NOTES

Plaque Formation by Strains of Spotted Fever Rickettsiae in Monolayer Cultures of Various Cell Types

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Some strains of spotted fever rickettsiae could be distinguished by their ability or inability to form plaques in monolayer cultures of various mammalian and avian cell types.

Since the development of tissue culture plaqueing methods for rickettsiae by Kordova (6) and McDade et al. (8), spotted fever rickettsiae have been grown and plaqueed in a variety of cell types of both vertebrate and arthropod origin (4, 5, 9). In some cell cultures, certain rickettsiae were found to grow well and form plaques, whereas in others, no plaques developed (5, 9). These reports suggested that plaque formation varied with the rickettsial strain, the cell culture, and the conditions employed and gave indications that a tissue culture host range pattern could be developed for these organisms. Such a pattern would be useful as an adjunct to other methods for the differentiation of strains and species. Present studies were undertaken to determine whether the formation of plaques or the lack of their development by spotted fever strains in a variety of cell types could be used for this purpose.

Cell lines used were obtained from the American Type Culture Collection (ATCC); the Certified Cell Line (CCL) number is noted in Table 1. Chicken embryo fibroblast cells were obtained from fertile chicken eggs supplied by SPAFAS, Inc., Norwich, Conn., and duck embryo fibroblast cells from duck eggs supplied by Truslow Farms, Chestertown, Md. Fibroblast cultures were prepared by the method of Wike et al. (11). Strains of spotted fever rickettsiae examined were: Sheila Smith (2), R strain (2), Iowa (2), and Bates (F. M. Bozeman, personal communication; isolated in 1956 at the Walter Reed Army Institute of Research) for *Rickettsia rickettsii*; *R. montana* (1) ATCC VR-611, and strains C (10) and maculatum (2) for *R. parkeri*. Seed stocks of all strains were propagated in embryonated eggs and maintained frozen at -70°C as 50% yolk sac suspensions in sucrose-phosphate-glutamate solution (3).

In experiments reported here, cell suspensions

in appropriate growth media were inoculated into 25-cm² screw-capped plastic tissue culture flasks and allowed to form confluent monolayers. Fluid was removed from the flasks, and serial dilutions of rickettsial stock cultures in brain heart infusion broth were added (0.2 ml per flask) and distributed over the cell monolayer. After incubation at 34°C for 1 h, 5 ml of modified Eagle medium with Earle salts (Grand Island Biological Co.) containing 0.25% agarose (SeaKem ME) was added to the cultures and allowed to solidify. The tightly capped flasks were incubated at 34°C for 7 days; the cell sheets were stained with 2 ml of 1:10 dilution of neutral red dye solution (Grand Island Biological Co.) and observed for plaques. Chicken embryo fibroblast cultures were included in each experiment as a control on the presence of viable rickettsiae.

Results of these experiments are presented in Table 1. A number of rickettsial strains could be differentiated by plaque formation in the various cell lines. The highly virulent strains of *R. rickettsii*, Sheila Smith and R, formed plaques in all cell lines tested except HeLa and HEp-2 cultures and could not be distinguished from one another. The less virulent Bates and Iowa strains also failed to form plaques in these and in one or more additional cell cultures. These last two rickettsial strains each developed plaques in a different set of cell cultures and could be differentiated from each other and from the virulent strains. The strains C and maculatum of *R. parkeri* also formed plaques in a unique set of cell lines that was identical for these two strains, but different from those for *R. rickettsii* and *R. montana*.

Seven strains representing three species of spotted fever organisms were employed in this study. Sheila Smith and R are highly virulent strains of *R. rickettsii*, whereas Bates and Iowa are of lower virulence. *R. montana*, a member

TABLE 1. *Plaque formation by rickettsial strains in various cell culture monolayers^a*

