JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1975, p. 345-352

Vol. 1, No. 4
Printed in U.S.A.

Fluorescent Antibody Studies in Chlamydial Infections

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Received for publication 11 December 1974

Irradiated McCoy cells infected with genital strains of *Chlamydia trachomatis* were grown in wells on slides coated with polytetrafluoroethylene. The inclusions produced in this system formed the antigen in an indirect immunofluorescence test, which detected group-specific chlamydial antibodies in sera from patients with psittacosis, as well as type-specific antibodies to *C. trachomatis* in sera from patients attending venereal disease clinics. Chlamydial antibodies were found more frequently and in higher titer in sera from women attending venereal disease clinics than in sera from a less promiscuous population attending a Family Planning Association clinic. Paired sera from 13 patients with non-gonococcal urethritis from whom chylamydiae had been isolated were tested against the homologous isolates; seroconversion was demonstrated in only one instance, and antibody was present in the first serum specimens of all the other patients. Chlamydia-specific immunoglobulin M was found in four of eight patients with psittacosis and in a proportion of sera from patients attending veneral disease and Family Planning Association clinics. The antigen for this immunofluorescence test can easily be prepared in laboratories with cell culture facilities for the isolation of *C. trachomatis*, and the test should be useful for laboratories which cannot undertake the micro-immunofluorescence test.

Infections with any member of the chlamydial genus may stimulate antibody production against the polysaccharide component which is common to both *Chlamydia psittaci* and *Chlamydia trachomatis* (5). Antibodies to this group antigen can be detected by the complement fixation (CF) test or the more sensitive but technically more complicated radioisotope precipitation test (8).

C. trachomatis can infect the eye and genital tract in man, but these infections (apart from the strains which cause lymphogranuloma venereum) are usually limited to the superficial epithelial surfaces and do not become systemic. This may explain why the antibody response to the group antigen in these trachoma-inclusion conjunctivitis (TRIC) agent infections, measured by the conventional CF test, is often weak or absent. Moreover, the demonstration of group antibodies does not by itself distinguish either between infections with *C. psittaci* and *C. trachomatis*, or between infections with the different strains of *C. trachomatis*. For these reasons, measurements of group antibodies alone in *C. trachomatis* (TRIC type) infections have proved to be of only limited value (19).

Type-specific antibodies against the different strains of *C. trachomatis* were originally demonstrated by the laborious mouse toxicity prevention test (1), which has recently been superceded by the micro-immunofluorescence (MIF) test of Wang and Grayston (21). In the latter test, yolk sac-grown elementary bodies of the known serotypes of *C. trachomatis* are placed in order in groups in slides so that 1 drop of serum can be placed on top of each group of antigens. Antibody reactions to each different serotype can then be measured by indirect immunofluorescence (IF). This test has been used both for serotyping new isolates of *C. trachomatis*, when early type-specific antibodies raised in mice are used (20, 23), and for measuring antibody responses to the different chlamydia serotypes in human sera and tears from patients with ocular or oculo-genital TRIC infections (7, 9). These workers found this test was both sensitive and specific since human antibodies directed against particular strains of *C. trachomatis* could be demonstrated. More recently, it has been shown that this (MIF) test can detect both group and type-specific antibodies in human sera from patients attending a venereal disease (VD) clinic (15).

However, the MIF test is an elaborate technique for many laboratories to perform. The purpose of this study was to devise an alternative indirect IF test, using as antigen the whole chlamydial inclusion rather than suspensions of elementary bodies. Irradiated McCoy cells infected with *C. trachomatis* (genital TRIC

strains) were grown in wells on slides coated with polytetrafluoroethylene (PTFE). Acetone-fixed preparations of these cells were found to be capable of detecting both group and type-specific antibodies. This test was then used in a study of the serological responses of patients to genital chlamydial infections.

MATERIALS AND METHODS

Chlamydial strains. The inclusions formed by a genital TRIC agent, T181, when grown in irradiated McCoy cell cultures, were used as antigen in all the experiments reported here, except for the tests on paired sera from 12 patients suffering from non-gonococcal urethritis (NGU) from whom chylamydiae were isolated [see sera (iv) below]. Each of these pairs of sera was tested against the inclusions of homologous isolate, and for pair 13, T181 was itself the homologous isolate.

