Immunological surrogates for Japanese encephalitis vaccine-induced protection

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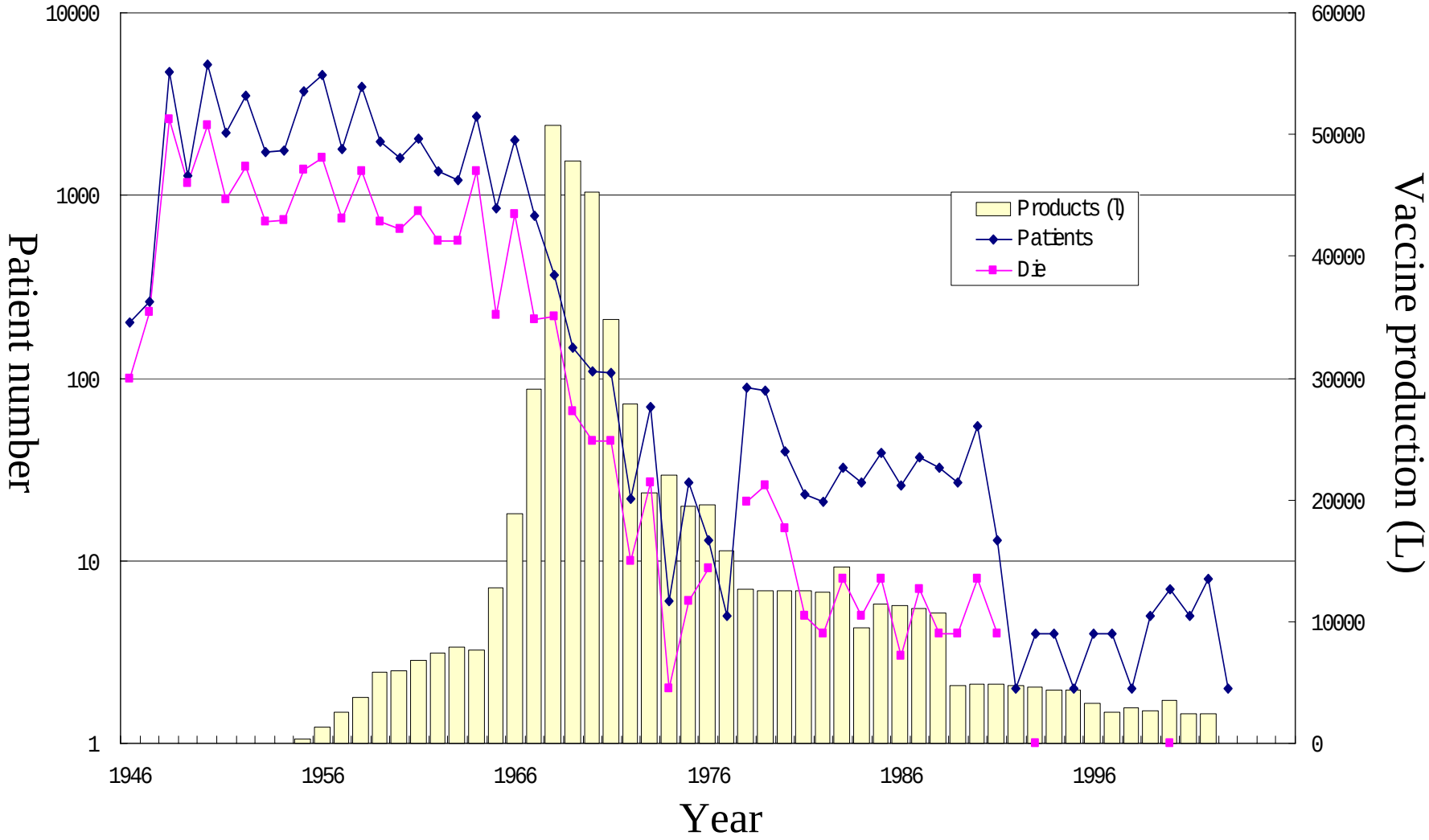
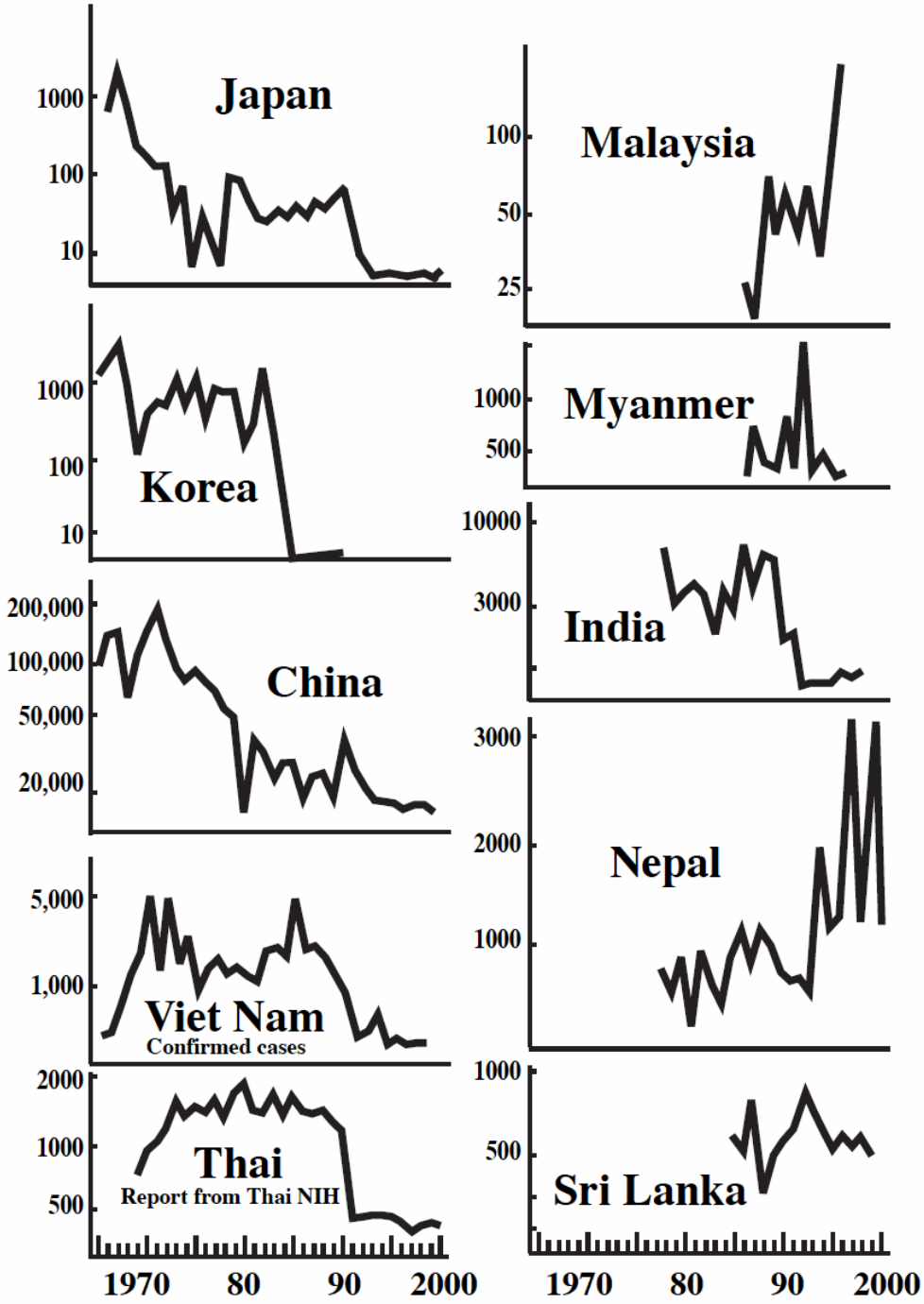
Japanese encephalitis virus (JEV.

