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Production and Partial Characterization of Monoclonal Antibodies to Four *Chlamydia psittaci* Isolates

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Monoclonal antibodies (MAbs) were produced to four *Chlamydia psittaci* isolates: NJ1 and TT3 from turkeys, VS1 from a parakeet, and B577 from an ovine abortion. MAbs were tested for reactivity with each isolate by the indirect immunofluorescent antibody technique and for neutralization by an inclusion reduction neutralization technique in tissue culture. Two genus-specific and 14 serovar-specific MAbs were produced. Genus-specific MAbs reacted with all avian and mammalian isolates; however, each failed to neutralize its homologous chlamydial isolate. Turkey isolates NJ1 and TT3 were antigenically similar; serovar-specific MAbs produced to each reacted equally with both isolates yet showed little or no reaction with other serovars. Serovar-specific MAbs to the parakeet and abortion isolates were distinct; no cross-reactions were seen with other serovars. None of the serovar-specific MAbs reacted with an ovine arthritis isolate. Of the 14 serovar-specific MAbs, 13 partially neutralized homologous strains with or without the addition of complement. Because of the high specificity, the serovar-specific MAbs used with the immunofluorescence technique provided a rapid and precise method to identify three serovars of *C. psittaci*

The genus *Chlamydia* is currently composed of two species, *C. trachomatis* and *C. psittaci*, on the basis of their susceptibility to sulfadiazine and their production of glycogen (9). *C. trachomatis*, known only to infect humans and mice, is composed of three biotypes on the basis of natural hosts, disease signs, and biological properties (9). The three biotypes are in turn composed of 15 immunotypes on the basis of various immunological tests.

C. psittaci comprises a diverse group of strains that have a broad host range and pathogenic potential. Attempts to group strains into serovars have produced limited success. Schachter et al. (16, 17), by using conventional antisera in a plaque reduction neutralization test, were able to classify several bovine and ovine isolates into two serological types. Spears and Storz (19), working primarily with mammalian isolates, identified eight biotypes on the basis of inclusion morphology and response to metabolic inhibitors added to the cell cultures. Later, Perez-Martinez and Storz (14) identified nine immunotypes by using conventional antisera with the microimmunofluorescence technique. These efforts have demonstrated that *C. psittaci* comprises a heterogeneous group of organisms and that efficient methods for serotyping the group have yet to be developed.

Monoclonal antibodies (MAbs) to *C. trachomatis* have been used quite successfully for both serotyping (2, 27) and laboratory diagnosis (20, 23). However, attempts to produce MAbs to *C. psittaci* have resulted in only limited success. There are reports of production of MAbs to an ovine abortion isolate and to a pigeon isolate (4, 24). A few of the MAbs have been serovar specific and have shown high specificity to the homologous isolate; however, most have been only genus specific.

The goal of this research was to produce MAbs that were serovar specific and that could be used for both diagnostic testing and immunotyping of *C. psittaci* isolates. MAbs were prepared against three avian isolates and an ovine abortion isolate. Hybridoma clones were selected which secreted antibody that produced bright fluorescence with the homologous strain by the indirect fluorescent antibody (IFA) technique and that lacked reactivity with heterologous isolates. MAbs were tested by the IFA technique for isolate specificity and by the inclusion reduction method for their ability to neutralize homologous strains.

MATERIALS AND METHODS

Chlamydial strains. Four *C. psittaci* isolates—NJ1 and TT3 from turkeys, VS1 from a parakeet, and B577 from an ovine abortion—were used to produce MAbs. The resultant MAbs were tested against 12 chlamydial isolates for strain specificity. The origin and other related information for each isolate are summarized in Table 1. Chlamydiae were adapted for growth in Vero cells by centrifuging the inoculum onto Vero cell monolayers in 25-cm^2 tissue culture flasks. Following adaptation for growth in Vero cells, chlamydiae were grown in 850-cm^2 roller bottles. Chlamydiae were harvested for use between their third and sixth passages in tissue culture.

