

Effect of Intravenous Inoculation of Bordetella  
Pertussis Vaccine on the In vivo Viremia  
and Antibody Response to Flavivirus  
Infection in Rhesus Monkeys

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#### OBJECTIVE

1. To determine if intravenous inoculation of bordetella pertussis vaccine "primes" Rhesus monkeys to develop exaggerated viremias and antibody responses when the animals are subsequently inoculated with infectious flaviviruses.

2. To compare the antibody response of the "pertussis primed" monkeys to that of normal uninoculated control animals and control animals experiencing a secondary type antibody response.

BACKGROUND : Typically Rhesus monkeys develop very mild infections when inoculated with wild or prototype dengue (D) and Japanese encephalitis (JEJ) strains, with no observable signs or symptoms and low level viremias (1-2 logs).

Whitehead et al. (1) reported preliminary observations of increased viremia in gibbons inoculated intravenously with bordetella vaccine; similar preliminary observations have been made in rhesus monkeys (Halstead, personal communication).

We reasoned that if in fact viremia levels could be augmented by prechallenge pertussis inoculation, this might provide a more rigorous model for vaccine efficiency testing.

Virus typing in the Department of Virology by the plaque reduction neutralization (PRNT) method for the past decade has employed monkey antisera raised against local Southeast Asian strains of dengue viruses. Although these sera have been used with great effectiveness, reference sera raised against the prototype strains of virus could be valuable for periodic validation of the typing technique. The high mouse passage prototype strains in this laboratory often provoke only a weak HI and neutralizing antibody response. As a separate control group for the pertussis primed monkeys, we also investigated the antibody response of monkeys on rechallenge with homologous prototype virus

six months after original challenge.

## METHODS

Rhesus monkeys: The rhesus monkeys challenged were divided into three groups (1) Normals: no previous flavivirus exposure, no pertussis inoculation (2) Immunes: previous inoculation with homologous prototype dengue or JEV prototype virus 6 months previously, no pertussis inoculation (3) Pertussis inoculated: no previous flavivirus exposure; 2.0 ml of pertussis vaccine intravenously on day-1 and 2.0 ml intravenously on day 2. All normal and pertussis inoculated monkeys were shown to be antibody free for dengue-1-4 and JEV by HAI and PRNT<sub>50</sub> before the start of the experiment. The antibody response of the "immunes" to original challenge six months previously is shown in Table 1.

Viruses: Dengue 1 (Hawaii) SM16 ( $2 \times 10^6$  PFU/.1 ml); Dengue 2 (New Guinea C) SM30 ( $2 \times 10^6$  PFU/.1 ml); Dengue 3 (H87) SM26 ( $9 \times 10^6$  PFU/.1 ml); Dengue 4 (H241) SM32 ( $8 \times 10^7$  PFU/.1 ml); JEV (Nakayama) SM13 ( $2 \times 10^8$  PFU/.1 ml). One ml of a 20% fresh mouse brain preparation of each virus strains in normal saline was inoculated into monkeys intravenously.

Vaccine: Plain Bordetella pertussis vaccine,  $20 \times 10^6$ /0.5 ml, lot 25 April 1979 (courtesy of Dr. Nathirat Sankavipa, Director, Virus Institute, Department of Medical Science, Ministry of Public Health).

Blood samples: Three ml of heparinized blood was obtained from each animal on days -1, 0, 2, 4, 6, 8, and 10 for determination of complete blood count and virus isolation studies. Blood without heparin for serologic studies was obtained on the following schedule: 10 ml on days -1, 0, 6, and 10; 25 ml on days 15 and 20; 30 ml on day 30, 35, and 59.

Viremia assays: A 1:1 mixture of plasma with buffy coat was assayed for virus by the direct and delayed plaque techniques in LLC-Mk2 cells and by the mosquito inoculation technique (*Toxorhynchites*). Detection of virus in inoculated mosquitoes was performed by examination of head squash preparations by direct fluorescence and by inoculation of thorax-abdomen suspensions into LLC-Mk2 cells for both direct and delayed plaque assays.

