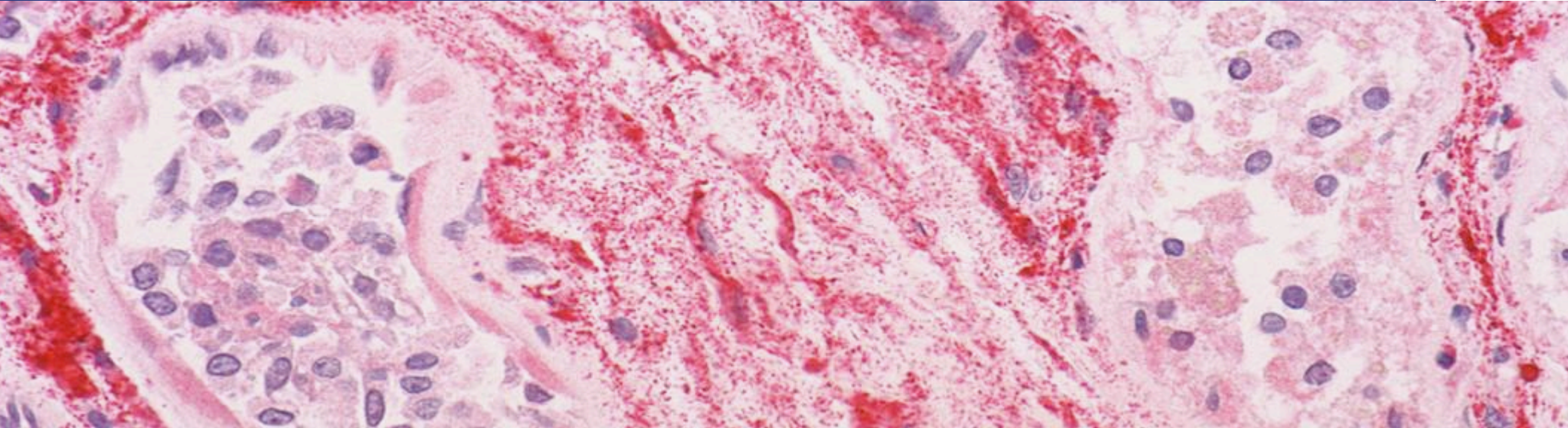


Viral Hemorrhagic Fevers



“Human destiny is bound to remain a gamble, because at some unpredictable time and in some unforeseeable manner nature will strike back.”

René J. Dubois (1901-1982)

VHF—in general

- Acute infection:
fever, myalgia, malaise; progression to prostration
- Small vessel involvement:
increased permeability, cellular damage
- Multisystem compromise (**varies with pathogen**)
- Hemorrhage may be small in volume
(indicates small vessel involvement,
thrombocytopenia)
- Poor prognosis associated with:
shock, encephalopathy, extensive hemorrhage

VHF Symptoms--General

- Combination of fever and hemorrhage that can be caused by a diverse group of human pathogens, including viruses, bacteria, protozoa, and fungi.
 - **Differential...**
- However, the term HF is reserved for systemic infections characterized by fever and hemorrhage caused by a special group of viruses transmitted to humans by arthropods and rodents.
 - Small lipid-enveloped RNA viruses.
 - Four different families.

VHF: Viruses

- Encapsulated, single stranded RNA viruses.
- Similar syndromes; different pathogenesis & treatment.
 - Ribavarin recommended for the treatment and prophylaxis of the arenaviruses and the bunyaviruses.
- Persist in nature: rodents, bats, mosquitoes.
- **Geographically restricted by host.**
- Potential infectious hazards from laboratory aerosols

Viral Hemorrhagic Fever Viruses

- **Filoviridae** Ebola, Marburg
- **Flaviridae** Yellow fever, Dengue, Kyasanur Forest
Omsk, Middle Eastern flavivirus
- **Arenaviridae** Lassa fever, New World Arenaviruses:
Junin, Machupo, Guanarito, Sabia
- **Bunyaviridae** **Rift Valley fever (RVF)**
Crimean Congo Hemorrhagic fever
(CCHF), Hantaan

VHF Viruses

- Persist in nature through **zoonotic** cycle although dengue and sometimes yellow fever may be maintained by the bite of a mosquito intermediate.
- With the exception of dengue viruses, All of these agents have a degree of **aerosol** infectivity.

Viral Hemorrhagic Fever Viruses- **Vectors**/Reservoir

- **Filoviridae** (-RNA) Unknown?
- **Flaviridae** (+RNA) **Mosquito**, Monkey, **Tick**
- **Arenaviridae** (-RNA) Rodent
- **Bunyaviridae** (-RNA) **Mosquito**, **Tick**, Rodent

VHF Clinical Features

- Febrile prodrome with myalgia and sometimes accompanied by gastrointestinal disturbance
- By the time medical attention is sought, patients have severe, acute illness, with evidence of abnormal vascular regulation and vascular damage.
- Systemically: abnormal vascular regulation manifests by mild hypotension in the early stages of the diseases and by shock in more severe and advanced infections.

VHF Clinical Features

- **Local** vascular abnormalities usually are visible as conjunctival suffusion, flushing over the face and thorax, and various exanthems (a skin eruption accompanying certain infectious diseases--rash.).
- Vascular damage may be evident as capillary leakage (periorbital edema, pulmonary edema).
- Thrombocytopenia is characteristic and usually accompanied by hemorrhage.
- Hemorrhage usually petechial and common in skin and mucus membranes
- Severe disease accompanied by profuse bleeding, central nervous disturbances and shock.

Conjunctival injection and petechiae



**SYMPTOMS VARY GREATLY
DEPENDING ON THE VHF AGENT**

Disease	Incubation	Case : Infection ratio	Case Fatality	Characteristic Features
South American HF	7-14	Most (>1/2) result in disease	15-30%	Hypotension, shock, bleeding, neurologic symptoms
Lassa fever	5-16	Mild infections probably common	~15%	Prostration, shock, less neurologic manifestations than South A. HF. Less thrombocytopenia, 20% deafness in convalescence.
RVF	2-5	~1%	~50%	Bleeding, shock, anuria , encephalitis, retinal vasculitis without overlap of HF syndrome
Crimean Congo HF	3-12	20-100%	15-30%	Most severe bleeding and ecchymoses of all the HFs.

Disease	Incubation	Case : Infection ratio	Case Fatality	Characteristic Features
Marburg or Ebola	3-16	High (particularly Zaire subtype of Ebola)	25-90%	Most severe of the HFs. Marked weight loss and prostration. Maculopapular rash common. Late sequelae: hepatitis, uveitis, orchitis
Yellow fever	3-6	80-95%	20%	Acute period , jaundice, renal failure.
Dengue HF	?	~1%	<1%	High fever for 3-5 days with development of shock lasting 1-2 days
Kyasanur Forest	3-8	Variable	0.5-9%	Typical biphasic disease with a febrile or hemorrhagic period often followed by CNS involvement.

Differential Diagnosis

The determination of which two or more diseases with similar symptoms is the one from which a patient is suffering based on an analysis of the clinical data.

- **Febrile tropical illnesses:**
 - **Malaria**
 - **Typhoid fever**
 - **Bacterial gastro-enteritis**
 - **Rickettsial diseases**
 - **Hepatitis**

Clinical Diagnosis

- **History**
- High fever, prostration, flushing, conjunctival injection.
- Postural hypotension.
- Axillary petichiae
- Thrombocytopenia (with the exception of Lassa).

