Use of a Monoclonal Antibody Specific for Wild-type Yellow Fever Virus to Identify a Wild-type Antigenic Variant in 17D Vaccine Pools

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(Accepted 6 March 1989)

SUMMARY

Monoclonal antibodies (MAbs) against the Asibi wild-type strain of yellow fever (YF) virus were prepared and characterized. One of the MAbs (designated MAb 117) was shown, by cross-immunofluorescence tests with flaviviruses, to be specific for wild-type YF virus. This MAb was used in indirect immunofluorescence tests to identify wild-type antigenic variants in several different YF vaccine pools. Simultaneously, a vaccine-specific MAb prepared previously (MAb 864) was used to identify YF strain 17D vaccine type variants in the wild-type Asibi virus preparation. One variant, isolated by plaque purification from a 17D vaccine pool, possessed the wild-type epitope and was neurovirulent in infant mice whereas other variants, lacking the wild-type epitope but with vaccine-specific epitopes (identified by MAb 411), were avirulent in infant mice. Avirulent variants were able to infect mice and induce antibody. Virus-specific antigen was still detected in the brains of these mice 4 weeks after inoculation, suggesting that persistent infections were developing. These results demonstrate the antigenic heterogeneity of 17D vaccine preparations. They also point to the potential risk of selection of wild-type variants in YF vaccine preparations and re-emphasize the need for modernization of techniques and more effective control measures to be taken during the production of YF vaccine.

In general, yellow fever (YF) vaccines have proved to be very safe and highly effective even though they were derived empirically by serial subculture in mouse and chick embryonic tissue (Mathis et al., 1928; Theiler & Smith, 1937). Notwithstanding, there have been some cases of encephalitis or other complications following immunization, and YF epidemics still occur in South America and Africa among non-immunized individuals. This has led to laboratory investigation of the vaccines and it is now well recognized that they consist of a heterogeneous population of variants differing in plaque morphology (Woodall, 1981; Liprandi, 1981), mouse virulence (Liprandi, 1981; Barrett & Gould, 1986), oligonucleotide fingerprint patterns (Monath et al., 1983) and antigenicity (Gould et al., 1985b; Buckley & Gould, 1985). In view of these and other factors, recommendations were made by Woodall (1981) to examine the genetic stability of the vaccine seed virus, and by the Pan American Health Organization (PAHO, 1984) to modernize the YF vaccine. These recommendations included the use of monoclonal antibodies (MAbs) to identify antigenic variations and the development of a tissue culture-derived vaccine to enable monitoring of plaque variants.

We previously prepared and characterized MAbs that exhibit specificity for, and can distinguish between, vaccine strains of YF virus (Gould et al., 1985a, b). We have now also

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Table 1. Results of cross-immunofluorescence tests with 54 flaviviruses and MAbs against YF Asibi virus

| Flaviviruses (group-arranged)* | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z |
| GLLMNPSCTACCDEJKKMMPRSSABIJJKKMNSSUWWYSZBIBERTBBERUDDDD | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| GGIFEGREYPBRNUNOOMPBAOLSLLEOOVJLETSSNFP IATTMADOGEBERE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| YTAGWEENOC | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| TTDDUDB | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| BKFGHGNKLEPRUL | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| OKGAUNUCSNNNNA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

* The names of the flaviviruses are abbreviated according to the recommendations of the International Catalogue of Arboviruses (Karabatsos, 1985) and their grouping is based on that of Porterfield (1980). CEE represents central European encephalitis virus.
two cells infected with YF 17D vaccine virus. To test whether or not these fluorescent cells represented wild-type variants within the vaccine pools, the IIF tests were repeated on vaccine preparations but the virus input multiplicity was increased to 1, producing a very high proportion of cells containing YF virus antigen. Eight 17D vaccine pools from different sources were examined in this way by IIF with MAb 117. All preparations contained a small proportion of variants expressing the wild-type epitope. At this stage no attempt was made to quantify accurately the proportion of antigenic variants but at high virus input multiplicities, one or two infected areas were observed on glass coverslips containing approximately $2 \times 10^5$ infected cells.