Cell cultures. Vero cells were cultured in Eagle minimum essential medium with Earle balanced salts, 20 mmol of HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)–5% fetal bovine serum per liter, 5.4 mg of glucose per liter, 292 mg of glutamine per liter, 2 μg of amphotericin B per ml, and 10 μg of gentamicin sulfate per ml. The same medium, with the addition of 0.5 μg of cycloheximide per ml, was used following inoculation of the Vero cells with chlamydiae.

Chlamydial antigens for production of MAbs. Partially purified ethylenimine (EI)-inactivated suspensions of chlamydial elementary bodies (EBs) were used to immunize mice. Chlamydial isolates were grown in Vero cell monolayers in tissue culture. Chlamydiae were harvested between 2 and 4 days following inoculation of the monolayer, depending on the isolate. Media and cells from four to six 850-cm^2 roller bottles were pooled and then centrifuged at 500 × *g* for 10 min to remove cellular debris. Chlamydiae were concentrated by centrifugation at 10,000 × *g* for 60 min, suspended

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TABLE 1. Isolates of *C. psittaci*

Chlamydial isolate	Origin	Yr isolated	Host	Disease	Reference or source
VS1	Ga.	1985	Parrot	Psittacosis	NVSL[a]
6BC	Calif.	1941	Parakeet	Psittacosis	ATCC[b]
VS2	Minn.	1985	Love Bird	Psittacosis	NVSL[a]
TT3	Tex.	1975	Turkey	Ornithosis	Page[c]
NJ1	N.J.	1959	Turkey	Ornithosis	10
SCT	S.C.	1973	Turkey	Ornithosis	12
VT	Va.	1965	Turkey	Ornithosis	Page[d]
B577	Utah	1962	Sheep	Abortion	21
EBA	Calif.	1959	Cattle	Abortion	22
OSP	Oreg.	1985	Sheep	Abortion	Andersen[e]
IPA	Iowa	1968	Sheep	Polyarthritis	11

[a] Diagnostic Virology number 85-37190 (VS1) or 85-36830 (VS2); National Veterinary Services Laboratory, Ames, Iowa.
[b] American Type Culture Collection, VR-125. No reference given.
[c] Isolated by L. Page from the 1974 to 1976 Texas outbreak (13).
[d] Isolated by L. Page.
[e] Isolated by A. Andersen from material supplied by O. Hedstrom, Corvallis, Oreg.

in distilled H_2O, and purified by centrifugation into a 30 to 50% step sucrose gradient at $10,000 \times g$ for 60 min. The layer containing chlamydial EBs was collected, diluted threefold in distilled H_2O, pelleted by centrifugation at $10,000 \times g$ for 60 min, and suspended in 5 ml of phosphate-buffered saline. The suspension contained approximately 10^8 inclusion-forming units per ml prior to inactivation.

Chlamydial EBs were inactivated by adding a sufficient quantity of a 1% solution of EI to obtain a final concentration of 0.05% (7). The mixture was incubated for 24 h at 37°C in a water bath. After incubation, a 40% thiosulfate solution was added to give a final concentration of 2% to neutralize the EI. The pH was adjusted to the 7.2 to 7.6 range with sodium bicarbonate. The inactivated EBs were used to immunize mice for hybridoma production and as antigens for typing of MAbs.

The chlamydiae used for neutralization tests were grown in roller bottles as described above. Following removal of cellular debris and concentration by pelleting, the chlamydial EBs were suspended as a 10× suspension containing 10^7 to 10^9 inclusion-forming units per ml in sucrose phosphate buffer (3), dispensed into ampoules, and frozen at −80°C.

Production of hybridomas and MAbs. BALB/c mice were immunized to the four *Chlamydia* isolates by intraperitoneal injection of 0.2 ml of the EI-inactivated chlamydial suspension. Blood samples were taken 3 weeks later and tested for seroconversion by the IFA technique described below. Seropositive mice were given an intravenous dose of 0.2 ml of antigen 3 days prior to cell fusion. Cell fusion and cloning of antibody-secreting hybridomas of interest were accomplished by using procedures described in detail previously (25, 26). Clones finally selected for production of MAbs were introduced into the peritoneal cavities of pristane-primed BALB/c mice for ascites production.

