Microcytotoxicity Tests on Human Cells in Culture: Effect of Contamination with Mycoplasma (37295)

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Cell culture techniques are widely used to study immunological aspects of human cancer. Investigations of antigens associated with human tumors and of the host immune response often use cell cultures derived from malignant and nonmalignant tissues. Contamination by mycoplasma (pleuropneumonia-like organisms = PPLO) is a major problem of tissue culture. When relying on cultured cells for biological studies, it should be considered that PPLO can influence the intrinsic surface or internal properties of the infected cells. Organisms can be localized intracellularly or at the cell surface (1). Although mycoplasma may not kill the infected cells, they influence the concentrations of nutrients in the culture medium (2), cause chromosomal abnormalities, change cell morphology (1), and reduce the efficiency of viral transformation (3). Fogh has also shown that SV-40 transformed human amnion cells support mycoplasma growth better than nontransferred cells (4). Data presented here show that mycoplasma infection may markedly affect the results obtained in tests for new antigens, and humoral antibody against cell surface antigens, on cultured human cells.

Materials and Methods. A large number of cultures derived from human tumors are used in the laboratory for immunological studies. Tests for PPLO contamination are performed routinely on all cultures. Cellular material from the cultures is streaked on nutrient agar plates specified for mycoplasma growth (Grand Island Biological Co., Berkeley, CA), plates are monitored for at least 10 days for growth of mycoplasma colonies. Contaminated cultures are either destroyed or isolated and treated with 50–100 μg/ml Aureomycin (Lederle, Pearl River, NY) for at least one month. Cultures are retested repeatedly beginning two weeks or more following cessation of treatment.

The present results were obtained in a serological study of human carcinomas using a complement mediated microcytotoxicity test, as previously described (5, 6). Rabbit serum was used as a source of complement. A cell line derived from an epidermoid carcinoma of the cervix, Me180 (7), has been an important target cell line in the present investigation. Early in the study it was found that a large number of sera had been tested against culture passages of Me180 which were infected with mycoplasma. The species was not identified. Cells were treated with Aureomycin, and no residual contaminating organisms have been detected following treatment to the present time. All sera were tested again for cytotoxicity against several mycoplasma-free passages of Me180. Most sera were tested on cells from more than one passage, thus ascertaining that any observed difference in titers was not attributable to a "normal" variation of the target cells in culture (6).

Results. Effect of mycoplasma contamination on Me180 target cells. Table I compares the results of the two series of cytotoxicity tests against Me180. It is obvious that many more sera were cytotoxic when the test cells were infected with mycoplasma. This was shown by the fraction of sera which showed increased titers when tested on PPLO-infected target cells. The effect was particularly striking with sera from patients with lymphomatous diseases. The fraction of these sera changing titer in the positive direction

TABLE I. Comparison of Cytotoxic Antibody Titers Against PPLO-Free Versus PPLO-Infected Me180 in Sera from Different Groups of Patients.

Groups of serum donors[a]	Antibody titers: On PPLO — Me180			On PPLO + Me180			Positive change	
	—	≤1:2	≥1:4	—	≤1:2	≥1:4	No. + changes/total	Percent
Breast Ca	7	—	2	6	—	3	1/9	11.1
Cervix Ca	3	—	5	1	1	6	2/8	25.0
Other female Ca	5	1	3	3	—	6	3/9	33.3
Lymphomas	16	1	3	5	2	13	11/20	55.0
B.L. (6) and NPC (2)	6	1	1	3	—	5	4/8	50.0
Hodgkin's	6	—	1	6	—	1	0/7	0.0
Digestive system Ca	6	3	—	6	—	3	3/9	33.3
Lung Ca	6	1	5	6	—	6	1/12	8.3
Melanoma	3	—	1	—	1	3	3/4	75.0
Sarcomas	11	1	3	9	—	6	3/15	20.0
Normal	22	2	6	18	1	11	5/30	16.7
Multipara	3	—	2	3	—	2	0/5	0.0
Total	94	10	32	66	5	65	33/136	24.3

[a] Abbreviations: Ca = carcinoma; B.L. = Burkitt's lymphoma; NPC = nasopharyngeal carcinoma.

