
**A TIME-LAPSE STUDY OF EFFECTS OF ANTICELLULAR ANTIBODY
ON MEMBRANE MOBILITY AND PHAGOCYTTIC ACTIVITY OF HELA CELLS**

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Phagocytosis is a recognized activity of most cells cultured *in vitro*, and HeLa cells are intermediate in phagocytic activity (2). The phagocytic activity of HeLa cells is inhibited by anticellular antibody, and this inhibition occurs with concentrations of anticellular antibody which are not cytotoxic in complement-free systems and do not alter uptake of tritiated thymidine and uridine (3).

Electron microscopic examination of HeLa cells reacted with anticellular antibody demonstrated a marked folding or agglutination of the plasma membrane (3). Easton, Goldberg, and Green (4) reported similar changes in the morphology of ascites tumor cells treated with anticellular antibody and they demonstrated ferritin-labeled antibody between the folds of the membrane. These observations suggest that anticellular antibody causes agglutination of contiguous portions of the plasma membrane which may fix the membrane and prevent evagination and invagination of the cell periphery essential for phagocytosis.

To evaluate this hypothesis, we studied the effect of anti-HeLa antibody on the mobility of the plasma membrane of HeLa cells with time-lapse cinematography. The effect of antibody on membrane mobility was compared with its effect on the capacity of the cells to phagocytose staphylococci. As antibody fragments obtained by papain digestion are univalent (5), they would combine with the plasma membrane but would not agglutinate. The effect of antibody fragments on phagocytosis and membrane mobility was evaluated also.

MATERIALS AND METHODS

HELA CELL CULTURES: Monolayers of HeLa cells grown in Eagle's Minimal Essential Medium (MEM) with 10% calf serum were trypsinized and cultured on coverslips in tissue culture dishes. After 18-24 hr at 36°C in 5% CO₂, the coverslips were assembled in Sykes-Moore chambers.

ANTICELLULAR ANTIBODY: Gamma globulin was isolated by ammonium sulfate precipitation of serum from rabbits immunized with repeated injections of washed, intact HeLa cells. Control gamma globulin was obtained from the rabbits prior to immunization. Univalent fragments were prepared as described by Porter (5), with modification. Gamma globulin in 0.1 M phosphate buffer, pH 7.4, was incubated for 2 hr at 36°C with 1% papain, 0.02 M cysteine-HCl, 0.004 M EDTA. The fragments were separated by carboxymethyl cellulose column chromatography and concentrated by pervaporation under vacuum at 2°C. Purity of the fractions was verified by gel diffusion with goat anti-rabbit F_{ab} and F_c.¹

Both intact gamma globulin and fragment preparations were adjusted to equivalent protein concentrations by dilution in MEM with 5% inactivated calf serum and were added to the HeLa cultures in assembled chambers. The effects of varying concentrations of these preparations on membrane activity and the capacity of cells to phagocytose staphylococci were studied in parallel cultures of HeLa cells.

TIME-LAPSE CONDITIONS: HeLa cell cultures in Sykes-Moore chambers were photographed with

¹ Carey, F. J., and C. K. Osterland. Unpublished observations.

