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VIRULENCE MARKERS OF DENGUE VIRUSES

ANNUAL REPORT

James L. Hardy and Srisakul C. Kliks

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19. ABSTRACT (continued)

The 72 strains of dengue-2 virus tested belong to six out of eleven genetically distinct topotypes recognized by the oligonucleotide fingerprint patterns. Each topotype is unique to its geographic location. The six geographic locations in this study represent dengue endemic areas with varying prevalence of DHF/DSS; being prevalent, sporadic or absent. The 33 strains of dengue-2 viral isolates with low monocyte-infectivity, typically associated with PUO/DF were distributed throughout the six topotypes. In contrast, the 39 dengue-2 viral isolates with high monocyte-infectivity, associated with DHF/DSS were distributed among four topotypes. They were topotypes unique to Thailand, the Philippines, Indonesia where DHF/DSS are prevalent and topotype unique to Jamaica/Puerto Rico post 1983 where DHF/DSS cases have occurred sporadically. The two topotypes excluded were those unique to locations where dengue infection is endemic but without incidence of DHF/DSS. These results suggest that 1) dengue viral virulence is one of the factors causing DHF/DSS from dengue-2 infection, 2) virulence of dengue-2 virus can be marked by the ability of the virus to infect monocytes, and 3) monocyte-infectivity as a virulence markers for dengue-2 virus is genetically determined.

I. Statement of the problem

Illnesses in humans caused by the four serotypes of dengue virus include pyrexia of unknown origin (PUO), classical dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The expression of these disease outcomes follows a discernable pattern in regions of the world where dengue viruses are endemic. Variations with virulence of dengue viruses, as defined by their ability to cause severe illness, is proposed to be one of the factors that contribute to the DHF and DSS disease outcomes. The manifestation of these severe outcomes is also related to host factors. For effective prevention and control of dengue diseases, it is important to identify virulent strains of dengue viruses and to elucidate mechanisms which result in multiple disease forms and patterns. The overall object of this research project is to establish whether monocytic infectivity of the virus can be used as a marker for dengue virulence. Subsequently, other biological markers which are unique to virulent viral strains and that can be mapped on viral glycoprotein by monoclonal antibodies, will be determined in order to establish the role, if any, of the viral glycoprotein in conferring virulence to the virus. Knowledge of how the viral glycoprotein is involved in the expression of virulence may contribute toward the development of an effective recombinant vaccine.

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Summary

The objective of the research project is to investigate whether monocyte-infectivity can be used as a virulence marker for dengue viruses. For our purpose, virulence is defined as the intrinsic ability of the virus to cause severe forms of dengue illness - DHF and DSS. DHF/DSS is the rare disease outcome subsequent to dengue viral infection that generally results in mild febrile illness or classical dengue fever (DF) . The prevalence of DHF/DSS varies in the different parts of the world where dengue infections are endemic or epidemic. Since the viral infection in human monocytes has been implicated by numerous epidemiological and experimental observations, as playing an important role in the development of the DHF/DSS, the ability of the virus to infect human monocytes may be related to the virulence of dengue virus.

Our approach has been to measure the viral infectivity for and multiplication in human monocytes in the presence and absence of dengue enhancing antibody and then determine if this correlates with disease outcome (DF or DHF/DSS). Our immediate goal is to verify monocyte-infectivity as a virulence marker for dengue-2 virus. Once the information regarding dengue-2 viral virulence and its in vitro marker is established, we will determine the appropriateness of the same marker for the virulence of other dengue serotypes.

To date, 86 dengue-2 viral isolates have been tested for their infectivity in human monocytes both in the presence and absence of enhancing antibodies. Of these, 72 isolates have the complete set of information required for the analysis. These isolates were analyzed to determine if there was an association between the clinical status of patients from which a virus was isolated and the infectivity of the virus in human monocytes. The results indicated that the probability of a viral isolate causing severe illness is correlated with monocyte-infectivity measured in the presence of enhancing antibodies. This suggests that a viral intrinsic property (i.e., monocyte-infectivity) is expressed through a mediation of an extrinsic host factor (i.e. enhancing antibodies). Further analysis for an association between the clinical manifestations (dengue fever and DHF I, II, III and IV) and monocyte-infectivity by viral strains from DHF/DSS prevalent regions, however, did not show a statistically significant association.

