NIAID Extramural Research Update: Recombinant Influenza Viruses and Biosafety

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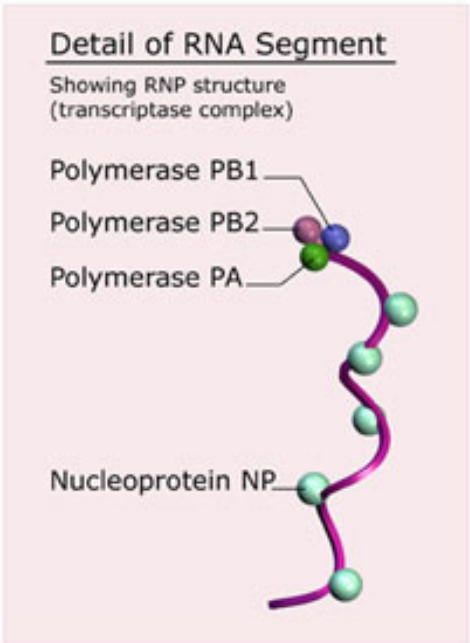
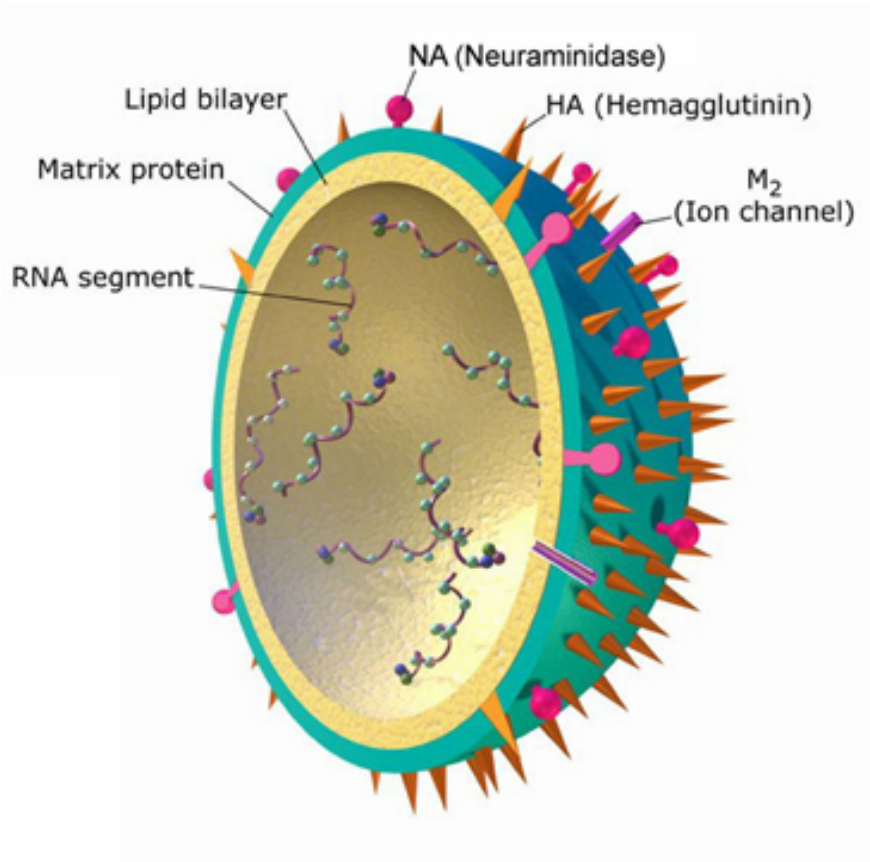
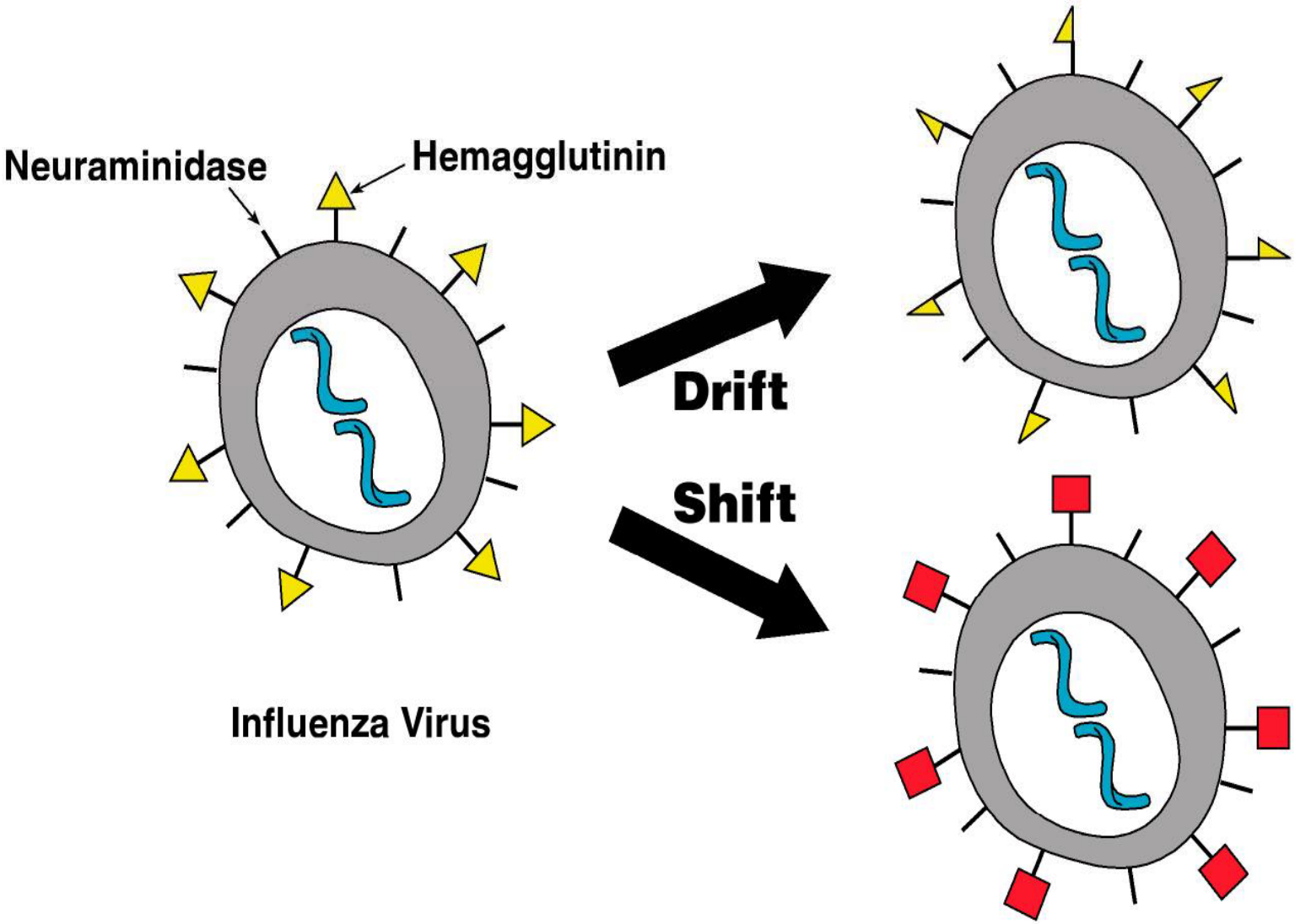
Influenza virus