Serology: Antibody responses were measured against D1-4 and JEV by a standard laboratory microtiter adaptation of the HAI method of Clark and Cassals, and by microtiter adaptation of the plaque reduction neutralization test on LLC-Mk2 cells.

## RESULTS

Hematology: The normal and immune monkeys developed a polymorphonuclear leukocytopenia on day 2 post infection; 4 of 5 monkeys in each group remained granulocytopenic for the duration of the study. The pertussis-inoculated group developed a prominent granulocytosis on day 0; although the total granulocyte counts fell in these monkeys, they never dropped below the pre-inoculation levels. Total lymphocyte counts remained unchanged in the normal and immune groups; in the pertussis inoculated group a relative lymphocytosis

appeared on day 6, was prominent on day 8, and returned to normal on day 10. Results are presented in Figure 1.

Viremia: Results of buffy coat-plasma mixture assays for virus are presented in Table II. Overall viremia was detected in 10 of the 15 study monkeys: 1 of 5 immunes, 4 of 5 normals, and 5 of 5 pertussis inoculated. A total of 16 blood specimens yielded virus isolates; virus was detected by the plaque assay on LLC-Mk2 cells in 3, by mosquito head squash fluorescence in 11, and by assay of thorax-abdomen suspensions on LLC-cells in 15.

Antibody response: The HAI antibody responses of infected monkeys are shown in Figure 2 and Table III. For every virus type the immune monkeys developed the most rapid and highest antibody response, the normal monkey produced a slower and somewhat lower response, and the pertussis primed monkey had the slowest and lowest response. Four of 5 normal monkeys had developed early antibody by day 6, compared to only 1 of 5 pertussis inoculated monkeys. Both the primary and secondary antibody responses of the D-2 challenged monkeys showed considerable HAI cross reactions to heterologous antigens, especially to dengue type-4.

Plaque reduction neutralization (PRNT) antibody titers are presented in Table IV. Titers were invariably highest against the homologous virus type. In every instance the normal monkey developed a greater PRNT antibody response than the corresponding pertussis immunized monkey, and the previously inoculated monkeys invariably developed the broadest antibody responses.

#### REFERENCE

1. Whitehead, R.H., Chaicumpa, V., Olson, L.C., Russell, P.K. Sequential Dengue Virus Infections in the White-Handed Gibbon (*Hylobates lar*). Amer. J. Trop. Med. Hyg. 19: 94-102, 1970.

Table I. Viremia and antibody response following primary inoculation of Indian Rhesus Monkeys with prototype dengue and JE viruses.

| Monkey No. | Inoculation c   | Viremia | Day after Inoc. | HI/NT   |       |        |        |         |
|------------|---|---------|-----------------|---------|-------|--------|--------|---------|
|            |   |         |                 | vs D1   | vs D2 | vs D3  | vs D4  | vs JE   |
| G-24/      | Den-1 (Hawaii)<br>SM16 (2x10 <sup>6</sup> PFU)            | -       | 15              | <10     | <10   | <10    | <10    | <10     |
|            |   |         | 30              | <10/<10 | <10   | <10    | <10    | <10     |
| G-327      | Den-2 (new Guinea-<br>C), SM 30 (1.7x10 <sup>6</sup> PFU) | -       | 15              | <10     | 40    | 20     | 40     | <10     |
|            |   |         | 30              | <10     | 40/40 | 20     | 40     | <10     |
| G-336      | Den-3 (H87)<br>SM26 (3x10 <sup>6</sup> PFU)               | -       | 15              | <10     | <10   | <10    | <10    | <10     |
|            |   |         | 30              | <10     | 10    | 20/<10 | 20     | <10     |
| G-337      | Den-4 (H241)<br>SM32 (8.5x10 <sup>6</sup> PFU)            | -       | 15              | <10     | <10   | <10    | 20     | <10     |
|            |   |         | 30              | <10     | <10   | <10    | 10/<10 | <10     |
| G-342      | JEV (Nak)<br>SM13 (9x10 <sup>7</sup> PFU)                 | -       | 15              | <10     | <10   | <10    | <10    | <10     |
|            |   |         | 30              | <10     | <10   | <10    | <10    | <10/<10 |