Testing and selection of hybridomas. Hybridoma cell culture fluids were screened for antibody by the IFA technique with confluent 24-h-old monolayers of Vero cells infected with *C. psittaci*. Cell monolayers were grown in 96-well multiwell dishes, inoculated with each *Chlamydia* strain at approximately 10^4 to 10^5 inclusion-forming units per 0.1 ml, and fixed with 50% acetone–50% methyl alcohol at 24 to 72 h postinoculation, depending on the time of optimal inclusion development by each particular strain. Hybridomas were screened for antibody by adding 75 μl of a 1:5 dilution of harvested cell culture fluid to each test well and then incubated for 1 h at room temperature, washed, and stained with 50 μl of fluorescein-conjugated anti-mouse immunoglobulin G (IgG) (heavy- and light-chain-specific) serum at a dilution of 1 to 30 (Organon Teknika, Malvern, Pa.) for 1 h. Wells were read by inverting the plates under an epifluorescence microscope and checking for staining of inclusions at a ×125 to ×160 magnification.

Clones positive for the homologous strain were retested for cross-reaction with heterologous strains by the same technique. The hybridomas selected were recloned once and then retested by the IFA technique for brightness of fluorescence and for specificity prior to production of ascites fluids. The MAbs were isotyped with a commercially available enzyme-linked immunosorbent assay kit (Bio-Rad).

Titration of ascites fluid. MAbs in ascites fluids were titrated by the IFA test as described above. The fluids were first screened at a 1:16 dilution, those testing positive were retested by using a twofold-dilution series. The highest dilution giving a clearly detectable fluorescence was recorded as the final dilution.

Neutralization test. MAbs were assayed for their neutralization activity by measuring the decrease in inclusion-forming units (8). Ascites fluids were heat inactivated at 56°C for 30 min immediately before each experiment. Serial 10-fold dilutions of ascites fluids were made in cell culture medium without serum. Equal volumes of *Chlamydia* EB suspensions were added after dilution in tissue culture media to provide a final 100 to 200 inclusions per 10 microscope fields. The diluent for the chlamydiae contained either 10% heat-inactivated fetal bovine serum or 10% fresh guinea pig serum to provide complement. The mixture was incubated at 37°C in a CO_2 incubator for 1 h, after which 0.1 ml was inoculated on each of three wells on a 24-h-old Vero cell monolayer grown in 24-well multiwell dishes. The multiwell dishes were centrifuged at $1,000 \times g$ for 1 h at 28°C, after which the inoculum was removed and replaced with 1.0 ml of cell culture media containing 0.5 μg of cycloheximide per ml. The monolayers were fixed with a 50% methanol–50% acetone mixture at 24 to 72 h, depending on the chlamydial isolate. Monolayers were stained and examined by the IFA technique by using a group-reactive MAb to B577 and a fluorescein-conjugated anti-mouse antiserum. The average number of inclusions for the 10 low-power (×160) microscope fields of the three wells for each dilution was compared with counts from a fetal calf control serum and from a normal ascites fluid at each dilution. A reduction of 50% or

TABLE 2. IFA reactions of different *C. psittaci* isolates mediated by MAbs to four isolates