Number of reported JE cases in Japan





Oya and Kurane

WHO Consultation on immunological endpoints for evaluation of Japanese encephalitis vaccines

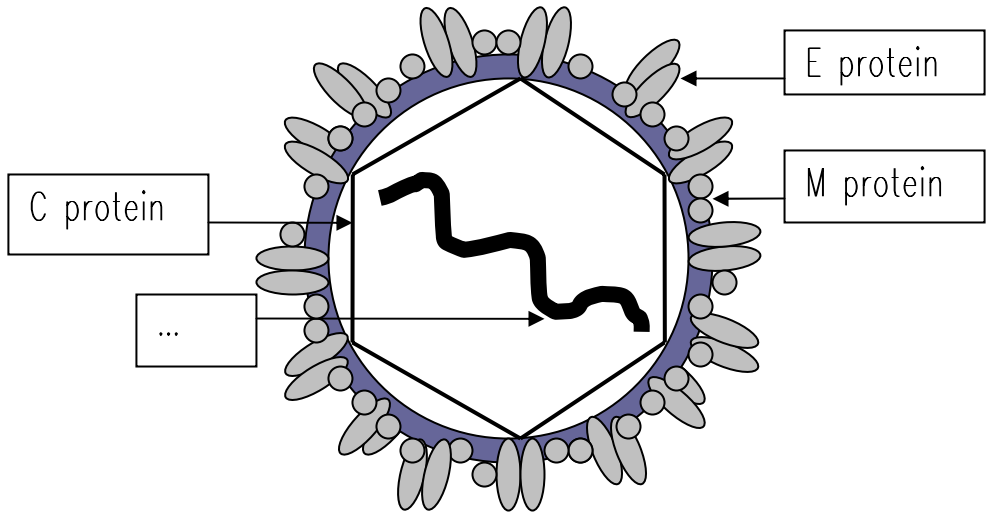
WHO, Geneva,
2-3 September 2004

Necessity of immunological surrogates

Several promising alternative vaccines The clinical evaluation of new JE vaccines for efficacy is complicated by the low incidence of JE. A placebo controlled trial would be difficult to justify on ethical grounds, and a comparative trial between licensed and new vaccine using the endpoint of prevention of clinical illness would require impractically large sample sizes.

