Gonorrhoea in the chimpanzee Serological testing

W. JERRY BROWN AND CHARLES T. LUCAS

Venereal Disease Research Section, Bacteriology Branch, Laboratory Division, Center for Disease Control, Health Services and Mental Health Administration, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia 30333, U.S.A.

This laboratory, with a special interest in the serodiagnosis of gonorrhoea, has become active in all aspects of serological testing of animal models as well as humans. Lucas, Chandler, Martin, and Schmale (1971) established the chimpanzee as the first animal model for *Neisseria gonorrhoeae*. They infected four male chimpanzees with human urethral exudates containing *N. gonorrhoeae*. Brown, Lucas, and Kuhn (1972) infected a male chimpanzee with an *in vitro* inoculum of *N. gonorrhoeae* and established the venereal transmission of that infection from male to female chimpanzee. Sequential serum samples were obtained from these six infected chimpanzees and tested for gonococcal antibody by a variety of serological assays.

Material and methods

The chimpanzees (*Pan troglodytes*) were bled approximately every 7 days. The sera were separated and stored in sterile vials at -70°C . until use.

One chimpanzee (JD) developed a unilateral purulent conjunctivitis as a result of autoinoculation on the 25th day. He was treated topically with a mixture of polymyxin B sulphate, neomycin sulphate, and bacitracin zinc (Neosporin Ophthalmic Ointment*) (Lucas and others, 1971). This same animal received 2.4×10^6 units aqueous procaine penicillin 8 weeks after infection, and was then re-infected at 21 weeks by the same procedure as that used by Lucas and others (1971).

The following serological procedures were used for the detection of gonococcal antibody:

- (1) Semiautomated complement-fixation test (Peacock, 1971).
- (2) Indirect fluorescent antibody (IFA) test for gonococcal antibody (Welch and O'Reilly, 1973).
- (3) Microflocculation test (Reising, 1971).
- (4) Sediment flocculation test (Lee and Schmale, 1970).
- (5) Semiautomated microhaemagglutination test (Logan, Cox, and Norins, 1970).

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*The use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

Results

Complement-fixing antibodies were first recorded 14 to 25 days after infection (Table). Complement-fixation (CF) reactivity was recorded up to 97 weeks in chimpanzees JD and RN, 63 weeks in LG, 54 weeks in LS, and 24 weeks in CH. JQ, the only female chimpanzee, never developed a positive reaction. Titres ranging from 2 to 64 were recorded (Fig. 1).

FIG. 1 Semiautomated complement-fixation test titres

TABLE Day of first recorded positive culture and reactive serology in six chimpanzees infected with *Neisseria gonorrhoeae*

Chimpanzee	Culture	Serological test				
		Semiautomated complement-fixation	Indirect fluorescent antibody	Microfloculation	Sediment flocculation	Semiautomated microhaemagglutination
JD	3	14	24	149	17	14
JD*	7	con't	con't	con't	ND	24
RN	3	25	30	175	24	95
LG	4	20	20	111	20	91
LS	21	15	NR	125	NR	35
CH	3	21	15	NR	ND	28
JQ	5	NR	18	88	ND	77

JD* = Treated and re-infected NR = Non-reactive ND = Not done Con't = Continued reactivity

With the IFA procedure for gonococcal antibodies, sera became reactive 15 to 30 days after the chimpanzees were inoculated (Table). One male chimpanzee (LS) never exhibited a positive IFA reaction. Sera of three animals (JD, RN, and LG) have been reactive up to 56 weeks, and sera of two animals (CH and JQ, with a shorter observation time) have remained reactive for up to 14 weeks. Titres up to 512 were recorded (Fig. 2).

Sera from five of the animals became reactive in the microfloculation assay between 88 and 175 days after the animals were inoculated (Table). One

animal (CH) never developed enough antibody for a reactive test. None of the sera were consistently reactive. The longest period of reactivity occurred in sera from JD and RN and lasted for 12 weeks (Fig. 3, opposite).

The sediment flocculation test was performed on sera from four of these animals (JD, RN, LG, and LS). Reactive tests were first recorded 17 to 24 days after inoculation (Table). The sera from one animal (LS) never became reactive. This test was used for only 18 weeks (Fig. 4, opposite).

The semiautomated microhaemagglutination procedure detected a one to three dilution rise from 2 to 6 weeks in sera from JD, and a one to two dilution increase in sera from the other five animals within 16 weeks (Fig. 5).

Discussion

All pre-inoculation cultures and sera from the chimpanzees were negative for *N. gonorrhoeae* and *N. gonorrhoeae* antibodies. Sera from the first four chimpanzees (JD, RN, LG, and LS) were reported by Lucas and others (1971) as having the first CF reactivity between 14 and 25 days after inoculation with titres as high as 64. It is interesting that CF antibodies were detected in sera from LS 6 days before his culture became positive. In the two chimpanzees infected with the *in vitro* strain of *N. gonorrhoeae*, CH had developed CF antibodies by 21 days after inoculation, with titres up to 8, and a positive CF test result was never obtained for JQ. CF antibodies were detected in the serum from CH about the same time that they were detected in sera from the previous four chimpanzees receiving the human exudate inoculum; the titre, however, was much lower. CH was inoculated approximately 9 months after the first four animals had been inoculated. This explains the shorter observation period. JQ was venereally infected by CH within 5 days after their cohabitation (Brown and others, 1972).

