INFECTION AND IMMUNITY, Nov. 1985, p. 595–597
0019-9567/85/110595-03$02.00/0
Copyright © 1985, American Society for Microbiology
Vol. 50, No. 2

Neutralization of *Chlamydia trachomatis* Cell Culture Infection by Serovar-Specific Monoclonal Antibodies

MARIANA E. LUCERO AND CHO-CHOU KUO*

Department of Pathobiology, University of Washington, Seattle, Washington 98195

Received 28 May 1985/Accepted 17 August 1985

Seventeen monoclonal antibodies (MAs) were tested. Five of seven serovar-specific MAs from five different serovars and two of five subspecies-specific MAs showed neutralizing activity as well as serovar specificity. No species- or genus-specific MAs showed neutralizing activity. No neutralization occurred without complement. Results indicate neutralization was serovar specific and complement dependent.

Monoclonal antibodies (MAs) against *Chlamydia trachomatis* are useful in many aspects of chlamydial research, including laboratory diagnosis (13, 15), serotyping (1, 17), antigenic analysis (14), and biology (4, 12). MAs against *C. trachomatis* show four distinctive reaction patterns of genus, species, subspecies, and serovar specificity by microimmunofluorescence (micro-IF) (14) and by the enzyme-linked immunosorbent assay (1, 14), with Formalin-treated elementary body preparations. To help understand some of the virulence factors of *C. trachomatis*, we tested 17 chlamydia-specific MAs for their ability to neutralize cell culture infectivity against seven different serovars from the trachoma and lymphogranuloma venereum biovars of *C. trachomatis*. A consistent pattern of serovar-specific neutralization by the serovar-specific MAs was observed and is presented here.

C. trachomatis strains B/TW-5/OT, H/UW-4/Cx, I/UW-12/Ur, K/UW-31/Cx (trachoma biovar), L1/440/Bu, L2/434/Bu, and L3/404/Bu (lymphogranuloma venereum biovar) were grown in HeLa 229 cell culture without cycloheximide (10). The organisms were suspended in sucrose-phosphate-glutamate medium at titers of approximately 5×10^8 inclusion-forming units (6) per ml for the trachoma biovar and 2×10^9 inclusion-forming units per ml for the lymphogranuloma venereum biovar, aliquoted, and stored at −75°C.

The MAs used in this study, with serovar specificity and cross-reactivity, micro-IF antibody titer (16), and immunoglobulin class, are presented in Table 1. These MAs have been described (1, 14, 17). All MAs, except those that are genus specific, were shown to recognize 40,000-molecular-weight (40K) major outer membrane proteins in immunobloting (J. J. Ma and C.-C. Kuo, unpublished data). Genus-specific MAs were not tested. An earlier study with another genus-specific MA showed reaction with an antigen of less than 10,000 molecular weight (14).

MAs were prepared by intraperitoneal inoculation of mice with hybridoma cells. Ascites were cleared by high-speed centrifugation (30,000 × *g*, 30 min), diluted 1:5 with phosphate-buffered saline, filter sterilized (0.22-μm pore size membrane filter; Millipore Corp., Bedford, Mass.), aliquoted, and stored at −75°C. Ascites produced by intraperitoneal inoculation of myeloma cells, originally used for making hybridoma, were processed similarly and used as an antibody control (control antibody).

Infectivity neutralization experiments were done in 24-h HeLa cell monolayers grown on cover slips (12-mm diameter) in flat-bottomed 1-dram (4-ml) Kahn vials with rubber stoppers. Inoculum dilutions that would yield 50 to 250 inclusions in 30 high-power fields (×400) were predetermined for each strain. Fresh guinea pig serum, as complement (GPC'), was obtained from a local supplier, distributed in small aliquotes, and stored at −75°C. GPC' was used at a final concentration of 5% (8, 11). Ascitic fluids were heat inactivated at 56°C for 30 min immediately before each experiment. Serial 10-fold dilutions of ascitic fluid were made in Hanks balanced salt solution. A 0.1-ml sample each of organism suspension, ascitic fluid dilution, and GPC' was added to a reaction vial containing Hanks balanced salts solution to make a total volume of 1 ml and incubated in a water bath at 37°C for 60 min. A 0.1-ml sample of the reaction mixture was then inoculated onto each of triplicate HeLa cell monolayers. After absorption for 60 min at room temperature, the inoculum was removed, 1 ml of culture medium (Eagle minimal essential medium containing 10% fetal calf serum) was added, and the vials were incubated at 35°C for 3 days with the trachoma biovar and at 37°C for 2 days with the lymphogranuloma venereum biovar. Cover slips were fixed with methanol and stained with May-Greenwald-Giemsa, and inclusions were counted under light microscopy as described by Furness et al. (6). Average numbers of inclusions in 30 high-power fields were obtained for the counts of each of the triplicate cover slips for each dilution. The percent neutralization was calculated by dividing the counts from the test antibodies by the counts of control antibodies in each 10-fold dilution. A reduction in the inclusion counts of more than 50% was considered positive neutralization (7, 8). Serial 10-fold antibody dilutions of 10^{-2} to 10^{-4} or higher (up to 10^{-7}), depending on the neutralization endpoint, were tested for each MA. The antibody dilution of 10^{-1} was dropped from testing after initial experiments showed that high ascitic fluid concentrations gave inconsistent neutralization results. A positive control, consisting of either TW-5 versus DD-1 or UW-4 versus LA-10, was included in each test. Each experiment was repeated at least twice.