MAb	Chlamydial isolate[a]										
	VS1	6BC	VS2	TT3	NJ1	ScT	VT	B577	EBA	OSP	IPA
VS1/C4	4,096	4,096	4,096	—	—	—	—	—	—	—	—
VS1/E8	8,192	8,192	4,096	—	—	—	—	—	—	—	—
VS1/F9	4,096	4,096	4,096	—	—	—	—	—	—	—	—
VS1/E3	4,096	4,096	4,096	—	—	—	—	—	—	—	—
TT3/C6	2,048	2,048	1,024	1,024	1,024	2,048	2,048	1,024	1,024	1,024	1,024
TT3/E11	32	—	16	2,048	8,192	4,096	4,096	—	—	—	—
TT3/A12	32	—	—	2,048	2,048	1,024	1,024	—	—	—	—
NJ1/B10	—	—	—	2,048	2,048	2,048	1,024	—	—	—	—
NJ1/C1	—	—	—	4,096	8,192	4,096	4,096	—	—	—	—
NJ1/A4	—	—	—	4,096	8,192	4,096	4,096	—	—	—	—
B577/D1	4,096	4,096	8,192	8,192	8,192	8,192	8,192	8,192	4,096	4,096	4,096
B577/E9	—	—	—	—	—	—	—	1,024	1,024	1,024	—
B577/B1	—	—	—	—	—	—	—	8,192	8,192	8,192	—
B577/D8	—	—	—	—	—	—	—	1,024	1,024	1,024	—
B577/F3	—	—	—	—	—	—	—	8,192	8,192	8,192	—
B577/A11	—	—	—	—	—	—	—	8,192	4,096	4,096	—

[a] Titer is the reciprocal of the dilution giving a clearly readable fluorescence of the inclusion bodies. —, No reaction detected at a 1:16 dilution.

more at a given dilution was considered positive. Each experiment was repeated at least twice.

RESULTS

One fusion for each chlamydial isolate was performed, yielding 100 to 150 primary hybridomas from 480 wells plated. Each fusion yielded 12 to 15 hybridomas which tested positive by the IFA technique to the homologous strain. The positive hybridomas were retested for reactivity for heterologous strains by the same technique. Approximately 70% of the positive hybridomas from each isolate were species specific, reacting only with the homologous isolate. Ultimately, three to six hybridomas from each fusion were selected on the basis of brightness of fluorescence and specificity of reactivity to the homologous strain. Two group-reactive hybridomas were also selected for monitoring growth of chlamydiae and for comparison studies; these were included in the study. The hybridomas selected were recloned once, retested for specificity, and introduced into mice for production of ascites fluids. The results of testing the MAbs against various isolates of avian and mammalian origin are shown in Table 2.

Two genus-specific MAbs (TT3/C6 and B577/D1) reacted with all isolates tested. The MAb TT3/C6 gave good fluorescence at a titer of 1:1,024 to 1:2,048 with all isolates, whereas the titers for the MAb B577/D1 ranged from 1:4,096 to 1:8,192. These isolates gave similar titers when tested for reactivity by the IFA test with the mouse pneumonitis and the lymphogranuloma venereum (LGV type II, strain 434) strains of *C. trachomatis* (data not shown).

The remaining 14 serovar-specific MAbs reacted with isolates of the homologous serovar at a dilution of 1:1,024 and at higher dilutions and failed to react at a dilution of 1:16 with isolates of heterologous serovars. The only exceptions to this were the two serovar-specific MAbs to TT3 (TT3/E11 and TT3/A12), which gave low-level (1:32) titers with the VS1 isolate; the fluorescence produced was of low intensity.

MAbs were tested for their ability to neutralize the homologous chlamydial strain by testing for the ability to reduce the number of inclusions following infection of tissue culture monolayers. The highest dilution giving a 50% or greater reduction, along with the percent reduction observed at that dilution, is given in Table 3. The two group-reactive MAbs, TT3/C6 and B577/D1, did not show any ability to neutralize the homologous strains. Also, a serotype-specific MAb, TT3/A12, failed to show neutralizing activity. The remaining 13 MAbs had neutralizing titers of 10^2 to 10^4, as shown in Table 3. Lower dilutions of the MAbs resulted in only a slight increase in ability to neutralize the homologous strain, and at no time was complete neutralization evident. The MAbs which demonstrated neutralizing ability were retested without complement. The results without complement were similar (data not shown).

MAbs were subtyped by the enzyme-linked immunosorbent assay technique. All were of the IgG type; the specific subtype for each is given in Table 3.

