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Action mechanism in vitro of sensitized regional lymph node cells on target cell. I. Anti-growth effect of regional lymph node cells of Ehrlich cancer transplanted mouse and that of normal lymph node cells induced by PHA, on Ehrlich cancer cell line (JTC-11)

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Action mechanism in vitro of sensitized regional lymph node cells on target cell. I. Anti-growth effect of regional lymph node cells of Ehrlich cancer transplanted mouse and that of normal lymph node cells induced by PHA, on Ehrlich cancer cell line (JTC-11)*

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Abstract

Under in vivo conditions JTC-II cells derived from Ehrlich ascites tumor are led to destruction by lymph node cells by two procees. The one is the interaction of lymph node cells of the C57BL (♀) mouse sensitized with Ehrlich ascites tumor cells, and the other is the interaction of normal C57BL (♀) mouse lymph node cells treated with PHA-M. In these two reaction systems the following differences have become clear. The regional lymph node cells from the C57BL (♀) mouse sensitized with Ehrlich ascites tumor cells show a marked inhibitory effect on the growth oflTC-II cells by 10 days after sensitization. In the observations under the phase contrast microscope these lymph node cells tend to adhere around the antigenic cells by culture hour 5-6, and by culture hour 24-48 they lead the latter to undergo cytolysis. The normal lymph node cells of C57BL (♀) mouse treated with PHA show anti-growth effect oflTC-II cells. PHA-M used proves to be effective in the concentration of 2% (v/v). Likewise after such normal lymph node cells are previously treated with 2% PHA-M for 12 hours, they also inhibit the growth of lTC-II cells when two cell groups are cultured together. In such intercellular reaction between the two cell groups there is no specificity. By observations under the phase contract microscopy, by culture hour 2-3 the adherence and aggregation of lymph node cells begin to occur, and by 18-24 hours of culture the target cells are led to undergo cytolysis. In this instance, lymph node cells are prone to adhere and aggregate on one side of the target cell.

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ACTION MECHANISM IN VITRO OF SENSITIZED REGIONAL LYMPH NODE CELLS ON TARGET CELL I. ANTI-GROWTH EFFECT OF REGIONAL LYMPH NODE CELLS OF EHRLICH CANCER TRANSPLANTED MOUSE AND THAT OF NORMAL LYMPH NODE CELLS INDUCED BY PHA, ON EHRLICH CANCER CELL LINE (JTC-11)

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Recently, relative to cancer immunity or homotransplantation immunity, studies have been carried out from both *in vivo* and *in vitro* aspects, and the essential part of such immunity has been demonstrated to lie in cellular immunity just as in the case of tuberculin type delayed hypersensitivity. In the series of our past experiments (1, 2) conducted by tissue culture methods, we have clarified that regional lymph node cells, aggregating onto their target cells, wield their anti-growth effect more markedly, and that this reaction has specificity (3, 4), which has been assumed to reflect an aspect of immune phenomenon *in vivo*.

As has been shown more recently by HELLSTRÖM *et al.* (5, 6) and MÖLLER *et al.* (7, 8), non-immune allogeneic lymphocytes under certain culture conditions, show a cytotoxic effect on their target cells by adhering and aggregating onto the latter and this effect is called allogeneic inhibition. The presence of this non-immune reaction mechanism as well as the cytotoxic effect in the immune reaction just mentioned seem to be important fundamental biological phenomena.

However, it remains still to be clarified whether these phenomena of apparent adherence and aggregation, common to *in vitro* immune reaction and allogeneic inhibition, simply show an approach of lymph node cells to target cells or cell specific immunological fusion, and by what mechanism the destruction of target cells is brought about. It seems possible to elucidate a part of cell immunity mechanism by analyzing similarity and dissimilarity of these phenomena in the two reactions and by studying to what extent allogeneic inhibition is involved in the reaction by sensitized lymph node cells *in vitro*. The author has studied the conditions for anti-growth action on Ehrlich cancer cells which would be the antigenic cells

in these two reactions, and present in this paper the findings as the author finds no specificity at all in allogeneic inhibition.

