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# Yellow Fever Vaccine: Direct Challenge of Monkeys Given Graded Doses of 17D Vaccine

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In rhesus monkeys a wide dosage range of 17D yellow fever (YF) vaccine extending to a level even below that recommended for vaccination of man elicited an immune response providing solid protection to challenge with virulent YF virus. Forty-three of 45 monkeys vaccinated with $10^{2.3}$ or greater weanling mouse mean lethal doses of 17D vaccine were resistant to challenge 20 weeks later with virulent Asibi strain YF virus. Monkeys given graded doses of lesser amounts of vaccine were progressively more susceptible to challenge. With a vaccine dose $\geq 10^{2.3}$ weanling mouse mean lethal doses, plaque neutralization (PN) seroconversion rates were 90% or greater, whereas hemagglutination-inhibiting (HI) and complement-fixing (CF) seroconversion rates were unrelated to vaccine dosage and were generally in the range of 20 to 80%. Ninety-six percent (51 of 54) of immune monkeys had PN titers $\geq 0.7$ $\log_{10}$ (fivefold) neutralization index as compared to approximately 55 to 65% who showed HI or CF titers $\geq 2$ $\log_2$ (fourfold) neutralization index. After challenge with Asibi strain YF virus, antibody titers of all three tests increased equally. In rhesus monkeys PN antibody titers were well correlated with YF immunity, whereas HI and CF antibody titers were not.

Studies with rhesus monkeys have provided much of the basic information regarding the usefulness of 17D yellow fever (YF) virus vaccine in man (4). Fundamental facts, such as the relationships between graded or single-level doses of 17D vaccine and the development of immunity (5, 9), between dosage and persistence of neutralizing (mouse protective) antibody (9), and between neutralizing antibody and hemagglutination-inhibition (HI) and complement-fixing (CF) antibodies (2), have come from studies with monkeys.

In light of the recent development of YF vaccine seed viruses free of specific avian leukosis viruses (ALV; references 1, 8) and the development of a sensitive and reproducible YF virus plaque neutralization (PN) test (6), we deemed it important to reexamine some of the fundamental aspects of 17D vaccination, antibody response, and immunity in rhesus monkeys. Furthermore, we felt that it would be advantageous to correlate the PN, HI, and CF antibody responses with the immunity induced in monkeys by graded doses of 17D vaccine in a single study rather than, as done previously, to acquire such information as a composite from many different investigations.

The first phase of this work involved evaluating the antibody responses of rhesus monkeys to graded doses of the ALV-free 17D YF virus vaccine (3) developed by Tauraso et al. (8). Optimal PN antibody responses were obtained with a vaccine dose as small as $10^{3.4}$ weanling mouse (WM) intracerebral mean lethal dose ($LD_{50}$). With this dose, PN antibody conversion was seen in better than 90% of the monkeys. The PN test was considered a more sensitive and specific indicator of YF antibody following vaccination than the HI or CF tests, but its relationship to immunity was unknown. The present study, therefore, was undertaken to determine the association between vaccine dosage, PN, HI, and CF antibody responses and the immune status of 17D vaccinated rhesus monkeys, as determined by direct challenge with the lethal Asibi YF virus strain.

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## MATERIALS AND METHODS

**Vaccine.** The ALV-free YF vaccine, lot no. 6678 manufactured by the National Drug Company and used in our previous study (3), was used throughout this investigation. The numbers of monkeys vaccinated and the dosage administered to each group are shown (Table 1). The methods of preparing vaccine dilutions and determining by mouse titration the actual dosages given to monkeys have been described (3).

**Animals.** Indian rhesus monkeys (*Macaca mulatta*) weighing (ca. 1.4 to 3.2 kg) were inoculated intramuscularly with 0.5 ml of YF vaccine 2 days after their arrival at the Primate Quarantine Unit, Laboratory Aids Branch, National Institutes of Health.

**Virulent challenge.** Monkeys were challenged with $10^{3.1}$ mouse intracerebral $LD_{50}$ (equivalent to $10^6$ monkey subcutaneous $LD_{50}$; P. Kosch, personal communication) of the virulent Asibi YF virus strain administered subcutaneously 20 weeks after vaccination with the 17D vaccine.

**Serological studies.** Sera were obtained from monkeys prior to (pre) and at 4, 8, and 20 weeks after vaccination (postchallenge). At 2 and 4 weeks after challenge with virulent YF virus, additional serum samples were collected from each monkey. The PN, HI, and CF antibody tests were performed as described (3).

