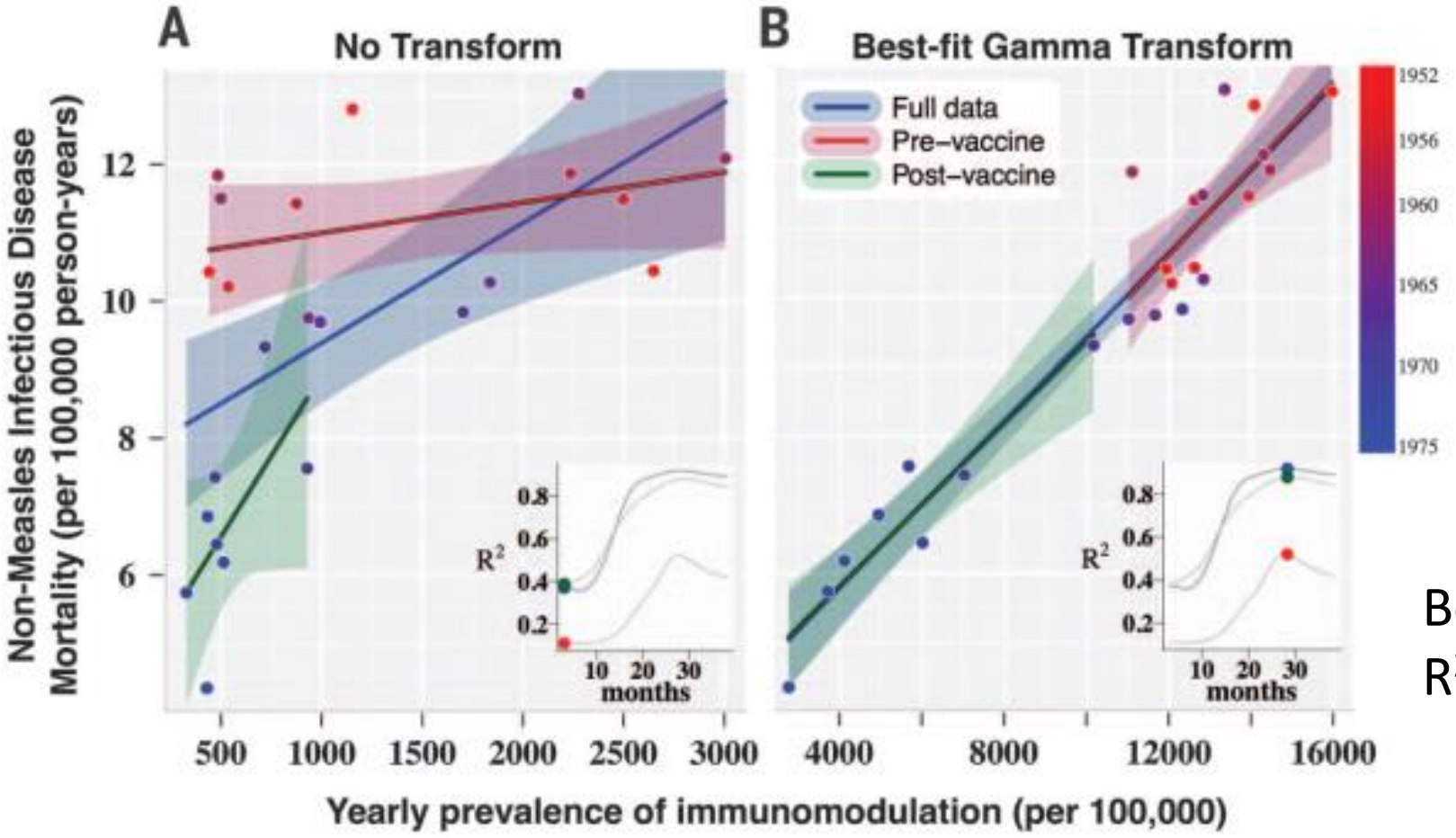
Results

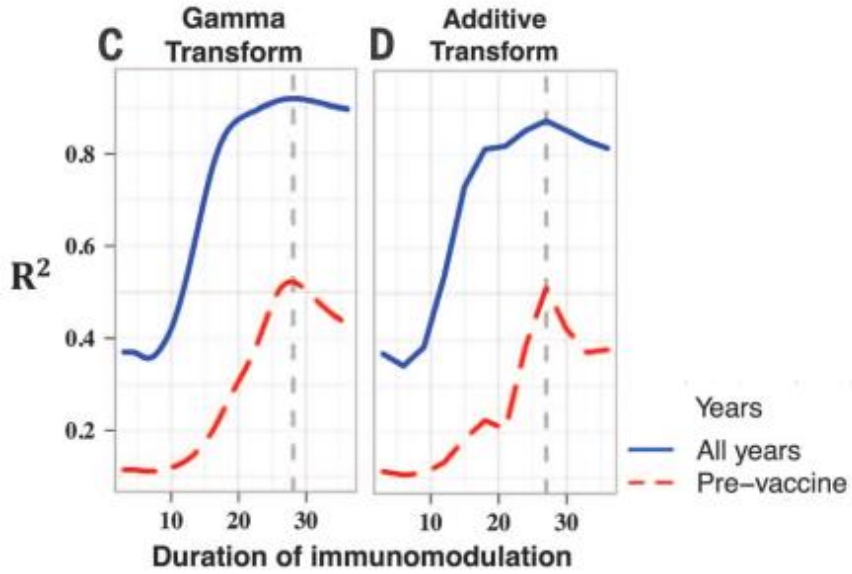


(Fig 1, Mina et al., 2015)

Results – England and Wales

- Annual incidence of nonmeasles infectious disease mortality regressed against the prevalence of MV immunomodulation