MATERIALS AND METHODS

Animals: The animals used were C57BL (♀) strain of mice bred and maintained at the Mouse Colony of Okayama University. These animals were fed on the solid feed (Oriental Industrial Co., Ltd.) and their age ranged 6–8 weeks old, weighing about 20g.

Tumor and immunization method: The tumor was Ehrlich ascites tumor maintained by successive passage through the peritoneal cavity of C57BL (♀) mice. After aspirating this tumor from the peritoneal cavity it was stained with 0.05ml of 1.0% trypan blue and the cell count of viable cells was taken by Bürkel-Türk hemocytometer. These viable cells 500×10^4 in number were injected subcutaneously on the back between scapulas of C57BL (♀) mouse (20 mice in all) to sensitize. The sensitized group was divided into 4 groups: one whose lymph nodes were taken out 5 days after sensitization, 2nd group whose lymph nodes taken out 7 days later; 3rd group whose lymph nodes taken out 10 days later, and fourth 14 days later.

Preparation of lymph node cells: From 3 mice, each bearing large tumor of about the same size of these 4 groups, axillary and cervical lymph nodes were taken out asceptically, minced with ophthalmic scissors in cold Hanks' solution, filtered through the 80-mesh filter to remove the residue, the filtrate was washed with cold Hanks' solution, and then it was centrifuged at 1,500 rpm for 10 min. This procedure was repeated 3 times to remove serum, and then the precipitate (cells) was again suspended in YLE culture medium (Earl's balanced salt solution containing 0.1% (w/v) yeast extract and 0.5% (w/v) lactalbumin hydrolysate). Normal mouse lymph nodes were obtained in similar manner from non-sensitized C57BL mice (♀).

Preparation of phytohemagglutinin (PHA): One vial (50mg) of phytohemagglutinin-M (Difco Lab.) was dissolved in 5ml YLE culture medium before the use.

Tissue culture cells:

1. *JTC-11 cells*: The cells used as antigenic cells are of a cell line established by SATO (4), HAMAZAKI (10) of Department of Pathology, Cancer Institute, Okayama University by passage of Ehrlich ascites tumor cells through mice. This cell line reproduces tumor in mice when injected and it is registered at Japan Culture Association as JTC-11 cell line. These cells were maintained in the YLE culture medium supplemented with 20% bovine serum for successive generations, and at the time of experiment, they were grown in TD-40 tubes, the cells were removed from the vessel wall with rubber cleaner, passed through the 80-mesh filter to remove the debris, and resuspended in the YLE medium for the use.

2. *A-strain cells*: This cell line has been derived from mammary cancer developed in C3H female mouse and maintained by cultures for successive generations. This cell line was maintained in the same manner as with JTC-11 cells, and for experimental use they were grown in TD-40 tubes, treated with

0.25% trypsin GKN solution (containing 0.1% glucose (w/v), 0.04% KCl, 0.8% NaCl dissolved in 0.25% (w/v) trypsin solution (2000E/g, Wako Pure Chemical Industrial Co., Ltd.)) and the cells on the vessel wall were scraped off, and resuspended in the YLE medium for the use.

3. *HeLa-S_3 cells*: This is a cell line derived from human uterine cancer and maintained by successive generations, being cultured in YLE medium supplemented with 50% bovine serum. For the experimental use the cells were first treated with 0.25% trypsin GKN solution and resuspended in YLE medium.