Laboratory Diagnosis

- ELISA
- PCR
- Isolation of virus from tissues

Filoviruses

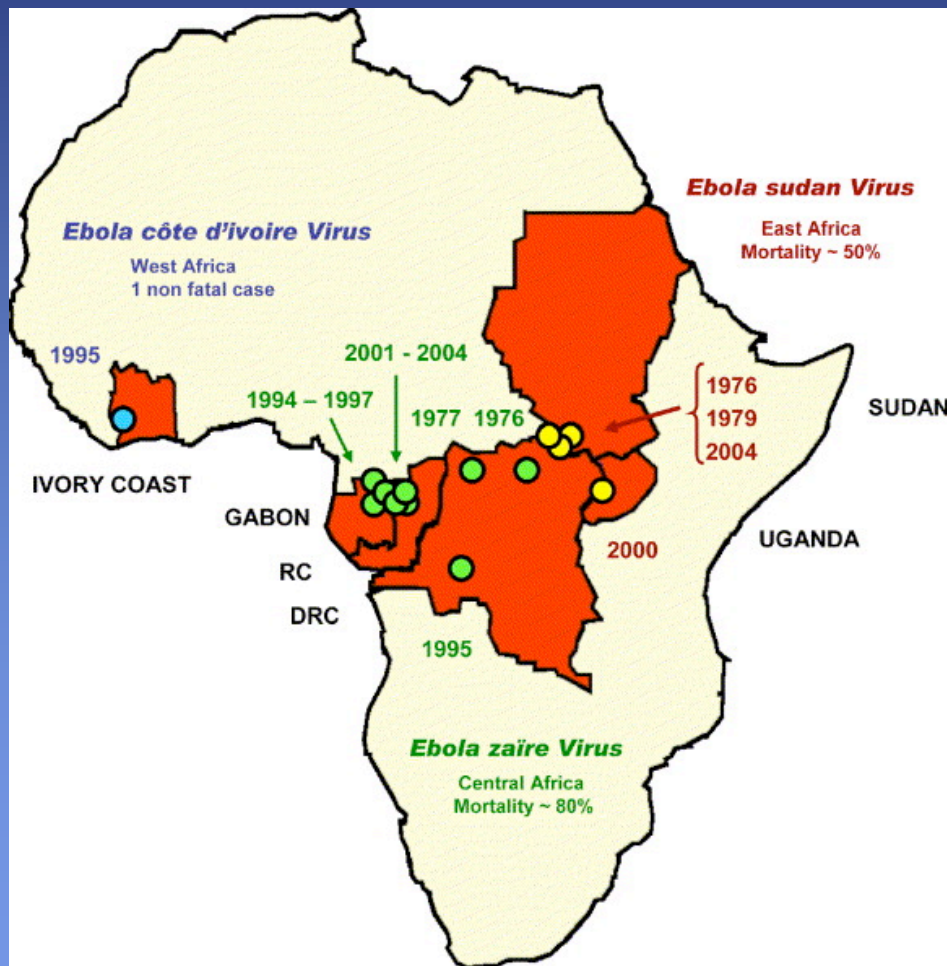
- Ebola (EBOV)
 - EBOV Zaire
 - EBOV Sudan
 - EBOV Reston
- Marburg

Ebola



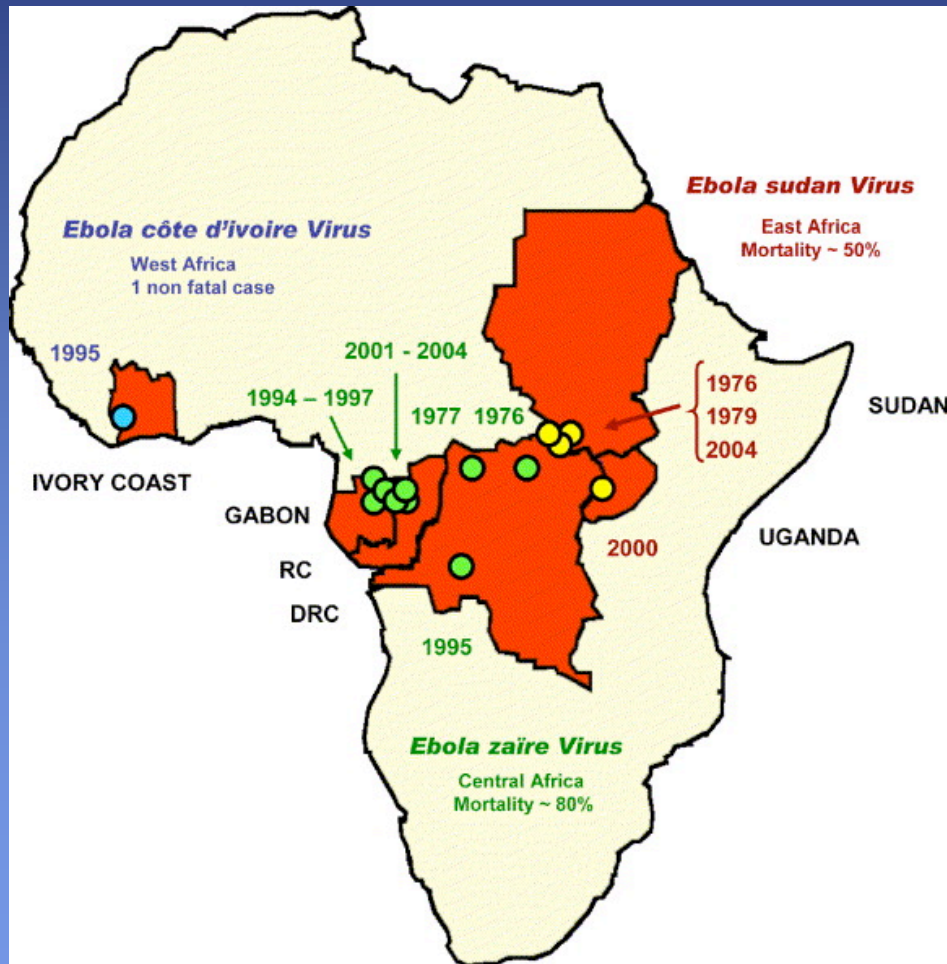
- 1-2 week incubation
- Abrupt onset fever, headache, myalgia
- GI symptoms, chest pain, delirium
- 53-88% case-fatality
- ~ 45% hemorrhage
- Person-to-person transmission
ape to person, other?
- African rainforest
- Unknown reservoir????

EBOV Outbreaks (1976)



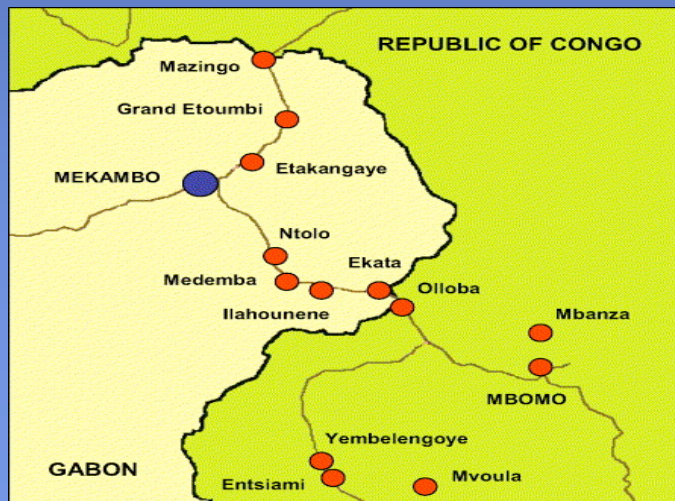
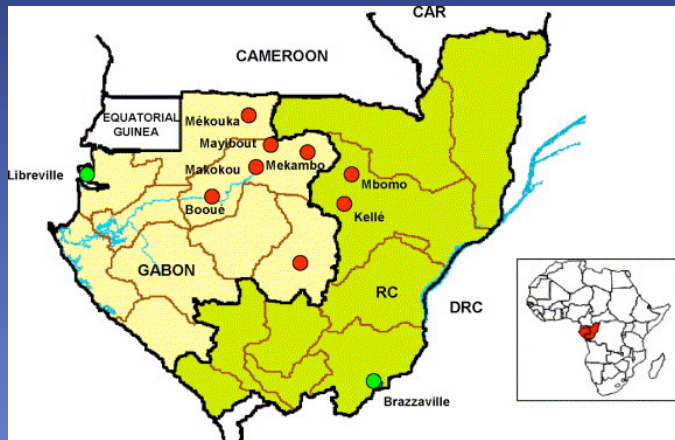
- First outbreak due to EBOV Sudan in 1976. 53% mortality (150/284).
- Second outbreak due to EBOV Zaire 1976 (named after river Ebola . 89% mortality (284/318).
- Third outbreak due to EBOV Sudan 1979. 65% mortality (22/34).

EBOV Outbreaks (1994-1997)



- After a 15-year period, Ebola re-emerged in 1994 for a 3-year period.
- The first outbreak killed 256/315 (81%) victims.
- 2 more outbreaks, then..
- In 1996, children from Mayibout II were the first victims. 18 children helped to carry and butcher a chimpanzee carcass found in the forest.