Five of seven serovar-specific MAs showed neutralizing activity as well as serovar specificity (Table 1). However, of two serovar B (DD-1 and DE-3) and K (KK-1 and KK-2) MAs tested, only DD-1 and KK-2 showed neutralizing activity against their respective serovar strains. Two of five subspecies-specific antibodies tested showed neutralizing activity: BB-3 against L2 and DA-2 against B and L2 strains.

* Corresponding author.

TABLE 1. Serovar-specific neutralization by MAs of *C. trachomatis* infection in cell culture

MAs				Neutralization[a] of *C. trachomatis* strain serovars					
Clone	Serovar specificity[b]	Immunoglobulin subclass	Micro-IF titer[c]	B	H	I	K	L2	L1/L3
DD-1	B	G3	400	+	−	−		−	
DE-3	B	G3	400	−					
LA-10	H	G2b	200	−	+	−			
PG-1	I	G2b	1,600	−	−	+			
KK-1	K	G2a	6,400	−			−		
KK-2	K	G2a	6,400	−			+		
155-35	L2	G1	6,400	−				+	−/−
DH-8	B, Ba, E	G1	800	−				−	
BB-3	B, Ba, L2	G2b	6,400[d]	−				+	−/
BB-11	B, Ba, E, D, L1, L2	G2b	6,400	−				−	−/
DA-2	B, Ba, E, D, G, F, L1, L2	G2b	6,400[d]	+	−	−		+	−/
LE-4	C, J, A, H, I	M	800	−	−				
AE-11	Species	G3	800	−				−	
KG-5	Species	G3	1,600	−					
2C1	Species	G3	1,600	−	−			−	
FC-5	Genus[e]	G3	800	−					
CH-3	Genus	G3	1,600	−	−			−	

[a] Antibody neutralization in the presence of GPC′. +, Neutralization; −, no neutralization; blank, not tested.
[b] Serological reactions to elementary bodies by micro-IF; underlines indicate strongest reactivity.
[c] Titers given are reciprocals of twofold dilution starting with 1:100 and are against the strongest reactors.
[d] Titers against each serovar are 32(B), 32(Ba), and 64(L2) for BB-3 and 64(B), 8(Ba), 8(E), 4(D), 8(G), 4(F), 2(L1) and 8(L2) for DA-2 × 100.
[e] Determined by enzyme-linked immunosorbent assay and reaction to reticulate bodies by micro-IF.

None of the three species-specific and two genus-specific MAs had neutralizing activity. The titers and percentages of neutralization of neutralizing antibodies are presented in Table 2.

To study the role of complement in neutralization, we retested all antibodies that neutralized infection in the presence of GPC′ against the homologous strain without complement. These antibodies included serovars B (DD-1), H (LA-10), I (PG-1), K (KK-2), L2 (155-35), and B-complex (BB-3 and DA-2). In addition, two MAs, KK-1 and AE-11, that did not show neutralizing activity were tested. KK-1 was tested against K strain, and AE-11 was tested against B and L2 strains. Serial 10-fold dilutions of antibody from 10^{-2} to 10^{-5} were tested. Neutralization was not observed in the absence of complement with any of the antibodies tested (data not shown).

The neutralization of chlamydia in cell culture by rabbit and mouse antisera has been shown to be strain specific (7, 8, 11) and complement dependent (8, 11). Similar findings were reported by Clark et al. (4) with murine MAs. However, only one strain, serovar A, was tested against four MAs, three which were serovar A specific and one which was subspecies specific (4). Our study with chlamydia-specific MAs confirmed serovar specificity and complement dependence for neutralization. In addition, our findings indicate that neutralizing activity resides mainly in serovar-specific antibodies.