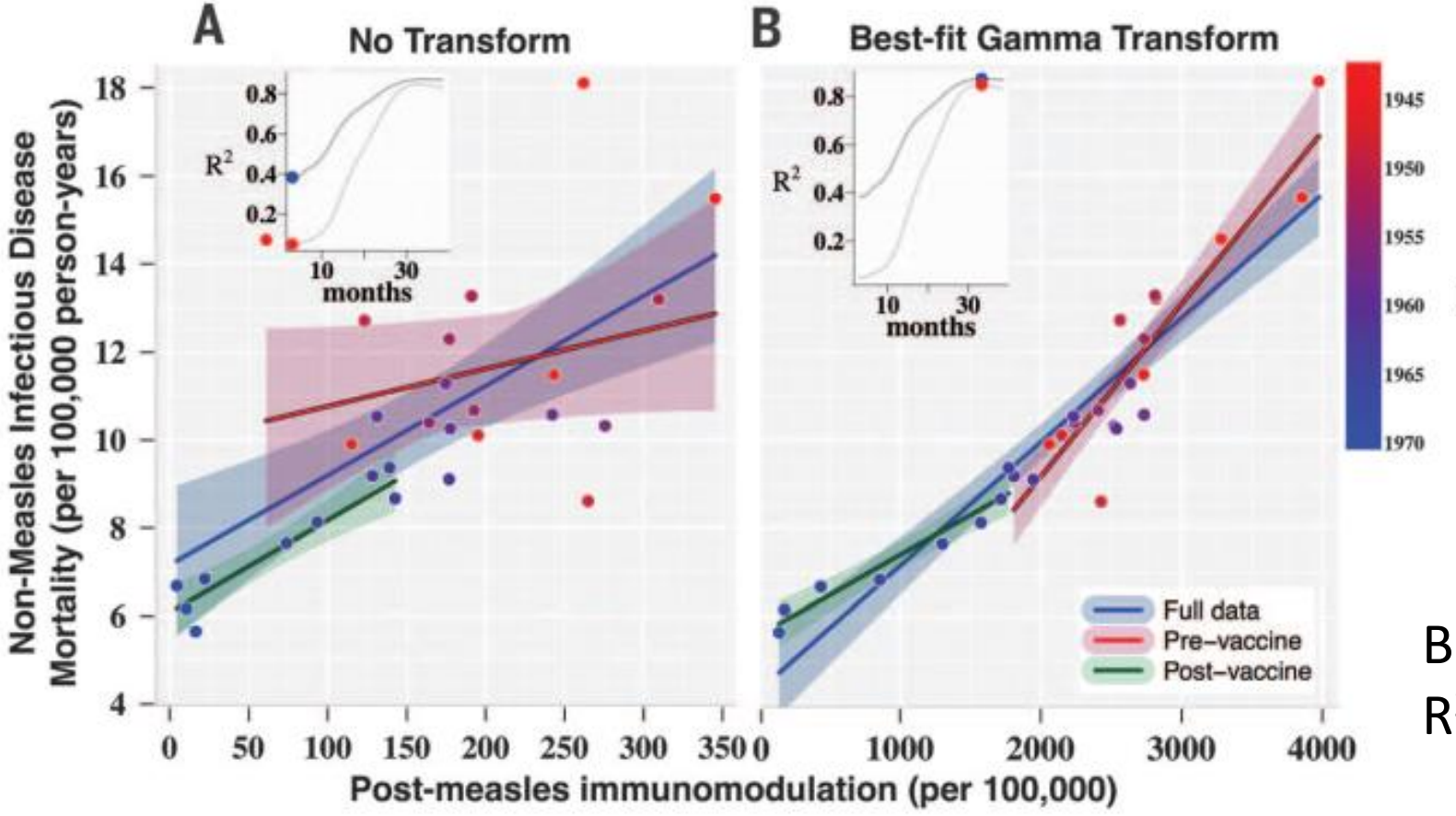


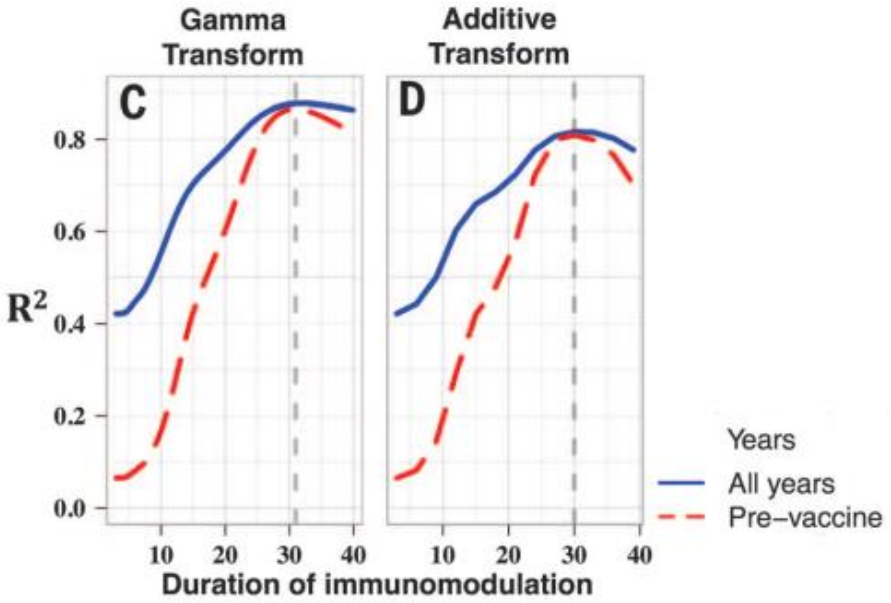
Best-fit duration = 28.3 months, R^2 (=0.92)

(Fig 2, Mina et al., 2015)