A search was also made for the MAb 864 17D specific epitope (Gould et al., 1985b) in YF wild-type virus preparations on Vero cells, using an estimated virus input multiplicity of 1. Very occasionally, small areas of MAb 864-positive cells were detected in YF Asibi-infected cultures.
suggesting that the Asibi virus does contain variants equivalent to the YF 17D vaccine virus. In contrast, none of 18 other wild-type YF isolates from either South America or Africa (Gould et al., 1985b) produced evidence of MAb 864 epitopes.

An attempt was then made to isolate a variant from 17D virus that contained the MAb 117 wild-type epitope. A large number of 17D plaques was produced in Vero cells under agarose overlay medium as described above. Well separated plaques were individually recovered in 1 ml aliquots of L15 medium containing 5% foetal calf serum and then grown in Vero cells on glass coverslips. Samples of infectious virus were collected from each dish and stored at −70 °C. The infected cells on coverslips were analysed by IIF for the presence of the MAb 117-reactive antigen. One plaque isolate designated YF 17D-1 produced fluorescence with MAb 117 and the proportion of fluorescent cells was equal to that obtained with a hyperimmune rabbit antiserum against 17D virus, demonstrating that most of the virus present in the preparation contained the wild-type epitope. Moreover, MAb 117 neutralized the infectivity of YF 17D-1 in plaque neutralization tests. This antigenic variant also produced fluorescence with 17D-specific MAb 864 but was negative with vaccine-specific MAb 411, suggesting that in acquiring the wild-type epitope (reactive with MAb 117) the E glycoprotein had lost some but not all of the antigenic characteristics of a vaccine virus. In IIF tests, no other plaques (total 50) produced fluorescence with MAb 117 but all were strongly positive with both MAb 864 and 411.

Attempts were also made, using plaque selection, to isolate variants from YF Asibi virus that possessed the MAb 864 17D vaccine-specific epitope. However, this did not prove possible, despite the fact that the presence of such variants had been demonstrated, albeit at a low level, by IIF.

Eight of the plaque-purified variants from 17D virus (designated 17D-1 to 17D-8) and appropriate control 17D vaccine virus (not plaque-purified) and Asibi virus, were compared for their ability to produce neurovirulent infections in newborn and 3- to 4-week-old (weanling) mice using the intracerebral route of inoculation. Groups of 10 mice were given 1, 10 or 100 p.f.u. of virus. In repeated experiments all the viruses were highly neurovirulent in newborn mice, killing at all three input doses but only the variant possessing the wild-type epitope (YF 17D-1) and the control non-plaque-purified and YF Asibi viruses were equally neurovirulent in weanling mice. None of the other plaque-purified viruses produced more than 40% lethality at any virus input concentration. Nevertheless, serum collected from surviving mice inoculated with the avirulent variants contained virus-specific antibody with a titre in IIF tests of at least 1/1000 implying that infection of the mice had occurred. Furthermore, IIF tests were also performed at 3, 5 and 7 weeks post-infection on smears of brains from mice inoculated with YF 17D-3 (non-virulent). Cells containing virus antigen were detected at each time interval, but no infectious virus was obtained from the brains beyond 3 weeks post-infection. These results imply that the YF 17D-3 virus infects and persists in the mouse brain without killing the mice. The highest yield of virus that could be obtained from mice injected with YF 17D-3 virus was usually not higher than 10^4 LD_{50}/ml of 10% brain suspension and was recoverable on day 10 post-infection. This contrasts with the much higher infectivities recovered from brains of mice infected with the virulent viruses (usually >10^7 p.f.u./ml).

