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Comparative Susceptibility of Eleven Mammalian Cell Lines to Infection with Trachoma Organisms

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Eleven mammalian cell lines, HeLa 229, HeLa M, Hep-2, FT, BHK-21, Vero, MK-2, MPK, L-WO5A2, McCoy, and L-929, were tested for their susceptibility to infection with Trachoma strains TW-3 (type C, ocular origin) and UW-5 (type E, genital origin). All the cell layers were pretreated with diethylaminoethyl-dextran before inoculation of the organisms, and the inocula were centrifuged onto the cell layers. HeLa 229 was found to be the most sensitive to infection as determined by inclusion counts. The next most susceptible were cell lines MK-2, Hep-2, McCoy, and HeLa M, in that order. Infectivity in these cells ranged from 89 to 12% of that observed in HeLa 229. The remaining cell lines, BHK-21, L-WO5A2, L-929, Vero, MPK, and FT, were much less susceptible with infectivity less than 10% that of HeLa 229. HeLa 229 cells and 5-iodo-2'-deoxyuridine-pretreated McCoy cells have been used most extensively with Trachoma organisms in our laboratories. Infectivity in these two cell culture systems, both pretreated with diethylaminoethyl-dextran, was compared using 13 Trachoma strains of both ocular and genital origins of different immunotypes. The two systems performed similarly except with two type C, three type I, and one type J strains. With the type C, I and J strains tested, considerably fewer inclusions were found in 5-iodo-2'-deoxyuridine-pretreated McCoy than in HeLa 229 because inclusion formation of these strains in McCoy cells was not enhanced by 5-iodo-2'-deoxyuridine pretreatment.

A group of organisms in the *Chlamydia trachomatis* which causes Trachoma, inclusion conjunctivitis, and genital infections of man has been called TRIC (3). We have proposed the term "Trachoma organisms" (J. T. Grayston and S. P. Wang, J. Infect. Dis., in press) in place of TRIC organisms to avoid confusion with *Trichomonas*, because we believe that the syndromes Trachoma and inclusion conjunctivitis are different stages of the same disease, and because we wish to emphasize that the same organisms that cause Trachoma cause genital infection (J. T. Grayston and S. P. Wang, J. Infect. Dis., in press). First isolated in the embryonated chicken egg, Trachoma organisms have since been successfully isolated and grown in cell culture. Gordon et al. (5) showed irradiated McCoy cells to be more sensitive than eggs for isolation of Trachoma organisms from human specimens. Jenkin (10) reported HeLa 229 to be susceptible to these organisms, whereas HeLa S3 and HeLa calf were not. HeLa 229 cells treated with diethylaminoethyl (DEAE)-dextran before infection were demonstrated to be as effective for isolation of Trachoma organisms as irradiated McCoy cells (12). In a direct comparison Gordon et al. (4) found substantially more inclusions in irradiated than in nonirradiated McCoy cells. Wentworth and Alexander (15) have established that exposure of nonirradiated McCoy cells to 5-iodo-2'-deoxyuridine (IUDR) for 3 days before infection rendered such McCoy cells as susceptible to Trachoma growth as irradiated McCoy cells. In a comparative study of fast- and slow-growing *C. trachomatis* agents, Blyth and Taverne (1) demonstrated that BHK-21 cells formed more inclusions and were as effective as irradiated McCoy cells in isolation of Trachoma agents from ocular and genital infections. Other direct comparisons of the effectiveness of different cell lines are not available.

The study reported here makes direct comparisons of 11 cell lines infected with two Trachoma strains. Additionally, two cell culture systems which have been extensively used in our laboratories, HeLa 229 and IUDR-treated McCoy cells, both pretreated with DEAE-dextran, were compared for their sensitivity to infection with different immunotypes of Trachoma strains from human ocular and genital origins.