Using monocyte-infectivity as a marker for virulence, we noted that the distribution of dengue-2 virulent strains appears to be geographically segregated. These virulent strains were detected in the geographic areas where DHF/DSS is prevalent, i.e. Thailand, The Philippines, and Indonesia. Further, it appeared that the virulent strains have been well established in Thailand for at least 25 years. The avirulent dengue-2 strains are distributed in the geographic areas where dengue infections are endemic but without the occurrence of DHF/DSS. Thus it can be concluded from these analyses that viral

infectivity in human monocytes in the presence of enhancing antibodies is a suitable marker for virulence of dengue-2 virus

The genetic diversity of dengue-2 viral strains has been established by Trent and associates (1983). Genetically distinct groups can be recognized by the oligonucleotide fingerprint patterns. These fingerprint patterns have been divided into 11 topotypes according to their degree of homology. These topotypes also segregate geographically. The 72 strains of dengue viral isolates evaluated in our study were found to belong to six different topotypes based on their fingerprint patterns. To determine if genetic variation and monocyte-infectivity of dengue-2 viral strains correlate in any way, we looked for common patterns of distribution of the virulent strains among various topotypes. Dengue-2 viral strains with high monocyte-infectivity were mostly distributed within topotypes unique to Thailand, The Philippines, and Indonesia where DHF/DSS is prevalent. The remaining isolates exhibiting high monocyte-infectivity, and not from Thailand, the Philippines or Indonesia, were in a topotype unique to Jamaica/ late Puerto Rico where DHF/DSS cases have occurred sporadically since 1983.

In order to develop a standardized and rapid method to measure viral monocyte-infectivity, we screened several human monocyte cell lines for dengue-2 viral susceptibility, using the in situ EIA method to detect infection. Monocyte cell line K-562 was chosen because the cells were permissive to dengue-2 infection in the absence of enhancing antibodies and adhered well to matrix of the tissue culture plates. A preliminary test comparing four virulent strains and four avirulent strains of dengue-2 virus indicated different rates of infection between both groups as measured by both the EIA and by titration of viral plaque forming units. Further evaluation for the reproducibility and reliability of the test to measure dengue-2 viral virulence is in progress.

Dengue viral serotypes 1,2 and 3 each have different geographic distributions from that of dengue-2 serotype and from one another. The epidemics of DHF/DSS associated with these serotypes are less well documented than those observed with dengue-2 serotype. Some of these serotypes were associated with clinical features slightly different from those associated with dengue-2 virus. It is not known if these dengue serotypes have the same pathogenetic basis for virulence as dengue-2 virus. Therefore, experiments are being done to determine if there is a correlation between the infectivity in monocytes and the clinical symptoms associated with selected viral isolates of each of the three dengue viral serotypes. The existence of a correlation provides evidence for a similar pathogenetic basis for dengue viral serotypes whereas a lack of correlation will suggest that a different mechanisms for viral virulence are involved. We have obtained 76 appropriate isolates of dengue serotypes 1,3 and 4 and, have prepared the viral stocks for the study. To date 35 dengue-1 and five dengue-3 strains have been tested for infectivity in human monocytes in the presence and absence of specific enhancing antibodies.

Foreword

For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.

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II. Background

There are two main hypotheses that can explain the occurrence of the disease severity (DHF/DSS) resulting from dengue viral infection. One hypothesis suggests that the main risk factor is the presence of preexisting dengue antibodies, acquired passively from the mother or actively from a prior infection with dengue virus of another serotype. (Halstead et al., 1970; Halstead, 1984). The other hypothesis (Rosen 1977) suggests that genetic variability within dengue viral populations gives rise to viral strains with varying degrees of virulence. Although evidence from epidemiologic and experimental studies have partially supported both of these hypotheses, conclusive evidence to prove either hypothesis to be the sole mechanism for the pathogenesis of DHF/DSS does not exist.

Our earlier observations relating the binding and replication of several strains of dengue-2 virus from Thailand suggested that the virus bound and replicated efficiently in a mosquito cell line, Ae albopictus. However, these viral isolates were unable to infect freshly isolated human monocytes due to poor binding. The viral binding was augmented by the addition of dengue polyclonal antibodies at the enhancing concentration which resulted in a greatly enhanced monocyte-infection. These results demonstrated the coexistence of two viral properties: the ability for monocyte-infectivity and paradoxically, low binding efficiency with dependency of the infection on enhancing antibodies. These observations, specific to dengue-2 virus in Thailand, suggest that the two alternative hypotheses about the occurrence of DHF/DSS are not mutually exclusive. In light of these findings, we investigated the ability of the virus to infect monocytes as the marker of virulence of dengue-2 virus within the context of the enhancing antibodies. The results of these experiments are presented in this progress report.

III. Assessment of monocyte-infectivity as a virulence marker for dengue-2 virus

A. Selection of viral strains

Previous research indicated that preexisting dengue enhancing antibody was an important host factor which increases the risk of developing DHF/DSS. The mechanism involves the augmentation of viral binding to monocytes by nonneutralizing antibodies. To examine for the impact of viral intrinsic factor(s) on the occurrence of DHF/DSS, careful consideration must be given to the selection of appropriate viral isolates to avoid extrinsic factors that could influence the results.