Tissue cultures:

Exp. I: Anti-growth effect of regional lymph node cells of sensitized mouse on JTC-11 cells

a) *Time-lapse changes of anti-growth effect of lymph node cells on JTC-11 cells after sensitization*:

The lymph node cells prepared from 4 groups (whose lymph nodes were taken out 5, 7, 10, 14 days after sensitization, respectively) were mixed with JTC-11 cells in the ratio of 40:1, i.e. 80×10^4 cells/ml: 2×10^4 cells/ml, to which 50 u./ml of potassium penicillin-G, (Takeda Pharmaceutical Co., Ltd.) was added, and suspended in 20% bovine serum-YLE medium to make the total volume 10 ml. As for the controls, non-sensitized lymph node cells were mixed with JTC-11 cells at the same ratio. One and half milliliters each of the mixture was pippetted into six short tubes and the replicate culture was carried out at 37°C by the method of EVANS *et al.* (11). At culture hour 24 and 48 the culture medium of 3 tubes of each group was decanted respectively, treated with 1.5 ml crystal violet solution (containing 100 ml distilled water, 2.1 g citric acid, and 50 mg crystal violet), incubated for 30 min at 37°C to lyse the cytoplasm. Next, the cells on the vessel wall were scraped off with a rubber cleaner, and by shaking a uniform turbid nuclear solution was obtained. Then with Bürkel-Türk hemocytometer the numbers of nuclei were counted, and taking such countings several times with each tube, average counts of nuclei was obtained. The result of this nuclear count decided the number of surviving JTC-11 cells.

b) *Observations under the phase contrast microscope*:

In similar manner lymph node cells obtained 10 days after sensitization were mixed with JTC-11 cells in the ratio of 80×10^4 cells/ml: 2×10^4 cells/ml and the mixture was suspended in 20% bovine serum-YLE medium. The cell suspension was then transferred to a TD-7 tube and was observed under the phase contrast microscope. Simultaneously, cinematographs of these cells were taken with the Bolex H16 Reflex operated by a cinetimer (Nikon/nac Product) and attached to the phase contrast microscope at the rate of 1 frame per 15 sec for 48 hours. At the same time with other tubes also containing identical cell mixture, 35-mm pictures were taken at random at 24 and 48 hours of the incubation, and the lymph node cells aggregated and attached on the target cell were counted.

Exp. II. Anti-growth effect of PHA-treated normal mouse lymph node cells on JTC-11 cells

a) *Preliminary experiment*:

In order to see the effect of PHA on JTC-11 cells, to 2×10^4 cells/ml of JTC-

11 cells was added PHA in the concentrations of 0.5, 1.0, 2.0 or 5.0% (v/v) and further by adding 20% bovine serum-YLE medium the final volume was adjusted to 10 ml. As the control there was set up a group without addition of PHA. Just as in Exp. I, 1.5 ml each of the mixture was put into 6 short tubes and incubated at 37°C. By the same procedures as in the preceding Exp. I, the nuclear counts were taken of each 3 tubes at 24 and 48 hours of the incubation and the average calculated.

b) *Effects of PHA on lymph node cells*

i) *Anti-growth effect of normal lymph node cells in the presence of PHA at varying concentrations on JTC-11 cells*:

For this experiment normal lymph node cells and JTC-11 cells were mixed in the ratio of 80×10^4 cells/ml to 2×10^4 cells/ml, and to this mixture PHA was added in concentrations of 0.5, 1.0, 2.0 and 5.0% (v/v), and finally by adding the 20% bovine serum-YLE medium the total volume was adjusted to 10 ml and cultured. The control group was cultured without PHA. With each of these groups the nuclear counts were taken in an identical manner as in the foregoing experiments.

ii) *Anti-growth effect of normal lymph node cells treated with PHA at varying lapse of time*:

To 80×10^4 cells/ml of lymph node cells PHA was added to make its concentration to 2.0% or 5.0% (v/v), and further YLE medium, 199-medium plus bovine serum were added in the ratio of 3:5:2, to make the final total volume to 70 ml to suspend these cells. As to the 2%-group and 5%-group each group was divided into 7 subgroups, and incubated for 30 min, 1, 2, 3, 6, 12, and 24 hours respectively at 37°C. After the incubation for respective periods each test tube was centrifuged at 1,500 rpm for 10 min to gather the lymph node cells. The cells so collected were washed with cold Hanks' solution, and then repeated this centrifugation-washing procedures 3 times. Next, to each of such tubes from which PHA was completely removed, 2×10^4 cells/ml of JTC-11 cells was added and this was suspended in 10 ml of 20% bovine serum-YLE medium. The nuclear counts were taken to determine the numbers of surviving cells as in the preceding experiments.