An initial rise in titre was observed between 2 and

FIG. 2 Indirect fluorescent antibody titres

FIG. 3 Microflocculation test results

4 weeks in the five animals which developed CF reactivity. All titres then declined only to rise again from 1 to 4 dilutions. JD was re-infected (at 21 weeks) as his serum titre was beginning a second rise. The titre continued to rise after re-infection.

Five chimpanzees developed antibodies which were detectable in the IFA test. The exception was LS whose serum was tested repeatedly, but never became reactive. His culture, however, remained positive for 27 weeks. This long period of infectivity might have been due to the fact that he was caged with a female chimpanzee that he infected. She is not included in this study, but could have been responsible for passing the organism back to LS. Gonococci from LS's own culture were used as antigen in the IFA test, but it failed to react with his serum sufficiently for the test to be called reactive. Tests are read as reactive at a 16 dilution or higher (Welch and O'Reilly, 1973). Titres were measured on only three animals for 15 weeks. The highest titres were recorded between 3 and 6 weeks after inoculation (Fig. 2).

Once the sera became reactive in the IFA test, they remained so. The last observations recorded on JD, RN, and LG showed that their sera were still reactive 56 weeks after inoculation (Fig. 2). Sera from CH and JQ were still reactive 14 weeks after inoculation (a shorter observation period). In two cases, the IFA test became reactive only a few days after the CF test.

In the microflocculation assay for detecting gonococcal antibody, sporadic weak reactions occurred in sera from five animals before the tests actually became reactive (Fig. 3). One chimpanzee, CH, never developed enough of this particular antibody to give a reactive test. Reising (1971) found 49.6 per cent. reactivity in human males with clinically diagnosed uncomplicated gonorrhoea and

FIG. 4 Sediment flocculation test results

79 per cent. reactivity in females, so that 50.4 per cent. of the infected males and 21 per cent. of the infected females were seronegative. Such may be the case in chimpanzee studies because of the length of time it takes for a detectable amount of this antibody to appear. The earliest detection of this antibody was 88 days after infection in JQ, the female.

The sediment flocculation test produced results comparable to those of the CF test for the earliest serological detection of gonococcal antibody. This

FIG. 5 Semiautomated microhaemagglutination test titres

test, however, has the disadvantage of not being able to be titred. Sera from only the first four chimpanzees (JD, RN, LG, and LS) were tested for 18 weeks after inoculation (Fig. 4).

The semiautomated microhaemagglutination procedure showed a small rise in serum titres in all animals after infection (Fig. 5).

In sera from one chimpanzee (JD), that was treated and re-infected, a threefold rise in titre was observed; this was followed by a decline to the normal baseline, and then a second threefold rise occurred which corresponded with the time of re-infection. For the other animals, the titres never rose more than two-fold. The baselines vary in the chimpanzees' sera because of different levels of nonspecificity in the test reactions. When absorbed with *N. sicca*, the sera had no reactivity.

B. B. Diena (1971) at the Canadian Communicable Disease Center examined some of our chimpanzee sera with a bentonite-flocculation procedure. He found a rise and decline in titre which will be reported elsewhere. T. M. Buchanan (1972) at Rockefeller University also found a similar rise and decline in quantitative measurements by using a radioimmune assay which measured 'anti-pili' antibody.

This study illustrates the fact that antigonococcal antibodies may be detected in the absence of a positive culture. Clearly, the time of examination is crucial because the culture may be the only positive result with non-reactive serological tests; the culture may be positive with one or more serological tests reactive; or the culture may be negative with one or more of the serological tests reactive (Fig. 6).

Although these serological procedures are designed for testing human sera, it seems that chimpanzee sera react very similarly. More sera, preferably human, must be tested in all new procedures as they are developed and modified in order to discover the earliest and most specific reaction.

Summary

Sera from chimpanzees infected with *Neisseria gonorrhoeae* from human exudate or *in vitro* passed gonococci became reactive earliest in the complement-fixation test, the indirect fluorescent antibody test, and the sediment flocculation test. All tests continued to show positive reactions in the absence of a positive culture.

The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

FIG. 6 Summary of reactive results of cultures and serological tests

CF Semiautomated complement-fixation test

IFA Indirect fluorescent antibody test

Microfloc Microflocculation test

Sed-floc Sediment flocculation test

MHA Semiautomated microhaemagglutination test

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Gonococcie du chimpanzé: épreuves sérologiques

SOMMAIRE

Les sérums de chimpanzés infectés par *Neisseria gonorrhoeae* à partir du pus humain ou à partir de gonocoques

entretenus *in vitro* devinrent très rapidement positifs pour les tests de fixation du complément, le test de l'anticorps fluorescent en technique indirecte et l'épreuve de floculation. Tous les tests gardèrent leur positivité en l'absence de culture positive.