Results – the United States

- Annual incidence of nonmeasles infectious disease mortality regressed against the prevalence of MV immunomodulation

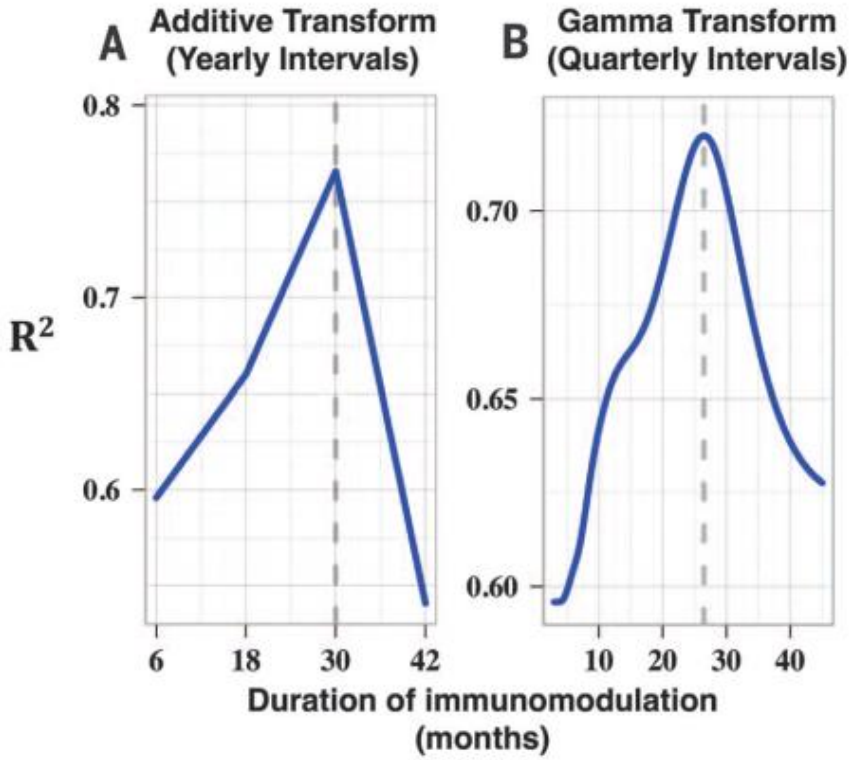




Best-fit duration = 30.9 months, R^2 =0.88

(Fig 3, Mina et al., 2015)

Results – Denmark

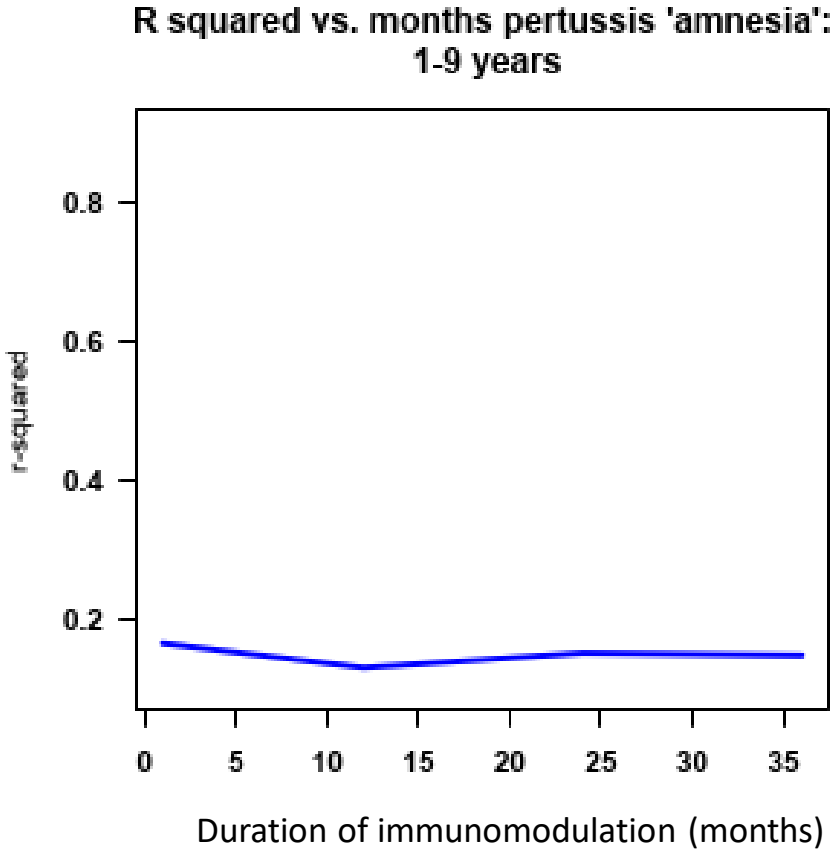


Best fit durations = 26.4 months

(Fig 4, Mina et al., 2015)

Results

- Data analysis on pertussis as control
  - Using England and Wales data set
  - Duration of immunomodulation tested from 0 to 48 months
  - No correlation between pertussis incidence and non-pertussis infectious disease mortality



(Fig S13, Mina et al., 2015)

2nd Study: Conclusion

- Measles infection
  - Caused roughly 2 to 3 years of prolonged impact on subsequent mortality due to immunomodulation
  - Implicated in nearly half of all childhood deaths from infectious disease

3rd study

RESEARCH ARTICLE | INFECTIOUS DISEASES

Incomplete genetic reconstitution of B cell pools contributes to prolonged immunosuppression after measles

Velislava N. Petrova[1,*], Bevan Sawatsky[2], Alvin X. Han[3,4], Brigitta M. Laksono[5], Lisa Walz[2,†], Edyth Parker[4], Kathrin Pieper…

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Science Immunology 01 Nov 2019:
Vol. 4, Issue 41, eaay6125
DOI: 10.1126/sciimmunol.aay6125