Cell type (ATCC no.)	Plaque morphol- ogy	Presence of plaques						
		<i>R. rickettsii</i>				<i>R. mon- tana</i>	<i>R. parkeri</i>	
		Sheila Smith	R strain	Bates	Iowa		Maculatum	Strain C
Human								
CCL 25 (WISH)	R	+	+	+	+	+	+	+
CCL 23 (HEp-2)	O	0	0	0	0	0	0	0
CCL 75 (WI-38)	S	+	+	0	+	ND	0	0
CCL 2 (HeLa)	O	0	0	0	0	0	0	0
Monkey								
CCL 26 (BS-C-1)	R	+	+	+	0	0	+	+
CCL 81 (Vero)	R	+	+	+	0	ND	0	0
CCL 7 (LLC-MK-2)	R	+	+	+	+	0	0	0
Hamster								
CCL 10 (BHK)	S	+	+	0	0	+	+	+
Mouse								
CCL 1 (L-929)	R	+	+	+	+	+	0	0
Chicken								
CEF (primary)	T	+	+	+	+	+	+	+
Duck								
DEF (primary)	T	+	+	+	+	+	0	ND

^a Symbols: +, plaques formed; 0, no plaques formed; ND, no data; R, round, clear plaques; S, stellate plaques; T, target-like plaques; CEF, chicken embryo fibroblast; DEF, duck embryo fibroblasts.

of the eastern Montana group that is avirulent for guinea pigs, appears closely related immunologically to *R. rickettsii*, but produces specific complement-fixing antibodies in mice. Lackman et al. (7) has proposed that this agent be elevated to a separate species, and this proposal, which is used in the *ATCC Catalog of Strains* (1975), has been followed here. Similarly, *R. parkerii*, as represented by strain C and maculatum strain, is related to *R. rickettsii* by cross-protection tests (7) but differs by toxin neutralization tests and by specific complement-fixing antibody produced in mice.

The rickettsial strains were all capable of multiplying at least to a limited extent in each cell line tested, whether or not plaques were formed. Assays of 7-day cultures showed infectivity titers 100 to 1,000 times greater than were found in the original inocula. The limited ability of rickettsiae to develop plaques in some cell lines was to some extent related to the conditions employed. A few plaque-like areas were seen occasionally in HeLa and HEp-2 cell cultures after long incubation when culture nutrients were nearly depleted and cell multiplication was reduced. However, under the standard conditions described, plaque production was never observed.

According to the *ATCC Catalog of Strains II*

(1975), two cell lines, HEp-2 (CCL-23) and WISH (CCL-25), may have been contaminated with HeLa cells. All other cell lines were reported to be properly identified. Identity tests were not conducted in our laboratory, but all cell lines were tested for plaque formation at several passage levels, ranging up to 12 passages from the original ATCC culture, with similar results obtained at all levels tested. Plaque formation in HEp-2 cell cultures was not observed, a result similar to that obtained in HeLa cell cultures. The possibility of contamination of this culture could not be excluded. However, WISH cell cultures allowed plaque formation by all rickettsial strains tested and appeared to be very different from the HeLa cell line.

Three morphologically different plaque types were observed in this study, the characteristics of which were associated with the type cell culture rather than with the rickettsial strain. In most cell lines, rickettsiae formed clear, round plaques varying in diameter from 0.5 to 2.0 mm (Fig. 1A). In WI-38 and BHK cell lines they formed stellate plaques (Fig. 1B), similar to those found by Osterman and Parr (9) for *R. conorii* in WI-38 cell cultures. In primary chicken and duck fibroblast cultures, rickettsiae developed round to slightly oval plaques meas-

FIG. 1. Plaque morphology developed in several tissue cell monolayers by the R strain of *R. rickettsii*. (A) round, clear plaques in BS-C-1 cell cultures; (B) stellate plaques in WI-38 cell cultures; and (C) target-like plaques in chicken fibroblast cell cultures.

uring 1.0 to 2.0 mm in diameter. These plaques frequently had a darker-staining inner ring that gave them a target-like appearance (Fig. 1C).

As present studies indicate, the formation or lack of development of plaques under a defined set of conditions in a variety of cell cultures may be a useful adjunct to other methods for distinguishing among spotted fever strains. Plaque formation may not have the status of an immunological or biochemical characteristic, but it may still be of value for differentiating among some rickettsial strains for which few other distinguishing properties are recognized.

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