c) *Observations under the phase contrast microscope*:

Samples were prepared in the same manner as in Exp. II, b-i) and 4 ml of each sample was placed in a TD-7 tube and was observed under the phase contrast microscope. Simultaneously, by taking 16 mm cinecamera attached to the microscope, the pictures were taken at rate of one frame per 15 sec, to observe the movements of the viable cells. With the other tubes 35 mm micrographs were taken at random at culture hour 24 and 48 to count the number of lymph node cells attached to and aggregated on the target cells.

d) *Non-specificity of the anti-growth effect of normal lymph node cells induced by PHA*:

To 1×10^4 cells/ml of JTC-11 cells, A-strain cells, HeLa-S_3 cells as the target cells, was added 40×10^4 cells/ml of normal lymph node cells, then PHA in the concentration of 2.0% (v/v) was added, and the culture medium was added

adjusting the final volume to 10 ml as before. As the controls non-PHA containing groups were prepared and the nuclear counts were taken at culture hour 24 and 48 as in the preceding experiments. After obtaining numbers of surviving cells in respective groups, the growth rate (%) was calculated by dividing the number of the target cells in the presence of PHA by the number of respective controls at culture hour 24 and 48.

RESULTS

Exp. I: Anti-growth effect of regional lymph node cells of sensitized mouse on JTC-11 cells.

a) *Time-lapse changes of anti-growth effect of lymph node cells on JTC-11 cells after sensitization*:

Compared with the group of JTC-11 cells alone or the group mixed with non-sensitized normal lymph node cells, all the 4 groups of sensitized regional lymph node cells in mixed cultures inhibited the growth of the target cells, JTC-11 cells, more markedly. Such an inhibitory effect reached its peak on 10th day after sensitization, which decreased in the order of the 14th, 7th and 5th day. With groups of 10th and 14th day after sensitization the inhibitory effect was clearly observed at culture hour 24. As to the difference from the control groups as proven by the t-test, there was a significant difference in $P<0.001$ (Table 1).

TABLE 1. ANTI-GROWTH EFFECT OF REGIONAL LYMPH NODE CELLS OF SENSITIZED MOUSE ON JTC-11 CELLS

Lymph node cell	Mean No.* of surviving JTC-11 cells after culture (Mean ± S.E.)		Significance of difference by t-test
	24 hr.	48 hr.	
sensitized (5th day)	4.07 ± 0.22	9.30 ± 0.80	$P<0.01$
sensitized (7th day)	3.44 ± 0.23	8.11 ± 0.69	$P<0.001$
sensitized (10th day)	2.43 ± 0.21	5.88 ± 0.23	$P<0.001$
sensitized (14th day)	2.78 ± 0.31	6.43 ± 0.50	$P<0.001$
normal	3.69 ± 0.41	10.13 ± 0.36	
none	3.48 ± 0.42	10.38 ± 0.88	

* $\times 10^4$

b) *Observations under the phase contrast microscope*:

The sensitized lymph node cells began to attach around JTC-11 cells by culture hour 5-6, by culture hour 24 the number of these lymph node cells attaching to the target cells increased, and JTC-11 cells gradually lost their cellular projections turning round in shape and losing their

mobility. At this stage 80-90% of JTC-11 cells had lymph node cells attached around them and 30-40% of them had the aggregation of lymph node cells on them. The target cells that had lost their mobility revealed increasing number of granules and vacuoles in their cytoplasm. By culture hour 30 to 40 the surface of JTC-11 cells gradually became irregular and then some of them underwent cytolysis. By culture hour 48, 90-95% of the target cells showed lymphocyte attachment and 60-65% of them the