Hypothesis

- Changes in composition of circulating B lymphocytes after MeV infection should be reflected in the genetic composition of the immune receptor repertoire of MeV-infected individuals

Methodology

1. Prospective study on the changes in genetic composition of human B lymphocytes after measles
2. Ferret model of measles-induced loss of acquired immunity

Methodology

1. Prospective study on human

Children subjects

- Aged 4 to 17 years
- Unvaccinated and without history of measles
- From 3 Orthodox Protestant schools in the Netherlands

Disease group

- Developed a course of laboratory-confirmed measles
- Blood collections:

1st: Before any symptoms of measles

2nd: Around 40 days after onset of rash

Uninfected control group

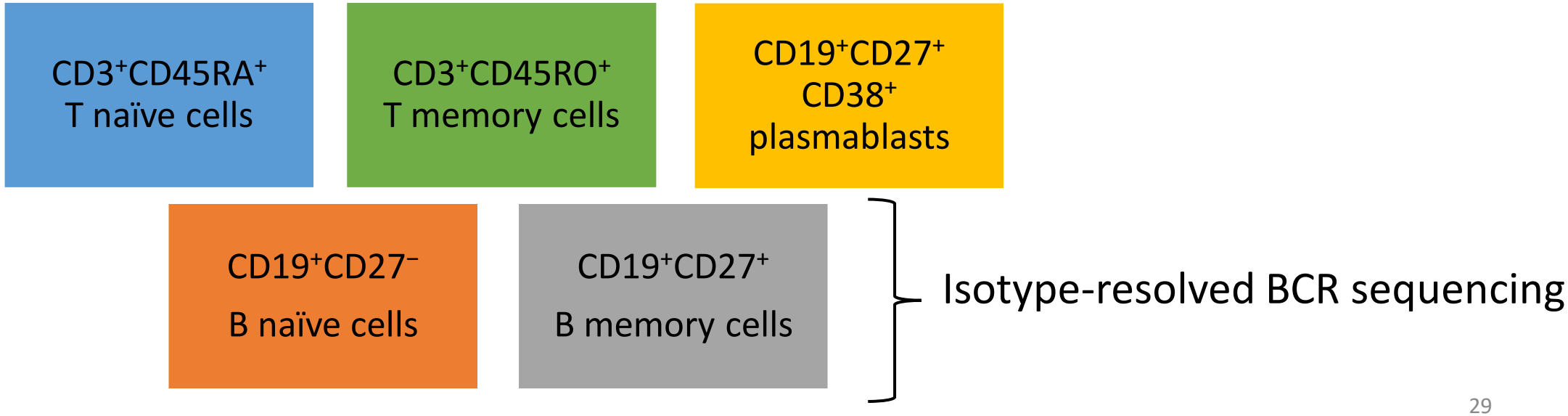
- Subjects remained seronegative to measles across the two time points

Vaccine control group

- Adults vaccinated with trivalent inactivated influenza vaccine (TIIV)
- Blood collected before and 40 days after vaccination

Methodology

- Human blood samples
  - Measles-specific antibody titre was determined
  - Peripheral blood mononuclear cells (PBMC) were isolated
- Fluorescence-activated cell sorting of PBMC
  - PBMC were stained with cell surface marker-specific antibodies and sorted in to five populations:

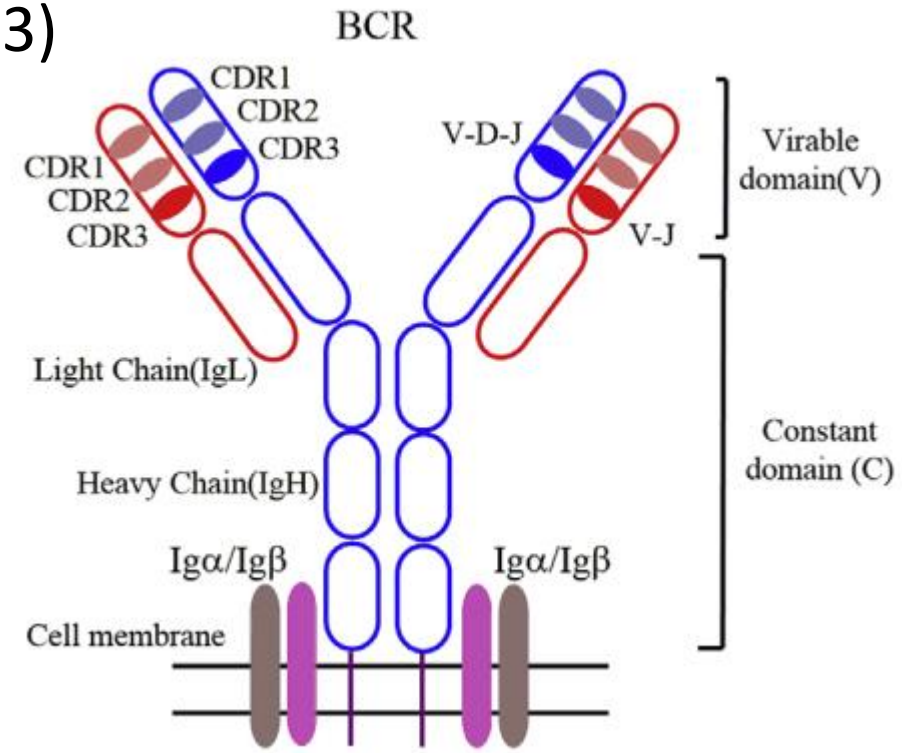


Methodology

- Isotype-resolved BCR sequencing
  - RNA extraction of B cell population
  - Library preparation
    - Reverse transcription with five IGHC region reverse primers
    - Amplification of cDNA with V-gene multiplex primer mix and “3′ universal” reverse primer using KAPA protocol
  - Sequencing
    - Performed using standard Illumina 300 bp paired-ended MiSeq protocols