EBOV Outbreaks (2001-2004)



- Most outbreaks linked to the handling of animal carcasses.
- Since its discovery in 1976, there have been 13 outbreaks in Africa (9 due to EBOV Zaire and 4 due to EBOV Sudan).
- 2 isolated cases of EBOV Ivory Coast.

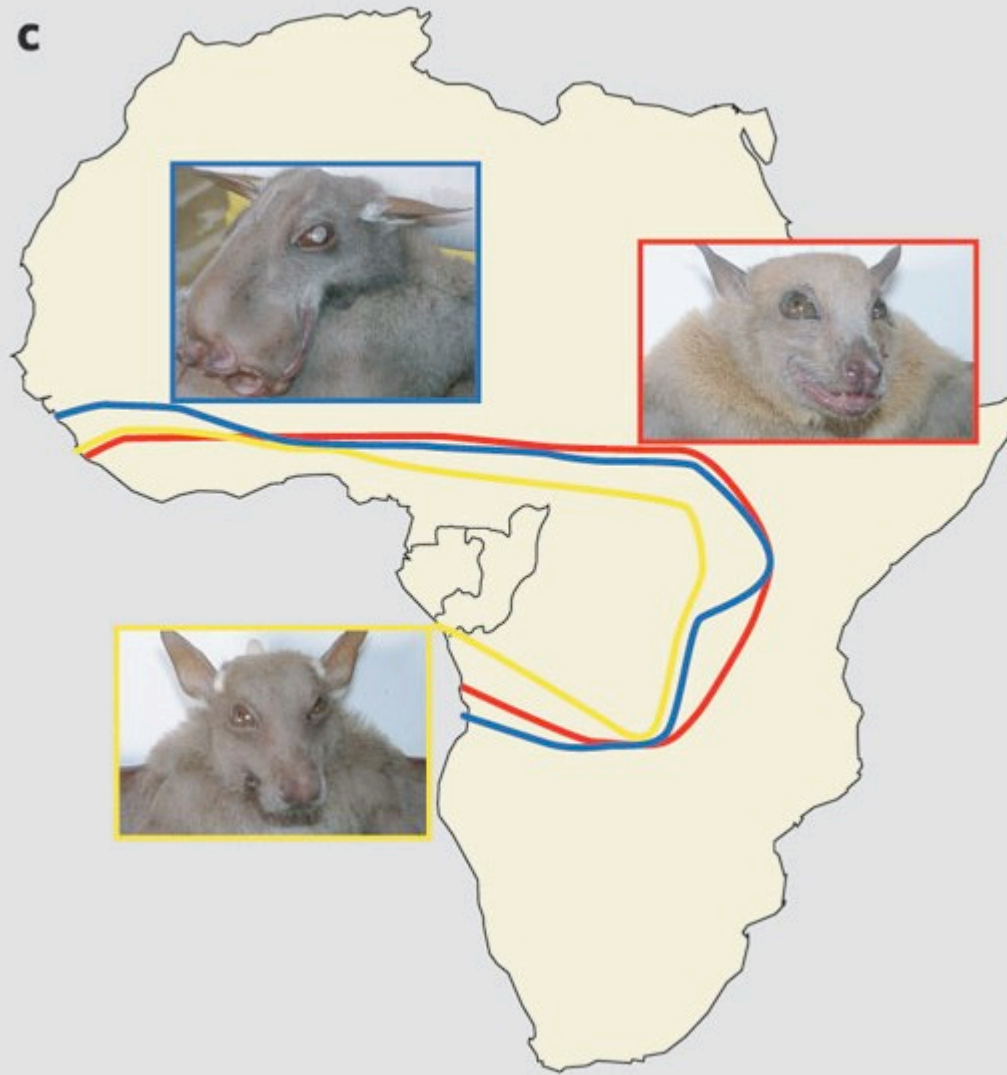
Transmission to Humans

- **Most outbreaks: no source identified.**
- **However, the 2001-2005 outbreaks in Gabon and the Republic of the Congo were linked to concurrent outbreaks that devastated local gorilla and chimpanzee populations.**

Transmission to Humans (data from 2005)

- EBOV reservoirs were identified by screening 1,013 animals captured in a trapping study near infected gorilla and chimpanzee carcasses.
- 679 bats, 222 birds 129 small terrestrial vertebrates were screened for EBOV.
- Only bats tested positive for EBOV by ELISA or PCR.

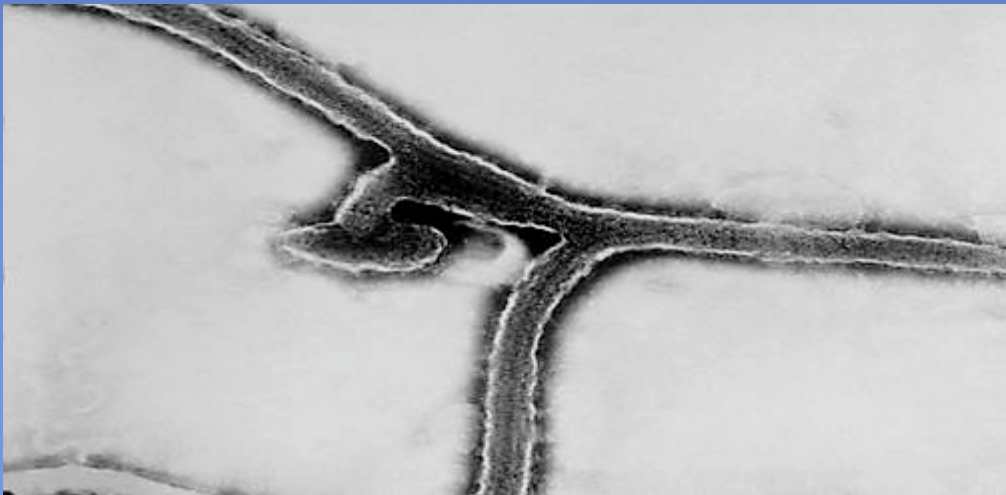
c





Marburg

- 1967
 - Marburg, Frankfurt, & Belgrade
 - 25 primary
 - 6 secondary
 - 7 deaths
 - African green monkeys from Uganda
- 1975
 - Australian traveller
 - Zimbabwe
 - 1 primary
 - 2 secondary
 - 1 death



Marburg

- 1980
 - Engineer
 - N.W. Kenya
 - 1 primary
 - 1 secondary
 - 1 death
- 1987
 - Danish traveller
 - W. Kenya
 - 1 primary
 - 1 death
- 1998-2000
 - Gold mine
 - N.E. DRC
 - 76 cases
 - 52 deaths
 - >150 cases through follow up