Dengue-2 virus is probably the most virulent serotype because of its frequent association with many DHF/DSS outbreaks. It was the predominant type associated with DHF/DSS in Thailand from the mid 1960's to the early 1980's in spite of the cocirculation of other serotypes (Hoke et al., 1983). It was the only serotype responsible for the large DHF/DSS outbreak in Cuba during the early 1980's (Guzman et al., 1984). Further, this serotype is widely distributed in various geographic areas, including those that are not known to be associated with the occurrence of DHF/DSS. Genetic diversity within the serotype has been confirmed and similar variants or topotypes are confined to certain geographic areas (Trent et al., 1983; Trent, personal communication). In addition to the diverse nature of dengue-2 serotype both genetically and epidemiologically, dengue-2 virus has been well studied with regard to the viral infectivity in monocytes (Halstead et al., 1977; Moren et al., 1984; Kliks et al., 1988). For these reasons, we initiated our study on the determination of monocyte-infectivity as a virulence marker using dengue-2 virus as a model.

The ideal approach would be to compare monocyte infectivity for viral strains isolated from DHF/DSS cases to those associated with pyrexia of unknown origin (PUO) or classical dengue fever from the same dengue epidemics. However, viral isolates obtained from DHF/DSS endemic regions tend to be limited to isolates from DHF/DSS cases while those from regions where DHF/DSS is sporadic or silent are mostly associated with PUO or classical dengue fever cases. In order to create a sufficiently large sample size of strains that are associated with a variety of disease outcomes, we obtained strains of dengue-2 virus associated with severe illnesses from regions where DHF/DSS is endemic. Conversely, most of the strains associated with PUO or DF included in our study were obtained from location where dengue infection is endemic but with sporadic cases or the absence of DHF/DSS. Attempts were made to include some of the isolates associated with PUO or DF from DHF/DSS prevalent areas. Likewise, we obtained as many isolates as possible of DHF/DSS cases from the DHF/DSS sporadic locations.

All of the isolates were from humans and, with one exception, had low viral passages of human isolates in mosquitoes and/or Aedes albopictus C6/36 cells. Information regarding their source of isolation, passage history, clinical symptoms, infection status and RNase fingerprint patterns were recorded in a data bank using the dBaseIII program. These viral isolates were coded and tested for their ability to infect freshly isolated human monocytes in the presence and absence of dengue enhancing antibodies.

B. Monocyte-infectivity test

One day old monocytes isolated by elutriation technique (Wahl et al., 1983) in the laboratory of Dr. L.H. Wahl at the National

Institutes of Health, Bethesda, MD, were airfreighted in wet ice overnight to our laboratory. Cells were counted and viability was determined by the trypan blue exclusion method. Cell aliquotes containing 6×10^5 cells were infected by each test viral strain at a multiplicity of infection (moi) of 0.05 and 0.5. The infection was performed in the presence and absence of mouse polyclonal antibodies to dengue-2 virus at the optimal enhancing dilution. After a four day incubation period, the infection was determined by 1) the quantity of virus produced in the monocyte culture and 2) the proportion of infected monocytes as measured by the indirect fluorescent antibody (IFA) technique. We noted variation in the intensity and staining pattern among IFA positive cells. Three main IFA patterns were noted; focal, granular and complete. The patterns appear to be characteristic of the locations of the tested viral isolates as well as specific, for the quantity of virus produced (PFU/culture).

C. Correlation between disease outcome and monocyte-infectivity

The variables used in our correlation analysis are listed (Table 1). The clinical diagnoses in this study are not standardized since the diagnosis was determined at the time of the outbreaks by different attending scientists or physicians. Most of the cases from Thailand and those from other countries diagnosed after 1975 are likely to conform with the WHO guideline criteria for diagnosis of DHF/DSS. Thus, although there may be some inconsistencies in reporting the clinical differentiation between PUO and DF vs DHF/DSS, we do not anticipate any systemic bias.

Our preliminary analysis of 72 dengue-2 viral isolates, using both Spearman and Pearson correlation procedures, suggested that the probability of a viral isolate causing severe illness is associated with 1) virus production in the presence of enhancing antibodies, 2) the enhanced quantity of virus produced and 3) the pattern of IFA staining of the infected monocytes regarding the distribution of the stain and the intensity of the stain (1+ to 4+) (Table 2). Further analysis using multiple logistic regression, which controlled for the variability of infection rate due to different donors, indicated that the occurrence of DHF/DSS was also associated with the three infection parameters mentioned above. The probability of a dengue-2 viral strain to cause DHF/DSS in a human infection increases by 15 %, 20 % and 33 % with a unit increase of the virus yield with enhancing antibodies, the enhanced quantity of virus yield and the IFA staining pattern, respectively (Table 2).