TABLE 1. *Protective effect of 17D YF vaccine in monkeys challenged with the virulent Asibi strain of YF virus*

| Vaccine dilution[a] | Vaccine dosage | | No. surviving per no. challenged[d] | PN[e] antibody titers of susceptible monkeys | % Survival |
|---|---|---|---|---|---|
| | NBM[b] | WM[c] | | | |
| Undiluted | 6.4[f] | 4.9[f] | 11/11 | | 100 |
| $10^{-1}$ | 5.5 | 4.7 | 11/12 | 0.0 | 92 |
| $10^{-2}$ | 3.4 | 3.2 | 11/11 | | 100 |
| $10^{-3}$ | 2.7 | 2.3 | 10/11 | Not tested | 91 |
| $10^{-4}$ | 1.7 | 0.0 | 10/12 | 0.0, 0.5 | 83 |
| $10^{-5}$ | 0.0 | 0.0 | 3/11 | 0.0, 0.0, 0.3, 0.3, 0.1, 0.1, 0.1, 0.9 | 27[*g] |

[a] Yellow fever vaccine lot no. 6678, National Drug Co. Each monkey was vaccinated intramuscularly with 0.5 ml of the appropriate dilution of vaccine.

[b] NBM = Newborn mice (1 to 3 days old).

[c] WM = Weanling mice (16–20 g; 24–28 days old).

[d] Each monkey was challenged with $10^{3.1}$ mouse intracerebral $LD_{50}$ of virulent Asibi strain yellow fever virus administered subcutaneously.

[e] Plaque-neutralizing (PN) titers expressed as difference in titer, $\log_{10}$, between pre- and postvaccination serum samples at the time of challenge (20 weeks after vaccination).

[f] Mouse intracerebral $LD_{50}$, expressed as $\log_{10}$ exponent per 0.5 ml of inoculum used to vaccinate monkeys.

[g] Significant difference, at 5% level, from value immediately above in table.

## RESULTS

**Protective effect of vaccination.** Table I shows the numbers and percentages of monkeys surviving challenge in relation to the dosage of vaccine administered. The dose of 17D vaccine theoretically able to protect 50% of the monkeys was $10^{1.3}$ (twenty) newborn mouse intracerebral $LD_{50}$. The individual plaque-neutralizing antibody titers of eleven monkeys who were susceptible to YF virus challenge are also shown in Table 1. At the time of challenge (20 weeks), one susceptible monkey had a PN antibody titer of 0.9 neutralization index (NI; equivalent to the difference in titer, $\log_{10}$, between pre- and postvaccination serum samples), but titers of the remaining ten animals were quite low and generally $\leq$ 0.3 NI. At 20 weeks after vaccination, 51 of 54 surviving monkeys tested had PN antibody titers $\leq$ 0.7 NI; three monkeys had PN titers of 0.6, 0.5, and 0.1 NI. Vaccine doses greater than or equal to 500 ($10^{2.7}$) newborn mouse or 200 ($10^{2.3}$) WM $LD_{50}$ resulted in immunity to challenge with virulent YF virus in 90 to 100% of the recipients.

The antibody titers of monkeys after vaccination are shown (Table 2). All monkeys were negative for PN antibody prior to vaccination as indicated by comparative titrations of the 17D YF virus on MA-104 embryonic rhesus monkey kidney cell cultures (3) in the presence and absence of monkey serum. The consistently significant differences between the PN antibody titers of monkeys vaccinated with the $10^{-3}$, $10^{-4}$, and $10^{-5}$ dilutions of 17D virus were related to the degree of protection afforded by vaccination (Table 1). In general, monkeys whose PN antibody titers were in excess of 0.7 $\log_{10}$ NI were resistant to Asibi challenge, whereas those whose titers were below 0.7 $\log_{10}$ NI were susceptible. Similarly, PN conversion rates below 40% (Table 3) seen in recipients of the $10^{-5}$ vaccine dilution were associated with the group showing significantly fewer resistant monkeys ($10^{-5}$ group, Table 1). PN tests were not done on the following: one $10^{-1}$ group, 20-week sample; one $10^{-2}$ group, 4-week sample; one $10^{-4}$ group, 20-week sample; one $10^{-3}$ group, 4-, 8-, and 20-week samples. These missing PN data account for the slight differences reported for the respective percent survival figures (Table 1) and the corresponding percent positive monkeys (Table 3).

