IN VIVO STUDIES ON VIRAL VIRULENCE

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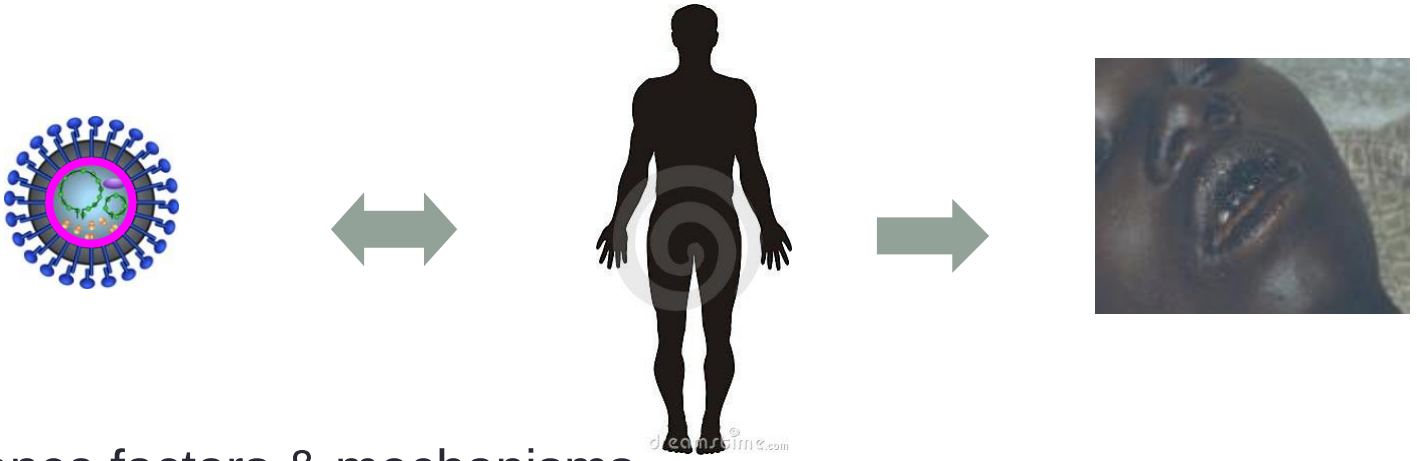
Viral Virulence

- Capacity of a virus to cause disease in a host

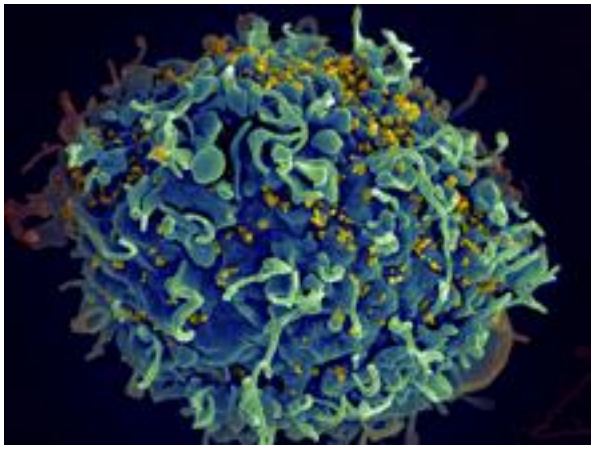
- Virulent vs avirulent or attenuated virus
  - A virulent strain causes significant disease
  - An avirulent or attenuated strain causes no or reduced disease



- Virulence factors & mechanisms
- Virus-induced immune suppression
- Pathogenesis

In vitro Studies

- *In vitro*: taking place in a test tube, culture dish or elsewhere OUTSIDE a living organism
- Cell lines, primary cells
- Assaying genes for essentiality to invasion, survival, replication, immune modulation and cytotoxicity



Multiple Virulence Determinants of Foot-and-Mouth Disease Virus in Cell Culture

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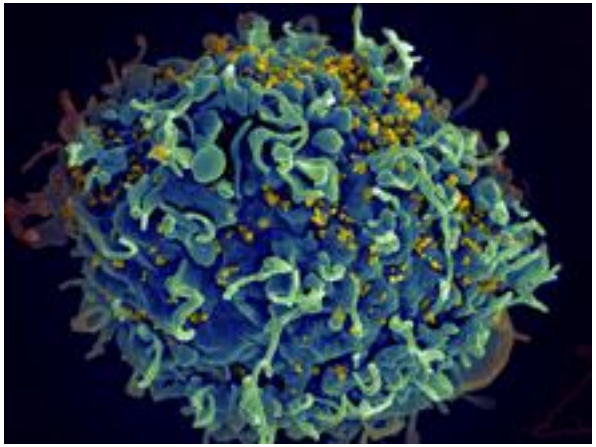
In vitro Studies