We ranked all 72 isolates according to the strongest correlate for monocyte-infectivity, i.e., virus production in the presence of enhancing antibodies. The results indicate that 100 % of the isolates were associated with a DHF outcome when the virus yield was $6 \log_{10}$ or greater, 94 % when the yield was $5 \log_{10}$ or greater and 68 % when the yield was greater than $4 \log_{10}$ (Table 3A). There were some discrepancy in the ranking due to the variability in monocyte donors. Thus, we

created an infectivity index by setting the virus yield of the reference viral strain from each experiment equal to 100. The viral yield of the tested strains were then expressed in relation to this reference index. After the standardization, only minor changes were observed (Table 3B). According to the adjusted ranking order, the isolates associated with DHF/DSS exhibited monocyte-infectivity index ranging from 45 to 135 with a mean of 81.92 and S.D of 20.68; while those isolates associated with PUO and DF exhibited the mean infectivity index of 48.17 with S.D. of 20.35. The difference in the monocyte-infectivity between the two groups was statistically significant ($p < 0.001$) (Figure 1).

It was of further interest to determine whether the degree of disease severity (i.e. DF and the different grades of DHF/DSS) was related to viral infectivity in monocytes. We tested 33 viral isolates from locations of high prevalence of DHF/DSS (Thailand, The Philippines and Indonesia) for an association between the clinical manifestations -DF, DHF I, II, III, IV and the viral infectivity in monocytes. The results from linear regression analyses of data computed separately for each country and combined altogether did not show a statistically significant association between monocyte-infectivity and the five grades of disease severity. However, the probability of each viral isolate from these three countries causing DHF of any grade was still associated with the extent of viral infection in the presence of enhancing antibodies.

The above findings suggest that 1) monocyte-infectivity is a virulence marker for dengue-2 virus; 2) virulence is defined strictly by the probability of the virus to cause DHF of any grade; 3) monocyte-infectivity is expressed through infection with the presence of enhancing antibodies and 4) although the development of DHF is associated with an infection by a virulent virus, the different severity within DHF may be related to other factors such as host.

D. Topotypes of virulent dengue-2 strains and their geographic distribution

Dengue-2 viral isolates included in this study varied genetically according to the RNase oligonucleotide fingerprint patterns (Trent, personal communication). These genetic variants have been organized into 11 topotypes based on the degree of the fingerprint homology (Trent, personal communication). The distribution of each topotype is confined within its unique geographic location (Trent et al., 1983; Trent, personal communication). It appears in our present study that the distribution of virulent strains of dengue-2 virus is also geographically related (Figure 2). Of 72 viral isolates analyzed, 39 came from DHF or DSS patients while 33 came from PUO or DF patients. The 39 viral isolates associated with DHF/DSS, exhibiting high monocyte-infectivity (index = 81.92 ± 41.32) belong to topotypes 1, 2,

3 and 6, specific for Thailand, the Philippines, Indonesia and Jamaica/late Puerto Rico, respectively (Trent, personal communication)(Figure 2A) . In contrast, the 33 isolates associated with PUO and DF were randomly distributed in all topotypes (Figure 2B). One isolate from Jamaica/late Puerto Rico topotype exhibited monocyte-infectivity higher than the mean + 2 S.D.(Figure 2B). The restriction of strains exhibiting high monocyte-infectivity among the topotypes unique to locations where DHF/DSS is prevalent or sporadic suggests 1) the role of viral genetics in the disease outcome and 2) the validity of the monocyte-infectivity as a virulence marker.

It may be of interest from an epidemiological view point to note the four anomalous strains with high monocyte-infectivity indices, from the Jamaica/late Puerto Rico topotype. They are: one isolate from Puerto Rico during the year 1986, associated with DHF; one isolate from Trinidad during 1986, associated with DF; and lastly, two isolates from Jamaica during 1983, of unknown clinical outcome (not included in the analysis). The time periods during which these isolates were detected coincides with the time in Puerto Rico when sporadic cases of DHF/DSS first emerged. In depth examination, with a larger sample size of isolates from this region, may enable us to detect a relevant genetic variation within the topotype or antigenic variation relevant to virulence. Such information may be important to an explanation for a changing disease pattern as exhibited by the emergence of DHF cases in the Caribbean region (CDC Dengue surveillance summary No. 51, 1988).