Primary isolation of both T181 and these other genital TRIC agents was made in irradiated McCoy cell cultures (17) from material obtained by swabbing the urethra. Infectivity was increased after primary isolation by further passage in irradiated McCoy cell cultures. Pools of each isolate made from heavily infected monolayers stored at −70 C in 10% sorbitol (17) were used in this study.

T181 was found to be a TRIC E serotype in the MIF test by J. Treharne, Institute of Ophthalmology, London, England. The 12 other strains used were not typed.

Slides. Chance slides, 0.8 to 1 mm in thickness, were thoroughly cleaned and stored in methanol. To prepare wells for the cells, the slides were flamed, and small, flat, sterile nail heads were placed flat side downwards on the slides, which were then sprayed with PTFE (Fluoro-glide, Chemplast Inc., N.J.). The resultant wells were about 4 mm in diameter. The usual arrangement consisted of 10 wells per slide arranged in two rows of five, about 1 cm between each well. The slides were then stored at 60 C until used.

Towards the end of this study, commercially prepared PTFE-coated slides with similar wells became available from C. A. Hendley & Co., Buckhurst Hill, Essex, England, and were used instead. These were rinsed in methanol, flamed, and stored at 60 C before use.

Preparation of antigen slides. Irradiated McCoy cell cultures, grown on 12-mm diameter cover slips in flat-bottomed plastic vials, 150,000 cells per tube, were inoculated with one of the genital TRIC agent pools described above at a dilution calculated to yield between 1,000 and 2,000 inclusions per cover slip. Tubes were centrifuged at about 2,500 × *g* for 1 h on an MSE super medium centrifuge, then incubated at 35 C. Later (42 to 44 h), the cover slip from one tube was fixed in methanol and stained with iodine to check that sufficient inclusions were present. The cells from the remaining tubes were removed from the cover slips by trypsinization, suspended in McCoy cell growth medium (17), and centrifuged at 1,000 rpm for 5 min on a bench MSE centrifuge. The pellet of cells was resuspended in growth medium, at three times the original cell concentration, to give an estimated cell concentration of 450,000 cells/ml. (Cell counts were not carried out at this stage.) The cells were gently dispersed in the medium with a Pasteur pipette, and drops of this cell suspension were then placed in the wells on the PTFE-coated slides. One large drop of cell suspension was added to each well, and 1 ml of cell suspension provided sufficient cells for 30 to 40 wells. The estimated number of cells per well was therefore between 11,000 and 15,000.

The slides were placed in moist boxes (17 by 11.5 by 6 cm), sufficient CO_2 was added to the atmosphere in the box to maintain the pH of the medium at about 7.0, and the box was sealed and incubated at 35 C. About 8 h later the slides were removed and rinsed once in phosphate-buffered saline, pH 7.2. One slide from each batch was fixed in methanol and stained with Giemsa to check the quality of the cell preparation and the amount of antigen (i.e., the number of inclusions) present. The remaining slides were fixed in acetone and stored at −70 C if they were to be kept for some months before use, or at −40 C if they were to be used within a few weeks.

Sera. The following groups of human sera were tested: (i) 16 sera from eight patients known to have complement-fixing antibodies to the chlamydial group antigen, in whom the diagnosis of psittacosis was likely on serological and clinical grounds. (ii) Sera from 58 female patients who attended a Family Planning Association (FPA) clinic for contraceptive advice. Chlamydiae were not isolated from any of these patients, and they were not suffering from other sexually transmitted infections. (iii) Sera from 116 female patients who attended VD clinics. Chlamydiae were isolated from 58 of these patients (chlamydia-positive patients) and a proportion were also found to be suffering from other sexually transmitted infections. (iv) Paired sera from 13 chlamydia-positive male patients who attended VD clinics suffering from NGU. Each pair was tested against the homologous isolate. For comparison, paired sera from 13 men with NGU and from whom chlamydiae were not isolated (chlamydia negative) were tested against T181. Each first serum specimen was obtained at the patient's initial attendance at the clinic, when urethral swabs for chlamydia isolation were taken, and the second specimen was usually collected 2 weeks later, after the patient had had a course of tetracycline. The patients with chlamydia-negative NGU were matched as far as was possible with the chlamydia-positive patients for the number of days between onset of urethritis and the collection of the first serum specimen.