TABLE 2. *Yellow fever virus PN, HI, and CF mean antibody titers of vaccinated monkeys before virulent challenge*

| Serological test | Post-vaccinal interval (wk) | Vaccine[a] dilution | | | | | |
|---|---|---|---|---|---|---|---|
| | | Undiluted | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| NI[b] | 4 | 3.1 ± .2 (11)[c] | 2.5 ± .4 (12) | 2.1 ± .2 (10) | 1.9 ± .2 (10) | 1.2 ± .2*[d] (12) | .76 ± .2 (11) |
| | 8 | 3.2 ± .3 (11) | 2.4 ± .4 (12) | 2.6 ± .2 (11) | 2.8 ± .3 (10) | 1.6 ± .3*[d] (12) | .67 ± .3*[d] (11) |
| | 20 | 3.3 ± .3 (11) | 2.4 ± .4 (11) | 2.5 ± .3 (11) | 3.0 ± .3 (10) | 1.7 ± .4*[d] (11) | .62 ± .2*[d] (11) |
| HI[e] | 4 | 3.1 ± .4 (9) | 2.7 ± .4 (11) | 2.7 ± .6 (7) | 2.9 ± .7 (12) | 2.7 ± .5 (10) | 1.6 ± .4 (8) |
| | 8 | 3.3 ± .5 (9) | 2.9 ± .5 (12) | 2.5 ± .3 (8) | 3.4 ± .6 (12) | 2.0 ± .3 (10) | 1.9 ± .4 (8) |
| | 20 | 3.2 ± .3 (10) | 3.2 ± .6 (9) | 2.5 ± .4 (11) | 3.2 ± .7 (10) | 2.8 − .5 (9) | 1.8 ± .4 (7) |
| CF[e] | 4 | 2.9 ± .3 (10) | 2.2 ± .5 (9) | 2.7 ± .7 (7) | 1.2 ± .6 (6) | 3.5 ± .6 (10) | .67 ± .5 (6) |
| | 8 | 2.4 ± .6 (7) | 1.9 ± .8 (8) | 2.0 ± .6 (9) | 2.0 ± 1.1 (6) | 2.3 ± .8 (10) | .67 ± .4 (6) |
| | 20 | 2.0 ± 1.0 (5) | 1.2 ± .5 (6) | 1.0 ± .3 (8) | 1.4 ± 1.2 (5) | 1.2 ± .7 (5) | 0.0 ± 0.0 (4) |

[a] Monkeys were vaccinated with the 17D yellow fever vaccine, lot no. 6678.

[b] NI = neutralization index (difference in titer between pre- and postinoculation serum titers expressed as $\log_{10}$ exponents ± standard error.

[c] Figures in parentheses = number of sera tested in group; includes both immune (YF virus resistant) and nonimmune monkeys.

[d] Significant difference, at 5% level, from value immediately to left in table.

[e] HI and CF antibody titers expressed as mean $\log_2$ exponents ± standard error.

TABLE 3. *Percent of monkeys positive for PN, HI, and CF antibodies after 17D vaccination and before challenge with Asibi strain YF virus*

| Serological test | Post-vaccinal interval (wk) | Vaccine dilution | | | | | |
|---|---|---|---|---|---|---|---|
| | | Undiluted | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| PN[a] | 4 | 100[b] | 92 | 100 | 100 | 83 | 36*[c] |
| | 8 | 100 | 83 | 100 | 100 | 83 | 27*[c] |
| | 20 | 100 | 82 | 91 | 100 | 73 | 36 |
| HI[d] | 4 | 78 | 40 | 57 | 33 | 60 | 25 |
| | 8 | 56 | 45 | 62 | 58 | 20 | 38 |
| | 20 | 78 | 44 | 45 | 60 | 44 | 14 |
| CF[d] | 4 | 90 | 56 | 62 | 17 | 90 | 17 |
| | 8 | 71 | 38 | 44 | 33 | 60 | 33 |
| | 20 | 40 | 33 | 38 | 20 | 25 | 0 |

[a] Percentage of monkeys developing a NI (difference in titer between pre- and postinoculation serum samples ≥0.7.

[b] Numbers of animals tested at each post-vaccinal interval and vaccine dilution are shown in the parentheses for the corresponding interval and dilution in Table 2.

[c] Significant difference, at 5% level, from value immediately to left in table.

[d] Percentage of monkeys developing a fourfold (2 $\log_2$) or greater rise in antibody titer.