IV. Search for a suitable mammalian monocytic cell line for the monocyte-infectivity test

Our study showed variability of monocyte-susceptibility to a standard strain of dengue-2 virus among different donors suggesting human biological variation as an added extrinsic factor in the present evaluation. We statistically controlled for donor variability in our analysis. It is prudent, however, to circumvent the donor-variability and to increase the rapidity and conveniency of the test. To accomplish this, we first developed an in situ enzyme immunoassay (EIA) to detect the viral infection expressed as cell associated antigens in the 96 well tissue culture plates. One day old BHK-21 cells grown in a 96 well plate were infected with varying amount (PFU) of dengue-2 viral strain 16681 from Thailand. After a 72 hour incubation period, cell monolayers were washed and fixed with cold methanol for the detection of cell associated viral antigens. We employed the method described by Yong-He and associates, (1984) using mouse polyclonal mouse ascitic fluid as the first antibody, goat anti-mouse Ig as the second antibody and 3'-3', 5'-5'-Tetramethylbenzidine (TMB) as the substrate for enzyme peroxidase. Satisfactory results were obtained when the infection was carried out at a multiplicity of infection ranging from 0.001 to 0.1 from Thailand (Figure 3).

We screened several mouse and human monocytic cell lines for their susceptibility for dengue infection and their ability to adhere to the plastic matrix. Human monocyte cell line K-562 was most suitable because of its susceptibility to dengue viral infection even in the absence of the enhancing antibodies. To promote cell adherence, we cultured the cells in specially treated tissue culture plates "Primaria" (Falcon product). Approximately 1×10^5 cells per well were plated into the 96 well "primaria" plates. Cell cultures were infected with four "virulent" and four "avirulent" isolates of dengue-2 at the moi of 0.1. After four days of incubation at 37 C, the culture fluids were collected for a plaque assay to determine the quantity of virus produced. The cell monolayers were fixed in cold methanol for 30 minutes before they were tested for the expression of viral antigen by the in situ EIA technique described above. The result (Table 4) demonstrated that the "virulent" strains may be distinguished from the "avirulent" strains by this rapid test-system. Further evaluation with a larger size of unknown and coded sample is planned to validate the reliability of this test.

V. Determination of monocyte-infectivity as a virulence marker for dengue-1,-3 and -4 viruses.

Observations and studies, implicating the infection of human monocytes in the presence of the enhancing antibodies to be important for the pathogenesis of DHF/DSS, were limited to dengue-2 viruses (Halstead et al., 1977; Burke et al., 1988 and Kliks et al., 1988). It has been assumed that DHF/DSS caused by other serotypes is associated with similar mechanisms of pathogenesis. However, the disease pattern of dengue-1 virus was different from that of dengue-2 virus; dengue-1 virus in Thailand was less virulent than dengue-2 and the occurrence of DHF/DSS by dengue-1 did not correlate with sequential infection (Burke et al., 1988). In addition, clinical manifestations of DHF/DSS found in Indonesia associated with dengue-3 infections exhibited slightly different clinical features (Eram et al., 1979); since they were more frequently associated with hemorrhage rather than hemoconcentration.

We will extend our investigation for a correlation between monocyte-infectivity of dengue viral isolates of serotype 1,3 and 4 and the associated clinical symptoms. Although the same approach as described with dengue-2 will be employed, only a preliminary assessment will be performed. We have obtained 35, 32 and 9 isolates of dengue-1,-3 and -4 virus respectively, for our study. Seventy percent of the virus stocks have been grown and prepared for the monocyte-infectivity test. To date 35 dengue-1 and three dengue-3 isolates have been tested.

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Table 1. Variables included in the correlation analyses

1. TEST NUMBER: represents each monocyte donor (NIH blood bank).
2. CODE: represents each viral isolate previously coded by CDC.
3. LOCATION: origin of viral isolates.
4. EPIDEMIOLOGICAL STATUS: 1 = no DHF
2 = sporadic DHF
3 = epidemic DHF
5. CLINICAL STATUS: 0 = PUO or DF
1 = DHF/DSS
6. MONOCYTE INFECTION WITHOUT AB: % FA positive monocytes.
7. MONOCYTE INFECTION WITH ENHANCING AB: % FA positive monocytes.
8. FA PATTERN: 1 = focal
2 = granular
3 = complete
9. ENHANCED MONOCYTE INFECTION: #7 - #6 in % FA positive monocytes.
10. VIRUS YIELD WITHOUT AB: Log_{10} PFU/ ml.
11. VIRUS YIELD WITH ENHANCING AB: Log_{10} PFU/ ml.
12. ENHANCED VIRUS YIELD: #10 - #11 in Log_{10} PFU/ml

Table 2. Correlation between DHF/DSS outcome and infection parameters

	Pearson		Spearman		Logistic Regression	
	r	p	r	p	p increment per unit increment	p
VYAb _@	0.476	<0.001	0.482	<0.001	32 %	<0.001
ENVY _*	0.366	<0.001	0.282	<0.05	15 %	<0.01
FA _#	0.409	<0.001	0.287	<0.05	20 %	<0.01