- Excellent tools for simple questions with limited variables
- Cells are easily manipulated
- Cost efficient
- Scalable

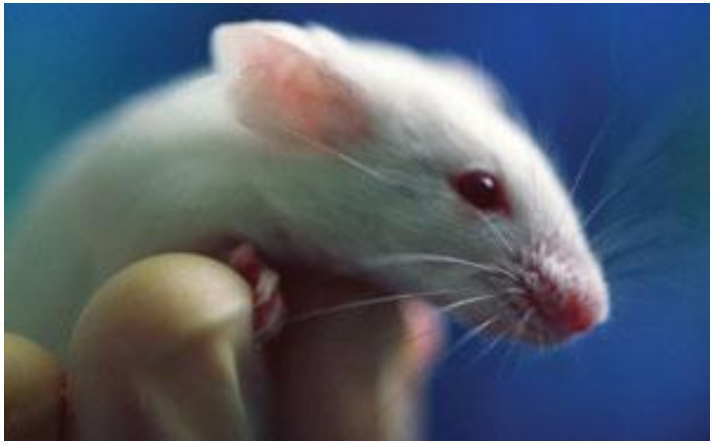
In vitro studies have given us a lot BUT…

- Focus on one specific aspect of the disease process only
- Cannot predict relevant phenotypes associated with pathogenesis

Models Systems to Study Viral Virulence: *In vitro* and *In vivo*



- *In vitro:* cell lines, primary cells



- *In vivo:* animals models

Mice with new receptors

Mice with complete viral genomes

Mice expressing individual viral genes

Study of virus infection and disease

Study of the host response to infection

Clonal T-cell receptor

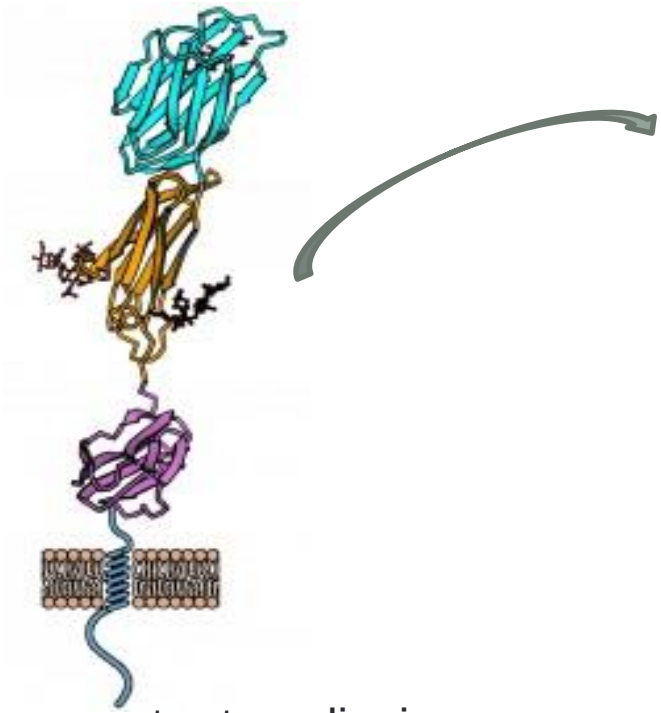
Immune mediator deletion

Immune cell deletion

Immune mediator overproduction

- Human viruses in animals
  - May need to manipulate the mouse
  - E.g. Transgenic poliovirus receptor mouse