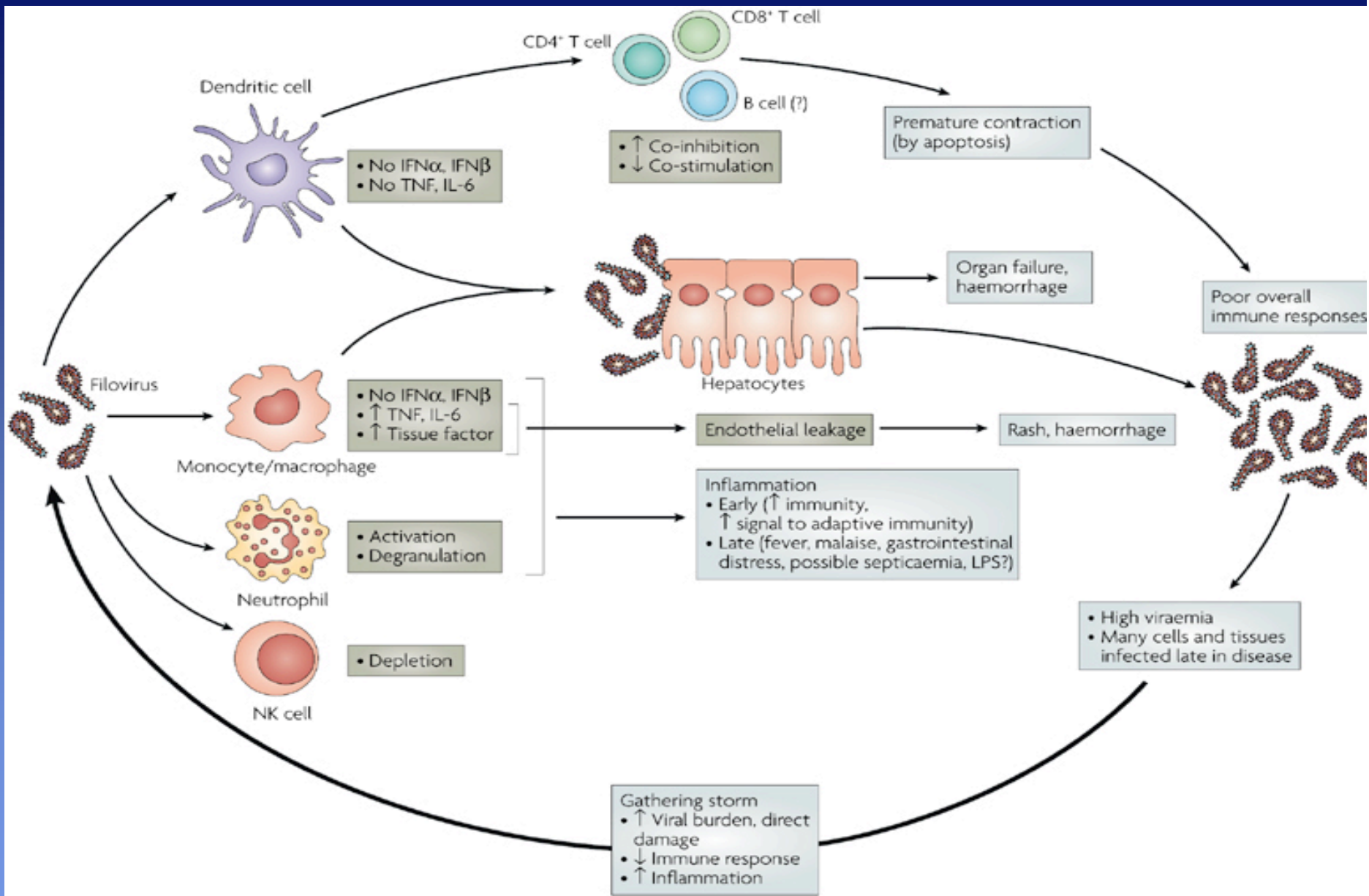
**How Ebola and
Marburg viruses
battle the immune
system.**

The Role of the Immune System

- **Central in the battle for survival against EBOV and MARV, respectively.**
 - **Loses the battle in nearly 90% of encounters in a matter of days.**
 - **Complex series of interrelated viral and immune events.**
- **EBOV and MARV relentlessly infect cells of the monocyte macrophage lineage, accelerating the release of pro-inflammatory cytokines.**

Pantropic Infection Profile

- Infecting all cell types in which only lymphocytes are spared.
- Triggering of neutrophils and other PMNs by interaction with TLRs.
- Replication of the virus, beginning in **dendritic cells, monocytes and various types of macrophages.**
 - In MARV infections, hepatocytes are particularly susceptible.
 - elevated liver enzymes--first telling signs
 - liver damage accounts for the coagulopathy.