(55.0%) differed significantly from that of all other sera (22/116 = 19.0%) with $p < 0.005$ by χ^2, and from that of sera from normal individuals (16.7%) with $p < 0.025$ by χ^2. Figure 1 shows the actual differences in cytotoxic antibody titers in sera from patients with lymphoid malignancies in tests against PPLO-infected Me180, versus Me180 in which no PPLO was detected. The effect also occurred in sera from patients with Burkitt's Lymphoma and nasopharyngeal carcinoma, and malignant melanoma, and to a lesser extent with sera from patients with miscellaneous carcinomas of the digestive system, or female reproductive system (see Table I).

Although the sample was small, it is interesting that a higher percentage of sera from patients with carcinoma of the cervix were reactive against the PPLO-negative Me180 target cells than sera from other groups. It is possible that this preferential reactivity reflects an antigenic specificity associated with cervical cancer.

Effect of mycoplasma contamination on skin fibroblast target cells. A skin fibroblast line (Coh) which was contaminated with mycoplasma was tested by cytotoxicity with 2 sera from cancer patients, and one serum from a normal individual. Titers were 1:8, 1:4, and —, respectively. All 3 sera were negative when tested on the same cell line after successful treatment with Aureomycin. The 2 sera which showed changed titers did not do so in the case of the Me180 target cells.

To ascertain that the deviation in cytotoxic antibody titers was attributable to PPLO-induced changes in the target cells, human skin fibroblasts, which had never been contaminated, were intentionally infected with mycoplasma. Two separate isolates of mycoplasma from tissue culture were used. The efficiency of infection was ascertained using nutrient agar plates, and infection of the fibroblasts was confirmed. A selected group of sera were tested in cytotoxicity tests against PPLO-infected and uninfected cells. The results shown in Table II show that after infection with mycoplasma, one cell line (Por) demonstrated heightened sensitivity to serum in the presence of complement. Comparable results were obtained

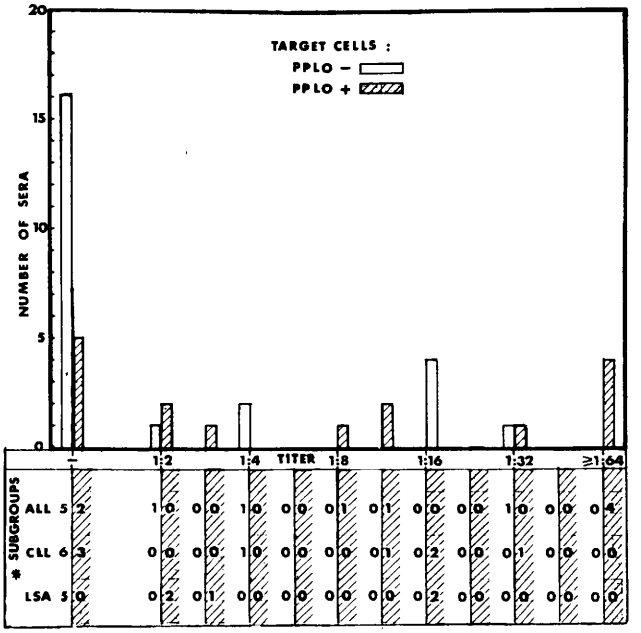

FIG. 1. Cytotoxic antibody titers in sera from lymphoma patients against Me180 cells contaminated with PPLO and against Me180 cells in which no PPLO could be detected. Titers represent that dilution of test serum which allowed 50% cell survival relative to an identical dilution of well-screened, noncytotoxic control serum. The difference in distribution of titers on infected versus uninfected target cells among groups of lymphoma patients is also shown. (ALL = acute lymphocytic leukemia; CLL = chronic lymphocytic leukemia; LSA = lymphosarcoma.)

when cells were tested 5 days and 3 weeks following infection. Results with a second skin fibroblast line (Ric) showed no influence by mycoplasma infection.

Anti-M. pneumoniae antibody. Six sera including 5 which demonstrated the greater sensitivity of mycoplasma infected target cells, were tested by complement fixation against *Mycoplasma pneumoniae*. All sera were negative for *M. pneumoniae* complement fixing antibodies. (See Table II, footnote *d*).

