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Laboratory Infection with Zika Virus after Vaccination against Yellow Fever

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Summary

One of the authors contracted a Zika virus infection during laboratory work. Subsequent studies revealed an immunological response of the anamnestic type due to preceding yellow fever vaccinations which rendered difficult etiological diagnosis had it not been possible to isolate the virus from the serum sample collected during the acute phase of the disease.

The authors comment on the possible importance of similar cases encountered in tropical countries when dignosis is based merely on the serological conversion from the acute phase to the convalescent phase of the disease.

1. Introduction

During laboratory work with arboviruses, one of the authors (C. M.) contracted a benign infection due to an infectious agent which was subsequently found to be Zika virus. The study of hemagglutination-inhibiting, complement-fixing and neutralizing antibodies found in the various serum samples collected from the same individual yielded results which seem to be of particular interest in respect to the antigenic relationship between yellow fever and Zika viruses.

In the case described here, the patient was vaccinated for the first time against yellow fever with the 17D vaccine some 11 years before contracting the Zika virus infection; he was revaccinated with 17D vaccine when he started to work in the arbovirus laboratory, that is, some two months before the onset of the infection. As the patient had to go to Africa during the same year and had lost his international yellow fever vaccination certificate he ought to have a further 17D vaccination about five months after the Zika virus infection.

The infection was rapid in onset with chills, fever of 38° C, sweating, retro-orbital pains, pains at the back of the neck and in the joints (1st day). There was no rash nor signs of involvement of the respiratory or digestive apparatus. No psychic anomalies were observed. On the following day (2nd day), the symptomat-

ology was the same and the morning temperature was 37.5° C rising at night to 38.3° C. On this day tetracycline was prescribed. The symptoms were unchanged on the third day, thereafter they gradually improved until one week later the patient was completely recovered.

2. Materials and Methods

Blood samples were collected from the patient on the first day of the illness (1st serum sample), on the seventeenth day (2nd serum sample), on the forty-third day (3rd serum sample), on the one hundred and sixty-eight day (4th serum sample) and on the two hundred and seventy-first day (5th serum sample).

As was stated above, the first Yellow Fever vaccination with 17D vaccine was given on 16. 8. 1961, the second on 14. 12. 1971 and the third on 17. 7. 1972, one week before the 4th serum sample was taken.

From the first blood sample, a virus could be isolated by i.c. inoculation in baby mice. The serological identification of the isolated virus (M virus) was carried out with hyper-immune mouse serum prepared in adult mice by 5 to 6 intraperitoneal inoculations of a 10 per cent suspension of mouse brain infected with the newly isolated agent at weekly intervals.

Hemagglutination-inhibition (HI), complement fixation (CF) and neutralization tests (NT) were carried out with the 5 serum samples collected from the patient and also with sera from mice immune to the yellow fever, Zika and M viruses. The HI tests were performed according to the technique of CLARKE and CASALS (4) with the microtest. The CF test was also performed using the microtest (7). The neutralization test (NT) with reference hyperimmune sera and patient serum samples were performed by mixing equal amounts of undiluted sera and 10 fold dilutions of the virus under study. The serum-virus mixtures were kept at 37° C for 1 hour before i.c. inoculation into suckling mice. The neutralization index expresses the difference between the titers of virus either in the diluent (Hanks solution with bovalbumine 0.4 per cent) and in the immune serum. Titers were determined according the KÄRBER method (8).

3. Results

After screening by HI tests with a large number of antigens from groups A, B, Bunyamwera, C, California, Sicily and Rift Valley Fever, the isolated virus was studied by HI, CF and N-tests with homologous mouse serum as well as with anti-yellow fever and anti-Zika mouse sera prepared in the same way. As seen in Table 1, these tests clearly demonstrated that the virus isolated from the patient's serum was Zika virus.

The serological reactions (cf. Table 2) obtained with the 1st and 2nd serum samples from the patient showed that it was of interest to collect further serum samples at larger intervals p.i. in order to study the immunological response against yellow fever and Zika viruses.

No HI antibodies could be found in the first serum whereas the CF test indicated a recent infection by a group B virus and the N-test showed a significant level of protective antibodies against yellow fever virus.

The 2nd sample collected 17 days after the onset of the disease showed in HI, CF, and N-tests an immunological response which was, however, essentially directed against yellow fever virus and not indicative for a recent infection by Zika virus.

On the whole, the reactivity of the 5 patient's serum samples indicated a secondary infection by a group B arbovirus. Based on HI and CF titers only, one would suppose that yellow fever virus might be most probably responsible for the laboratory infection. That such an assumption, however, is erroneous, was clearly proved by the successful isolation of Zika virus from the patient's serum during the acute phase of disease. Thus, the outstanding high and early occurring antibody titer against yellow fever virus ought to be interpreted as an anamnestic response to the preceding 17D vaccinations stimulated by the antigenically closely related Zika virus. With this assumption, the results obtained in the N-test are in line, since they show that the antibody titer against M and Zika virus steadily increased from the 2nd to the 5th serum sample and finally equalled that of yellow fever virus.