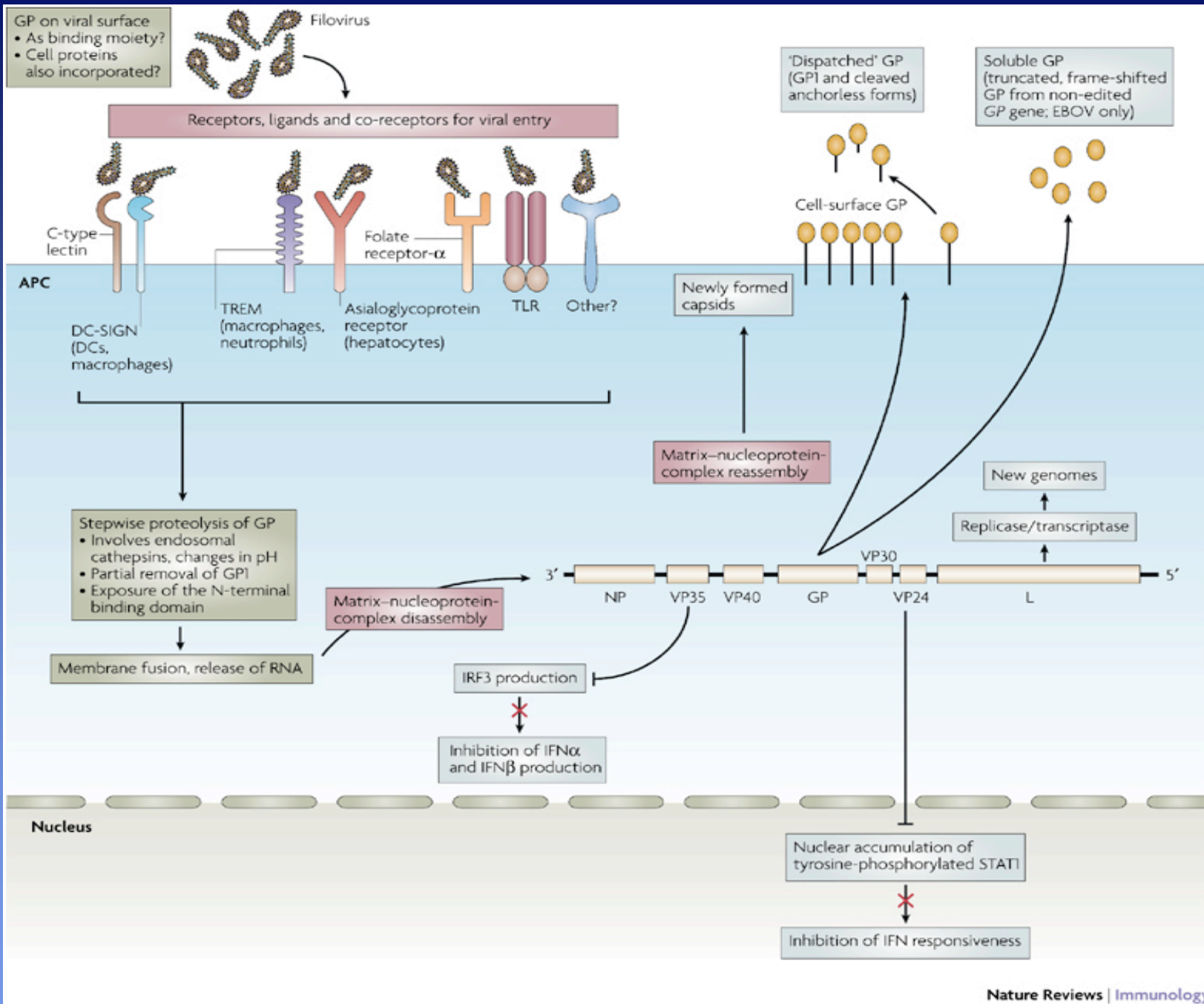


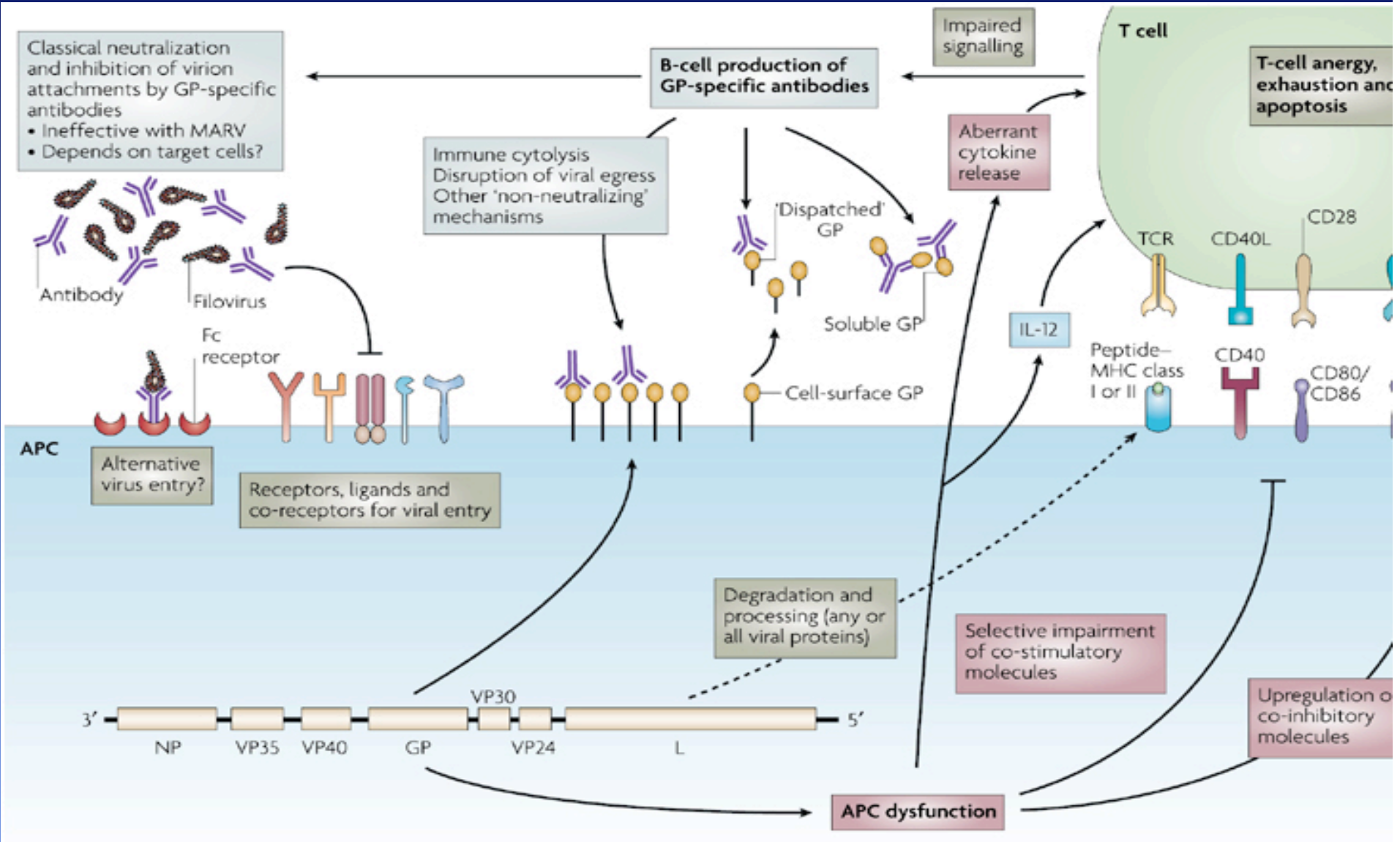
Initial Innate Immune Activation

- VP35-mediated prevention of type I INFs (INF- α and INF- β).
- VP24 interferes with the ability of INF- α , - β and - γ to induce an antiviral state in cells.
- High viral burdens correlated to INF dysregulation.
 - INF dysregulation also affects DC maturation, lack of NK and T cell activation.
 - Late in the infection process, elevated INF levels may be detected.
- **Big picture: dysregulation of cytokine networks=cytokine storm.**

Disordering of Adaptive Immunity

- **Dysfunctional antigen presentation.**
 - **filoviruses have been shown to silence active co-stimulatory molecules in DCs (such as CD40, CD86, and IL-12).**
- **Pronounced indications of apoptotic death in lymphocyte populations in peripheral blood and lymph nodes.**

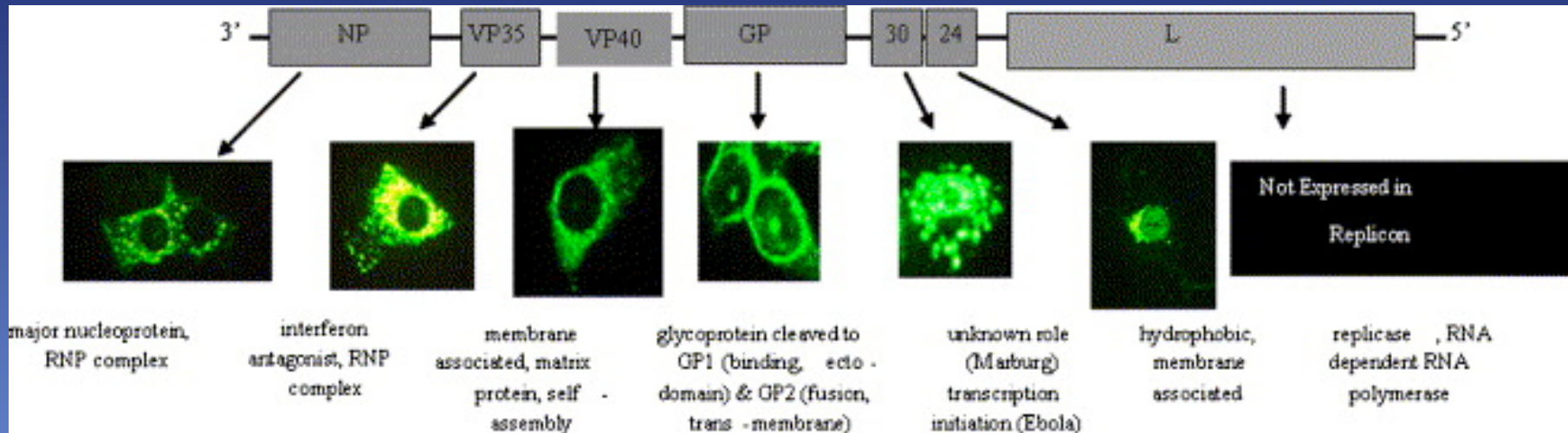




Status of Filovirus Vaccines

- VP40 and GP share sufficient homology between different filoviruses that heterologous virus-like particles can be generated which express the VP40 of one virus and the GP of another.

Status of Filovirus Vaccines



- Genomes of all filoviruses are composed of a nonsegmented, negative sense, single-strand RNA (-19 kb) encoding genes NP (major nucleoprotein), VP35, VP40 (matrix protein), **GP** (glycoprotein), VP30 (minor nucleoprotein), and VP24 and L (RNA-dependent RNA polymerase).

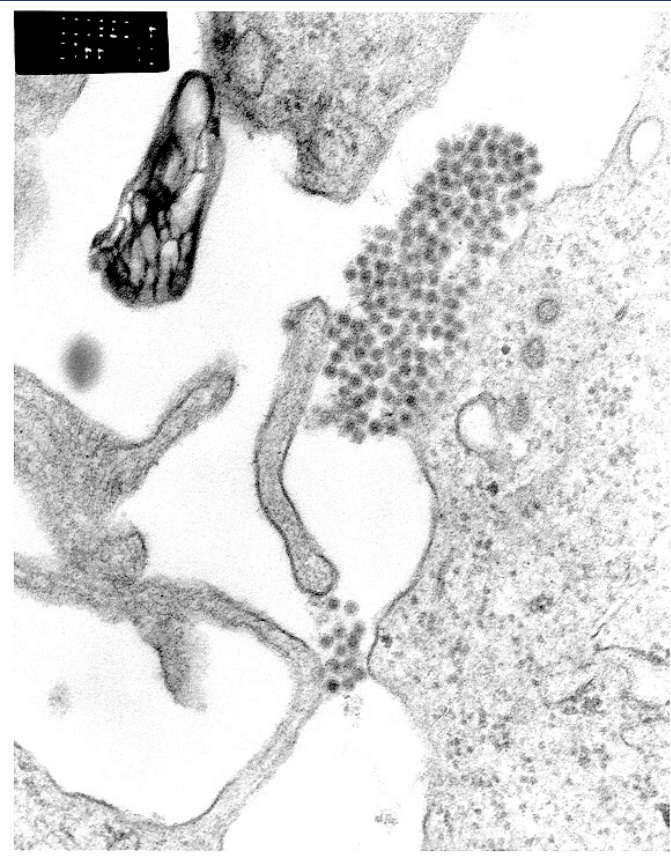
Status of Filovirus Vaccines

- **Classical approaches**
 - **attenuation**
- **New approaches**
 - **DNA vaccines**
 - **Vector-based vaccines**
 - **virus-like particles**
 - **adenovirus-based vaccines**
 - **vesicular stomatitis virus-based vaccines**
 - **IVIG**