MATERIALS AND METHODS

Trachoma strains. The strains employed were: A/G-17/OT, B/TW-5/OT, C/TW-3/OT, C/ND-3/OT, D/G-1/OT, E/Bour/OT, E/UW-5/Cx, F/UW-6/Cx, H/UW-4/Cx, I/UW-12/Ur, I/UW-29/Cx, I/870/OC, and J/475/Cx. Strains 475 and 870 were obtained from Julius Schachter, University of California, San Francisco. The strain names include: immunologic type/abbreviation of country or laboratory and number/site of isolation. Abbreviations are OT (ocular Trachoma), Cx (cervix), Ur (urethra), and OC (ocular conjunctivitis). These strains have been described previously (14). Strain 475 is tentatively classified as type J which is closely related to type C. All Trachoma organisms used have been serially passed only in embryonated eggs. For testing infectivity in various cell lines, dilutions of infected-yolk sac suspension were used as inoculum. A 40% (wt/vol) infected-yolk sac suspension of each strain was prepared in sucrose-phosphate-glutamate (sucrose, 75 g; KH_2PO_4, 0.52 g; Na_2HPO_4, 1.22 g; glutamic acid, 0.72 g; water to 1 liter; pH 7.4 to 7.6), dispensed in 1-ml volumes in screw-cap vials, and kept at −70 C until used. Two dilutions, 10^{-3} and 10^{-4} (or 10^{-2} and 10^{-3}), were inoculated on cell monolayers. A 10% suspension was regarded as a 10^{-1} dilution. Two of the strains were titrated in eggs: TW-3 (50% egg infective dose of $10^{-8.1}$/g of yolk sac) and UW-5 (50% egg infective dose of $10^{-7.9}$/g of yolk sac).

Clinical specimens. Conjunctival swab specimens were collected from active Trachoma cases as a part of our epidemiological study of Trachoma being conducted on Taiwan (7). Thirteen specimens from which an isolate was obtained and the immunologic types determined were included in this study. These specimens have been frozen and thawed at least once and some were diluted if the amount of remaining specimen was not enough for the test.

Cell lines. The 11 cell lines used are listed in Table 1 along with the source.

Cell culture. All cells were grown in monolayers in a medium consisting of Eagle minimum essential medium supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) buffered with 0.04% $NaHCO_3$ and 10 mM β-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid. For infection, 12-mm diameter cover slips were placed in the bottom of 1-dram (4 ml) glass vials followed by addition of 2×10^5 to 3×10^5 cells in 1 ml of cell culture medium containing streptomycin but without penicillin. The vials were incubated at 35 C overnight to give confluent monolayers, or for 3 days in the case of IUDR pretreatment. IUDR pretreatment (15) involved covering monolayers of 2×10^5 cells with 1 ml of culture medium containing streptomycin and 10 μg of IUDR per ml (Stoxil, 0.1% opthalmic solution, Smith, Kline and French Labs., Philadelphia, Pa.).

Pretreatment with DEAE-dextran. In these experiments all cell cultures were routinely pretreated with DEAE-dextran before inoculation (12).

Determination of inclusion counts. Monolayers of each cell line were infected as described previously (12). Briefly, the culture medium of each vial was removed, the monolayer was rinsed twice with DEAE-dextran (30 μg/ml), 0.1 ml of inoculum was added, and the mixture was centrifuged at $900 \times g$ at 20 C for 60 min. Four cover slip monolayers were inoculated for each dilution of Trachoma suspension. After centrifugation, the inoculum was removed and 1 ml of culture medium added. The vials were incubated at 35 C for 3 days. The cover slips were then removed, stained with Giemsa, and examined at ×400 magnification with a micrometer (7 by 7 mm) centered in the ocular lens. Inclusions were counted in 30 fields and the results expressed as the average of the four cover slips per dilution (2). Each experiment was performed twice in successive weeks using the same pool of inoculum. For comparison, HeLa 229 was included in every experiment and infected in parallel with other cell lines. To express infectivity of a given cell line, an index value was obtained by dividing the average inclusion count of that cell line by the average inclusion count of HeLa 229 cells inoculated at the same time. An index value of 1.0 indicates that the cell line showed the same number of inclusions as HeLa 229. Values more or less than 1.0 state that the cell line produced greater or fewer inclusions.

In one experiment, iodine staining of 3-day inclu-

TABLE 1. *Cell lines: sources, and history*

Cell line	Heteroploid (H) or diploid (D)	Origin	Source[a]
HeLa 229	H	Human, cervix, carcinoma	Kuo
HeLa M	H	Human, cervix, carcinoma	Cooney
Hep-2	H	Human, larynx, carcinoma	Cooney
FT	D	Human, tonsil, fetal	Cooney
BHK-21	H	Hamster, kidney	Cooney
Vero	H	Monkey, kidney	Jenkin
MK-2	H	Monkey, kidney	Jenkin
MPK	H	Minipig, kidney	Jenkin
L-W05A2	H	Mouse, connective tissue	Jenkin
McCoy	H	Mouse[b]	Alexander
L-929	H	Mouse, connective tissue	ATCC

[a] M. K. Cooney, University of Washington, Seattle; H. M. Jenkin, Hormel Institute, Austin, Minn.; E. R. Alexander, University of Washington, Seattle; ATCC, American Type Culture Collection.