Methodology

- Analysis on genetic properties of isotype-specific BCR repertoires
  - IGHV-J gene frequencies
    - % of sequences a certain IGHV-J combination to the total BCR repertoire
  - Complementarity determining region 3 (CDR3)
    - Amino acid length
    - Mutation rate from germline
  - B cell “clone”
    - Defined as BCR sequences with identical IGHV and IGHJ annotation and CDR3 length



(Fig 1A, Ye et al., 2018)

Methodology

2. Ferret model of measles-induced loss of acquired immunity
   - Three groups of 4 male ferrets

     Group 1: LAIV vaccination

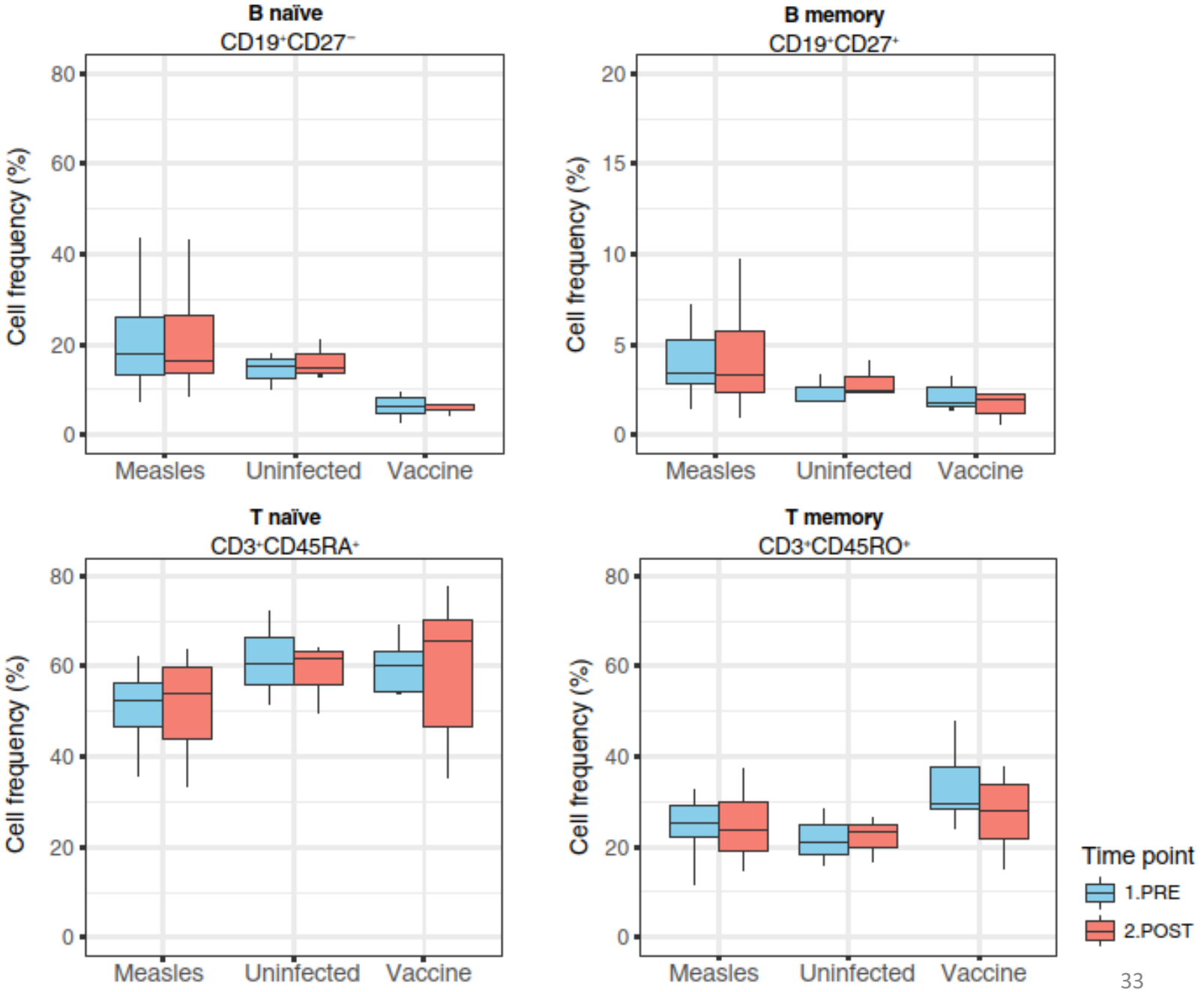
     Group 2: LAIV vaccination + CDV infection

     Group 3: Control (No LAIV vaccination and CDV infection)
     - LAIV: Tetravalent seasonal live attenuated influenza vaccine
     - CDV infection : Canine distemper virus (CDV) infection four weeks after LAIV
       - Used as a surrogate model for measles infection
   - Influenza A/INDRE/Mexico/4487/2009 challenge
     - For all groups ten weeks after CDV infection
     - Animals were infected intranasally with virulent 2009 pandemic H1N1 influenza virus strains