TABLE 2. Neutralization titers of MA against *C. trachomatis* in cell culture

MA		*C. trachomatis* (serovar/strain)	Neutralization	
Clone	Serovar specificity		%[a]	Highest titer[b]
DD-1	B	B/TW-5	67–85	10^{-2}
LA-10	H	H/UW-4	78–82	10^{-2}
PG-1	I	I/UW-12	72–75	10^{-2}
KK-2	K	K/UW-31	58–61	10^{-2}
155-35	L2	L2/434	57–74	10^{-2}, 10^{-3}
BB-3	B, Ba, L2	L2/434	81–82	10^{-2}
DA-2	B-complex[c]	B/TW-5	73–85	10^{-3}, 10^{-4}
		L2/434	69–72	10^{-2}

[a] Maximum percentages in any 10-fold dilutions in tests repeated two to three times.
[b] Maximum dilutions in serial 10-fold dilutions that gave more than 50% reduction in inclusion counts in repeated tests.
[c] Reaction against B, Ba, E, D, G, F, L1, and L2.

Serovar-specific MAs DE-3 and KK-1 were not neutralizing antibodies. Because epitope recognition of MAs is specific, it is rare for two randomly chosen clones to produce antibodies with identical epitope specificity (2). It is possible that DD-1 and DE-3 (B specific) and KK-1 and KK-2 (K specific) recognize different epitopes in the region of serovar determinant(s) but that only DD-1 and KK-2 recognize epitope critical for infectivity neutralization. A competitive inhibition assay can be used to test whether the neutralizing and nonneutralizing antibodies bind to the identical epitope. However, the complete answer can be obtained only after the serovar-specific and neutralizing antigens are isolated and analyzed for amino acid sequence. The nonneutralizing serovar-specific MA for chlamydia has also been reported by Clark et al. (4). Their immune electron microscopy showed that the neutralizing antibodies recognized the antigen on the elementary body surface in homogeneous distribution and nonneutralizing antibodies in irregular patchy distribution. The findings from these studies indicate that serovar-specific MAs should be screened for their neutralizing activity before they are used for functional or structural studies.

Differences in the complement-binding capacity of individual murine immunoglobulin subclasses could also explain the observed abilities to neutralize infectivity. Differential binding of GPC′ by the mouse immunoglobulin G1 subclass has been reported (5, 9). However, all immunoglobulin G subclasses are represented in both neutralizing and nonneutralizing antibodies in this study.

Two subspecies-specific MAs that had neutralizing activ-

ity also showed serovar specificity. It is possible that these two antibodies interact with regions capable of inhibiting chlamydial infectivity in addition to cross-reacting with other cell surface antigens detected by micro-IF.

Failure of three species-specific MAs to neutralize was in contradiction to the study of Peeling et al. (12), who reported that species-specific MA AE-11 produced complement-independent neutralization of serovars I and L2. We tested AE-11 against B and L2 strains with and without complement but were not able to demonstrate neutralization. The reason for this discrepancy is unknown.

Using rabbit anti-serovar L2 major outer membrane protein antiserum, Caldwell and Perry (3) showed that the neutralization of L2 strain in HeLa 229 cell culture occurred because the antiserum interfered with chlamydial development after its internalization, rather than blocking the process of attachment. Because only the homologous strain was tested in this study, it is not known whether the neutralization was serovar specific.

The enhancing effect of complement on chlamydial neutralization is unknown. Whether complement in the presence of specific antibody kills chlamydia by extracellular lysis, as with other gram-negative bacteria, has not been investigated. As reported by Howard using rabbit and human hyperimmune sera (8), 5% of guinea pig serum, in either the presence or the absence of normal mouse sera, caused no inactivation of chlamydia (data not shown). Therefore, the action of complement indicates specific enhancement. Because each experiment contained an internal control for GPC′, i.e., the neutralization was determined by comparing test antibody against control antibody in each serial dilution, both containing GPC′, the neutralization measured was specific.

In conclusion, this study reaffirmed the findings that antibody directed against serovar-specific antigen mediates resistance against chlamydial infection. Serovar-specific MAs can be used to study protective antigens and mechanisms of pathogenesis.

This work was supported by Public Health Service grant EY-00219 from the National Eye Institute and a grant (284-0116) from the Edna McConnell Clark Foundation.

We thank Robertina Swanson for technical assistance.