[b] Reference 4, Gordon et al.

sions was also done according to the method described (15).

RESULTS

Variation of inclusion counts. The expected variation in inclusion counts was determined with ocular TW-3 and genital UW-5 strains in HeLa 229 cells. Three 10-fold dilutions (10^{-3}, 10^{-4}, and 10^{-5}) were employed. The test was repeated 10 times over a period of 4 weeks using the same inoculum pool. The variation of inclusion counts is presented in Table 2. The standard deviation of the mean inclusion count was from 21 to 48% and the standard error 7 to 15%. There was a linear relationship between concentration of inoculum and the inclusion count.

Infection of 11 cell lines with TW-3 and UW-5. None of the cell lines evidenced deleterious effects from the manipulations or culture conditions. At the time of infection, the 1-day-old monolayers of each cell line appeared mostly confluent. Every cell line produced demonstrable inclusions, although the numbers of inclusions ranged widely. The size of inclusions also varied, but for the most part, the larger the cell cytoplasm the greater the size of the inclusion it contained. The larger inclusions did not necessarily contain more elementary bodies or suggest a potential for greater infectivity.

Tables 3 and 4 present the results of the comparative studies of the 11 cell lines inoculated with TW-3 (type C) and UW-5 (type E). Inclusion counts with UW-5 were usually higher than with TW-3. The rank order of infectivity indices for the cell lines, whether infected with TW-3 or UW-5, was about the same. When infected with either TW-3 or UW-5, HeLa 229 was unsurpassed in formation of inclusions. With TW-3, MK-2 was the closest to HeLa 229 with indices of 0.89 at the 10^{-3} dilution and 0.65 at 10^{-4}. With UW-5, Hep-2 was the closest with indices of 0.43 and 0.37. With both Trachoma strains, MK-2, Hep-2, McCoy, and HeLa M comprised the group of cell lines showing medium susceptibility compared to HeLa 229. The remaining cell lines were much less susceptible.

Infectivity of various Trachoma strains in HeLa 229 and IUDR-pretreated McCoy cells. Table 5 shows the results of comparing 13 different Trachoma strains for their ability to form inclusions in HeLa 229 versus IUDR-pretreated McCoy cells. An index value greater

TABLE 2. *Variation of inclusion counts in 10 cell culture titrations over 4 weeks in HeLa 229 cells with Trachoma strains TW-3 and UW-5*[a]

Inoculum dilutions	Inclusion counts per 30 fields							
	C/TW-3/OT				E/UW-5/Cx			
	Avg	SD	% SD	% SE	Avg	SD	% SD	% SE
10^{-3}	375	99	26.4	8.4	552	155	28.1	8.9
10^{-4}	63	30	47.6	15.1	107	51	47.7	15.1
10^{-5}	8	2	25.0	7.9	14	3	21.4	6.8

[a] $SE\text{-}SD/\sqrt{N}$, where N = 10, SD is standard deviation, and SE is standard error.

TABLE 3. *Infectivity of TW-3 in 10 different cell lines compared to HeLa 229*

Cell line	10^{-3} Dilution			10^{-4} Dilution		
	Inclusion counts		Index	Inclusion counts		Index
	Cell line	HeLa 229		Cell line	HeLa 229	
HeLa 229			1.00			1.00
MK-2	235	264	0.89	41	63	0.65
Hep-2	251	482	0.52	22	60	0.37
McCoy	126	376	0.34	33	80	0.41
HeLa M	50	415	0.12	16	79	0.20
BHK-21	8	247	0.03	4	40	0.10
L-W05A2	9	344	0.03	6	69	0.07
L-929	9	261	0.03	2	59	0.03
FT	6	431	0.01	2	79	0.03
Vero	3	282	0.01	3	70	0.04
MPK	1	339	<0.01	1	78	0.01

TABLE 4. *Infectivity of UW-5 in 10 different cell lines compared to HeLa 229*

Cell line	10^{-3} Dilution			10^{-4} Dilution		
	Inclusion counts		Index	Inclusion counts		Index
	Cell line	HeLa 229		Cell line	HeLa 229	
HeLa 229			1.00			1.00
Hep-2	271	630	0.43	69	187	0.37
MK-2	185	504	0.37	44	136	0.32
McCoy	135	454	0.30	37	138	0.27
HeLa M	168	619	0.27	25	212	0.12
LW05A2	64	586	0.11	22	214	0.10
L-929	28	384	0.07	6	108	0.06
BHK-21	9	472	0.02	5	120	0.04
Vero	4	257	0.02	3	219	0.01
MPK	1	575	<0.01	1	264	<0.01
FT	3	619	<0.01	1	212	<0.01