- Animal viruses that resemble human infection



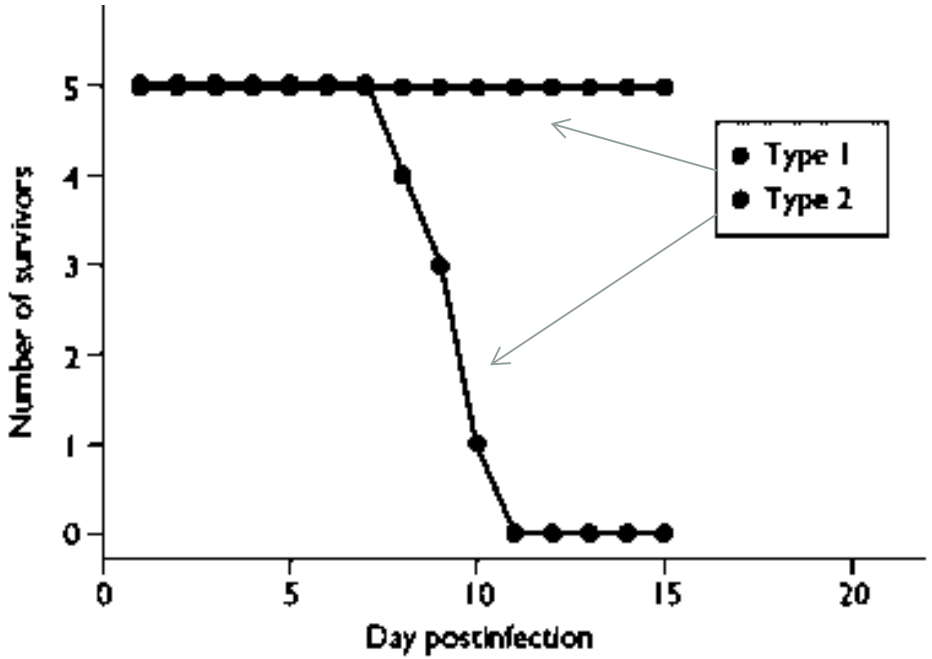
human receptor to poliovirus (hPVR)

Transgenic poliovirus receptor mouse (TgPVR)

Virulence can be Quantitated

- Meantime to death
- Meantime to appearance of symptoms
- Measurement of fever, weight loss
  - Influenza infection
- Measurement of pathological lesions
  - Poliovirus infection
- Reduction in blood CD4+ lymphocytes
  - HIV-1 infection

General Approach



What makes type 2 virus more virulence than type 1?
Are there any genetic differences that affect viral virulence?

Principles of Virology, ASM Press

Virulence Genes

- Major goal of virology is to identify virulence genes that determine virulence

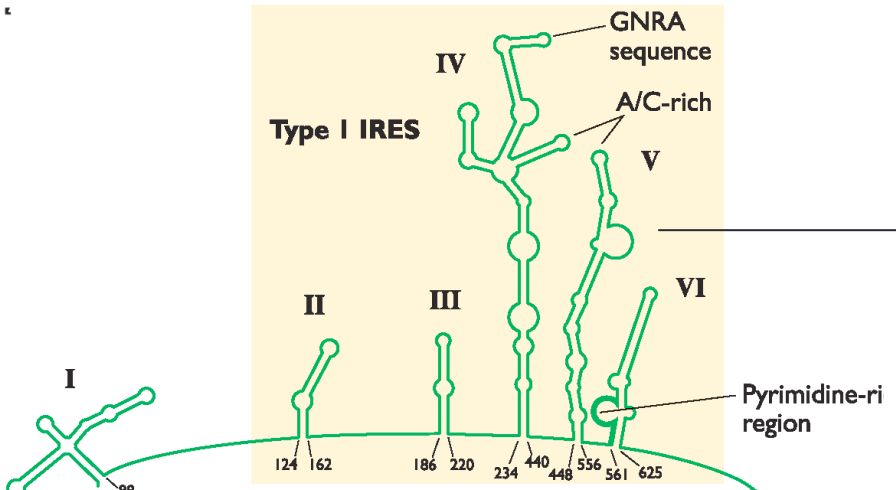
- Viral virulence genes involved in
  - Viral replication
  - Defeat host defense mechanisms
  - Invasiveness
  - Intrinsic cell killing effects