Vaccine type*	Comments	Principal concerns
Killed filovirus	Early vaccine efforts and recent proofs of concept; inadequate efficacy [†] in non-human primates	Safety [‡] ; potency [§] ; observed disease exacerbation
Live attenuated filovirus	Only as proofs of concept with natural or passaged viruses; high risk could theoretically be mitigated by reverse-genetics approach	Live vaccine; balance of safety and potency incomplete attenuation or reversion
Live vaccinia vectored	Proof of concept, deprioritized along with other live pox-vectored vaccines	Live vaccine balance of safety and potency; vector immunity [¶]
Expressed protein, baculovirus	Incomplete efficacy in guinea pigs, no reported efficacy in non-human primates	Potency, adjuvant requirement; altered glycosylation
Defective VEE replicon	Excellent efficacy in rodents, first demonstration of efficacy against MARV in non-human primates, minimum protective dose about 10 ⁵ IU in non-human primates	Vector immunity; safety at doses high enough to achieve potency
DNA	Adequate in rodents; incomplete non-human-primate efficacy with MARV and none reported with EBOV; touted for immunological priming	Potency
Defective adenovirus	Excellent efficacy if doses 10 ¹⁰ IU or higher. First demonstration of non-human-primate efficacy with EBOV, including one-dose protection	Vector immunity, safety at doses high enough to achieve potency
Virus-like particles	Good rodent efficacy; safety and possible potency advantage compared with killed virus particles	Potency; adjuvant requirement
Live recombinant VSV	Excellent rodent and non-human-primate efficacy with both MARV and EBOV; single-shot vaccine, rapid immunity; no overt illness from live vaccine itself; in recombinant vaccine, filovirus glycoprotein replaces VSV glycoprotein	Live vaccine; balance of safety and potency; environmental release ^{**}
Live recombinant parainfluenza	Good efficacy against Ebola virus in guinea pigs and non-human primates; contains both parainfluenza virus and EBOV glycoproteins	Live vaccine; balance of safety and potency; environmental release

*Vaccines are listed in approximate chronological order of their first public descriptions of significant efficacy; the first four genetic vaccines (vaccinia through DNA) became known roughly simultaneously in 1996. [†]Here, vaccine efficacy for filoviruses is operationally defined by prevention of disease in a susceptible inoculated with an amount of virus (usually 10⁷ to 10⁸ plaque-forming units ≈ LD₅₀) associated with ordinary exposure. [‡]Safety concerns might arise in many itself (as with killed viruses), but more often refer to anticipated difficulties in achieving manufacture suitable for regulated human trials, and then an expectation of adequate safety in large trials and diverse populations. [§]Potency is interpreted as the capacity to elicit a protective immune response as defined by a quantitative assay that correlates with protection. [¶]For live and replication-defective recombinant vaccines, vector immunity refers to pre-existing immunity to the vaccine vector, which can arise naturally or by successive vaccination with the same vector, and acts to diminish the effective dose and therefore potency of vaccine delivered. ^{**}Environmental release is an additional concern for new replicating vaccines, requiring assessment of possible consequences of vaccine transmission to humans or other animals, including arthropods. EBOV, Ebola virus; IU, infectious units for replication-defective vectors that infect cells without further spread; MARV, Marburg virus; VEE, Venezuelan equine encephalitis virus; VSV, vesicular stomatitis virus.

Dengue

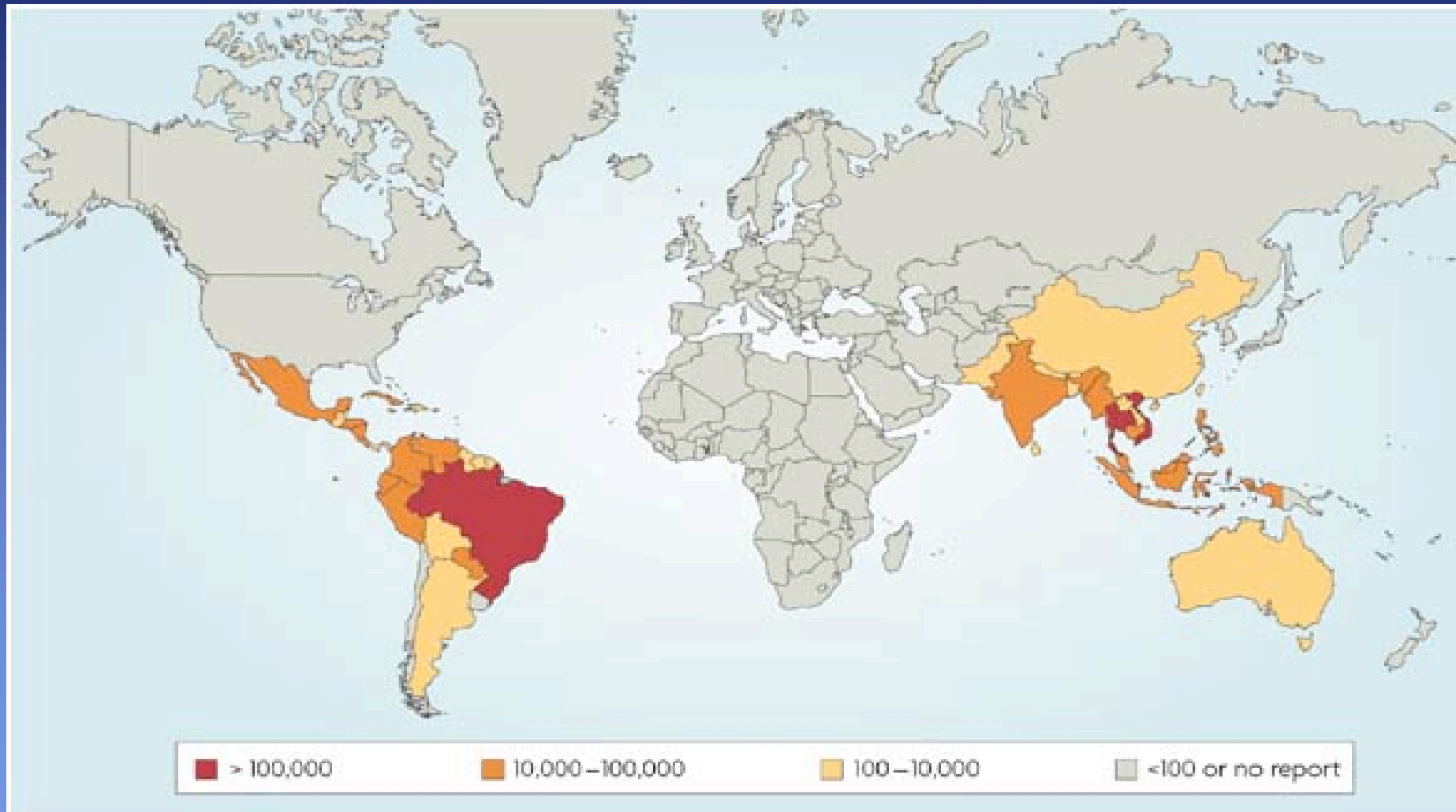


The incidence and geographical Distribution of dengue has gradually Increased over the last 3 decades.

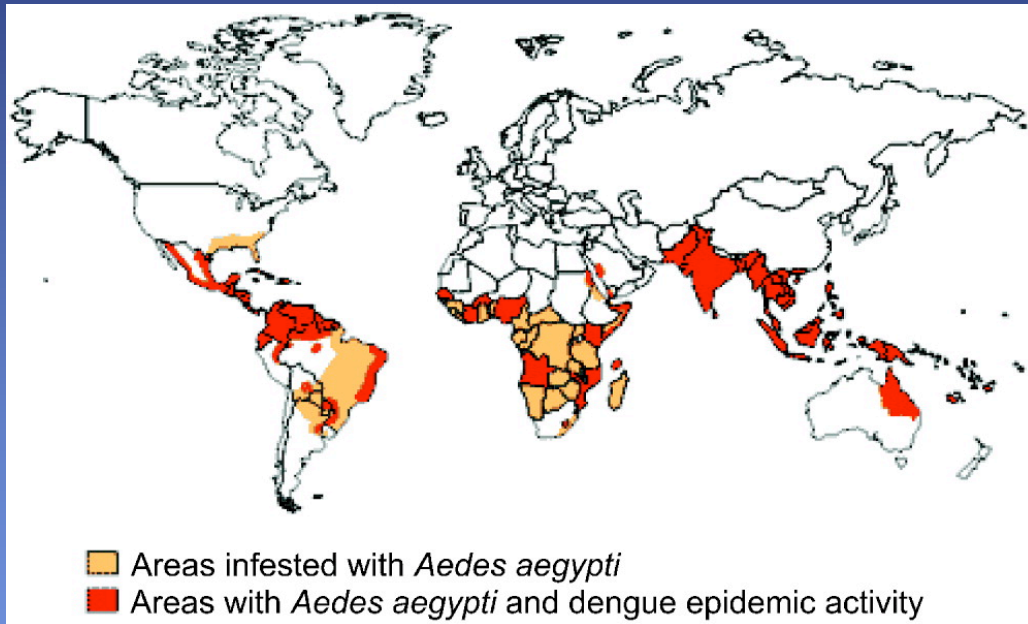
Dengue Virus (DENV)

- Flavivirus (YFV, WNV, JEV, TBEV)
- Four serotypes: DENV-1, DENV-2, DENV-3, DENV-4.
- Each is capable of causing a full spectrum of disease.
- No cross-protection, instead, exposure to one serotype intensifies disease symptoms following infection with a second serotype.