Results

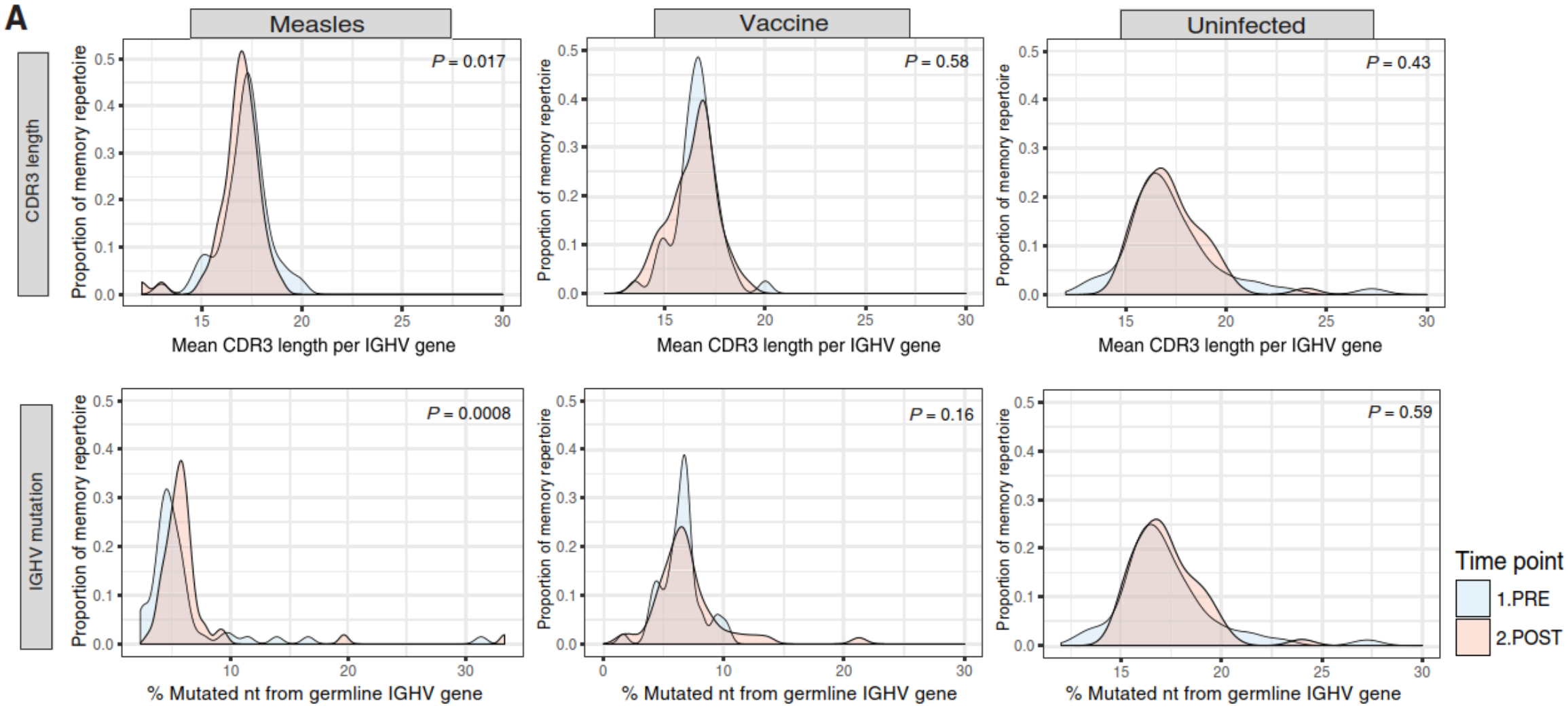
Prospective study on human

- Disease group, n= 26
- Uninfected control, n = 3
- Vaccine control group, n =7



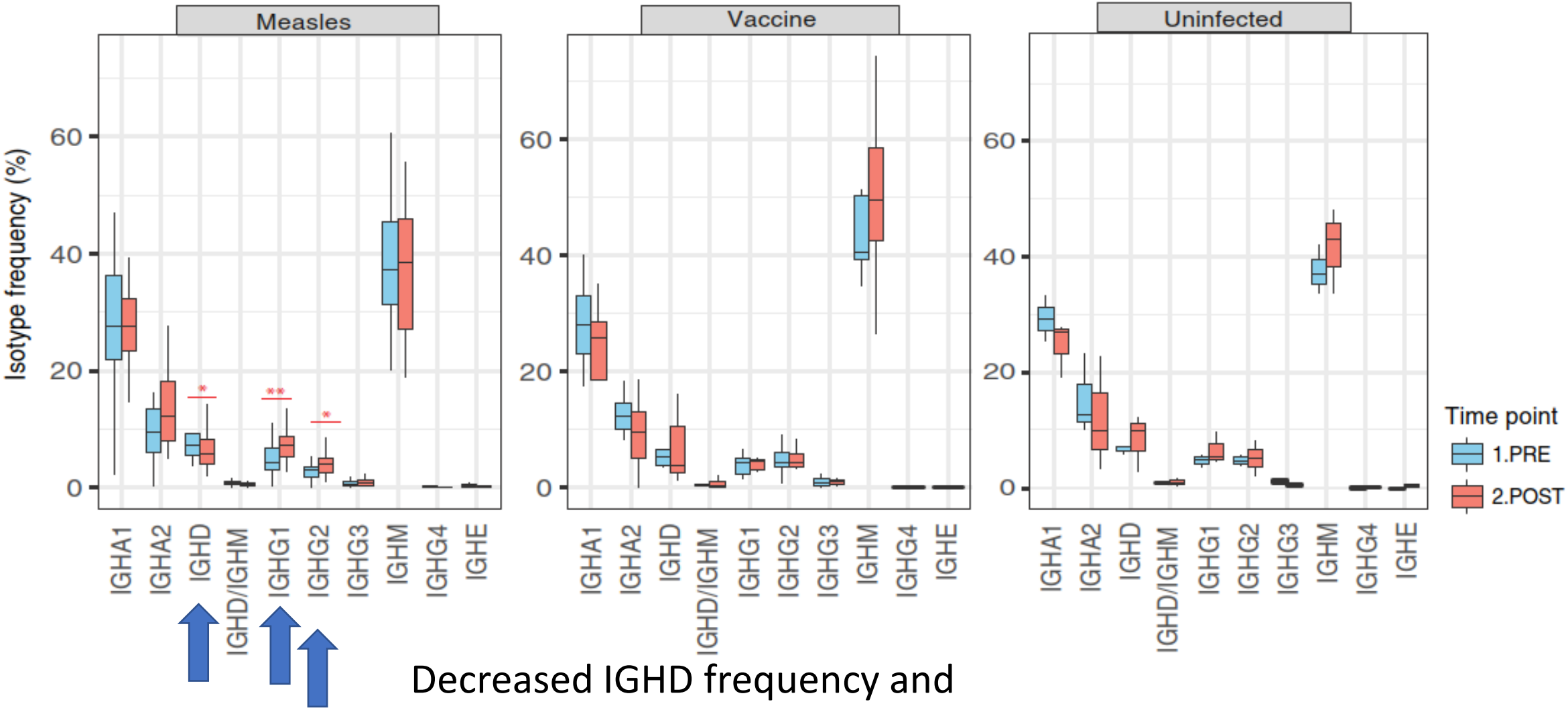
(Fig 1B, Petrova et al., 2019)