The patients from groups (ii), (iii), and (iv) formed part of a larger group of patients who were studied between 1971 and 1973 (10, 18).

All sera were obtained between 2 months and 3 years before the present test were separated within 24 h of being taken and stored at −20 C.

Indirect IF test for chlamydia-specific immunoglobulins (Ig). Twofold dilutions of the test sera were made in microtiter plates (Cooke Engineering Co. Ltd.). Phosphate-buffered saline was used as diluent. One drop of appropriate serum dilution was placed on each well of an antigen slide which was then incu-

bated in a moist container for 1 h at 37 C. It was then washed thoroughly in phosphate-buffered saline on a Luckham mechanical shaker for 10 min and air-dried thoroughly, and fluorescein-conjugated, rabbit anti-human globulin (Wellcome Research Laboratories, Beckenham, England), previously standardized for optimal staining, was added at 1 drop per well. The slide was then re-incubated for 1 h at 37 C, washed twice in fresh phosphate-buffered saline (10 min each wash), mounted, and read using a Reichardt Zetopan microscope with a wide-field dark-ground condenser, a HBO200 mercury vapor lamp, a Reichardt E2 interference filter, and SP2 barrier filter.

Fluorescence was graded nil to +++. Faint but definite fluorescence, mainly around the inside of the margin of the inclusion, was taken as the end point. Slides were prepared by one of us (S.J.R.) and read blind by the other (E.O.C.). All sera were originally screened at dilutions of 8 and 16, and subsequently were titrated out to appropriate dilutions if necessary. Titers of <8 were regarded as negative. Positive and negative serum controls were included with each batch of antigen slides used.

Detection of chlamydia-specific IgM in IF test. The procedure was as described in the IF test above, except that a fluorescein-conjugated swine anti-human IgM (Nordic Pharmaceuticals & Diagnostics, distributed by Fraburg Ltd., Maidenhead, Berks., England) was used. Serum from a patient with psittacosis which consistently gave fluorescence in this system was fractionated on a sucrose-density gradient (2) and both 7*S* and 19*S* fractions were collected. The 19*S* fraction stored at 4 C was used as the positive IgM control. This fractionated serum was also used to test the specificity of the conjugate (see Results).

In addition, IgM was looked for in all sera from groups (i) and (iv), and in 60 sera from groups (ii) and (iii) by screening unfractionated sera at 1/8. Any serum in which IgM was demonstrated was tested for rheumatoid factor by the latex agglutination test, to exclude false positive reactions. Rheumatoid factor was not demonstrated in any of these sera.

CF test. A 4-volume (0.025 ml/volume) micromethod with overnight fixation at 4 C was used. Antigen (the agent of ovine chlamydial abortion, supplied by Standards Laboratory, Central Public Health Laboratory, Colindale, UK) was diluted to its optimal peak dilution and complement (Richardson's preserved) diluted to contain 3 U (HD_{50}) for use. Sera from groups (i) and (iv) and 42 sera from groups (ii) and (iii) were tested in the CF test. All sera were tested against normal yolk sac antigen to detect possible false positive reactions, and sera from the same patients were always tested in parallel. Titers were expressed as reciprocals of the serum dilutions giving 50% fixation.

RESULTS

Growth of irradiated McCoy cells on slides. Suspensions of McCoy cells grown in wells on PTFE-coated slides attached to the glass and flattened out within 6 to 8 h. This occurred both with uninfected cells and with cells containing chlamydial inclusions. About 100 inclusions per well, which gave several inclusions per field at times 400 magnification, provided sufficient antigen for the IF test, and uninfected McCoy cells acted as control cells for the system. In practice, it was often possible to achieve larger amounts of antigen (up to 400 inclusions per well) without producing the cytopathic effect which occurs when irradiated McCoy cells are heavily infected with chlamydiae and which would impair the quality of the cell monolayers in these preparations.