Dengue--Distribution



Dengue--Distribution

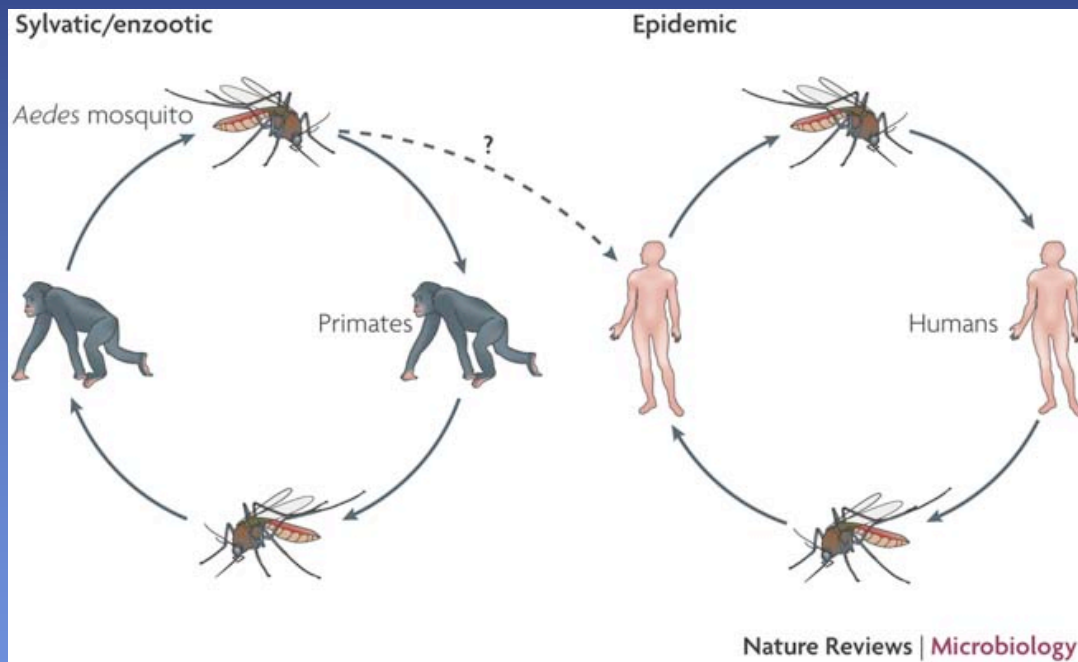


-Change in ecology caused by WWII expanded the geographical distribution of the *Aedes* mosquito

-Endemic in more than 100 tropical countries

-50-100 million cases/year

Dengue—Life Cycle



Dengue virus unique among arboviruses in that it does not require an enzootic cycle for the maintenance of epidemic transmission in humans.

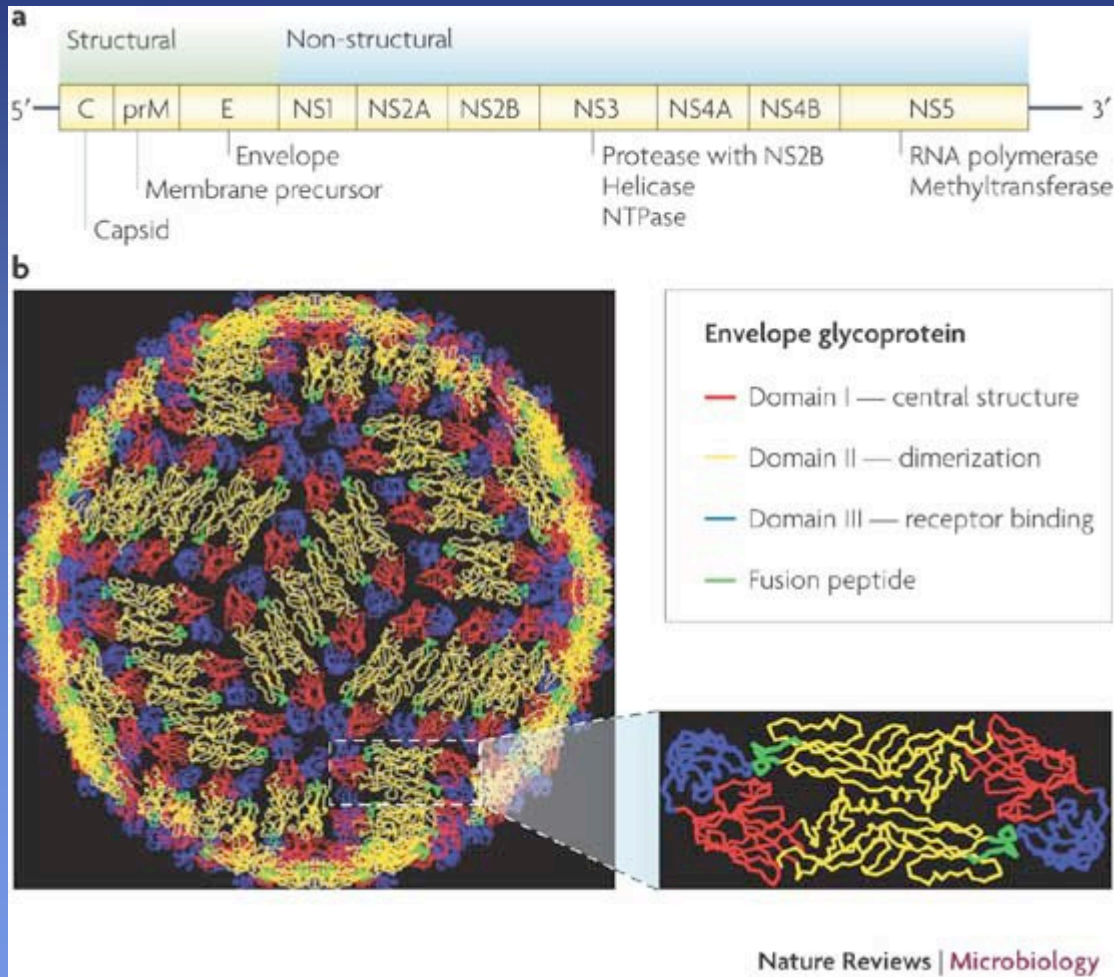
Sylvatic-A form of disease that occurs in wild animals.

Dissemination in the Human Host



Following skin inoculation, the virus replicates in local dendritic cells, with subsequent systemic infection of macrophages and lymphocytes, followed by viraemia entry into the blood stream
-Up to 10^5 - 10^6 infectious units/ml.

The Dengue Virus Genome and Virus Particle



-E glycoprotein mediates viral attachment to cells.

-Also the target of protective antibodies

-3 Structural domains

Clinical Disease

- Inapparent
- DF (Dengue Fever)
- DHF (Dengue Haemorrhagic Fever)
- DSS (Dengue Shock Syndrome)
 - **The degree of vascular leak and haemorrhagic manifestation generally differentiate the clinical syndromes.**

Clinical Disease-DF

- Classic DF.
- Incubation of 3-14 days.
- Sudden fever onset accompanied by headache, pain behind the eyes, general myalgias, skin **rashes**.
- Milder in younger children.
- Leukopenia, thrombocytopenia and elevation of serum transaminases common laboratory abnormalities.