- Decreased CDR3 length and increased IGHV mutation in the B memory compartment following measles



(Fig 3A, Petrova et al., 2019)

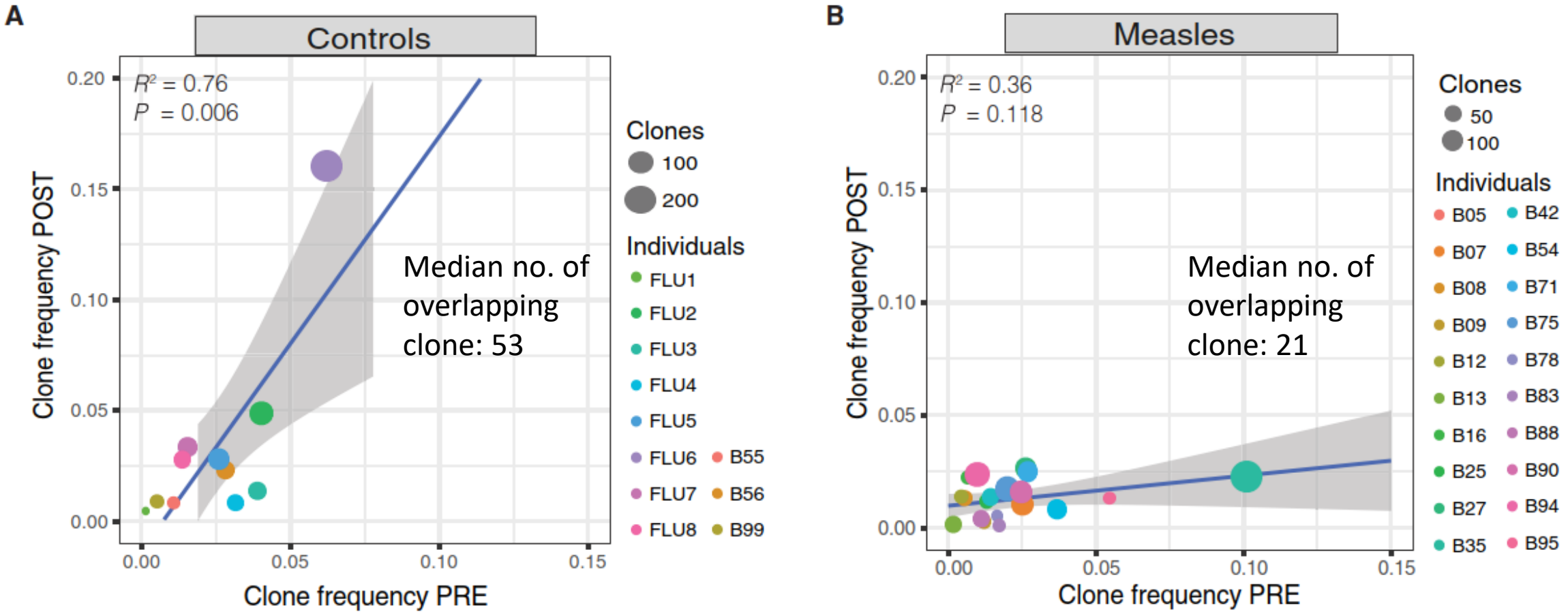
- Isotype profile in the B memory compartment following measles



Decreased IGHD frequency and
increased IGHG1 and IGHG2 frequencies in measles group

(Fig 3B, Petrova et al., 2019)

- Lower number of overlapping clone in measles group
- Reduced frequency of overlapping B cell clones after measles