Appearance of fluorescence. Inclusions in cells harvested at about 52 h post-inoculation appeared as discrete, brightly fluorescing bodies in the IF test when positive sera were used, and were easily detected at times 400 magnification against a background of nonfluorescing, uninfected cells. The distinctive morphology of the inclusion provided a check on the specificity of fluorescence in this system. The source of fluorescence seemed to be the elementary bodies within the inclusion, rather than the matrix, for the same regular granularity which is seen when inclusions are Giemsa stained was also observed in this fluorescence system.

Detection of group-specific antibodies in sera from patients with psittacosis. The tests on sera from patients with psittacosis showed that the IF test was capable of detecting group-reactive antibodies, and that the titers obtained were comparable to those obtained in the CF test (Table 1). Specific IgM was also found in the sera from four of these eight patients with psittacosis.

Specificity and sensitivity of the test for IgM. The specificity of the IgM conjugate used in this study was demonstrated by (i) the absence of any fluorescence in the 7*S* fraction of the second serum from patient SA, Table 1, and (ii) the absence of fluorescence in the 19*S* fraction of the same serum when this was pretreated with 2-mercaptoethanol (2).

The unfractionated serum from patient SA also demonstrated specific IgM, and incubation of this with antigen for 2 or 3 h at 37 C, or at 4 C overnight, did not affect the IgM titer when compared with that obtained after 1 h of incubation at 37 C. There was thus no evidence that the IgG antibodies present in this serum reduced the reactivity of the IgM antibodies by steric interference, unlike results found in sera from monkeys inoculated intramuscularly with chlamydial suspensions (12).

Paired sera from patients with chlamydia-positive NGU. A primary infection with *C. psittaci* in humans is accompanied by sero-con-

TABLE 1. *Chlamydial antibody titers in patients with psittacosis, measured by the IF and CF tests*

Patient	Age	Sex	Clinical details	Date serum collected	CF test titer	IF test	
						Ig[a] titer	IgM present[b]
KN	69	M	Pyrexia. Chest infection. Kept pigeons.	7.9.72	8	32	No
				21.9.72	128	512	Yes
				29.11.72	1,024	2,048	NT
SA	44	F	Influenza-like illness and cough 6 weeks previously. Erythema multiforme on 8.2.74	8.2.74	512	128	Yes
				21.3.74	256	256	Yes
				18.4.74	256	256	Yes
GW	44	M	Bronchopneumonia. Sick parrot.	27.10.72	<8	8	No
				8.11.72	32	64	No
BU	56	M	Pericarditis. ?Myocardial infarction.	13.10.72	<8	<8	No
				24.11.72	32	32	No
				16.11.72	16	16	No
CP	50	M	Acute myocarditis	18.2.74	<8	<8	No
				19.3.74	32	32	No
				2.4.74	64	32	No
NL	28	F	Died of pneumonia. Other members of family also ill. Kept psittacine birds.	10th day of illness	128	128	Yes
HW	60	F	Pyrexia and cough. Kept parrot and budgerigars.	13.8.74	256	128	No
				23.8.74	>512	128	No
CL	43	M	Ill for 6 weeks with pyrexia and cough. Kept an aviary.	29.7.74	128	64	Yes

[a] Ig, Chlamydia-specific immunoglobulin detected with fluoroscein-conjugated rabbit anti-human globulin.
[b] NT, Not tested.

version, chlamydial antibodies being absent in serum collected early in the illness, and later specimens demonstrating increasing titers of antibody. It might therefore be expected that paired sera collected from patients with NGU at appropriate times in the illness would demonstrate sero-conversion if a primary infection of the urethra with *C. trachomatis* were associated causally with NGU. In 12 of 13 paired sera from chlamydia-positive cases of NGU, the first serum specimens were collected up to 7 days after onset of the urethritis and in only one pair was sero-conversion demonstrated in the IF test (Table 2, patient no. 7). All other first specimens had antibodies to the homologous isolate, even when the serum was taken on the day of onset of the urethritis (Table 2, patient no. 1). A significant rise of antibody was found in only two of the 13 patients. Patient number 1 showed a fourfold rise from 8 to 32, and number 7 demonstrated a rise from <8 to 16. Specific IgM was demonstrated in 8 of 13 of these patients and in three of these it was present only in the second specimen. Four patients had a previous history of urethritis (Table 2) but their antibody levels did not appear to differ from the pattern found in the nine patients with no past history of NGU.