It is now well recognized that YF virus vaccines contain a heterogeneous population of plaques (Liprandi, 1981; PAHO Report, 1984) and that plaque variants can be selected from the 17D vaccine which show differing neurovirulence characteristics in mice (Liprandi, 1981; Barrett & Gould, 1986). In this paper, we have identified antigenic variants that differ in neurovirulence for mice. Whether these changes would be reflected by changes in viscerotropism for primates remains to be seen. Despite the effectiveness and the comparative safety of YF vaccines, occasional cases of encephalitis have been associated with YF vaccination in humans, and different sublines of vaccine have been shown to produce different levels of encephalitis in laboratory monkeys. On the other hand, some batches of vaccine have shown poor immunogenicity, which has largely been attributed to increased laboratory passage levels of these vaccines (WHO, 1981). The 17D vaccine was derived empirically from the Asibi wild-type virus (Theiler & Smith, 1937) and has never been subjected to plaque purification or other selective procedures. Thus, wild-type variants may still be present in 17D vaccine pools and this seems to be supported by our data.
Evidence of a variant in Asibi virus possessing a vaccine-associated epitope was also found. It is interesting that Mr Asibi, the person from whom Asibi virus was isolated, showed only mild clinical symptoms when infected with YF virus (Theiler, 1951). Thus it is perhaps inaccurate to refer to the Asibi isolate as virulent YF virus. By demonstrating the occurrence of vaccine epitopes in Asibi, our results suggest that it may have always contained variants with relatively low virulence for humans. This is also supported by the report that subsequent attempts to reproduce the derivation of a 17D vaccine from other YF isolates failed (Theiler, 1951). This could be explained if the isolates were chosen from virulent human YF infections, since these preparations may not have contained variants of the type described above.

We have previously reported that MAbs prepared against YF 17D vaccine virus show antigenic specificities with flaviviruses ranging from unique to broadly cross-reactive (Gould et al., 1985b). As reported here, this was also the case with MAbs prepared against the Asibi wild-type virus. The epitope represented by MAb 117, however, was detected in all wild-type YF viruses available for analysis but none of the recognized YF vaccines and no other flaviviruses produced significant fluorescence in conventional immunofluorescence tests. Thus MAb 117 can be considered diagnostic for wild-type YF virus.

Evidence of variants in vaccine virus pools possessing the YF virus wild-type epitope was found only when cell monolayers were deliberately infected with relatively high virus input concentrations, suggesting that all or most of the 17D vaccine seed lots contain only a low proportion of antigenic variants with wild-type epitopes. One such variant (YF 17D-1) was more neurovirulent for weanling mice than variants lacking the wild-type epitope (YF 17D-2 to -8). Uncontrolled laboratory passage of such heterogeneous virus preparations or inadvertent selection of plaque variants with altered virulence characteristics could lead to variability in the performance of vaccine seed pools when safety-tested in monkeys.

As yet there is no explanation for the difference in virulence between vaccine and wild-type YF virus. However, our results from this report and previous publications (Gould et al., 1985b; Cammack & Gould, 1986) show that at least four epitopes of the YF envelope glycoprotein (those identified by MAbs 864, 411, 427 and 117) can either be absent, present or relocated in the E protein and also seem to be associated with changes in the virulence of the virus. We have also recently shown by immunoblotting of YF viruses with monoclonal and polyclonal antibodies that significant levels of intact 17D or 17DD E protein fail to accumulate in infected cells and this appears to arise as a result of breakdown of E. In contrast, the wild-type viruses accumulate large quantities of intact E protein (Cane & Gould, 1989).

In view of this, we compared the 17D-1 and 17D-3 plaque variants by immunoblotting under non-reducing conditions but found that both have retained the vaccine characteristic, i.e. the E protein remains labile (unpublished observations) as described by Cane & Gould (1989). Moreover, serial subculture of the 17D seed pool in newborn mouse brains has so far failed to reveal a stable variant equivalent to wild-type virus. It would be interesting to test very early preparations of 17D vaccine (with low serial passage level) to see whether or not they contain variants with wild-type characteristics.

Although it is recognized that the mouse model of YF virus virulence does not mimic the human situation, further investigations seem justified to determine whether MAbs and neurovirulence in weanling mice can be used to identify variants of YF virus that might serve as potential vaccine strains before testing in more expensive laboratory animal systems.

REFERENCES


Short communication


(Received 12 December 1988)