Table II. Detection of viremia in experimental rhesus monkeys. MF = virus detected by Mosquito Inoculation with fluorescent staining of head squash preparation; MP = virus detected by mosquito inoculation with inoculation of thorax-abdomen suspension onto LLC-Mk2 cells and observing for plaques; D = virus detected by the delayed plaque technique on LLC-Mk2 cells

| Monkey Number | Virus Inoculated | Monkey History | Day post inoculation |         |   |   |    |
|---------------|------------------|----------------|----------------------|---------|---|---|----|
|               |                  |                | 2                    | 4       | 6 | 8 | 10 |
| G241          | D-1              | prev infect    | -                    | -       | - | - | -  |
| G246          | "                | pertussis      | MF,MP                | MF,MP   | - | - | -  |
| G220          | "                | normal         | MF,MP                | D,MF,MP | - | - | -  |
| G327          | D-2              | prev infect    | -                    | -       | - | - | -  |
| G262          | "                | pertussis      | D,MF,MP              | MF,MP   | - | - | -  |
| G347          | "                | normal         | MF,MP                | D,MF,MP | - | - | -  |
| G336          | D-3              | prev infect    | -                    | -       | - | - | -  |
| G308          | "                | pertussis      | MP                   | -       | - | - | -  |
| G349          | "                | normal         | MF,MP                | MP      | - | - | -  |
| G337          | D-4              | prev infect    | -                    | -       | - | - | -  |
| F339          | "                | pertussis      | MP                   | -       | - | - | -  |
| G310          | "                | normal         | -                    | -       | - | - | -  |
| G342          | JEV              | prev infect    | MP                   | -       | - | - | -  |
| G348          | "                | pertussis      | MF,MP                | D       | - | - | -  |
| G346          | "                | normal         | MF,MP                | -       | - | - | -  |

Table III. Results of HAI serology. (0= 1/10, 1=1/10, 2=1/20, 3=1/40, 4=1/80, 5=1/160, 6=1/320, 7=1/640).