Paired sera from patients with chlamydia-negative NGU. Chlamydial antibodies were low or absent in paired sera from 12 patients with chlamydia-negative NGU, both in the IF test (when T181 was used as antigen) and in the CF test. In patient 13, the titer rose from 64 to 256 in the IF test and IgM was present in the second serum specimen. It is possible this was a case of chlamydia-positive NGU in whom isolation failed. None of the three chlamydia-negative patients with a previous history of NGU had antibodies to T171 in their sera.

Sera from patients attending FPA and VD clinics. Table 3 shows the distribution of antibody titers against T181 found in the IF test in sera from female patients attending FPA clinics and VD clinics. There was a significant difference in antibody distribution in sera from patients attending FPA clinics, where the titer in 64% of the patients was <8, compared with the sera from patients attending VD clinics ($P < 0.001$). In the latter, 47% of chlamydia-negative patients and only 12% of chlamydia-positive patients had a titer of <8. The geometric mean titer in sera where the chlamydial antibody titer ⩾8 was lower in the FPA clinic patients than it was in either the chlamydia-negative or the chlamydia-positive VD clinic

patients. Genital chlamydial infections are sexually transmitted, and these VD clinic patients were shown previously to be more promiscuous than these FPA clinic patients (10). The serological results reflect the greater incidence of chlamydial infections in the more promiscuous population.

Specific IgM was not found in 14 sera with a total specific globulin titer ≤ 32; nine of these sera were from chlamydia-positive patients and five from chlamydia-negative patients. It was demonstrated in 20 of 30 sera from chlamydia-positive patients and in 8 of 16 sera from chlamydia-negative patients, where the total specific globulin titer was ≥ 64.

Forty-two sera with total specific globulin titers ≥ 64 in the IF test were also tested in the CF test, and a very wide variation in titers between the two tests was found (Fig. 1). In eight sera (19%), the CF and IF titers were not significantly different, but in 27 sera (64%) there was more, and often much more, than a fourfold difference between the IF and CF titers in any one serum, the CF titer being consistently the lower. The lack of correlation between the IF and CF titers of these sera, compared with the generally good correlation obtained with sera from patients with psittacosis (Table 1), is probably due to type-specific chlamydial antibodies present in variable amounts in the sera from patients attending VD clinics.

DISCUSSION

At present little is known about serological reactions to genital chlamydial infections. It is important, as these data are accumulated, to know what each test can detect. The CF test and radioisotope precipitation test, both of which use *C. psittaci* organisms as antigen, will measure only group-reactive antibodies. The IF tests, whether using yolk sac suspensions of elementary bodies or whole chlamydial inclusions grown in cell culture as antigen, use *C. trachomatis* serotypes and may therefore be capable of detecting both group- and type-specific antibodies.

TABLE 2. *Chlamydial antibody titers found in IF and CF tests in paired sera from chlamydia-positive cases of NGU*

Patient no.	Days since onset of urethritis when serum was obtained	Past history of urethritis	CF test titer	IF test[a]	
				Ig[b] titer	IgM present
1	0	No	<8	8	No
	15		<8	32	No
2	2	Gonorrhoea several	<8	128	Yes
	17	months ago	<8	128	Yes
3	2	No	<8	32	No
	35		<8	32	No
4	3	No record	16	32	Yes
	17		32	32	Yes
5	4	No	<8	16	No
	18		<8	32	Yes
6	6	No	<8	16	No
	13		<8	32	Yes
7	7	NGU 30 years ago	<8	<8	No
	21		<8	16	No
8	7	NGU 1 year ago	16	32	No
	19		8	32	Yes
9	7	NGU 15 months ago	16	16	Yes
	21		16	16	Yes
10	7	No	<8	16	No
	21		<8	16	No
11	7	NGU 3 years ago	16	32	No
	21		16	32	Yes
12	14	No	Anticomplementary	32	Yes
	28		Anticomplementary	32	Yes
13	[c]	No	32	128	Yes
	[c]		64	256	Yes

[a] Each serum pair was tested against a homologous isolate.
[b] Ig, Chlamydia-specific immunoglobulin detected with fluorescein-conjugated rabbit anti-human globulin.
[c] Not complaining of a urethral discharge. Second serum obtained 14 days after first.