Discussion. Mycoplasma infection of cultured cells may significantly influence data derived using those cells for cytotoxicity tests. Although only one immunological test was used in the present study, this finding might be extended to other immunological tests and test systems. Increased sensitivity in cytotoxicity tests could be induced *de novo* by infecting a line of human skin fibroblasts (Por) cells with PPLO. The increased sensitivity to cytotoxic sera could be reversed by successful treatment of the mycoplasma infection. This was shown with Me180 cervix carcinoma cells, as well as with a line of skin fibroblasts (Coh).

The increased sensitivity to cytotoxic sera could be due to PPLO antigens, PPLO-induced antigens, unmasking of cellular antigens, or nonspecific changes in the target cells due to the surface localization of the organisms. It is doubtful that appearance of PPLO antigens was responsible for heightened target cell killing, since none of five sera which showed titer differences on mycoplasma-infected versus uninfected target cells had detectable complement-fixing antibody against *M. pneumoniae*. The possibility of a nonspecific change in the target cells is likewise unlikely since cells did not become proportionately more sensitive to all sera tested. Furthermore, sera showing changed titers on one of the PPLO-infected target cell lines did not necessarily show such a change with another line. The PPLO-induced change in the different target cells was, therefore, quali-

TABLE II. The Effect of Mycoplasma Infection on the Sensitivity of Skin Fibroblast Cells to Selected Sera Plus Complement.[a]

Serum	Diagnosis	Cytotoxic antibody titer[b]			
		Showed difference on PPLO-infected Me180	Against skin fibroblast cells infected with[c]		
			0	I	II
102[d]	ALL	yes	—	1:4	1:2-4
268[d]	CaOv (anti-HL-A)[e]	yes	1:16	1:32	1:64
38[d]	Normal	yes	—	—	—
93	LSA	yes	—	—	—
94[d]	CLL	yes	—	—	—
95	RCS	yes	—	—	—
231[d]	CaCx (anti-HL-A)[e]	no	1:32	1:128	1:128
98[d]	ALL	no	—	—	—
183	ChMyoSa	no	—	—	—

[a] Abbreviations: ALL = acute lymphocytic leukemia; CaOv = carcinoma of the ovary; anti-HL-A = polyvalent anti-HL-A serum; LSA = lymphosarcoma; CLL = chronic lymphocytic leukemia; RCS = reticulum cell sarcoma; CaCx = carcinoma of cervix; ChMyoSa = chondromyosarcoma.

[b] Titer = dilution of test serum allowing 50% survival relative to an identical dilution of a noncytotoxic control serum.

[c] Skin fibroblasts (Por) were left uninfected (0), or infected with each of 2 tissue culture isolates of PPLO (I; II), and used as target cells. (—) indicates no cytotoxicity.

[d] These sera were tested by complement fixation for anti-*M. pneumoniae* antibody by the U.C.L.A. Hospital Clinical Laboratory.

[e] Anti-HL-A was determined although these sera had antibodies against multiple HL-A antigens, as determined by cytotoxicity through the courtesy of Dr. P. Teraski and Dr. M. Takasugi, U.C.L.A., occurrence of anti-HL-A activity in test sera did not correlate with the demonstration of mycoplasma induced changes in the target cells.

tatively or quantitatively different, and perhaps dependent upon individual properties of each target cell line. The present data could be explained by the mycoplasma making previously cryptic cellular determinants available to antibody and complement, or by PPLO inducing a change in an already exposed cellular antigen.

Certain groups of sera showed significantly more striking changes in titers than other groups on the PPLO-infected versus uninfected Me180 target cells. Clearly, mycoplasma infections can interfere significantly with serological analysis, and should not be disregarded in any tumor immunology studies which involve cultured cells.

Summary. Mycoplasma infection of human cell cultures significantly changed results of tests for complement-dependent antibody. Infected Me180 cells were lysed with a larger number of sera and with higher serum dilutions than the corresponding uninfected cells. Certain groups of sera (*e.g.*, sera from the patients with lymphomas) showed a more striking difference when tested against the contaminated versus uncontaminated Me180 cells. A similar change in target cell sensitivity was also observed with two lines of human skin fibroblasts. Mycoplasma infection is an important factor to consider in interpreting the results of experiments dependent on cultured cells.

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