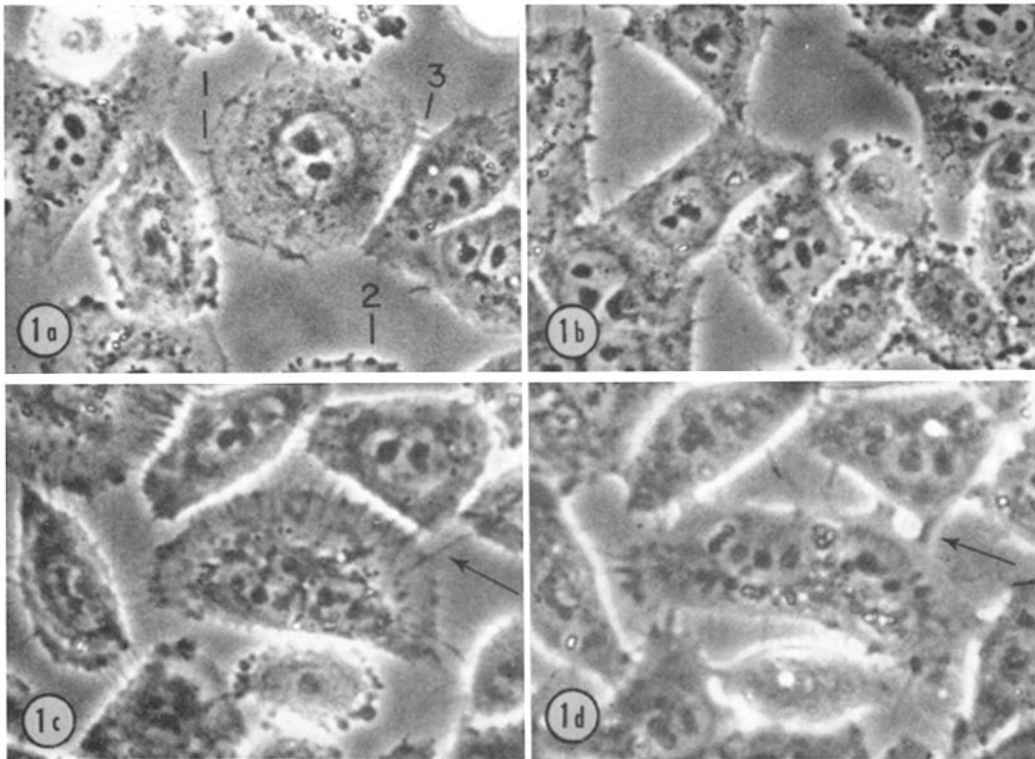


FIGURE 1 *a* HeLa cells and normal gamma globulin, 1.0 mg/ml, 2 hr, 36°C. \times 750 phase. 1. Filamentous type projections. 2. Broad footlike processes. 3. Intercellular bridges.

FIGURE 1 *b* HeLa cells and papain-split anticellular gamma globulin 1.5 mg/ml, 2 hr, 36°C. \times 750 phase.

FIGURE 1 *c* HeLa cells and anticellular gamma globulin, 1 mg/ml, 7 min after addition of antibody fraction, 36°C. \times 750. Arrow indicates cytoplasmic process which straightened, thickened, and became immobile.

FIGURE 1 *d* Same field as *c* 2 hr after addition of antibody fraction. Arrow indicates cytoplasmic process as mentioned in 1 *c*.

phase optics at \times 166 in an upright microscope at 36°C. Experiments were recorded on 16-mm film at a speed of 32 frames per minute. Selected fields of cultures were photographed intermittently over a 24-hr period.

PHAGOCYTOSIS OF STAPHYLOCOCCI: The techniques used to determine the effects of anticellular antibody on phagocytosis of *Staphylococcus aureus* by HeLa cells have been described previously (3). The per cent of cells which phagocytosed staphylococci was obtained by direct count of fixed and stained preparations.

MEASUREMENT OF ANTIBODY ACTIVITY: Previous studies demonstrated that the concentration of antibody required to inhibit phagocytosis exceeded that of antibody needed to produce necrosis in the

presence of complement. Antibody activity was determined as previously described (3). The concentrations of antibody selected for study inhibited phagocytosis, but were not cytotoxic in the absence of complement. The combination of univalent antibody fragments with HeLa cells was verified with fluorescent antibody techniques and ^{125}I -labeled fragments.¹

RESULTS

Control cultures incubated with normal gamma globulin or normal fragments exhibited two types of membrane activity: (1) active protrusion and withdrawal of cell processes in broad, footlike formations; (2) the continuous undulating move-

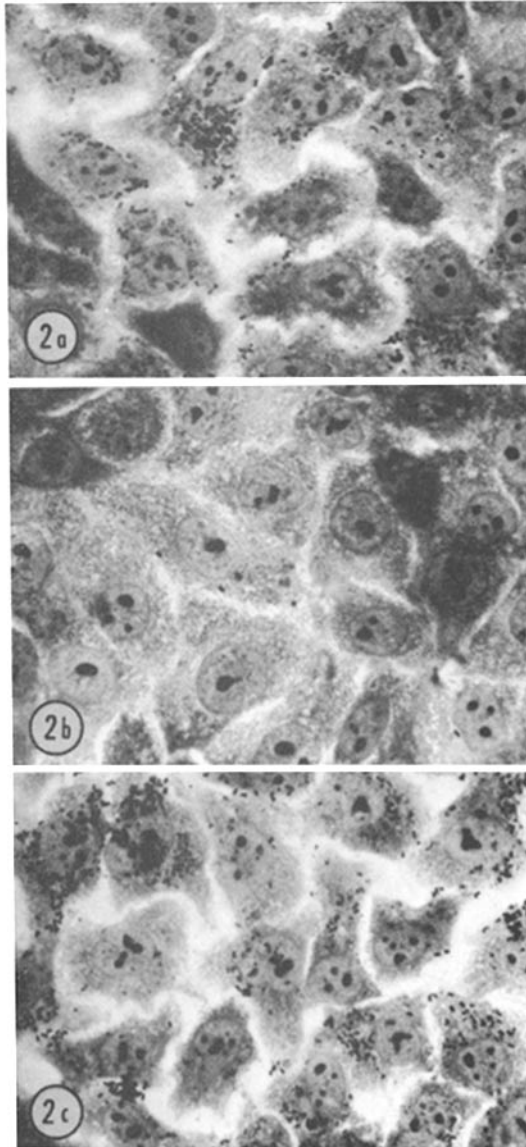


FIGURE 2 HeLa cells exposed to gamma globulin fractions for 4 hr, followed by staphylococci for 18 hr. Giemsa stain. $\times 600$.