Consistently significant differences were not seen in the HI or CF antibody titers (Table 2) of different dosage groups. Consequently, there was no direct relationship between mean HI or CF antibody titers and resistance to virulent challenge. Similarly, the group HI and CF conversion rates (Table 3) were not directly related to vaccine dose or immunity. The 17D vaccination was responsible for increasing HI titers to WN and Langat group B arboviruses. In no case did the titers to heterologous antigens exceed those to 17D.

The relation between YF-PN antibody titer and the resistance of monkeys to YF challenge is shown in further detail (Table 4). The PN antibody titers immediately before challenge at 20 weeks are of most interest here, but additional data on the relative levels and persistence of antibody has been included for comparison. Nonimmune monkeys developed average PN antibody titers of less than 0.5 $\log_{10}$ NI, and between 73 and 91% of these monkeys had PN titers of less than 0.7 $\log_{10}$ NI before challenge at 4, 8, and 20 weeks after vaccination. Five prechallenge PN antibody titrations of sera from nonimmune monkeys showed titers ≥ 0.7 $\log_{10}$ NI. Two of these values were recorded for one monkey at 4 and 8 weeks, and the other three values were seen in one of the three prechallenge tests of serum from each of three other monkeys in the nonimmune group. A significant contrast was seen in the case of immune monkeys, where approximately 95% developed PN antibody titers greater than or equal to 0.7 $\log_{10}$ NI, and average titers were greater than or equal to 2.2 $\log_{10}$ NI. Only 2 of 54 immune monkeys had more than one PN

TABLE 4. *Relationship between antibody titer of monkeys and immunity to yellow fever virus*[a]

| Test | Weeks after vaccination | Immune | | | | Nonimmune | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. tested | Titer[b] | ≥ Critical NI | | No. tested | Titer[b] | ≥ Critical NI | |
| | | | | No.[c] | Percent[c] | | | No.[c] | Percent[c] |
| PN | 4 | 55 | 2.2 ± 0.9*[d] | 53 | 96 | 11 | 0.5 ± 0.5 | 3 | 27 |
| | 8 | 56 | 2.6 ± 1.0*[d] | 54 | 96 | 11 | 0.2 ± 0.3 | 1 | 9 |
| | 20 | 54 | 2.7 ± 1.0*[d] | 51 | 94 | 11 | 0.2 ± 0.3 | 1 | 9 |
| HI | 4 | 47 | 3.0 ± 1.6*[d] | 27 | 57 | 9 | 1.0 ± 0.0 | 0 | 0 |
| | 8 | 49 | 3.0 ± 1.5*[d] | 27 | 55 | 9 | 1.1 ± 0.3 | 0 | 0 |
| | 20 | 43 | 3.1 ± 1.4*[d] | 24 | 56 | 7 | 1.1 ± 0.4 | 0 | 0 |
| CF | 4 | 43 | 2.6 ± 1.8*[d] | 29 | 67 | 5 | 0.8 ± 1.3 | 1 | 20 |
| | 8 | 41 | 2.1 ± 2.1 | 21 | 51 | 5 | 0.4 ± 0.9 | 1 | 20 |
| | 20 | 25 | 1.1 ± 1.6 | 8 | 32 | 3 | 0.0 ± 0.0 | 0 | 0 |

[a] Each monkey challenged with $10^{3.1}$ mouse intracerebral $LD_{50}$ of the virulent Asibi strain of yellow fever virus.

[b] Expressed as $\log_{10}$ exponent of the NI ± standard deviation (SD) for PN titers and as $\log_2$ titer ± SD for HI and CF tests.

[c] ≥ Critical NI = 0.7 $\log_{10}$ (fivefold increase) for PN titers and 2 $\log_2$ units (fourfold increase over pretiter) for HI and CF tests.

[d] Significant difference, at 5% level, from mean titer of nonimmune group.

titer less than 0.7 $\log_{10}$ NI after vaccination. It does not appear as though the positive PN titers seen in nonimmune monkeys were due to cross-reacting antibodies, since HI tests using several group B arbovirus antigens were negative both before and after vaccination in the case of sera from three monkeys that were available for testing. The possibility of naturally occurring plaque neutralizing serum inhibitors in monkey serum has not been ruled out. Seven of 161 prechallenge PN antibody tests of sera from immune monkeys gave values below 0.7 $\log_{10}$ NI. These seven values were distributed as follows: six were in sera obtained from two monkeys at 4, 8, and 20 weeks after vaccination, and one was in one of the three prechallenge tests done on a third serum sample. The PN antibody titers of the remaining 51 immune monkeys were all ≥ 0.7.