|            |     | HAI Antibody Response |   |   |   |   |        |   |   |   |   |           |   |   |   |   |
|------------|-----|-----------------------|---|---|---|---|--------|---|---|---|---|-----------|---|---|---|---|
| Virus type | Day | Normal monkey         |   |   |   |   | Imune  |   |   |   |   | Pertussis |   |   |   |   |
|            |     | Dengue                |   |   |   |   | Dengue |   |   |   |   | Dengue    |   |   |   |   |
|            |     | 1                     | 2 | 3 | 4 | J | 1      | 2 | 3 | 4 | J | 1         | 2 | 3 | 4 | J |
| D-1        | 0   | 0                     | 0 | 0 | 0 | 0 | 1      | 0 | 0 | 0 | 0 | 0         | 0 | 0 | 0 | 0 |
|            | 6   | 0                     | 0 | 0 | 0 | 0 | 5      | 3 | 4 | 5 | 4 | 0         | 0 | 0 | 0 | 0 |
|            | 10  | 2                     | 0 | 2 | 1 | 0 | 7      | 5 | 6 | 6 | 6 | 1         | 0 | 0 | 0 | 0 |
|            | 15  | 3                     | 1 | 3 | 3 | 3 | 6      | 4 | 5 | 6 | 5 | 2         | 0 | 0 | 0 | 0 |
|            | 20  | 3                     | 1 | 2 | 2 | 2 | 6      | 4 | 4 | 5 | 5 | 2         | 0 | 0 | 0 | 0 |
|            | 30  | 3                     | 0 | 0 | 2 | 2 | 6      | 4 | 5 | 6 | 5 | 2         | 0 | 0 | 0 | 0 |
|            | 35  | 2                     | 0 | 0 | 2 | 1 | 6      | 3 | 5 | 5 | 5 | 2         | 0 | 0 | 0 | 0 |
|            | 60  | 3                     | 1 | 2 | 2 | 0 | 5      | 4 | 5 | 5 | 4 | 4         | 0 | 0 | 0 | 0 |
| D-2        | 0   | 0                     | 0 | 0 | 0 | 0 | 0      | 0 | 0 | 0 | 0 | 0         | 0 | 0 | 0 | 0 |
|            | 6   | 1                     | 3 | 1 | 2 | 1 | 1      | 1 | 3 | 1 | 2 | 1         | 0 | 0 | 1 | 0 |
|            | 10  | 3                     | 4 | 3 | 5 | 3 | 3      | 4 | 3 | 5 | 3 | 1         | 4 | 2 | 3 | 2 |
|            | 15  | 3                     | 4 | 4 | 5 | 4 | 3      | 4 | 4 | 5 | 4 | 1         | 4 | 4 | 3 | 3 |
|            | 20  | 3                     | 4 | 4 | 4 | 4 | 3      | 4 | 4 | 4 | 4 | 1         | 4 | 4 | 3 | 3 |
|            | 30  | 3                     | 4 | 4 | 4 | 5 | 3      | 4 | 4 | 4 | 5 | 2         | 4 | 5 | 4 | 3 |
|            | 35  | 2                     | 4 | 3 | 4 | 4 | 2      | 4 | 3 | 4 | 4 | 1         | 3 | 3 | 3 | 2 |
|            | 60  | 3                     | 5 | 4 | 4 | 3 | 4      | 5 | 4 | 5 | 4 | 2         | 4 | 2 | 3 | 2 |
| D-3        | 0   | 0                     | 0 | 0 | 0 | 0 | 0      | 0 | 1 | 0 | 0 | 0         | 0 | 0 | 0 | 0 |
|            | 6   | 0                     | 0 | 2 | 1 | 0 | 4      | 4 | 6 | 6 | 6 | 0         | 0 | 0 | 0 | 0 |
|            | 10  | 1                     | 1 | 4 | 3 | 2 | 5      | 4 | 7 | 6 | 5 | 1         | 0 | 4 | 2 | 1 |
|            | 15  | 2                     | 1 | 5 | 3 | 2 | 4      | 3 | 5 | 5 | 5 | 3         | 2 | 5 | 4 | 4 |
|            | 20  | 2                     | 1 | 4 | 3 | 2 | 4      | 3 | 5 | 5 | 4 | 3         | 2 | 5 | 4 | 4 |
|            | 30  | 1                     | 0 | 4 | 3 | 2 | 3      | 3 | 5 | 5 | 4 | 2         | 2 | 4 | 4 | 4 |
|            | 35  | 1                     | 0 | 3 | 2 | 1 | 3      | 3 | 5 | 4 | 4 | 2         | 1 | 3 | 3 | 3 |
|            | 60  | 2                     | 1 | 3 | 2 | 1 | 2      | 2 | 3 | 3 | 1 | 1         | 0 | 2 | 2 | 0 |
| D-4        | 0   | 0                     | 0 | 0 | 0 | 0 | 0      | 0 | 0 | 0 | 0 | 0         | 0 | 0 | 0 | 0 |
|            | 6   | 0                     | 0 | 0 | 4 | 0 | 3      | 4 | 5 | 6 | 5 | 0         | 0 | 0 | 2 | 0 |
|            | 10  | 0                     | 0 | 3 | 5 | 2 | 4      | 4 | 6 | 7 | 5 | 0         | 0 | 0 | 4 | 1 |
|            | 15  | 1                     | 1 | 3 | 4 | 3 | 4      | 4 | 5 | 6 | 5 | 1         | 1 | 3 | 5 | 3 |
|            | 20  | 1                     | 1 | 3 | 4 | 3 | 4      | 3 | 5 | 6 | 5 | 1         | 1 | 2 | 4 | 3 |
|            | 30  | 1                     | 1 | 3 | 4 | 3 | 4      | 3 | 5 | 5 | 4 | 1         | 0 | 2 | 4 | 3 |
|            | 35  | 1                     | 0 | 3 | 4 | 3 | 3      | 2 | 4 | 5 | 4 | 1         | 0 | 2 | 4 | 2 |
|            | 60  | 1                     | 1 | 2 | 3 | 1 | 2      | 3 | 4 | 4 | 2 | 1         | 1 | 1 | 3 | 0 |