FIGURE 2 *a* Normal gamma globulin, 1.0 mg/ml.
 FIGURE 2 *b* Anticellular gamma globulin, 1.0 mg/ml.
 FIGURE 2 *c* Univalent anticellular fragments, 1.5 mg/ml.

ment of thin, filamentous projections from the cell surface (Fig. 1 *a*). The entire membrane appeared to be involved in this activity. Between adjacent cells cytoplasmic bridging was observed, and these

TABLE I
Effect of Gamma-Globulin Preparations on Membrane Mobility and Phagocytosis

Gamma-globulin and Concentration	Phagocytosis	Membrane mobility
<i>mg/ml</i>		%
Intact antibody		
1.0	$\frac{22^*}{264}$ (8)	5-10‡
0.66	$\frac{78}{277}$ (28)	20-30
0.50	$\frac{149}{256}$ (58)	50
0.40	$\frac{253}{278}$ (84)	80-90§
Univalent antibody		
1.5	$\frac{255}{287}$ (89)	100
0.75	$\frac{252}{275}$ (91)	100
Normal gamma-globulin		
1.0	$\frac{225}{242}$ (93)	100
0.66	$\frac{208}{226}$ (92)	100

* Numerator, number of cells containing staphylococci; denominator, number of cells examined; number in parenthesis indicates per cent.

‡ Estimated per cent of total membrane actively moving as visualized in time-lapse films.

§ Majority of inactive membrane limited to inter-cellular processes.

connecting processes were in constant motion. These activities persisted during the period of observation, although the footlike processes were more pronounced in young cultures.

Shortly after the addition of intact anticellular antibody, changes in cell surface and mobility occurred. The fine filamentous projections straightened, thickened, and the undulating activity diminished. The effect was observed as early as 10 min after the addition of intact antibody and increased on further incubation, reaching a

maximum by 2–4 hr (Fig. 1 *c, d*). At this time, the processes appeared to be rigid; this was especially marked in processes extending between the cells. The formation of thickened footlike pseudopodia also gradually ceased during the same period. Not all cells nor all portions of the membrane of each cell showed complete inhibition of activity. As the majority of the membrane became inactive, isolated short segments of the cell developed vigorous wavelike motion. Although the alterations in the membrane mobility can be visualized only in the time-lapse film, the thickened and straightened intercellular processes are evident in the prints from the film (Fig. 1 *d*).

Concentrations of antibody which inhibited membrane activity of the majority of cells prevented phagocytosis of staphylococci by 92% of the cells in corresponding cultures (Fig. 2 *a–b*). The degree of inhibition of membrane activity and phagocytosis was directly related to the concentration of antibody added. With decreasing concentrations of antibody, the degree of inhibition of membrane activity was less marked and the percentage of cells phagocytosing staphylococci was greater (Table I).

When the supernatant medium containing the antibody was removed and replaced with MEM free of antibody, membrane mobility and phagocytic activity returned within 12–24 hr. The period of recovery was dependent on the antibody concentration originally added to the culture. With high concentrations of antibody, repeated changes of fresh medium were required.

The anticellular antibody fragments did not alter mobility of the membrane; nor did they inhibit phagocytosis. The activity of the membrane of cells incubated with fragments was indistinguishable from that of the membrane of cells observed in control cultures. (Table I; Figs. 1 *b, 2 c*)

Cell division was observed to continue in all cultures even in the presence of antibody.

SUMMARY

These time-lapse studies demonstrate that intact anticellular antibody inhibited the movement of cell surface and that the extent of inhibition was dependent upon the concentration of antibody in the culture.

The inhibition of membrane mobility was associated with inhibition of phagocytosis, and these effects were directly related.

Univalent antibody fragments did not alter membrane mobility or phagocytic activity.

These observations indicate that anticellular antibody inhibited the phagocytic activity of HeLa cells by immobilizing the plasma membrane. The previously published observation that antibody agglutinates the cell membrane (3, 4) and the above observations with univalent fragments indicate that immobilization of the cell periphery may be due to agglutination of the plasma membrane by intact antibody.

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