DISCUSSION

Sixteen antibody-secreting hybridomas, including 14 serovar-specific and 2 genus-specific clones, were produced to

TABLE 3. Neutralization titers of MAbs against the homologous *C. psittaci* isolate in cell cultures

MAb	Immunoglobulin subclass	*C. psittaci* strain	Neutralization:	
			%[a]	Titer[b]
VS1/C4	IgG2a	VS1	54–78	10^{-3}
VS1/E8	IgG2b	VS1	76–82	10^{-3}
VS1/F9	IgG2a	VS1	80–84	10^{-3}
VS1/E3	IgG2a	VS1	75–83	10^{-2}
TT3/C6	IgG3	TT3	<10	—
TT3/E11	IgG3	TT3	68–80	10^{-3}
TT3/A12	IgG3	TT3	<10	—
NJ1/B10	IgG3	NJ1	63–85	10^{-3}
NJ1/C1	IgG2b	NJ1	70–76	10^{-4}
NJ1/A4	IgG2b	NJ1	80–85	10^{-4}
B577/D1	IgG1	B577	<10	—
B577/E9	IgG3	B577	68–83	10^{-4}
B577/B1	IgG2b	B577	78–85	10^{-3}
B577/D8	IgG3	B577	69–75	10^{-3}
B577/F3	IgG2a	B577	63–79	10^{-4}
B577/A11	IgG2a	B577	58–77	10^{-3}

[a] Maximum percentages in any 10-fold dilutions in tests repeated twice.
[b] Maximum dilutions in serial 10-fold dilutions that gave more than 50% reduction in inclusion counts in repeated tests. —, No neutralization at 10^{-1}.

four isolates of *C. psittaci*. The four isolates represented three serovars (two avian serovars and one ovine abortion serovar). By using the IFA technique to detect chlamydial inclusions in infected tissue culture monolayers, three distinct serovars of *C. psittaci* were identified with the MAbs produced. They are represented by the MAbs TT3 and NJ1, which reacted with the four virulent turkey isolates (TT3, NJ1, VT, and SCT); the MAbs to VS1, which reacted with psittacine isolates VS1, 6BC, and VS2; and the MAbs to B577, which reacted with abortion isolates EBA, B577, and OSP. One isolate, IPA, an ovine arthritis isolate, failed to react with any of the serovar-specific MAbs. The results show that the serovar-specific MAbs are highly strain specific, with little or no reaction with heterologous strains, and that they can be used to serotype chlamydial isolates both rapidly and accurately.

The results described above are in agreement with reported attempts to biotype or serotype *C. psittaci* isolates with conventional (polyclonal) antisera. The abortion isolate is of immunotype/biotype 1, on the basis of earlier studies (5, 14, 16–19); IPA, which is an ovine arthritis isolate and likely of immunotype/biotype 2, would not be expected to react with it. Previous biotyping or serotyping has not included avian isolates other than an occasional reference strain. In those attempts, the avian isolates have also been in a separate group. Banks et al. (1) proved that neutralizing antibody to avian *C. psittaci* isolates could be measured by the plaque reduction neutralization test. Their results indicated that there were at least two avian serotypes of *C. psittaci* and that virulent turkey isolates formed a potential group that was serologically distinct from the other isolates.

There are two reports on production of MAbs to *C. psittaci*. DeLong and Magee (4) reported on the production of MAbs to an ovine abortion strain. They succeeded in producing eight MAbs following three fusions. One MAb produced by in vitro immunization was serovar specific; the remaining MAbs were genus specific. The serovar-specific MAb was able to identify the abortion isolates and was nonreactive with the ovine arthritis isolates. Toyofuku et al. (24) produced five MAbs to a pigeon isolate. Two were serovar specific and reacted only with the homologous strain. By using the MAbs, they classified 9 *C. psittaci* isolates of pigeon origin into three serological groups and 16 isolates from budgerigars into four serological groups. Classification was based on titer levels obtained with the MAbs in the enzyme-linked immunosorbent assay, microimmunofluorescence test, and complement fixation test. Because these researchers used different chlamydial strains and primarily group-reactive MAbs, the results should not be compared with our results.