HAI Antibody Response

| Virus<br>type | Day | <u>Normal monkey</u> |   |   |   |   | <u>Immune</u> |   |   |   |   | <u>Pertussis</u> |   |   |   |   |
|---------------|-----|----------------------|---|---|---|---|---------------|---|---|---|---|------------------|---|---|---|---|
|               |     | Dengue               |   |   |   |   | Dengue        |   |   |   |   | Dengue           |   |   |   |   |
|               |     | 1                    | 2 | 3 | 4 | J | 1             | 2 | 3 | 4 | J | 1                | 2 | 3 | 4 | J |
|               | 0   | 0                    | 0 | 0 | 0 | 0 | 0             | 0 | 0 | 0 | 0 | 0                | 0 | 0 | 0 | 0 |
|               | 6   | 0                    | 0 | 0 | 0 | 3 | 4             | 4 | 5 | 5 | 7 | 0                | 0 | 0 | 0 | 0 |
|               | 10  | 2                    | 1 | 3 | 4 | 6 | 4             | 3 | 5 | 5 | 7 | 0                | 0 | 0 | 1 | 4 |
| JEV           | 15  | 2                    | 2 | 5 | 4 | 6 | 3             | 3 | 4 | 4 | 7 | 1                | 1 | 2 | 2 | 5 |
|               | 20  | 3                    | 2 | 4 | 4 | 7 | 3             | 2 | 4 | 4 | 7 | 1                | 1 | 2 | 3 | 5 |
|               | 30  | 3                    | 2 | 4 | 4 | 7 | 3             | 2 | 3 | 4 | 6 | 1                | 0 | 2 | 2 | 5 |
|               | 35  | 2                    | 1 | 3 | 4 | 6 | 2             | 2 | 3 | 3 | 6 | 0                | 0 | 2 | 2 | 5 |
|               | 60  | 2                    | 2 | 3 | 3 | 5 | 1             | 2 | 2 | 2 | 4 | 1                | 0 | 1 | 2 | 4 |

Table IV. Plaque reduction neutralization test of monkey sera against dengue 1, 2, 3, 4 and JE by Micro-technique.

| Virus Type | Monkey No. | Date blood collected | PRNT Titer |      |     |     |      |
|------------|------------|----------------------|------------|------|-----|-----|------|
|            |            |                      | D1         | D2   | D3  | D4  | JE   |
| D1         | G-220      | 30 days              | 590        | 125  | 19  | <10 | <10  |
|            |            | 60                   | 1000       | 17   | 25  | <10 | <10  |
| D1*        | G-241      | 30                   | >640       | 50   | 43  | 16  | <10  |
|            |            | 60                   | >640       | 84   | 100 | 25  | <10  |
| D1**       | G-246      | 30                   | 190        | 23   | 10  | <10 | <10  |
|            |            | 60                   | 400        | <10  | <10 | <10 | <10  |
| D2         | G-347      | 30                   | 18         | 600  | 12  | <10 | <10  |
|            |            | 60                   | 62         | 1850 | 20  | <10 | <10  |
| D2*        | G-327      | 30                   | 65         | 1650 | 70  | 14  | 10   |
|            |            | 60                   | 50         | 2400 | 50  | 10  | <10  |
| D2**       | G-262      | 30                   | 10         | 36   | 21  | <10 | <10  |
|            |            | 60                   | <10        | 65   | 30  | <10 | <10  |
| D3         | G-349      | 30                   | <10        | 22   | 103 | <10 | <10  |
|            |            | 60                   | 10         | 22   | 500 | <10 | <10  |
| D3*        | G-336      | 30                   | 100        | 35   | 640 | 14  | <10  |
|            |            | 60                   | 100        | 39   | 240 | <10 | <10  |
| D3**       | G-308      | 30                   | <10        | <10  | 78  | <10 | <10  |
|            |            | 60                   | <10        | <10  | 96  | <10 | <10  |
| D4         | G-310      | 30                   | <10        | <10  | <10 | <10 | <10  |
|            |            | 60                   | <10        | <10  | <10 | 48  | <10  |
| D4*        | G-337      | 30                   | 10         | 18   | 28  | 23  | <10  |
|            |            | 60                   | <10        | 27   | 11  | 12  | <10  |
| D4**       | F-339      | 30                   | <10        | <10  | <10 | 21  | <10  |
|            |            | 60                   | <10        | <10  | <10 | 41  | <10  |
| JEV        | G-346      | 30                   | <10        | <10  | <10 | <10 | 2000 |
|            |            | 60                   | <10        | <10  | <10 | <10 | 1000 |
| JEV*       | G-342      | 30                   | <10        | <10  | <10 | <10 | 290  |
|            |            | 60                   | <10        | 15   | 10  | <10 | 570  |
| JEV**      | G-348      | 30                   | <10        | <10  | <10 | <10 | 1500 |
|            |            | 60                   | <10        | <10  | <10 | <10 | 680  |