Clinical Disease-DHF, DSS

- Characterized by fever, thrombocytopenia, haemorrhagic manifestations and evidence of increased vascular permeability with leakage of intravascular fluid into interstitial space
- Viraemia is generally 10-100-fold greater in DHF and DSS than in DF.
- **More likely in areas where all 4 serotypes are found.**

Clinical Disease-DHF, DSS

- Primarily a disease found in children under the age of 15 in hyper-endemic areas.
- Haemorrhagic manifestations include: capillary fragility; petechiae, ecchymoses or purpura; bleeding from the mucosa, GI tract or other sites.
- Around the time of defervescence, the patient's condition deteriorates, with advent of haemorrhagic manifestations with or without symptoms of hypovolemia.

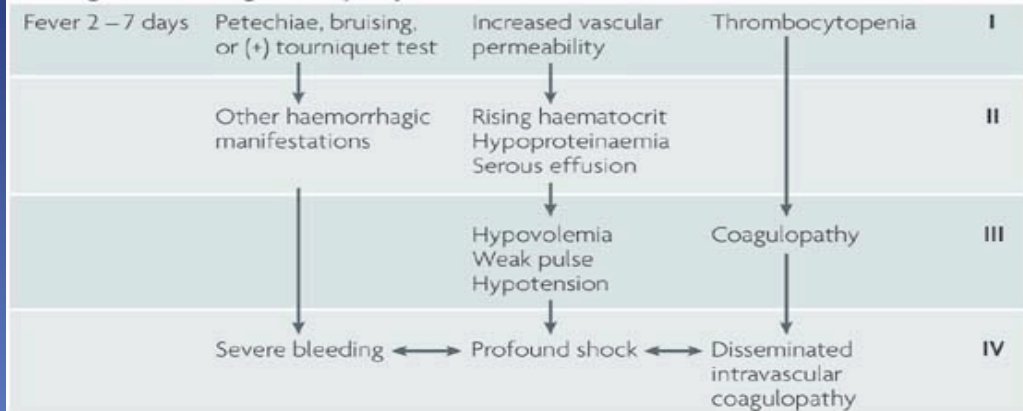
The Range of Dengue Disease

a Dengue fever (DF)

Febrile illness with 2 or more of the following: headache, retro-orbital pain, myalgia, arthralgia, rash, leukopenia, haemorrhagic manifestations; and virus recovery, serological response or temporal occurrence with other cases

b Dengue haemorrhagic fever (DHF)

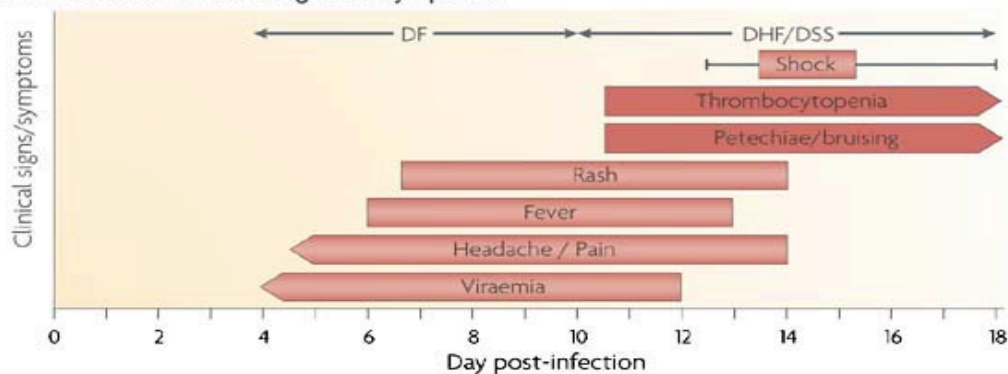
Grade



c Dengue shock syndrome (DSS)

Rapid/weak pulse and narrow pulse pressure; or manifestations of hypotension, cold, clammy skin and restlessness

d Time course of clinical signs and symptoms



Immunity to DENV Infection

- Adaptive immunity contributes to the resolution.
- Major role in protection against re-infection.
- **CONVERSELY:** it also is believed to have a crucial role in the enhancement of disease severity seen in DHF or DSS patients.
- Therefore, immunization against DENV must address both the issues of protective immunity and the proposed pathogenic role antibodies.

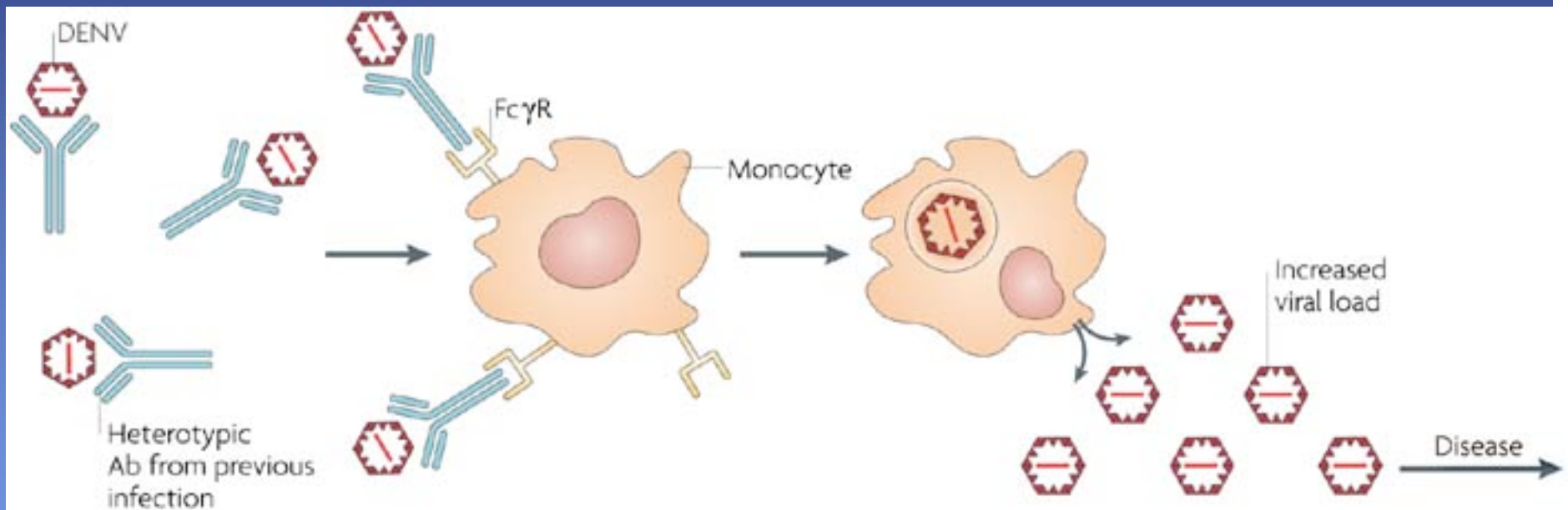
Immunity to DENV Infection

- Robust neutralizing antibody responses develop after DENV infection.
- Provide life-long immunity against re-infection with the same serotype and short-lived protection against a heterologous DENV serotype.
- This short period of crossprotection has been associated with the presence of crossreactive neutralizing antibodies that wane rapidly after infection.

Immunity to DENV Infection

- Antibodies against the virus E protein are protective.
- Both neutralizing (anti E protein antibodies) and non-neutralizing antibodies are generated.
- Non-neutralizing antibodies mediate the pathogenesis of DHF and DSS by a process called **Antibody-Dependent Enhancement (ADE)**.

ADE



Laboratory Diagnosis

- ELISA (IgM)
- RT-PCR
 - Advantage:
 - Detection during acute phase
 - Distinguish between serotypes
- Isolate virus

Vaccine type	Vaccine developer(s)	Clinical testing status
Live attenuated	WRAIR/GSK Biologicals	Tetravalent, Phase II
Live attenuated	Mahidol University/sanofi pasteur	No current testing
Live attenuated, chimeric	NIAID, NIH	Monovalent (DENV-1 – 4), Phase I/II
Live attenuated, chimeric	Acambis/sanofi pasteur	Tetravalent, Phase I
Live attenuated, chimeric	CDC/InViragen	Preclinical
Inactivated virus	WRAIR	Preclinical
Subunit	Hawaii Biotech	Begins 2007
DNA	Navy Medical Research Center	Monovalent (DENV-1), Phase I

GSK, GlaxoSmithKline; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health; WRAIR, Walter Reed Army Institute of Research.