There are no previously published reports on the ability of MAbs to *C. psittaci* to show neutralizing ability. This is in part due to the problems associated with producing serovar-specific MAbs. In this study, it was found that 13 of the 14 serovar-specific MAbs neutralized the homologous strain in tissue culture and that the neutralizing ability was independent of complement. This is in contrast with published reports on MAbs to *C. trachomatis*, in which complement is reported to be required (8). Previous reports on neutralization tests with *C. psittaci* that used murine and chicken sera do not indicate a need for complement for neutralization (1, 6, 15).

Mice used for the cell fusion procedure were immunized with EI-inactivated whole chlamydiae, and the resulting clones were selected by testing for inclusions in chlamydia-infected tissue culture monolayers which were fixed with a methyl alcohol-acetone mixture. The choice of EI as an inactivant was fortunate for the production of serovar-specific MAbs because, in subsequent tests, the resulting serovar-specific MAbs reacted only poorly or not at all with chlamydiae in tissues or tissue cultures fixed with 10% Formalin. Because many of the MAbs that were selected were serovar specific and would also neutralize the homologous isolate, this would indicate that Formalin treatment of chlamydiae may destroy many of the serovar-specific antigenic sites needed to produce neutralizing antibodies. Formalin has been used extensively for the inactivation of chlamydiae for vaccines and for production of hyperimmune sera, and the destruction of these antigenic sites may explain some of the problems encountered in producing neutralizing antibody.

Fragility of the serovar-specific antigenic sites may explain the results in two earlier reports on production of MAbs. DeLong and Magee (4), using Formalin as an inactivant, reported production of six genus-specific MAbs and only one serovar-specific MAb; Toyofuku et al. (24), using beta-propiolactone as an inactivant, produced three genus-specific and two serovar-specific MAbs. It is clear that a critical evaluation is needed of chemicals used for the inactivation of *C. psittaci* for production of vaccines, hyperimmune sera, and MAbs.

This study demonstrates that serovar-specific MAbs can be produced to different *C. psittaci* isolates and that these MAbs can be used to classify the *C. psittaci* group definitively and accurately into distinct serovars. The serovar-specific MAbs give an all-or-none reaction when used at proper dilutions; when a positive reaction is detected, the specificity is such that one can be confident that the isolate is of the same serovar. Also, because of the specificity and ease of performing the IFA test, little training is needed to interpret the results. The serovar-specific MAbs also have potential for adaptation to a number of diagnostic techniques which can rapidly and simultaneously provide both a diagnosis of *Chlamydia* infection and serovar identification.

LITERATURE CITED

1. **Banks, J., B. Eddie, M. Sung, N. Sugg, J. Schachter, and K. F. Meyer.** 1970. Plaque reduction technique for demonstrating neutralizing antibodies for *Chlamydia*. Infect. Immun. **2**:443–447.
2. **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.
3. **Bovarnick, M. R., J. C. Miller, and J. C. Snyder.** 1950. The influence of certain salts, amino acids, sugars, and proteins on the stability of rickettsiae. J. Bacteriol. **59**:509–522.
4. **DeLong, W. J., and W. E. Magee.** 1986. Distinguishing between ovine abortion and ovine arthritis *Chlamydia psittaci* isolates with specific monoclonal antibodies. Am. J. Vet. Res. **47**:1520–1523.
5. **Eb, F., and J. Orfila.** 1982. Serotyping of *Chlamydia psittaci* by the micro-immunofluorescence test: isolates of ovine origin. Infect. Immun. **37**:1289–1291.
6. **Hahon, N., and R. M. Nakamura.** 1964. Quantitative assay of psittacosis virus by the fluorescent cell-counting technique. Virology **23**:203–208.
7. **Larghi, O. P., V. L. Savy, A. E. Nebel, and A. Rodriquez.** 1976. Ethylenimine-inactivated rabies vaccine of tissue culture origin. J. Clin. Microbiol. **3**:26–33.
8. **Lucero, M. E., and C.-C. Kuo.** 1985. Neutralization of *Chlamydia trachomatis* cell culture infection by serovar-specific monoclonal antibodies. Infect. Immun. **50**:595–597.
9. **Moulder, J. W., T. P. Hatch, C.-C. Kuo, J. Schachter, and J. Storz.** 1984. Genus *Chlamydia*, p. 729–739. *In* N. R. Krieg (ed.),

Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.