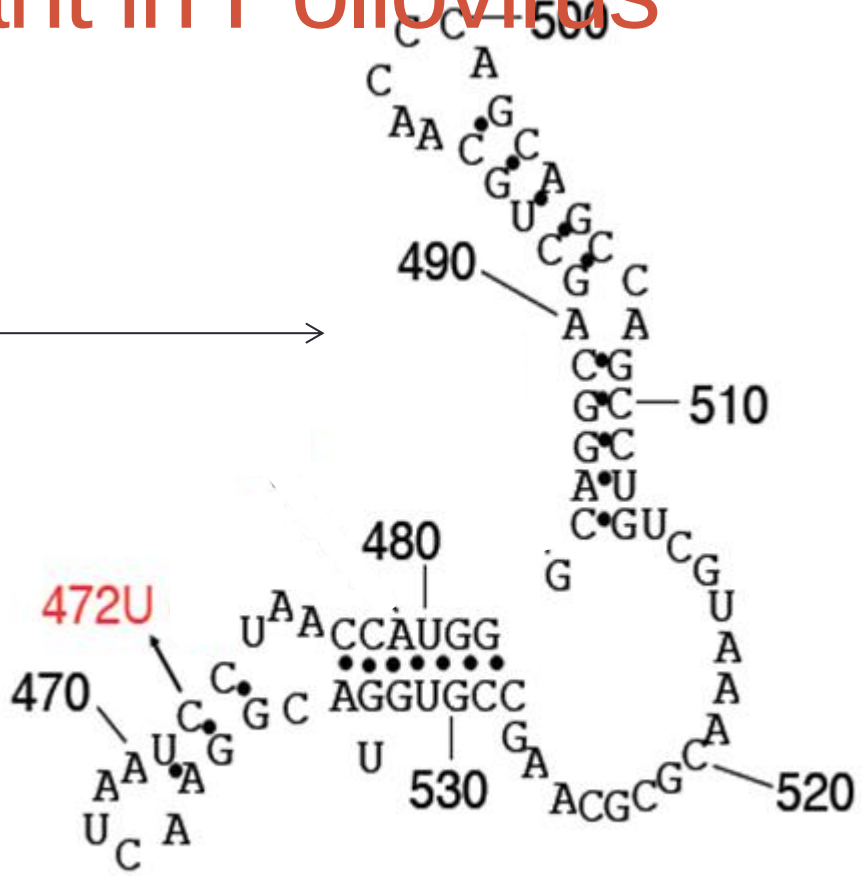
- Virulence genes usually identified by mutation: a virus that causes reduced or no disease in a specified system

Virus	Growth in cell culture	Effect on mice	Virulence phenotype
Wild type		 Replication	Neurovirulent
Mutation leading to a general defect in replication		 Poor replication	Attenuated
Mutation in a gene specifically required for virulence		 Poor replication	Attenuated