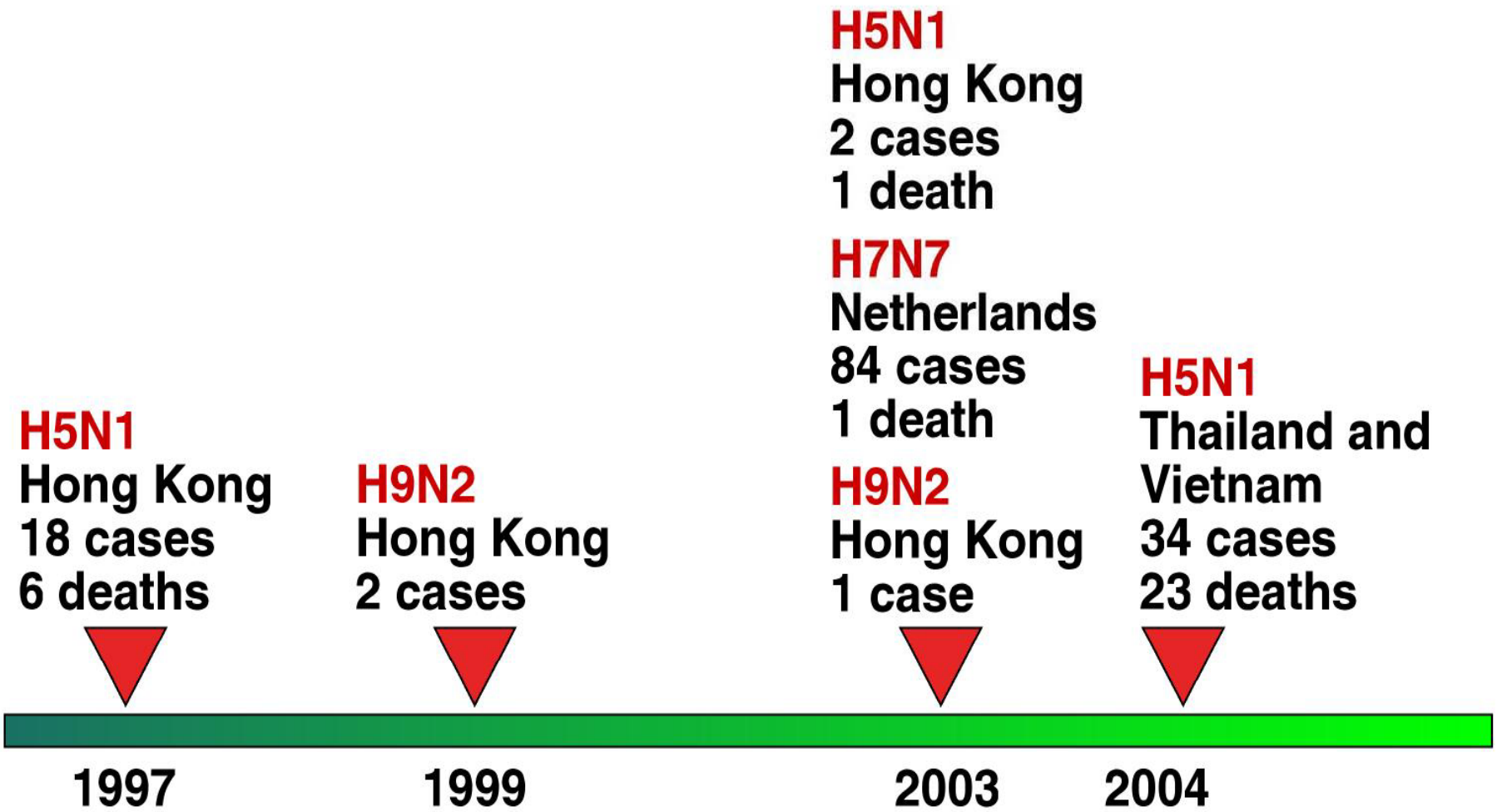
Influenza: Antigenic Drift and Shift



Past Antigenic Shifts

1918	H1N1	“Spanish Influenza”	20-40 million deaths
1957	H2N2	“Asian Flu”	1-2 million deaths
1968	H3N2	“Hong Kong Flu”	700,000 deaths
1976	H1N1	“Swine Flu”	No pandemic
1997	H5N1	“Bird Flu”	Pandemic averted?