72 Observations

r = correlation coefficient

p = probability

@ virus yield in the presence of enhancing antibodies

* enhanced virus yield

FA staining pattern

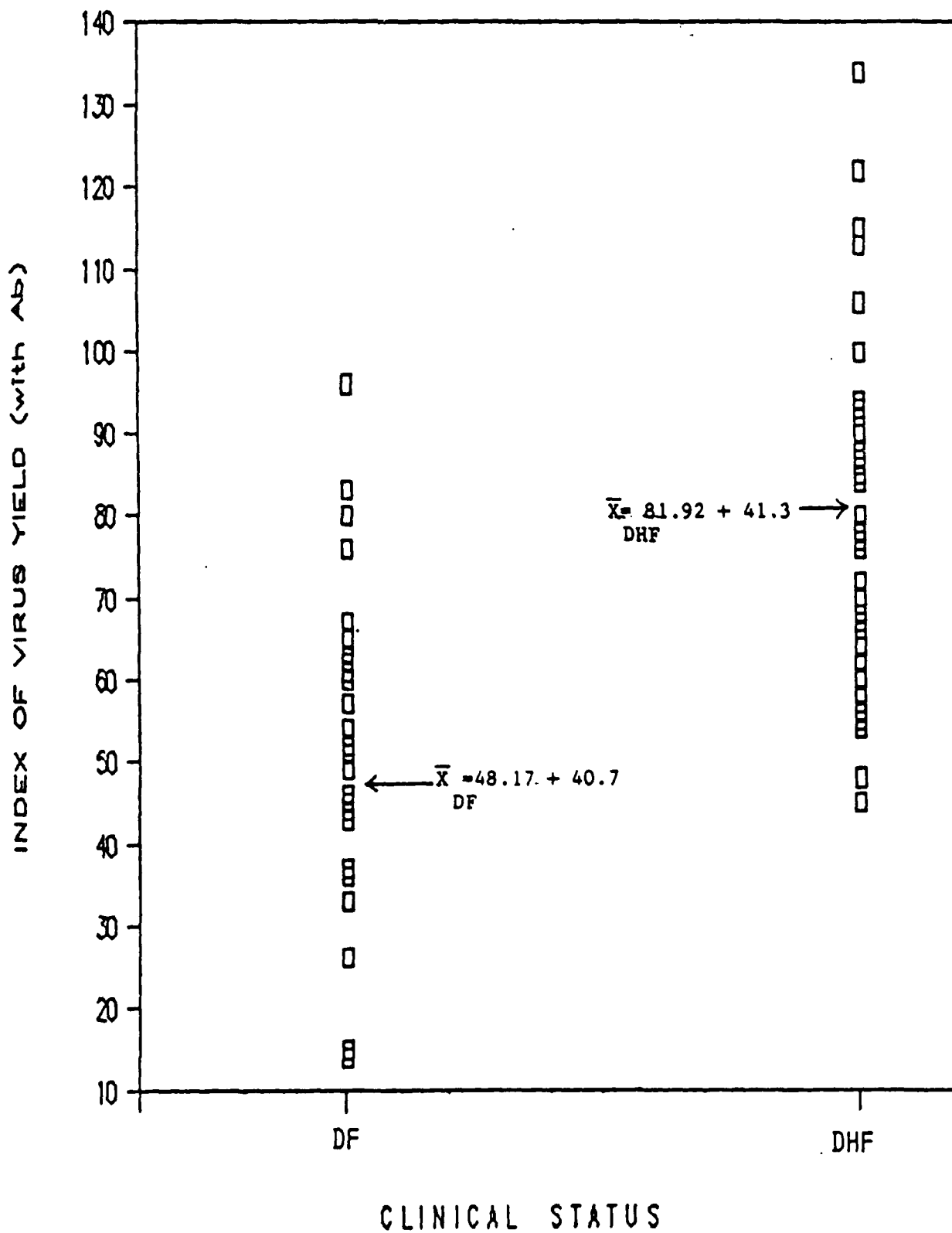
Table 3 Dengue-2 isolates ranked from high to low monocyte-infectivity.