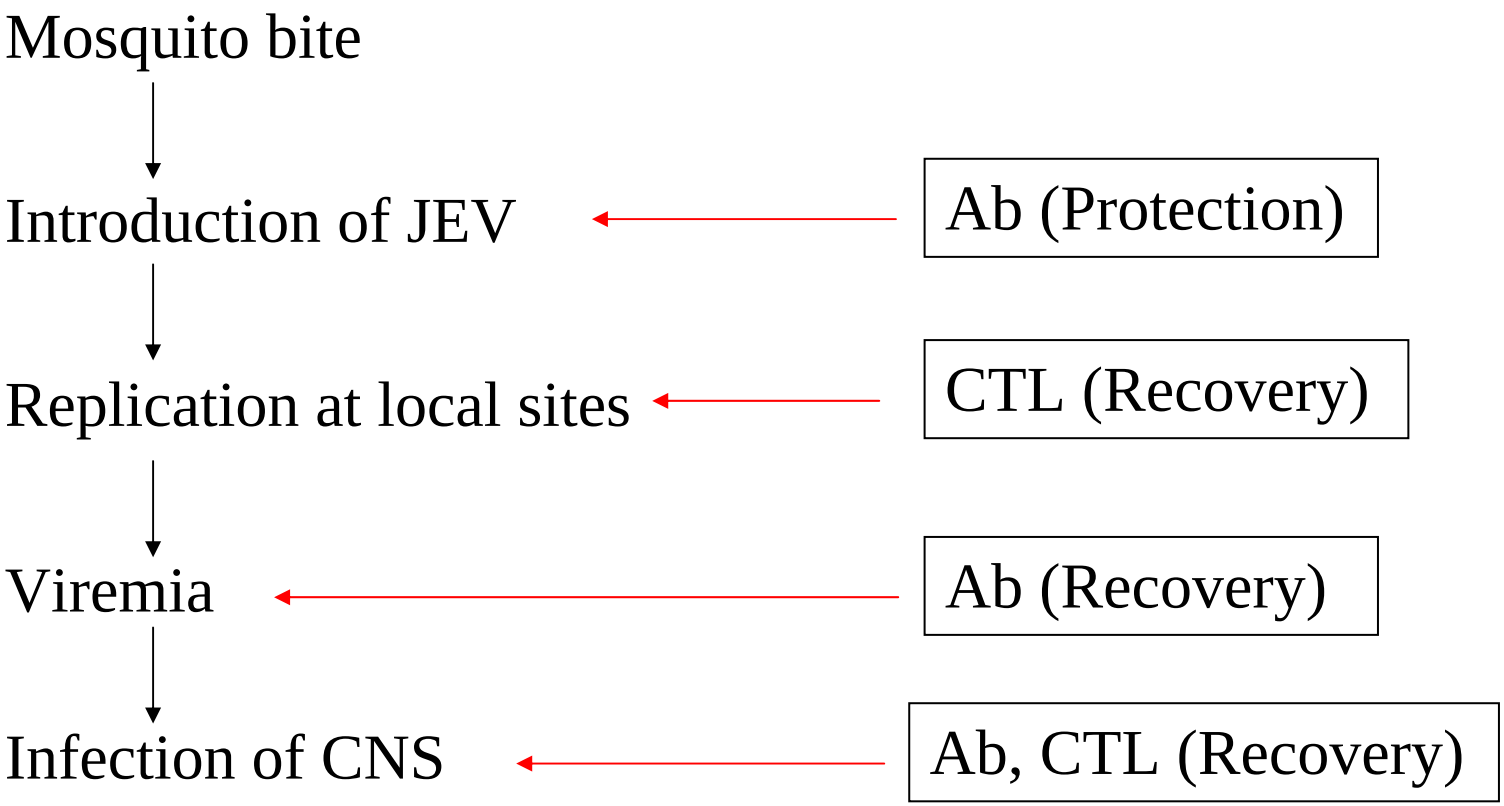
Necessity of immunological surrogates (continued)

There is a need to determine a vaccine-induced immunological readout that may be used as a surrogate for protection from disease. The availability of a universally accepted surrogate would promote clinical development and evaluation of new vaccines.



Schematic presentation of Japanese encephalitis virion

JEV infection



Role of neutralizing antibody in protection against and recovery from JEV infection

Passive protection study-1

Oya et al. (Naika 17: 905-909, 1966, Acta Paediatric Jpn 30:175-184, 1988)

1) Groups of 2-week old mice (10 mice) were s.c. injected with 0.2 ml of serially diluted hyperimmune mouse serum against Nakayama-NIH strain of JEV.

2) One day later, blood samples were collected from eyelids for antibody tests.

3) Mice were i.p. challenged with JaTH 160 strain of JEV.

4) Mice were observed for 2 weeks and LD_{50} titers were calculated in each group.

5) Protection doses were determined by the differences in LD_{50} between abtibody-treated and control groups.

Results

Serum dilution	HI	NT	LD_{50} difference
1:1	80	40	8.5
1:3	20	10	6.6
1:10	10	<10	5.3
1:30	<10	<10	5
1:100	<10	<10	3
1:300	<10	<10	2
1:1000	<10	<10	3

Passive protection study-2

Lubiniecki AS et al. Am J Trop Med Hyg 22: 535-542, 1973

1) Female mice were immmunized by 4 time s.c. injections of JEV vaccine. Mice were mated 1 week after the 4^{th} immunization. At various ages, progeny of immunized and control (non-immune) mothers were challenged ip with JEV.

2) Aliquots (0.2ml) of either 1:2 or 1:20 dilutions of the immune maternal serum were administered i.p. to normal 3-4 week old mice. On one or 15 day following the serum injection, these mice were challenged I.p. with various dilutions of JEV.