LITERATURE CITED

1. **Barnes, R. C., S.-P. Wang, C.-C. Kuo, and W. E. Stamm.** 1985. Rapid immunotyping of *Chlamydia trachomatis* with monoclonal antibodies in a solid-phase enzyme immunoassay. J. Clin. Microbiol. **22**:609–613.
2. **Benjamin, D. C., J. A. Berzofsky, I. J. East, F. R. N. Gurd, C. Hannum, S. J. Leach, E. Margoliash, J. G. Michael, A. Miller, E. M. Prager, M. Reichlin, E. E. Sercarz, S. J. Smith-Gill, P. E. Todd, and A. C. Wilson.** 1984. The antigenic structure of proteins: a reappraisal. Annu. Rev. Immunol. **2**:67–101.
3. **Caldwell, H. D., and L. J. Perry.** 1982. Neutralization of *Chlamydia trachomatis* infectivity with antibodies to the major outer membrane protein. Infect. Immun. **38**:745–754.
4. **Clark, R. B., I. Nachamkin, P. F. Schatzki, and H. P. Dalton.** 1982. Localization of distinct surface antigens on *Chlamydia trachomatis* HAR-13 by immune electron microscopy with monoclonal antibodies. Infect. Immun. **38**:1273–1278.
5. **Ey, P. L., G. J. Russell-Jones, and C. R. Jenkin.** 1980. Isotypes of mouse IgG. I. Evidence for non-complement-fixing IgG_1 antibodies and characterization of their capacity to interfere with IgG_2 sensitization of target red blood cells for lysis by complement. Cell. Immunol. **17**:699–710.
6. **Furness, G., D. M. Graham, and P. Reeve.** 1960. The titration of trachoma and inclusion blenorrhoea viruses in cell culture. J. Gen. Microbiol. **23**:613–619.
7. **Graham, D. M., and J. E. Layton.** 1971. The induction of chlamydia group antibody in rabbits inoculated with trachoma agents and demonstration of strain specific neutralizing antibody in sera. Excerpta Med. Int. Congr. Ser. **233**:145–157.
8. **Howard, L. V.** 1975. Neutralization of *Chlamydia trachomatis* in cell culture. Infect. Immun. **11**:698–703.
9. **Klaus, G. G. B., M. B. Pepys, K. Kitajima, and B. A. Askonas.** 1979. Activation of mouse complement by different classes of mouse antibody. Immunology **28**:687–695.
10. **Kuo, C.-C., S.-P. Wang, and J. T. Grayston.** 1977. Growth of trachoma organisms in HeLa 229 cell culture, p. 328–336. *In* D. Hobson and K. K. Holmes (ed.), Nongonococcal urethritis and related infections. American Society for Microbiology, Washington, D.C.
11. **Pardini, S., and R. C. Ballard.** 1982. Detection of type-specific antibody to lymphogranuloma venereum serotypes of *Chlamydia trachomatis* with a micro-neutralization test. Curr. Microbiol. **7**:205–208.
12. **Peeling, R., I. W. MacClean, and R. C. Brunham.** 1984. In vitro neutralization of *Chlamydia trachomatis* with monoclonal antibody to an epitope on the major outer membrane protein. Infect. Immun. **46**:484–488.
13. **Stephens, R. S., C.-C. Kuo, and M. R. Tam.** 1982. Sensitivity of immunofluorescence with monoclonal antibodies for detection of *Chlamydia trachomatis* inclusions in cell culture. J. Clin. Microbiol. **16**:4–7.
14. **Stephens, R. S., M. R. Tam, C.-C. Kuo, and R. C. Nowinski.** 1982. Monoclonal antibodies to *Chlamydia trachomatis*: antibody specificities and antigen characterization. J. Immunol. **128**:1083–1089.
15. **Tam, M. R., W. E. Stamm, H. H. Handsfield, R. S. Stephens, C.-C. Kuo, K. K. Holmes, K. Ditzenberger, M. Krieger, and R. C. Nowinski.** 1984. Culture-independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. N. Engl. J. Med. **310**:1146–1150.
16. **Wang, S. P., and J. T. Grayston.** 1971. Classification of TRIC and related strains with micro-immunofluorescence. Excerpta Med. Int. Congr. Ser. **233**:305–321.
17. **Wang, S. P., C.-C. Kuo, R. C. Barnes, R. S. Stephens, and J. T. Grayston.** 1985. Immunotyping of *Chlamydia trachomatis* with monoclonal antibodies. J. Infect. Dis. **152**:791–800.