A				B			
CODE	CLINST	VYAB	LOCATION	CODE	CLINST	ADJYAB	LOCATION
680	1	7.3	THAI	990	1	135	THAI
124	999	6.6	JAMAICA	62	1	122	THAI
37	1	6.5	THAI	124	999	122	JAMAICA
565	1	6.4	THAI	742	1	115	PR
828	1	6.2	THAI	658	1	113	PHIL
381	1	6.2	THAI	680	1	106	THAI
990	1	6.2	THAI	1261	1	106	INDO
973	1	6.2	THAI	293	1	100	THAI
670	1	6.1	THAI	16681	1	100	THAI
529	1	6	THAI	801	0	96	TRINIDAD
982	1	5.8	THAI	37	1	94	THAI
62	1	5.6	THAI	565	1	93	THAI
133	0	5.5	PR	973	1	92	THAI
16681	1	5.5	THAI	381	1	90	THAI
460	1	5.3	THAI	828	1	90	THAI
742	1	5.3	PR	737	1	88	THAI
658	1	5.2	PHIL	670	1	88	THAI
293	1	5	THAI	529	1	87	THAI
901	0	4.9	TRINIDAD	745	1	85	THAI
1256	0	4.9	INDO	982	1	84	THAI
1013	1	4.8	INDO	294	0	83	THAI
729	1	4.7	PHIL	616	1	80	THAI
294	0	4.5	THAI	463	1	80	PHIL
782	0	4.5	SRI	133	0	80	PR
491	0	4.5	FIJI	519	1	78	PHIL
737	1	4.4	THAI	460	1	77	THAI
884	1	4.4	THAI	629	0	76	SRI LANKA
658	1	4.4	PHIL	34	1	76	INDO
1208	1	4.4	INDO	1013	1	72	INDO
669	0	4.2	PR	42	1	72	INDO
728	0	4.2	PR	46	999	70	BURMA
519	1	4.2	PHIL	729	1	70	PHIL
10099	1	4.2	PHIL	884	1	68	THAI
4	0	4.2	FIJI	399	1	67	THAI
629	0	4.1	SRI LANKA	491	0	67	FIJI
616	1	4	THAI	1208	1	66	INDO
654	0	4	PHIL	782	0	65	SRI LANKA
463	1	4	PHIL	658	1	64	PHIL
745	1	3.9	THAI	144	999	63	TRINIDAD
1016	1	3.9	INDO	4	0	63	FIJI
1209	0	3.8	INDO	669	0	62	PR
40979	1	3.7	BURMA	728	0	62	PR
399	1	3.6	THAI	10099	1	62	PHIL
42	1	3.6	INDO	22	0	61	BURMA
46	999	3.6	BURMA	220	999	61	INDO
202	0	3.5	PR	654	0	60	PHIL
34	1	3.5	INDO	742	1	60	PR
144	999	3.4	TRINIDAD	419	999	58	TRINIDAD
483	0	3.4	PHIL	1016	1	58	INDO
71	0	3.4	FIJI	1209	0	57	INDO
419	999	3.15	TRINIDAD	655	1	56	INDO
22	0	3.1	BURMA	40979	1	55	BURMA
742	1	3	PR	32	0	54	TAHITI
1261	1	3	INDO	153	1	54	THAI
1122	1	3	INDO	263	0	52	THAI
54	1	3	DOMREP	202	0	52	PR
153	1	2.9	THAI	71	0	51	FIJI
32	0	2.9	TAHITI	483	0	49	PHIL
731	0	2.9	PHIL	889	1	48	PHIL
263	0	2.8	THAI	525	0	46	FIJI
220	999	2.8	INDO	1256	0	45	INDO
655	1	2.8	INDO	1122	1	45	INDO
889	1	2.6	PHIL	254	0	45	PR
525	0	2.5	FIJI	54	1	45	DOMREP
254	1	2.3	PR	872	0	44	JAMAICA
555	0	2.2	PR	555	0	44	PR
872	0	2.2	JAMAICA	731	0	43	PHIL
160	0	1.8	PR	975	0	37	SRI
975	0	1.7	SRI LANKA	160	0	36	PR
860	0	1.7	PR	850	0	33	PR
411	0	1.7	PR	411	0	33	PR
44	0	1.4	MEX	411	0	26	MEX
718	0	0.7	TONGA	32	0	15	TAHITI
16	0	0.7	TAHITI	563	0	14	MEXICO
22	0	0.7	TAHITI	718	0	14	TONGA
563	0	0.7	MEXICO	16	0	14	TAHITI
545	0	0.7	FIJI	545	0	14	FIJI

Table 4. Comparison of monocyte-infectivity in K-562 cells between dengue-2 virulent and avirulent strains.

	dengue-2 strains	EIA O.D.readings	log 10 PFU/ml
VIRULENT	Thailand-64 16681	1.049	5.70
	Thailand-64 565	1.061	5.20
	Thailand-81 973	1.218	4.20
	Trinidad-82 801	0.497	3.40
AVIRULENT	Mexico-83 044	0.154	2.70
	Mexico-86 086	0.013	0.07
	Puerto Rico-86 411	0.104	2.08
	Fiji-71 491	0.102	2.40

Figure 1. Difference in monocyte-infectivity between viral isolates associated with DF and with DHF (range and mean + 2 S.D.).