TABLE 5. *Infectivity of various Trachoma strains in HeLa 229 and IUDR-pretreated McCoy cells*

Trachoma strains	Lower dilution[a]			Higher dilution[a]		
	Inclusion counts		Index	Inclusion counts		Index
	IUDR-McCoy	HeLa 229		IUDR-McCoy	HeLa 229	
A/G-17/OT	301	334	0.90	83	97	0.86
B/TW-5/OT	150	249	0.60	45	34	1.32
D/G-1/OT	675	600	1.13	237	317	0.75
E/Bour/OT	347	395	0.88	85	102	0.83
E/UW-5/Cx	330	454	0.73	85	138	0.62
F/UW-6/Cx	222	189	1.17	37	42	0.88
H/UW-4/Cx	158	187	0.84	72	73	0.99
C/TW-3/OT	119	376	0.32	28	80	0.35
C/ND-3/OT	89	412	0.21	24	107	0.22
I/UW-12/Ur	71	175	0.41	14	34	0.41
I/UW-29/Cx	58	278	0.21	6	32	0.19
I/870/OC	42	253	0.17			
J/475/Cx	12	53	0.23	3	10	0.30

[a] Inocula (two 10-fold dilutions): 10^{-2} and 10^{-3} for strains UW-4, ND-3, and 870; 10^{-3} and 10^{-4} for other strains.

than 1.0 means that more inclusions were counted in IUDR-pretreated McCoy cells than in HeLa 229 cells. Although there were variations in results with the dilutions of inoculum, the results suggest that with the strains tested of Trachoma immunotypes A, B, D, E, F, and H the ability of the two cell cultures to support growth was about the same. On the other hand, the six type C, I, and J strains grew much better in HeLa 229 cells.

Effect of IUDR pretreatment on McCoy and HeLa 229 cells susceptibility to Trachoma organisms. Studies of the effect of IUDR treatment on the ability of McCoy cells to support growth of several immunotypes of Trachoma organisms is shown in Table 6. An approximate two-fold increase in inclusion count was observed with D/G-1/OT and E/UW-5/Cx after IUDR treatment. However, no enhancement was found with the type C, I, and J strains tested: C/TW-3/OT, C/ND-3/OT, I/UW-12/Ur, and J/475/OC.

IUDR pretreatment of HeLa 229 cells was studied with TW-3 and UW-5 strains. Infectivity indices ranged from 0.90 to 1.16 with UW-5 and 0.61 to 0.65 with TW-3 indicating no enhancement of infection.

Comparative isolation of Trachoma organisms from conjunctival swab specimens in HeLa 229 and IUDR-pretreated McCoy cells. Since laboratory established type C strains persistently showed fewer inclusions in IUDR-pretreated McCoy than in HeLa 229, a comparison of the two culture systems for isolation of

TABLE 6. *Effect of IUDR pretreatment of the cell culture on growth of several Trachoma strains in McCoy cells*

Trachoma strains	Lower dilution[a]			Higher dilution[a]		
	Inclusion counts		Index, IUDR+/-	Inclusion counts		Index, IUDR+/-
	IUDR-McCoy	McCoy		IUDR-McCoy	McCoy	
D/G-1/OT	273	141	1.94	57	33	1.70
E/UW-5/Cx	335	135	2.48	64	37	1.73
C/TW-3/OT	119	126	0.94	28	33	0.85
C/ND-3/OT	32	53	0.60	9	11	0.82
I/UW-12/Cx	47	68	0.69	7	8	0.88
J/475/OC	9	20	0.45	4	6	0.67

[a] Inocula (two 10-fold dilutions): 10^{-2} and 10^{-3} for strains ND-3 and 475; 10^{-3} and 10^{-4} for other strains.

wild-type C strains was undertaken. Isolation of Trachoma organisms from known positive conjunctival swab specimens was attempted simultaneously in HeLa 229 and IUDR-pretreated McCoy. Four tubes were inoculated per specimen. At the first passage, one tube was stained with Giemsa and another with iodine. The remaining two tubes were harvested and serially passaged to four new tubes. Isolation was terminated on the second passage, two tubes of which were stained with Giemsa and two with iodine.