Table 1. *Serological Differentiation between Isolated Virus M, Zika- and Yellow Fever (Asibi) Viruses by Neutralization (N), Hemagglutination Inhibition (HI) and Complement Fixation (CF) Tests*

Tests	Sera[a]	Virus antigens		
		M	Zika	Y.F.
I. N-test	anti M	3.8[b]	3.4	1.7
	anti Zika	3.7	3.6	1.4
	anti Y.F.	1.6	1.2	2.7
II. H-I test	anti M	1/160[c]	1/80	0
	anti Zika	1/40	1/40	0
	anti Y.F.	1/40	1/40	1/80
III. CF test	anti M	16/128[d]	32/512	16/128
	anti Zika	16/256	32/512	16/128
	anti Y.F.	8/32	8/32	64/256

[a] Immune sera from mice.
[b] $\log_{10}$ neutralization index.
[c] Reciprocal of titer.
[d] Serum titer over antigen titer.

4. Discussion

The role played by certain viruses in human pathology has been elucidated mainly through the study of laboratory infections. Knowledge obtained in this way has been of great importance for the study of the pathogenicity of arbovirus infections. Of the 312 viruses which have so far been isolated and classified as arboviruses, about 82 are known to be the cause of human infections (12) although, as far as it is known, only some 39 are responsible for diseases frequently observed. Thus, the knowledge of the pathogenic potential of about half of the arboviruses capable of causing symptomatic disease in humans has been acquired mainly from the study of laboratory infections. One of the arboviruses whose pathogenic role was determined in this way was the Zika virus (11).

The role of Zika virus in illness occurring in nature is not accurately known. In serological tests carried out with arboviruses on different occasions, whenever

Table 2. *Reactivity of Patient Serum Samples with Isolated*

No.[a]	Collect. (days)[b]	H-I test Viruses[c] M	Z	YF	W	B
1	1	0	0	0	0	0
2	17	80[d]	40	320	20	40
3	43	80	40	160	20	40
4	161	40	20	160	20	20
5	271	40	20	80	0	20

[a] Samples No. 1—3 taken after 1st and 2nd 17D vaccination; No. 4, 5 taken after 3rd 17D vaccination.
[b] After onset of disease.
[c] M isolated virus strain; Z Zika; YF Yellow fever (Asibi); W Wesselsbron; B Banzi.
[d] Reciprocal of titer.
[e] Serum titer over antigen titer.
[f] $\log_{10}$ neutralization index.

the yellow fever and Zika viruses are involved, high percentages of HI antibodies to both these viruses appear in a large number of cases. Sometimes it is difficult to say with certainty which of the viruses—Zika or yellow fever—is present in a given place (10, 1, 5, 6). In cases of secondary infection with group B viruses, the finding of HI antibodies to Zika or yellow fever seems merely to indicate that the Zika virus antibodies detected can only be the result of a serological overlap which is much greater between these two viruses than among other group B viruses. The risk of false interpretations of serological data due to cross reactions between antigenically closely related arboviruses, particularly in cases of superinfections, has been already emphasized by CASALS (2).

The extremely high percentage of Zika virus antibodies which have been found in some serological surveys (1) make it logical to assume that in some areas this virus is very probably associated with illness in human subjects. However, this has not yet been demonstrated with certainty.

In the case described here, the patient was vaccinated against yellow fever some 11 years before and again about 2 months before developing Zika virus infection. The antibody response was therefore of the anamnestic type already observed by other workers (3, 9, 13) in secondary infections due to group B viruses. Thus, the etiology of our current infection could be only ascertained by the isolation of Zika virus, this in contrast to the first case of a primary laboratory infection with Zika virus (11) which was accurately diagnosed by serological tests.

The conclusion which can be drawn from our observations may serve as a reminder of the care which is needed in the interpretation of serological results obtained during epidemiological surveys in areas where several arboviruses are present and essentially in those areas where jungle yellow fever probably exists. In cases of superinfections with group B viruses only the isolation of the virus can be considered to provide a reliable etiologic diagnosis, at least until the serological techniques will be improved.

M Virus and Other Antigenically Related Arboviruses

CF-Test					N-Test		
Viruses					Viruses		
M	Z	YF	W	B	M	Z	YF
0/0	4/4[e]	8/8	0/0	0/0	0.9[f]	0.6	2.3
16/64	8/32	32/32	8/8	8/16	2.7	2.0	3.7
8/32	8/16	32/16	4/4	8/8	3.6	3.0	4.0
8/16	16/16	4/8	4/4	8/8	3.5	3.7	4.1
4/8	8/32	32/64	0/0	4/8	4.6	4.5	4.8

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