• Figure 2. Distribution of viral isolates from DF and DHF/DSS patients among different topotypes. 1 = Thailand/Burma, 2 = the Philippines, 3 = Indonesia, 4 = Sri Lanka, 5 = Early Puerto Rico/ Mexico and 6 = Jamaica/late Puerto Rico. Closed squares (■) represent viral isolates associated with DHF, opened squares (□) represent viral isolates associated with DF.

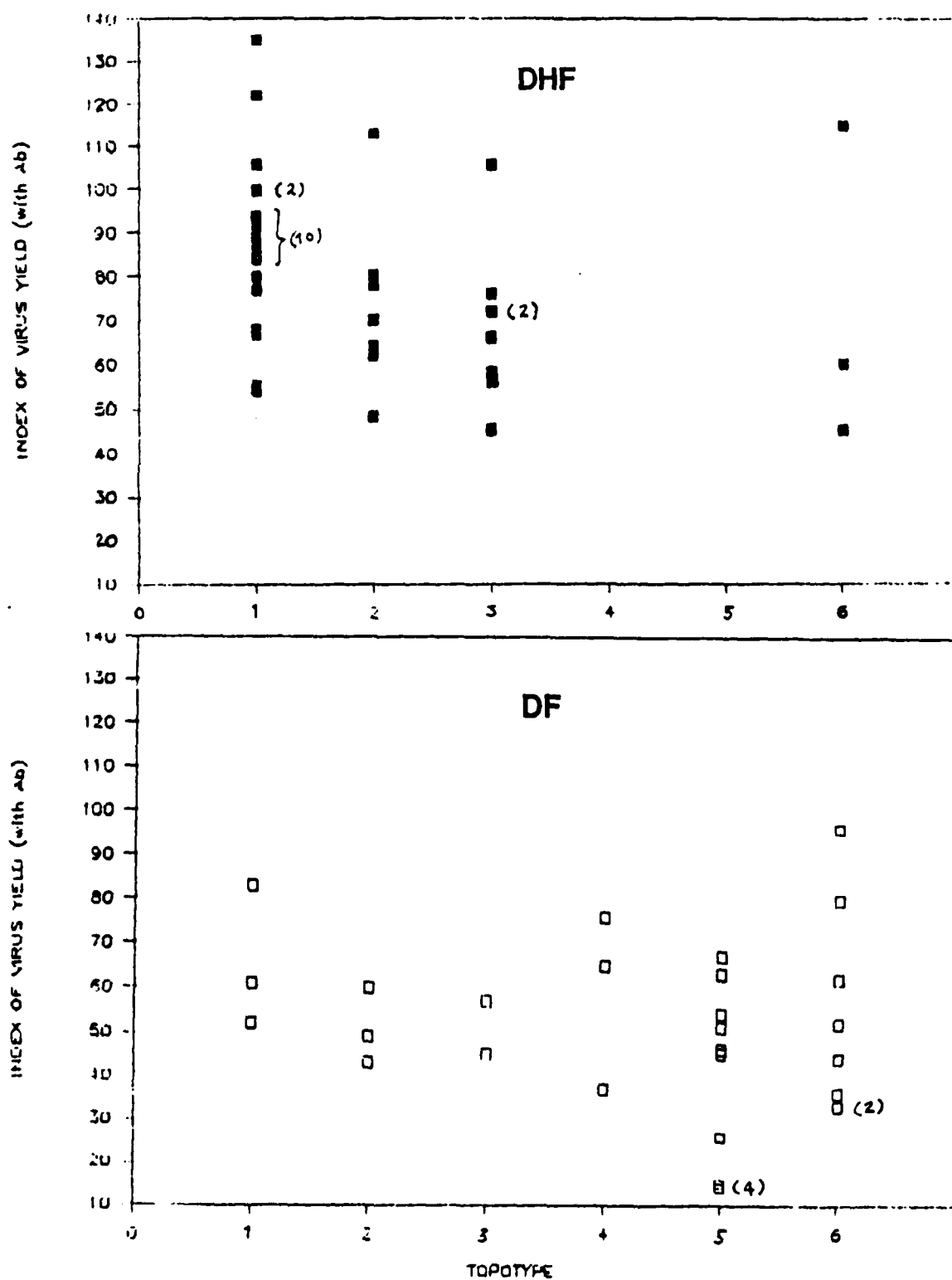


Figure 3. Dengue-2 infection at various MOI as detected by the in situ after 72 hrs incubation period. Each data point represents a mean value from three experiments and the bars represent one standard error.