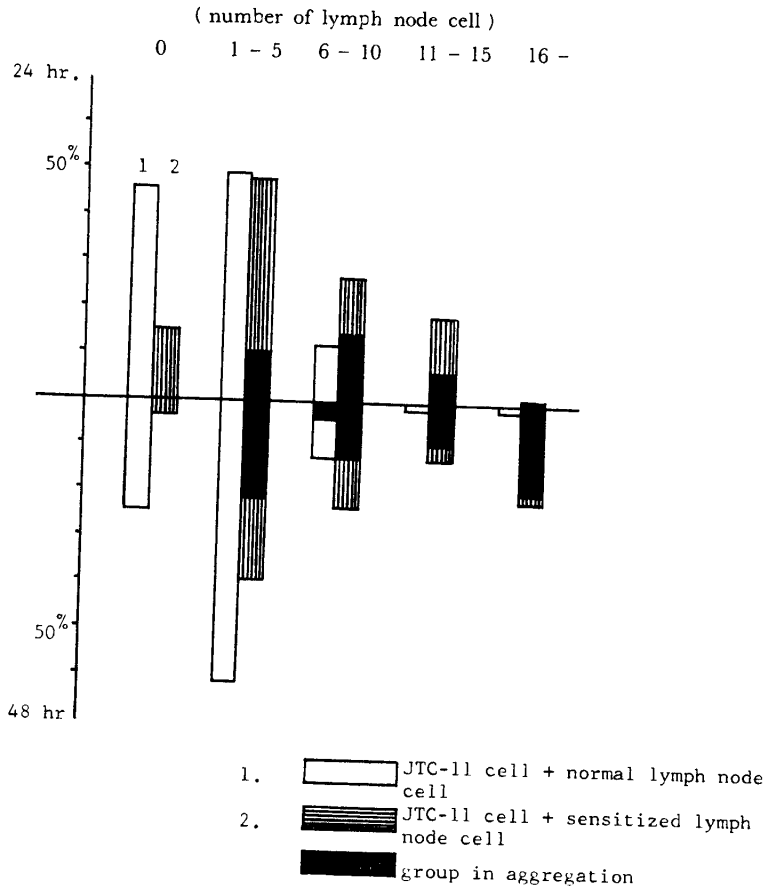
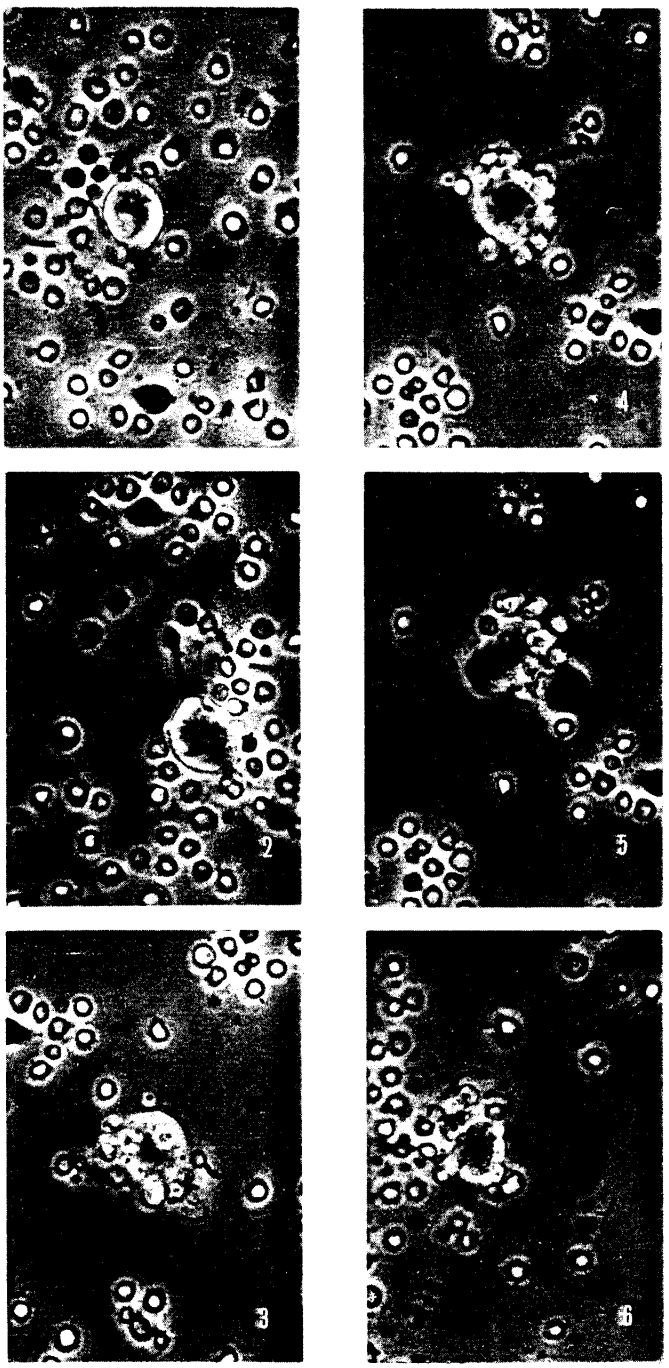