Principles of Virology, ASM Press

Virulence determinant in Poliovirus

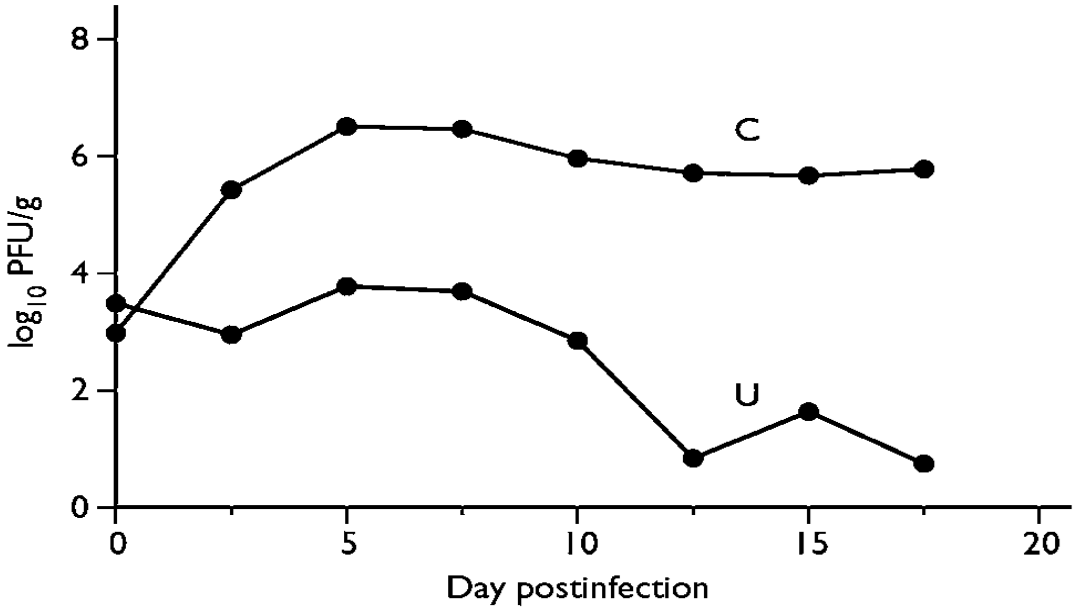




Poliovirus genome

- Poliovirus replication in mouse brain

Virus	Base at 472	LD_{50}
PRV7.3	U	$>2 \times 10^7$
PRV8.4	C	9×10^3



- One base change is enough to change the virulence of poliovirus
- Implication: develop oral polio vaccine (OPV)

Other Factors Affecting Viral Virulence in Mice

- Dose
- Route of infection
- Age of host

Virus	No. of virions needed to kill 50% of animals			
	Suckling mice		Adult mice	
	Intracerebral infection	Subcutaneous infection	Intracerebral infection	Subcutaneous infection
Wild-type La Crosse virus	~1	~1	~1	~10
Attenuated La Crosse virus mutant	~1	$>10^5$	$>10^6$	$>10^7$

- Species
- Gender (males slightly more susceptible to viral infections than females)

Age of host

Gender

Species

Route of inoculation

Dose

Animal experiments. Five- to 6-wk-old female BALB/c mice were obtained from a commercial supplier (Charles River Laboratories, Wilmington, Massachusetts, United States). All mice were housed in [illegible]ages and allowed to acclimatize for 5 days prior to use in experiments.

To assay virulence, groups of three to six mice were each inoculated i.p. at two different sites with 10 FFU of virus in 0.1 ml of DMEM. Following infection, mice were observed daily for clinical symptoms and their weights were recorded for 11 d postinoculation. All surviving animals were observed for at least 21 d (three times the average duration of survival of the control animals).

The MLD_{50} was determined by i.p. inoculation of mice (three to six per group) with serial 10-fold dilutions of virus and monitoring of the survival rates.

To assess virus growth characteristics in mice, groups of 12 animals were inoculated i.p. with 5 FFU of virus (corresponds to approximately 500 MLD_{50} for MA-ZEBOV). On days 1, 2, 3, and 5 postinfection, spleen, liver, and blood were collected from three infected mice, and the spleen and liver samples were homogenized. Viral infectivity titers were determined by use of a focus-forming assay in Vero E6 cells [36].

Ebihara, H., Takada, A., Kobasa, D., Jones, S., Neumann, G., Theriault, S., ... & Kawaoka, Y. (2006). Molecular determinants of Ebola virus virulence in mice.*PLoS pathogens*, *2*(7), e73.

Benefits and Problems of Using Animal Model

Benefits

- Show human-like clinical signs and pathology of disease
- Suitable for transmission experiments, vaccine research
- Helps to understand some certain mechanism of disease and therapeutic agent observed in animal model.

Problems

- Practical considerations
- Absence of small animal model
- Never mimics exactly what happens in people

Future Prospects

- To increase knowledge of molecular mechanism of virus replication and mechanism of pathogenesis in viral disease further.
- To investigate antibody enhancement of virus infection, induction of auto immunity and development of immune cell dysfunction and tumours
- To develop in the field of small animal models
- Efficacy assessment and pre-clinical evaluation of novel virus vaccine constructs

Molecular Determinants of Ebola Virus Virulence in Mice

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Mike Bray[9], Heinz Feldmann[3,8], Yoshihiro Kawaoka

PB1-mediated virulence attenuation of H5N1 influenza virus in mice is associated with PB2

Jing Li,† Yongqiang Li,† Yi Hu, Guohui Chang, Wei Sun, Yinhui Yang, Xiaoping Kang, Xiaoyan Wu and Qingyu Zhu

te of Microbiology and

The Virulence of 1997 H5N1 Influenza Viruses in the Mouse Model Is Increased by Correcting a Defect in Their NS1 Proteins[▽]

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Heterogeneous virulence of pandemic 2009 influenza H1N1 virus in mice

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Mutations in the Envelope Protein of Japanese Encephalitis Virus Affect Entry into Cultured Cells and Virulence in Mice

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Influenza A Virus PB1-F2 Protein Contributes to Viral Pathogenesis in Mice

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THANK YOU