Mortality following JEV challenge in 5-day old progeny

Virus(Log_{10})	No. of dead mice (n=10)			
	Immunized		Control	
	M	F	M	F
5	2	0	9	6
6	1	0	9	8
7	0	1	10	7
8	1	3	7	8
9	8	5	10	9
10	10	9	10	10

Results of these studies

1) Passively transferred antibody proptects mice from lethal JEV infection.
2) There is a linear correlation between levels of passively acquired NT antibody titers and protection levels.

Passive protection study-3

Beasley DW, et al. Vaccine 22:3722-3726, 2004

Methods: MHAFs raised against Chimeri-Vax-JE, and JE-Vax were examined for the ability to protect mice from lethal challenge with wild type JEV strains representing 4 major genotypes.

Results: Protective capacity of antibody was demonstrated, including resistance to challenge with heterologous JE virus genotypes. Protection level depended on titre of antibody, virulence of the challenge strain, and compatibility of genotype of immunogen and challenge.

Role of immunological memory

Konishi et al. J Virol 73: 5527-5534, 1999

1) Anamnestic neutralizing antibody response is critical for protection of mice from lethal JEV infection.Ten mice were immunized with pcDNA3 JEME. Six and four showed detectable and undetectable levels of neut Ab.

2) Eight of these animals showed a rapid rise in neut antibody following challenge with 10,000 LD_{50} of JE virus. These surviving animals developed anti-NS1 antibody, suggesting that JEV were propagated after challenge.

3) Induction of CTL response alone were not sufficient to prevent death.

Anamnestic antibody responses

In some situations, anamnestic neutralizing antibody response rather than the levels of pre-exsisting neutralizing antibody is critical for protection of mice from lethal JEV infection.

Role of T lymphocytes in recovery from JEV infection

Role of T lymphocytes

1) Both CD4 and CD8 T cells were required for protection against intracranial inoculation of JEVusing passive transfer experiments. (Murali-Krishna K, et al. J Gen Virol.77:705-714, 1996)

2) CD8 -/- mutant mice were more likely to die, with higher CNS viral burden after WNV infection , suggesting that T cells have a role in controlling and clearing viral spread in the tissue. (Shrestha B, et al. J Virol. 78:8312-8321, 2004)

Scientific rationale for the selection of correlates for protection

1. NT antibodies provide the best evidence that protective immunity has been established.
2. The functional assay of neutralization shows correlation with protection, as demonstrated in multiple passive transfer studies in animals.
3. A linear titer-protection relationship exists, and data from efficacy trials corroborate the role of NT antibodies in protection.

Scientific rationale for the selection of correlates for protection (continued)

4. NT antibody mediated protection has been demonstrated for homotypic and also for heterotypic challenge.

5. On the other hand, low antibody titres do not exclude protection.

Conclusion:

Neutralizing antibody is considered to be the best immunological surrogate for JE vaccine-induced protection

What is the “protective antibody level” of 10?

Protective neutralizing antibody level of 10 does not necessarily assure sterile immunity, but neutralizing antibody level of 10 is a reliable and practical marker which represents the entire immune system including memory B cells and CD4+ T cells (and maybe CTLs), induced by JE vaccine.

Primary endpoint of the evaluation of candidate vaccines

1) A quantitative analysis of NT antibodies should be done in a head-to-head comparison with a registered vaccine, following a non-inferiority trial design. A reasonable threshold antibody level for protection is a 1:10 dilution in a 50% PRNT.
2) Non-inferiority should be measured as percentage of seroconversion, but the provision of GMT data is encouraged.

Things to be considered

Strain-specific and cross-reactive Ab responses

Vaccine A: NT Ab to strain A > strain B

Vaccine B: NT Ab to strain B > strain A

Which challenge virus should be used for assessing NT Ab titers?

1. One challenge virus strain
   Homologous to strain A or strain B
2. One challenge virus strain
   Strain C that is heterologous to A and B
3. Two challenge virus strains
   Strain A for vaccine A
   Strain B for vaccine B