Fig. 1 The number of regional lymph node cells attached to JTC-11 cells and that in aggregation at 24- and 48-hour tissue culture.

Photoplates show JTC-11 cells in the mixed cell culture with regional lymph node cells obtained the C57BL mice 10 days after transplantation of 500×10^4 Ehrlich ascites cells.

1: At culture hour 6
2: At culture hour 18
3: At culture hour 24
4: At culture hour 30
5: At culture hour 38
6: At culture hour 40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3
4
5
6

aggregation. Throughout the course, sensitized lymphocytes all tended to adhere around the target cells, and as the number of adhering lymphocytes increased, the percentage of aggregation also increased (Fig. 1, Photos 1-6).

Exp. II: Anti-growth effect of PHA-treated normal mouse lymph node cells on JTC-11 cells

a) Preliminary experiment: *Effect of PHA on JTC-11 cells*:

In comparing the growth of JTC-11 cells in culture in the presence or absence of PHA, it was found that only the group cultured with PHA in the concentration of 5.0% (v/v) showed a slight growth inhibition, but the groups added with 0.5-2.0% (v/v) PHA there could be observed no inhibitory effect ($P>0.05$, Table 2).

TABLE 2. EFFECT OF PHA ON JTC-11 CELLS

Concentration of PHA (% v/v)	Mean No.* of surviving JTC-11 cells after culture (Mean ± S.E.)		Significance of difference by t-test
	24 hr.	48 hr.	
0.5	4.08 ± 0.39	10.14 ± 0.93	$0.01<P$
1.0	3.38 ± 0.46	9.32 ± 0.79	$0.05<P$
2.0	3.85 ± 0.39	8.87 ± 0.87	$0.05<P$
5.0	3.49 ± 0.30	7.97 ± 0.45	$P<0.001$
none	3.55 ± 0.36	9.19 ± 0.88	

* $\times 10^4$

b) *Anti-growth effect of normal lymph node cells in the presence of varying concentrations of PHA on JTC-11 cells*:

The anti-growth effect of normal lymph node cells on the target cells

TABLE 3. ANTI-GROWTH EFFECT OF NORMAL LYMPH NODE CELLS IN THE PRESENCE OF VARYING CONCENTRATIONS OF PHA ON JTC-11 CELLS

Concentration of PHA (% v/v)	Mean No.* of surviving JTC-11 cells after culture (Mean ± S.E.)		Significance of difference by t-test
	24 hr.	48 hr.	
0.5	4.41 ± 0.50	12.13 ± 1.02	$0.01<P$
1.0	4.23 ± 0.29	12.74 ± 0.83	$0.01<P$
2.0	3.46 ± 1.03	11.05 ± 0.65	$P<0.001$
5.0	2.68 ± 0.26	7.23 ± 0.64	$P<0.001$
none (JTC-11+nLy.)	5.37 ± 0.51	14.16 ± 0.63	
JTC-11 cell only	5.07 ± 0.33	14.59 ± 1.00	

* $\times 10^4$

in the presence of PHA, as compared with that of the control without PHA, was enhanced, and such inhibitory effect was marked with 5.0% and 2% (v/v) PHA, but such effect decreased as the concentration of PHA was lessened, and at 1.0% or 0.5% (v/v) there was no significant difference from the controls. Between the groups with 5.0% or 2.0% PHA and the controls according to t-test there could be recognized a significant difference ($P<0.001$, Table 3).