Documented Human Infection with Avian Influenza Viruses: A Timeline

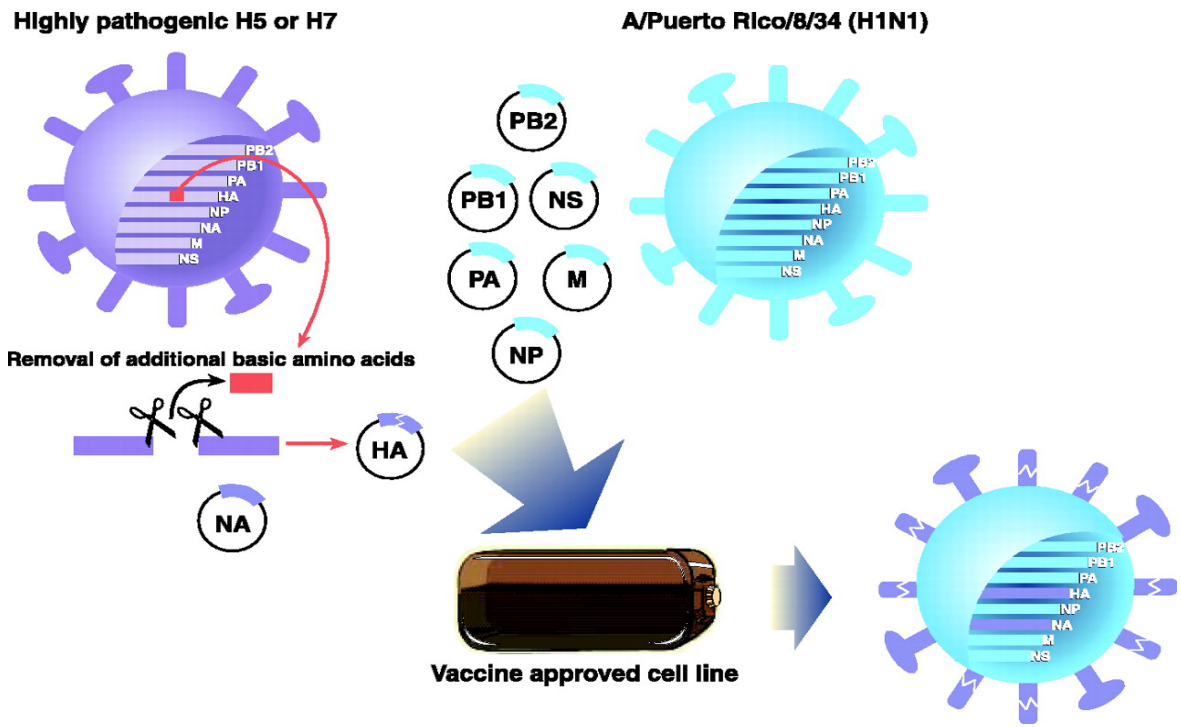


Source: WHO

DMID Influenza Program

- Basic research (40 grants)
  - Viral replication
  - Structure and assembly
  - Pathogenesis
  - Virus-host interaction
  - Viral Evolution
- Diagnostics (7 grants)
- Drug Discovery (3 grants)
- Vaccine development (10 grants)

Genetic rescue of influenza virus from cDNAs



From: Webby, R. and Webster R. Science 2003. 1519-1522

With the advent of reverse genetics…

- Powerful tool to elucidate the biology of the virus:
  - Custom-make recombinant influenza viruses containing specific genes.
  - Introduce selected point mutations/deletions to evaluate the function of viral protein domains.
- Potentially faster and easier to generate of vaccine reference strains (used to make Vietnam H5N1 reference viruses).
- Eliminates the need for working directly with the clinical isolate.
- Can engineer HA of pandemic virus strains without the basic amino acids at the cleavage site (associated with high virulence).

Molecular basis for high virulence of 1997 H5N1

- The H5N1 human isolates when tested in mice were divided into two groups: those strains that caused systemic lethal infection in the mice and those strains that were attenuated.
- Using reverse genetics the investigators systematically swapped the genes from the highly virulent viruses into the attenuated ones. The pathogenicity of these recombinant viruses was tested in mice.
- The PB2 gene was found to confer high lethality to the virus. Further testing revealed that a nucleotide change within the PB2 gene appeared to be key to the virus's virulence.
- The same mutation was observed in the H7N7 strain isolated from the lethal human case in the Netherlands and in the H5N1 virus isolated from lethal human cases in Vietnam in 2004.