From 10 specimens previously yielding type C organisms, reisolation was made from five in both cell systems. Two reisolations were accomplished in HeLa 229 only, one on the first passage and one on the second passage. There was one reisolation in the McCoy system only on the second passage when a single inclusion was identified in all four slides. Two type B and one type D specimen were positive in the first passage in both HeLa 229 and McCoy. With Giemsa stain, inclusions in HeLa 229 cells were more easily seen than in McCoy cells. Conversely, with iodine stain the inclusions of McCoy cells were more easily detected. Often in HeLa 229 and once in McCoy cells a large portion of inclusions seen by Giemsa were not stained by iodine. Inclusions were generally larger in HeLa 229 than in McCoy cells, but the numbers counted were similar in the two cell systems.

DISCUSSION

There has been a paucity of reports dealing with direct comparisons of Trachoma inclusion formation in various cell lines. Workers in the field have frequently relied upon limited information in choosing a cell culture system for growth and/or isolation of Trachoma agents. An example is the prevalent supposition that irradiated McCoy cells (5) or the equivalent, IUDR-pretreated McCoy cells (15) constitute the best system available for working with Trachoma agents. However, two essentially different systems have been developed using HeLa 229 (12) and BHK-21 (1) cells which have the common advantage over McCoy of not requiring pretreatment with IUDR or irradiation. These two systems have shown to be at least equal to the McCoy systems in growth and isolation of Trachoma agents. Even McCoy cells without any treatment have been used with some success (9). It appears that centrifugation of the inoculum onto the cell layer provides the most effective enhancement of infectivity.

In the present report, experiments were conducted in uniform fashion so that results obtained for the tested cell lines would be comparable. However, it may be that each cell line has its own optimum conditions for the growth of Trachoma organisms which were not found and utilized. Besides centrifugation, DEAE-dextran treatment was added to the procedure, because it was simple and effective. The concentration of DEAE-dextran (30 μg/ml) was not found to cause noticeable harm to cells (11), and similar concentrations (20 to 30 μg/ml) have been used by others in McCoy (13, 15) and Hep-2 (8) to enhance chlamydia infection.

The use of Giemsa for observing inclusions is well suited for cell lines such as HeLa 229, HeLa M, Hep-2, MK-2, and FT which have relatively large cytoplasma with easily detectable inclusions. McCoy, Vero, BHK-21, and L cells are less suitable because of their more compact morphology and inclusions are not easily differentiated with Giemsa stain. Although Giemsa can be used with such cell lines, iodine staining, because observation is easier and quicker, has been preferred. However, one has to be aware of the possibility of inclusions not being stained by

iodine. Iodine stains glycogen in the inclusions. Ample glycogen is present only in certain stages of the developmental cycle of the organisms (6).

The unexpectedly low level of inclusion formation by TW-3 and UW-5 in BHK-21 compared to HeLa 229 might have been because the particular line of BHK-21 used in these experiments possessed basic differences of susceptibility from the BHK-21 line used by others (1). This could be analogous to the contrasting results obtained here using two HeLa lines, HeLa 229 and HeLa M. Jenkin (10), in addition, reported that of three HeLa cell lines, HeLa 229, S3, and calf, only HeLa 229 was susceptible to infection with two Trachoma strains employed.

Further unexpected results came from pretreating HeLa 229 and McCoy cells with IUDR. Not only did IUDR fail to enhance infectivity in HeLa 229, but caused a slight decrease in inclusions after infection with TW-3 (but not UW-5). This inhibition may result from alteration of HeLa 229 cells due to the IUDR; or it may simply be a consequence of the advanced age of the cell monolayer at the time of infection, since the IUDR pretreatment requires 3-days incubation before infection. The most significant finding was the failure of IUDR pretreatment to enhance infection with type C, I, and J strains. This failure probably explains the relatively poor growth of these strains only in IUDR-pretreated McCoy cells when compared to HeLa 229.

Using no treatment other than rinsing with DEAE-dextran, HeLa 229 consistently performed better than any of the other cell lines. IUDR-pretreated McCoy cells produced results closely comparable to HeLa 229 with most Trachoma types and with the few isolation studies from conjunctival specimens. However, the cells relative insensitivity to type C, I, and J strains provides serious concern over this widely used culture system. More comparative isolation studies from clinical specimens would be necessary before concluding that IUDR-treated McCoy cells are less effective for isolation of type C, I, and J strains. In addition to the consistently high sensitivity to infection of all known types of Trachoma organisms, we prefer the HeLa 229 cell culture system with DEAE-dextran pretreatment for growth and isolation of Trachoma organisms because of the simplicity and rapidity of monolayer preparation.

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