TABLE 4. ANTI-GROWTH EFFECT OF NORMAL LYMPH NODE CELLS TREATED WITH 2% (v/v) PHA VARYING LAPSES OF TIME

Time of treating	Mean No.* of surviving JTC-11 cells after culture (Mean ± S.E.)		Significance of difference by t-test
	24 hr.	48 hr.	
30′	2.61 ± 0.33	9.39 ± 0.94	$0.1<P$
1 hr.	2.59 ± 0.35	8.07 ± 0.84	$0.05<P$
2 hr.	2.45 ± 0.32	8.01 ± 0.53	$0.05<P$
3 hr.	2.47 ± 0.30	8.12 ± 0.48	$0.05<P$
6 hr.	2.86 ± 0.32	8.40 ± 0.41	$0.01<P$
12 hr.	2.53 ± 0.22	7.54 ± 0.69	$P<0.001$
24 hr.	2.71 ± 0.26	6.65 ± 0.69	$P<0.001$
JTC-11 cell + nLy. +PHA (2.0% v/v)	2.85 ± 0.36	6.74 ± 0.39	
JTC-11 cell + nLy.	3.49 ± 0.40	9.81 ± 0.71	
JTC-11 cell only	3.21 ± 0.30	8.83 ± 0.50	

* $\times 10^4$

TABLE 5. ANTI-GROWTH EFFECT OF NORMAL LYMPH NODE CELLS TREATED WITH 5% (v/v) PHA VARYING LAPSE OF TIME

Time of treating	Mean No.* of surviving JTC-11 cells after culture (Mean ± S.E.)		Significance of difference by t-test
	24 hr.	48 hr.	
30′	4.01 ± 0.39	10.15 ± 0.81	$0.05<P$
1 hr.	3.83 ± 0.24	11.54 ± 0.80	$0.05<P$
2 hr.	3.42 ± 0.24	10.32 ± 0.82	$0.05<P$
3 hr.	3.12 ± 0.40	10.03 ± 0.85	$0.01<P<0.05$
6 hr.	2.72 ± 0.20	6.86 ± 0.66	$P<0.001$
12 hr.	2.94 ± 0.33	7.21 ± 0.70	$P<0.001$
24 hr.	3.03 ± 0.33	6.88 ± 0.37	$P<0.001$
JTC-11 cell + nLy. +PHA (5.0% v/v)	3.94 ± 0.40	5.76 ± 0.59	
JTC-11 cell + nLy.	3.49 ± 0.30	9.81 ± 0.71	
JTC-11 cell only	3.21 ± 0.30	8.83 ± 0.50	

* $\times 10^4$

i) *Anti-growth effect of normal lymph node cells treated with PHA at varying lapses of time* :

In the group treated with 2.0% PHA there occurred no change within 30 minutes to 6-hour treatment, but the lymph node cells acquired anti-growth effect by 12-hour treatment. In the group treated with 5.0% PHA there could be seen no change within 30 minutes to 3-hour treatment while anti-growth effect was acquired by 6-hour treatment ($P < 0.001$, Tables 4, 5).