Overlapping clone: Clone detected in both time points with same identity

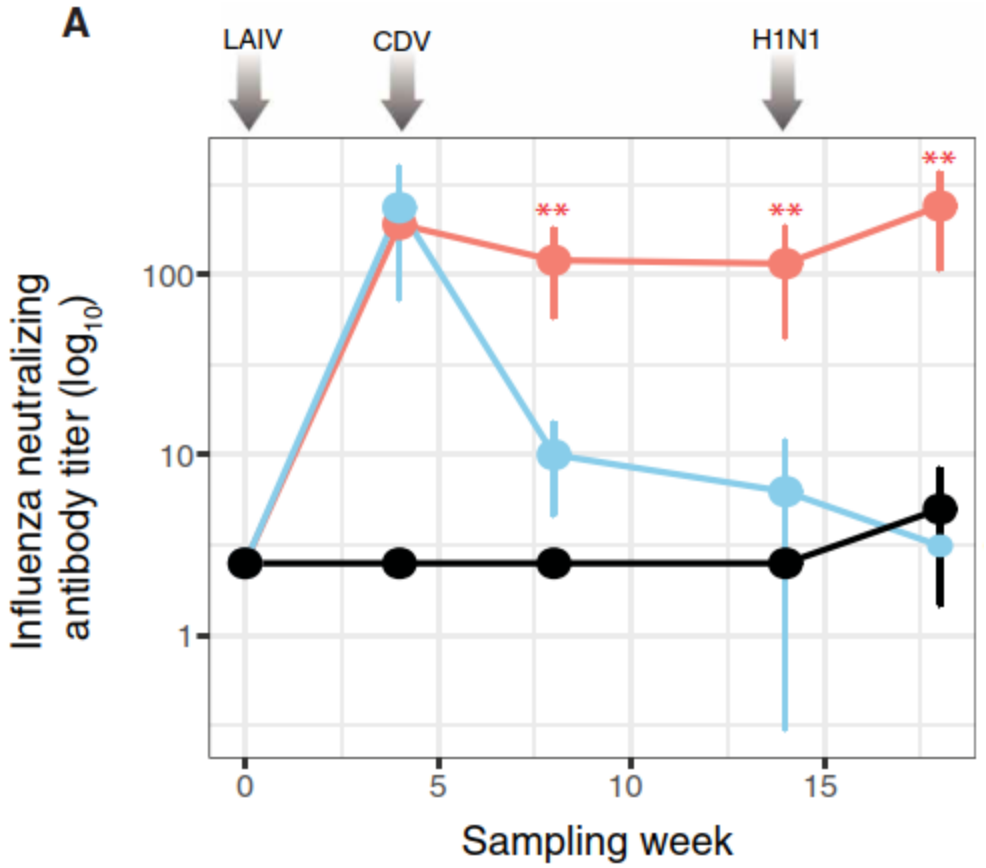
Clone frequency: No. of overlapping clone/ Total no. of clone per individual

Dot size: No. of overlapping clone of the individuals

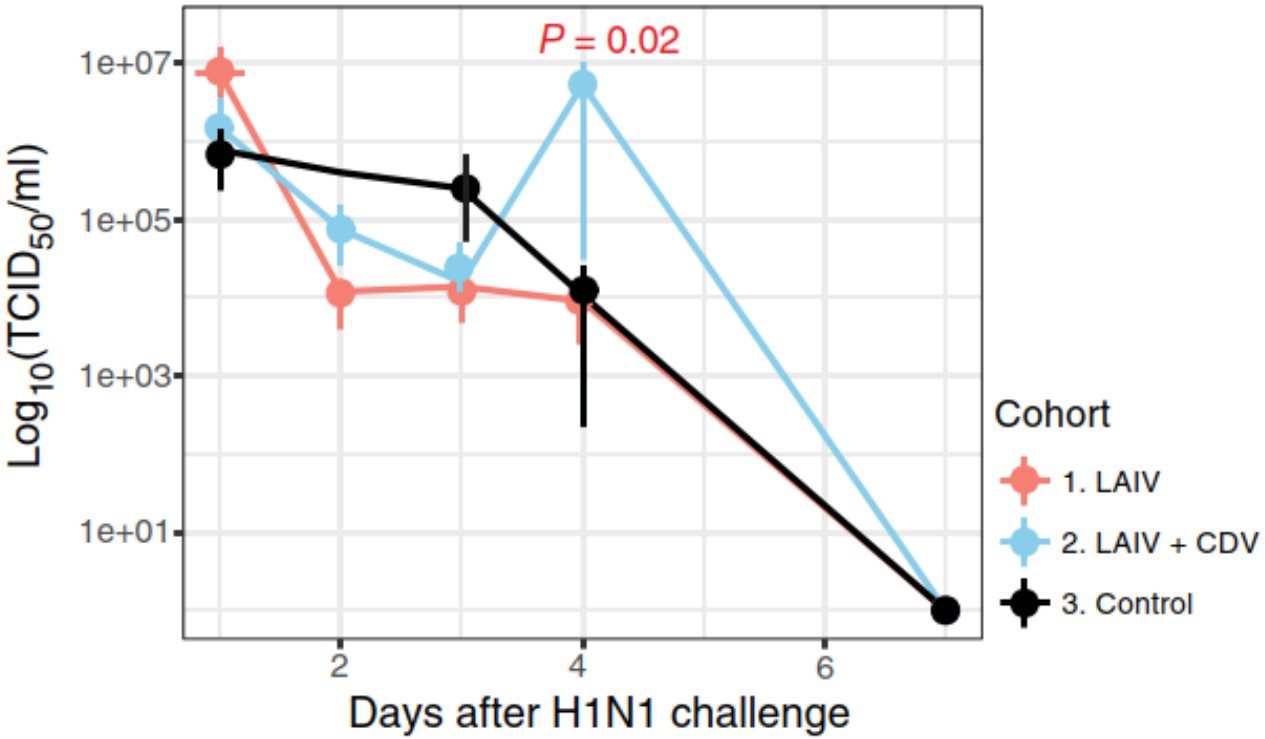
(Fig 5A, Petrova et al., 2019)

2. Ferret model of measles-induced loss of acquired immunity

Influenza-neutralizing antibody titers



Titers of influenza H1N1 virus in nasal swabs



(Fig 6, Petrova et al., 2019)

3rd Study: Conclusions

1. Changes in genetic composition suggested previously generated B memory populations depleted after measles infection in human
2. Vaccine-acquired immunity was lost after CDV infection in ferret

Take home messages

- “Immune Amnesia” hypothesis
  - Long-term immunosuppression after measles infection is caused by the loss of acquired immunological memory due to depletion of pre-existing memory lymphocytes during acute infection
  - Supported by evidences from
    - Animal experiments
    - Epidemiological data analysis
    - Genetic analysis of lymphocytes
- Importance of measles vaccination
  - Not only to protect against measles
  - To maintain both individual and herd immunity to other pathogens

Q & A

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