TABLE 3. *Distribution of chlamydial antibody in sera from patients attending VD and FPA clinics, measured in IF test*

Source of sera	No. of sera	Titer									Mean titer[a]
		<8 (%)	8	16	32	64	128	256	512	1024	
Chlamydia-negative patients attending FPA clinic	58	37 (64)	13	5	0	1	0	0	2	0	15.49
Chlamydia-negative patients attending VD clinics	58	27 (47)	9	7	2	6	0	4	2	1	36.75
Chlamydia-positive patients attending VD clinics	58	7 (12)	6	12	2	4	8	10	5	4	78.75

[a] The geometric mean titer of patients with a titer >8.

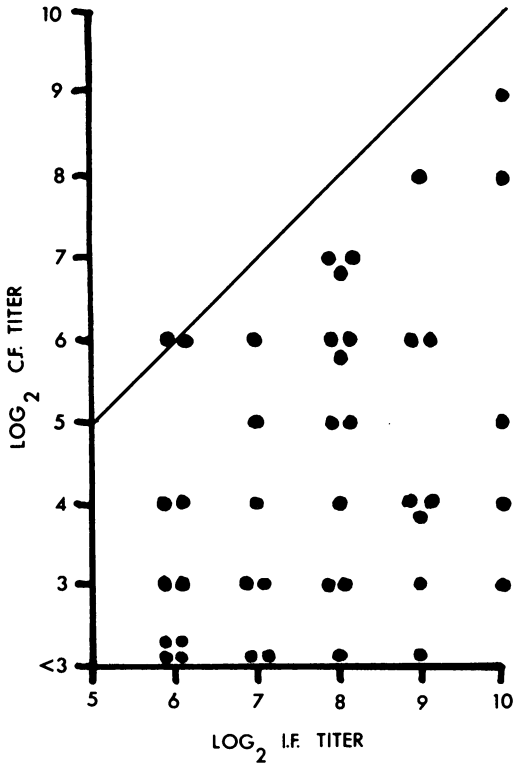

FIG. 1. *Comparison of chlamydial antibody titers found by CF and IF tests in 42 sera from patients attending VD and FPA clinics. Only sera with titers >64 in the IF test are compared. Each point on the diagram refers to one serum. Sera with the same titers in the IF and CF tests would fall on the line shown in the diagram.*

The chlamydial group antigen has been fairly well characterized (5) and located within the elementary body cell wall (4). Much less is known about the type-specific antigens, but like the group antigens they are acetone stable (6). Therefore, both type and group antigens should theoretically be present in acetone-fixed elementary body preparations.

The MIF test, when used for serotyping chlamydial isolates, detects type-specific reactions by utilizing the type-specific nature of early antibody raised in mice against the different chlamydial strains. When the MIF test is used for testing human sera, it is likely that it will also detect any group antibody present. Type-specific reactions have been demonstrated in human sera with this test; for instance, in Tunisian children with active trachoma (9) and in sera from patients with trachoma in Iran and Tunisia (7). But both these groups of workers also found sera, particularly from patients with oculo-genital chlamydial infections and lymphogranuloma venereum, which reacted broadly with all the antigens in the MIF test, and it is likely that these results reflected the combination of group- and type-specific antibodies that were present in the sera. Recently Philip and his colleagues, testing sera from patients with lymphogranuloma venereum and urethritis in the MIF test, have shown that the group antibody present can be absorbed out with heterologous *C. trachomatis* serotypes (15).

The IF test used in this study was as sensitive as the CF test in detecting group-reactive antibodies in serum from patients with psittacosis, and it is suggested that the greater discrepancies between these two tests found in many of the sera from VD clinic patients were due to type-specific antibodies which reacted with T181 in the IF test. The difference between the titer found in the IF test compared with those found in the CF test will therefore depend on the proportions of group- and type-specific antibodies present in any serum.