\* Previous inoculation with homologous strain.

\*\* Pertussis immunized.

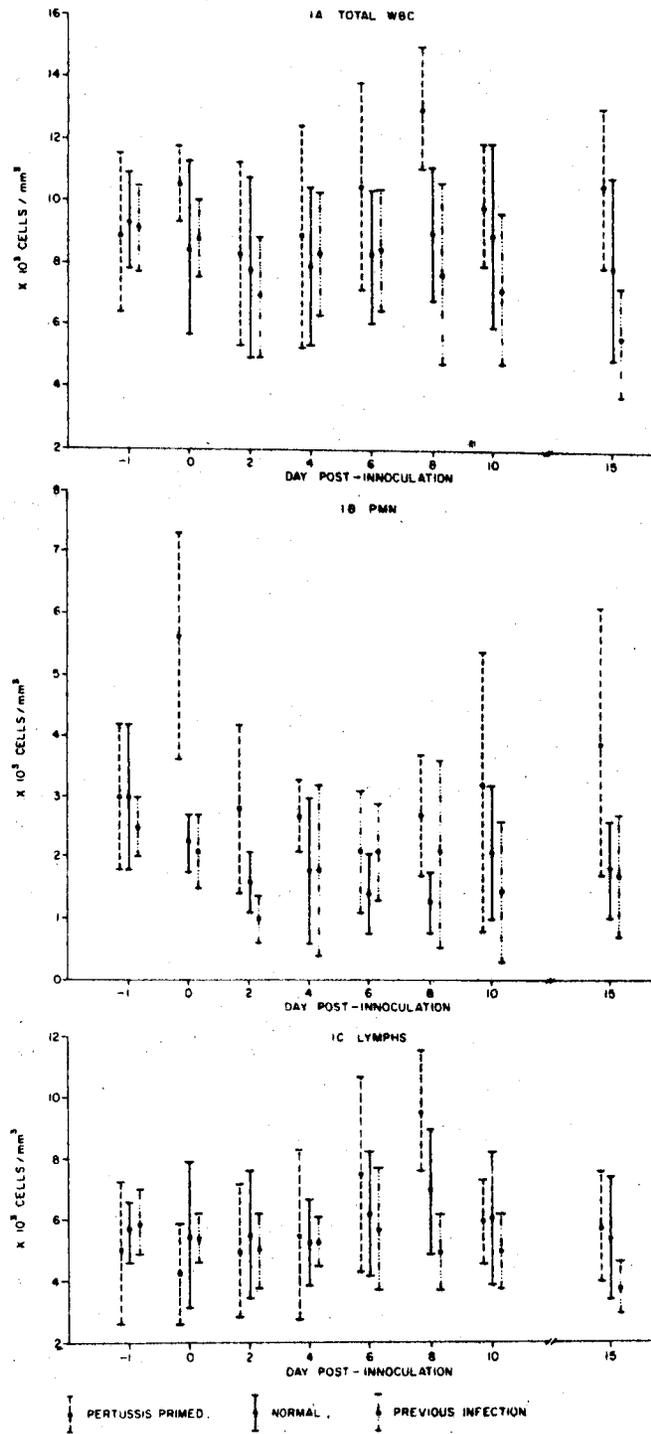


Figure 1. Leukocyte Responses in Normal Monkeys, Previously Infected Monkeys, and Pertussis Primed Monkeys Challenged with Prototype Dengue Viruses.