Hatta, M., Gao, P., Hoffmann, P., and Kawaoka, Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science* 293(5536):1840-42 (2001).

H5N1 influenza virus gene suppresses the host immune responses

- The NS1 gene from a non-virulent viral strain was replaced with one from a 1997 H5N1 strain. Pigs infected with the recombinant virus experienced significant illness compared to the animals infected with the wild type strain.
- These results show that the H5N1 viruses that emerged in Hong Kong appear to have an NS1 gene that allows the virus to bypass the host defenses.
- These results provide an important target for ongoing surveillance efforts aimed at trying to identify the emergence of influenza strains with pandemic potential.

Seo S, Hoffmann E, and Webster RG: Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. Nature Medicine 9:950 954, 2002.

NIAID seeks clues to the 1918 influenza pandemic

Why was this strain so infectious and so lethal?
Where did the virus come from?
What are the genetic basis of its high virulence?

- In 1998 NIAID partially supported an expedition by an international team of scientist to obtain and study tissue samples from the bodies of 6 Norwegian miners who died of influenza in 1918 and were buried in the permafrost.

Sequencing of the1918 influenza virus

- Aim 1: complete sequencing of 1918 influenza genes.
  - Completed: HA, NA, NS, NP and M genes.
  - Nearly done: PB1, PB2 and PA.
  - HA sequences from 6 different isolates from around the world have identified 1 mutation in HA.
- Aim 2: characterize pre- and post-1918 viruses to understand origin and evolution of this pandemic strain
  - Data suggests that donor viruses for HA and NP genes was an “avian” influenza virus of unknown source.
  - Crystal structure of 1918 HA protein, confirms that this protein had structural features primarily found in avian viruses.

Stevens, J., Corper, A., Basler, C., Taubenberger, J., Palese, P., and Wilson, I: Structure of the uncleaved human H1 hemagglutinin from the extinct 1918 influenza virus. Science, 303(5665):1866-70, 2004.

Characterization of recombinant influenza viruses containing five genes of the 1918 strain.

- Recombinant viruses containing the two major surface viral glycoproteins, HA and NA, of the 1918 strain are highly lethal in mice.
- Mice immunized with an inactivated influenza vaccine were completely protected from lethal challenge.
- This study provides important information on the pathogenicity of a pandemic virus and on the identification of vaccine strategies against emerging pandemic influenza strains.

Tumpey, T., Garcia-Sastre, A., Taubenberger, J., Palese, P., Swayne, D., and Basler, C.: Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus. PNAS 101(9):3166-71, 2004

Upcoming studies

- Further characterization of the 1918 strain
- Generation of new recombinant influenza viruses containing genes from highly pathogenic avian and human viral strains.
- Review of new grant applications = biohazard concerns + need guidance from NIH-OBA.

Current biosafety guidelines to work with influenza virus.

- Biosafety in Microbiological and Biomedical Laboratories, 4th edition (May 1999).
  - Agent: Influenza
    - Activities Utilizing Non-contemporary virus strains:

      "Research or production activities utilizing contemporary strains may be safely performed using Biosafety Level 2 containment practices. Susceptibility to infection with older noncontemporary human strains, with recombinants, or with animal isolates warrant use of Biosafety level 2 containment procedures."

NIAID Reverse Genetics Workshop, July 2001

- Goal: Discuss several issues relating to biosafety of the natural and recombinant influenza viruses and the potential to generate 1918-like strains and recombinants.
- Outcome: Complex issue, rely on local IBCs, update BMBL.
- Future directions: “another consensus meeting on the 1918 virus be held at a future time when more data would be available.”

Summary and future directions

- BMBL currently being updated (Dr. Wilson, NIH Occupational Safety and Health Branch).
- More information available - NIAID seeking guidance from OBA and RAC committee on appropriate biocontainment level to be used when working with recombinant influenza viruses, especially those containing one or more genes from highly pathogenic avian and 1918 virus strains.
- Safety symposium being organized by OBA - Sept 2004