HI antibody titers, like the PN titers, were significantly higher among immune monkeys than among nonimmune animals. Significant differences between CF antibody titers of immune and nonimmune groups were seen only at 4 weeks after vaccination. Unlike PN antibody titers, a very large proportion of low (<fourfold increase) HI and CF antibody titers were seen in monkeys who were immune to virulent YF virus challenge. Thus 40 to 45% and 33 to 68%, respectively, of immune animals had HI or CF antibody titers below 2 $\log_2$ NI. Susceptibility, on the other hand, was clearly related to low HI or CF antibody titers as well as to low PN titers. All of the YF virus-susceptible monkeys had HI antibody titers below 2 $\log_2$ NI, and only 2 of 13 samples from susceptible monkeys were CF positive (titer ≥ fourfold NI). The data indicate that monkeys whose HI or CF antibody titers increase fourfold or more following vaccination will probably be immune to infection with Asibi strain YF virus. In this study survival after YF challenge was observed in 100% of monkeys whose HI titers and in 80 to 100% of monkeys whose CF titers increased fourfold or more following vaccination. This does not infer that the reverse situation is true, i.e., that low HI and CF titers are indicative of YF susceptibility. In fact, quite the opposite result was seen wherein nearly 70 and 80% of the monkeys with low (< fourfold increase) HI or CF titers, respectively, were also resistant to YF challenge. Low PN antibody titers, in contrast, were indicative of YF susceptibility with approximately 80% mortality seen in monkeys whose titers were less than 0.7 $\log_{10}$ NI.

The PN, HI, and CF antibody responses of vaccinated monkeys following challenge with Asibi virus are shown (Table 5). Increased antibody titers of 2 to 3 log units ($\log_{10}$ values for PN results; $\log_2$ values for HI and CF results) were seen with all of the serological tests (compare with Table 2). The HI and CF antibody titers displayed greater decrease between 2 and 4 weeks after challenge than did the PN antibody titers. There were no indications that the PN, HI, or CF responses follow-

ing challenge were influenced by vaccination with different doses of 17D virus.

## DISCUSSION

We previously reported that a dose of $10^{3.4}$ WM intracerebral $LD_{50}$ of 17D YF vaccine would elicit PN antibody in 90% or more of vaccinated rhesus monkeys (3). The present study confirms our earlier observation and extends the significance of the PN antibody response by relating it to protection. The lowest dose of 17D vaccine capable of stimulating PN antibody conversion in over 90% of the monkeys was $10^{2.3}$ WM $LD_{50}$. This dose, which is only 1/100th of the dose recommended for man, protected over 90% of the vaccinated monkeys against a challenge that is lethal for 100% of nonprotected monkeys. Reduced PN antibody conversion rates accompanied by increased mortality following challenge were seen in groups of monkeys receiving less than $10^{2.3}$ WM $LD_{50}$ of 17D vaccine. PN antibody titers were similarly related to immunity. Ninety-one to 100% of 44 monkeys from groups with average PN antibody titers $\geq 10^2$ were resistant to challenge. Lower dosages of vaccine resulted in progressively reduced PN antibody titers which were accompanied by progressively lower survival rates.

Whereas PN antibody titers were related to immunity, HI or CF antibody titers were not. This was clear from the fact that only PN antibody titers were directly related to survival and from the fact that titers above a "critical" level ($\geq$ fivefold NI) were better correlated with resistance to YF virus than were "critical" ($\geq$ fourfold increase) HI or CF antibody titers. "Critical" antibody titers were selected on the basis of experimental observation and in keeping with a workable definition for this term. It seemed reasonable, if the antibody titer were indicative of immunity, to expect NI values of at least 2 $\log_2$ (fourfold increase) in immune animals. This figure was therefore selected as the critical titer for HI and CF antibody levels. It can be seen that the value of 0.7 $\log_{10}$ NI (fivefold increase) used for PN titers was a useful figure and at the same time one that was consistent with experimental findings. Almost all of the susceptible monkeys had titers below $10^{0.7}$ NI and, more significantly, over 95% of immune monkeys had titers above this value. This high degree of relatedness to immunity distinguished the critical PN antibody titer from either the HI or CF critical NI. An even better correlation between PN response and the immune status of monkeys could have been accomplished by raising the critical PN-NI from 0.7 to 1.0. This change would reduce the number of false positive PN responses, i.e., positive PN titers seen in nonimmune monkeys, to near zero while retaining greater than 90% PN conversion in immune monkeys. But raising the critical titers of HI and CF responses would not actually be useful since fewer titers of immune monkeys would equal or exceed the new critical antibody level and the correlation between positive responses and immunity would be poorer than presently indicated. As with PN titers, essentially all monkeys with HI or CF titers above the critical level were immune, but in the case of the HI and CF tests positive responders were seen in only 32 to 67% of immune monkeys in contrast to a PN conversion rate $\geq$ 90%. This fact was reflected in the finding that HI or CF antibody levels below the critical titer, in contrast to low PN antibody titers, were not indicative of YF