The whole chlamydial inclusion has been

used as antigen previously, in both direct and indirect immunofluorescence tests, for the diagnosis of TRIC agent infections; the inclusions can be recognized in scrapings of conjunctival and genital epithelium (19), and in cell culture (13), with hyperimmune animal sera. Antibodies to chlamydiae in human sera have also been measured in tests that use whole inclusions as antigen; for these, monolayers of cells infected with chlamydiae grown on cover slips have been used (3). The method of growing chlamydia-infected McCoy cells on slides which was used in the present study is technically very much easier than handling infected monolayers on cover slips, and the testing of large numbers of sera becomes possible. This IF test also has certain advantages over the MIF method: the inclusion is a more convenient size for use in a fluorescence test than individual elementary bodies; antigen slides can be prepared from cell cultures without the use of fertilized eggs; preparations of a new isolate can usually be obtained on slides within 2 to 3 weeks of primary cultivation; and by testing sera from patients against the homologous isolates, the need for serotyping the isolate can be avoided.

T181, the strain used as antigen in most of this work, is a TRIC E serotype of *C. trachomatis*. The D and E type TRIC agents, which appear to be very similar antigenically when serotyped in the MIF test (21, 23), are together the most common genital strains found in England (7) and they both provoke a similar antibody response in humans. Screening sera against an E serotype in the IF test, in addition to measuring any chlamydial group antibody that is present, should also detect any type-specific antibody against the D and E strains. Moreover, the frequency and titer of antibodies to serotype E should reflect fairly reliably the distribution of antibodies to genital chlamydial infections in general, in different populations, and in different clinical states.

The data obtained when paired sera from chlamydia-positive NGU patients were tested against the homologous isolates provided little evidence that a primary chlamydial infection is the initial cause of NGU, since sero-conversion was demonstrated in only 1 of 13 patients. The existence of antibodies at the time the first serum specimen was taken, even when this was obtained early in the attack of urethritis, suggests either that the chlamydial infection had existed sufficiently long before symptoms developed to allow antibodies to develop, or that a previous silent chlamydial infection had occurred which caused a rise in antibody without producing any symptoms (since eight of these patients, all with antibody in their first serum specimens, had no past history of any NGU). These findings do not conflict with the theory that was put forward to explain the results of earlier studies of chlamydial isolations in patients attending VD clinics. This postulated that chlamydiae may form chronic, often quiescent, infections in the human genital tract which may be reactivated by urethritis in the male (18) and by gonorrhoea or oral contraception in the female (10).

Chlamydial IgM was found in sera from four patients with psittacosis; this IgM is therefore likely to be group specific. It was also found in a proportion of the sera from patients attending VD clinics, and we did not determine whether this was type or group reactive. Philip and his colleagues, however, showed that the IgM present in sera from patients with urethritis was type specific, since it was reactive only against specific strains, rather than against the whole range of serotypes used in the MIF test (15). It therefore seems likely that IF test are capable of detecting both type and group IgM if the appropriate strains are used as antigen.

Specific IgM has not been found in serum or tears from patients with experimentally induced inclusion conjunctivitis (11), but it has been reported previously in sera from patients attending VD clinics (15, 16). The significance of this IgM is not yet clear; it may indicate a primary infection with a particular *C. trachomatis* strain, as is suggested by some of the work on experimental infections in animals (22). However, in other experiments, when killed suspensions of elementary bodies were injected intravenously at intervals into rabbits, specific IgM was demonstrated (by CF tests on fractionated sera) after each challenge, not only after the first injection (14). On the basis of these results, these workers suggested that chlamydiae provoke an antibody response which is similar to that produced by gram-negative bacteria and rickettsiae, where IgM production persists beyond the acute primary stage of infection. The relevance of these findings in animals, which have been experimentally infected or inoculated with chlamydiae, to the host response in the naturally occurring chlamydial infections in man has yet to be determined; our results, which demonstrated IgM in 8 of 21 sera from female patients who were chlamydia negative, as well as in 20 of 39 sera from chlamydia-positive patients, perhaps support the concept of a persistent, rather than a transient, production of IgM in some genital chlamydial infections.

The IF test described in this study should be

useful in laboratories which need a sensitive method of detecting antibodies to chlamydiae, but which cannot undertake the MIF test. It will detect both group-specific chlamydial antibody and also type-specific antibodies against whatever particular strain is used as antigen.

ACKNOWLEDGMENTS

We are grateful to A. L. Hilton, J. D. Milne, and F. Hindley for their clinical collaboration, to C. M. P. Bradstreet for the CF test antigen, and to Suzanne K. R. Clarke for much helpful criticism and advice.

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