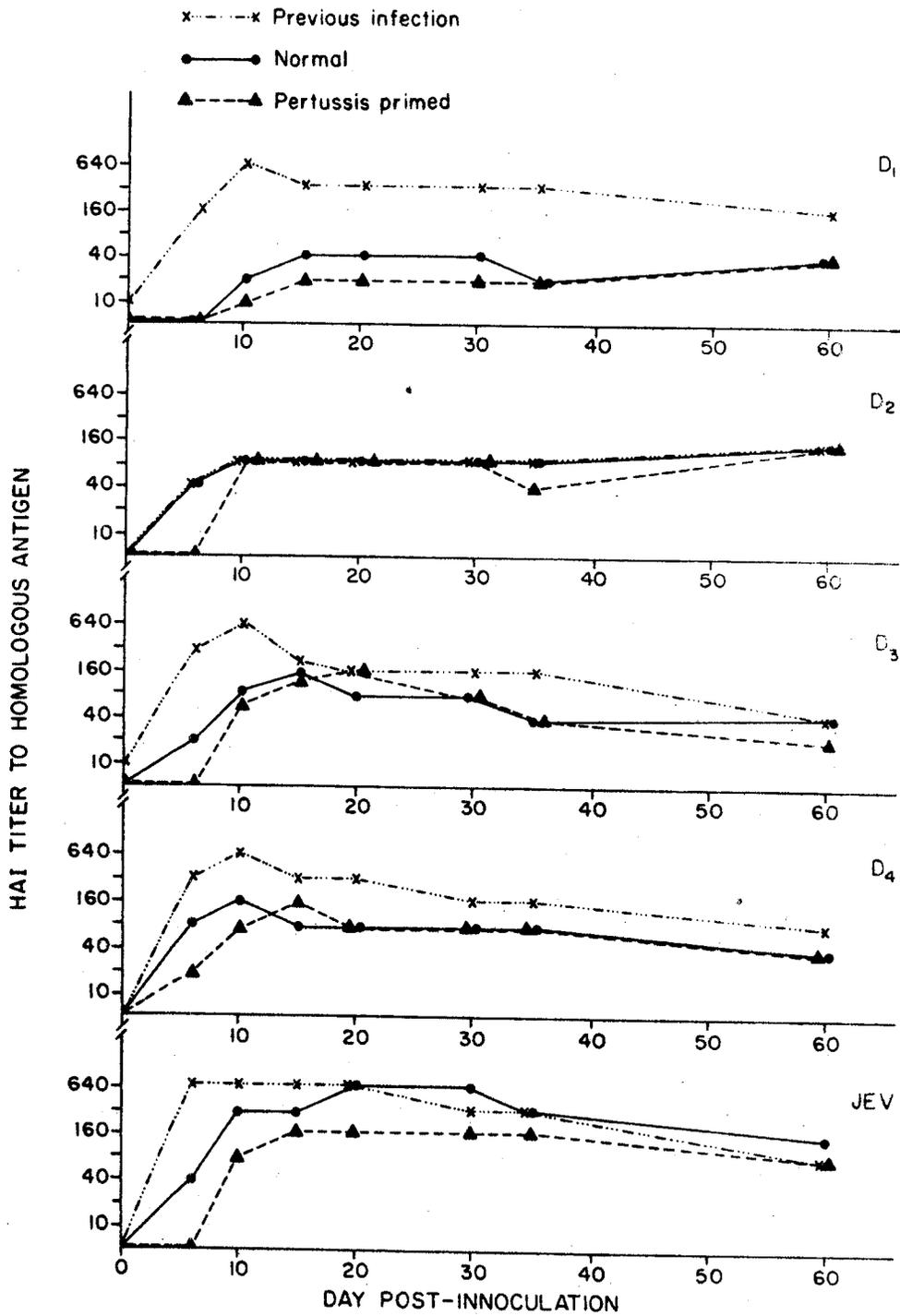


Figure 2. HAI Antibody Responses in Normal Monkeys, Previously Infected Monkeys, and Pertussis Primed Monkeys Challenged with Prototype Dengue Viruses.