10. **Page, L. A.** 1959. Experimental ornithosis in turkeys. Avian Dis. **3:**51–66.
11. **Page, L. A., and R. C. Cutlip.** 1968. Chlamydial polyarthritis in Iowa lambs. Iowa Vet. **39:**10–18.
12. **Page, L. A., W. T. Derieux, and R. C. Cutlip.** 1975. An epornitic of fatal chlamydiosis (ornithosis) in South Carolina turkeys. J. Am. Vet. Med. Assoc. **166:**175–178.
13. **Page, L. A., and J. E. Grimes.** 1984. Avian chlamydiosis (ornithosis), p. 283–307. *In* M. S. Hofstad (ed.), Diseases of Poultry, 8th ed. Iowa State University Press, Ames.
14. **Perez-Martinez, J. A., and J. Storz.** 1985. Antigenic diversity of *Chlamydia psittaci* of mammalian origin determined by microimmunofluorescence. Infect. Immun. **50:**905–910.
15. **Piraino, F., and C. Abel.** 1984. Plaque assay for psittacosis virus in monolayers of chick embryo fibroblasts. J. Bacteriol. **87:** 1503–1511.
16. **Schachter, J., J. Banks, N. Sugg, M. Sung, J. Storz, and K. F. Meyer.** 1974. Serotyping of *Chlamydia*. I. Isolates of ovine origin. Infect. Immun. **9:**92–94.
17. **Schachter, J., J. Banks, N. Sugg, M. Sung, J. Storz, and K. F. Meyer.** 1975. Serotyping of *Chlamydia*: isolates of bovine origin. Infect. Immun. **11:**904–907.
18. **Spears, P., and J. Storz.** 1979. Biotyping of *Chlamydia psittaci* based on inclusion morphology and response to diethylaminoethyl-dextran and cycloheximide. Infect. Immun. **24:**224–232.
19. **Spears, P., and J. Storz.** 1979. *Chlamydia psittaci*: growth characteristics and enumeration of serotypes 1 and 2 in cultured cells. J. Infect. Dis. **140:**959–967.
20. **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.
21. **Storz, J.** 1966. Psittacosis-lymphogranuloma infection of sheep. Antigenic structures and interrelations of PL agents associated with polyarthritis, enzootic abortion, intrauterine and latent intestinal infections. J. Comp. Pathol. **76:**351–362.
22. **Storz, J., D. G. McKercher, J. A. Howarth, and O. C. Straub.** 1960. The isolation of a viral agent from epizootic bovine abortion. J. Am. Vet. Med. Assoc. **137:**509–514.
23. **Tam, M. R., W. E. Stamm, H. H. Handsfield, R. 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. New Engl. J. Med. **310:**1146–1150.
24. **Toyofuku, H., I. Takashima, J. Arikawa, and N. Hashimoto.** 1986. Monoclonal antibodies against *Chlamydia psittaci*. Microbiol. Immunol. **30:**945–955.
25. **Van Deusen, R. A.** 1984. Making hybridomas, p. 15–25. *In* N. J. Stern and H. R. Gamble (ed.), Proceedings of USDA seminar on hybridomas in agricultural and veterinary research. Rowman and Allanheld Publishers, Totowa, N.J.
26. **Van Deusen, R. A., and C. Whetstone.** 1981. Practical aspects of producing and using anti-viral monoclonal antibodies as diagnostic reagents. Proc. Annu. Meet. Am. Assoc. Vet. Lab. Diag. **24:**211–228.
27. **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.