TABLE 5. *Yellow fever virus PN, HI, and CF mean antibody titers of vaccinated monkeys after virulent challenge*[a]

| Vaccine dilution | NI[b] | | HI[c] | | CF[c] | |
|---|---|---|---|---|---|---|
| | 2 wk | 4 wk | 2 wk | 4 wk | 2 wk | 4 wk |
| Undiluted | 5.1 ± 0.1 (11)[d] | 4.8 ± 0.2 (11) | 6.9 ± 1.0 (8) | 6.2 ± 0.3 (10) | 4.8 ± 0.2 (9) | 3.2 ± 0.4 (9) |
| $10^{-1}$ | 5.0 ± 0.1 (10) | 4.8 ± 0.2 (10) | 7.7 ± 0.5 (9) | 6.5 ± 0.4 (11) | 4.6 ± 0.5 (9) | 4.0 ± 0.7 (6) |
| $10^{-2}$ | 5.0 ± 0.2 (11) | 4.8 ± 0.2 (11) | 8.3 ± 0.9 (10) | 6.8 ± 0.6 (11) | 5.0 ± 0.6 (10) | 5.0 ± 0.7 (6) |
| $10^{-3}$ | 4.8 ± 0.2 (10) | 4.5 ± 0.4 (9) | 6.6 ± 0.7 (10) | 6.3 ± 0.4 (10) | 4.8 ± 0.9 (6) | 3.7 ± 0.8 (6) |
| $10^{-4}$ | 4.7 ± 0.2 (9) | 4.7 ± 0.2 (9) | 6.6 ± 0.4 (8) | 5.9 ± 0.5 (10) | 6.0 ± 0.6 (8) | 4.9 ± 0.9 (7) |
| $10^{-5}$ | 5.0 ± 0.1 (3) | 4.9 ± 0.4 (3) | 9.5 ± 0.5 (2) | 7.7 ± 1.2 (3) | 2.5 ± 0.5 (2) | 1.0 ± 0.7 (2) |

[a] Monkeys were vaccinated with the 17D YF vaccine, lot no. 6678 (see Table 1 for dosages), and 20 weeks later were challenged with $10^{3.1}$ intracerebral mouse $LD_{50}$ doses of the virulent Asibi YF virus strain.

[b] NI = plaque neutralization index (difference in titer between pre- and postinoculation serum samples) expressed as $\log_{10}$ exponent ± standard error.

[c] HI and CF antibody titers expressed as $\log_2$ exponent ± standard error.

[d] Numbers in parentheses = number of sera tested.

virus susceptibility. These observations are considered convincing evidence that HI and CF antibody titers are not meaningfully related to immunity in contrast to PN antibody titers which appear to be.

The PN, HI, and CF mean antibody titers were dramatically increased following Asibi challenge: HI antibody levels were enhanced approximately 16- to 25-fold, whereas the PN and CF responses generally were increased $10^2$ to $10^4$ and 4- to 16-fold, respectively. There appeared to be little relation between the original vaccine dose and the antibody level of any of the tests following challenge. The single exception may have been two monkeys that received the $10^{-5}$ dilution of 17D vaccine and whose CF antibody titers failed to show the degree of increase seen in their PN and HI antibody titers.

Since it would be impossible to perform similar studies to relate vaccine dosage and antibody response to the immune status of 17D vaccinated volunteers, direct virus-challenge studies in nonhuman primates become particularly relevant. The results of experiments done with YF virus vaccine in nonhuman primates have related to the human situation (4) but cannot substitute for knowledge gained from studies of this vaccine in man. The reactogenicity and antibody responses of volunteers given a constant dose of ALV-free 17D YF vaccine have already been examined (7). No attempt was made in the trial in man to determine the dose of vaccine which would give an optimal antibody response. The results of the present study in which it was shown that the PN antibody response in rhesus monkeys is related to immunity suggests that updated methodology might be applied advantageously to studies of the dosage-limits of the 17D vaccine-induced antibody response in man.

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