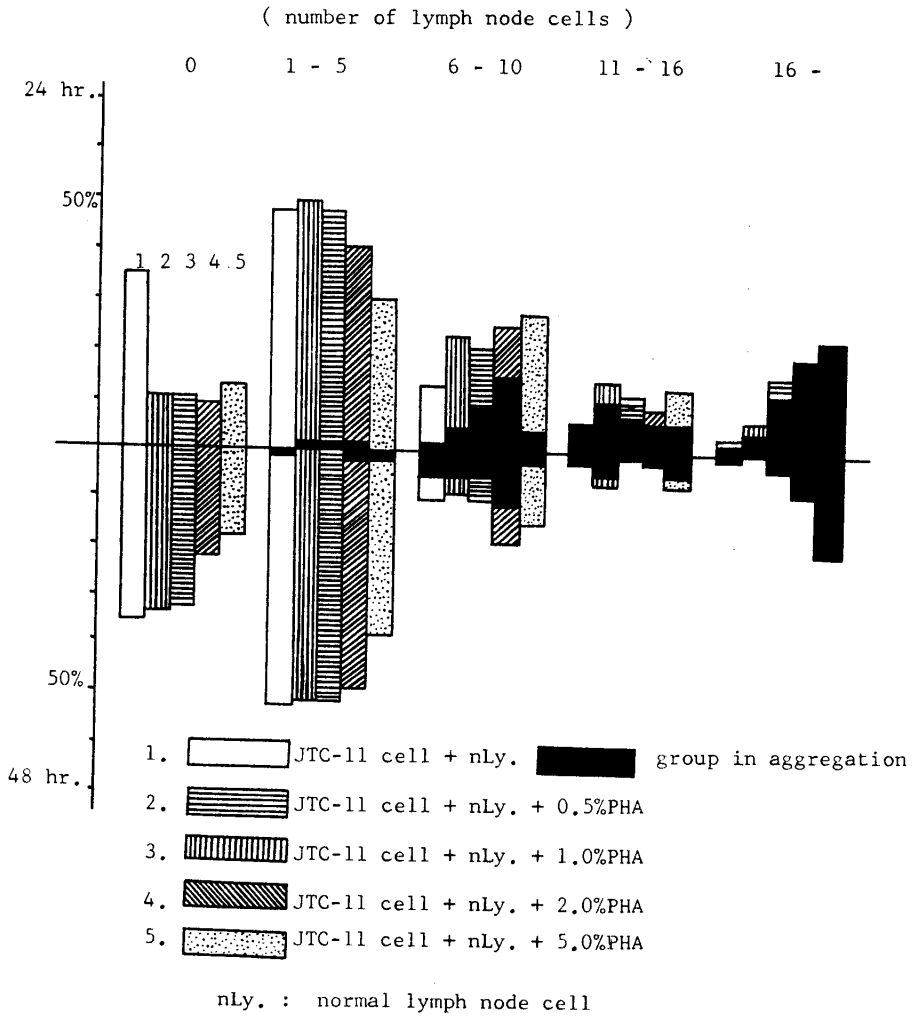


Fig. 2 The number of PHA-treated normal lymph node cells attached to JTC-11 cells and that in aggregation at 24- and 48-hour tissue culture.

c) *Observation under phase contrast microscope*:

Lymph node cells added with 2.0% PHA began to adhere to JTC-11 cells by culture hour 2-3, and the number of such adhering cells increased by culture hour 7-10, by 14-18 hours JTC-11 cells began to retract their cellular projections, gradually became spherical in shape, losing their mobility, and by culture hour 18-24 their cytoplasm showed increasing number of granules and their cell membrane became rough, losing their cellular structure, they underwent cytolysis. By culture hour 24 there could be seen the attachment of lymph node cells on 70-90% and the aggregation on 30-40% of the target cells. By 48 hours the attachment around the cells could be seen on 90-95% and the aggregation on 40-50% target cells. As the concentration of PHA was increased from 0.5% up to 5.0% the number of lymph node cells attaching increased and the anti-growth effect became greater, showing concomittant increase of aggregation of the target cells. In any case the lymph node cells treated with PHA tended to aggregate and adhere on one side of the target cell (Fig. 2, Photos 7-12).

d) *Non-specificity of anti-growth effect of normal lymph node cells induced by PHA*:

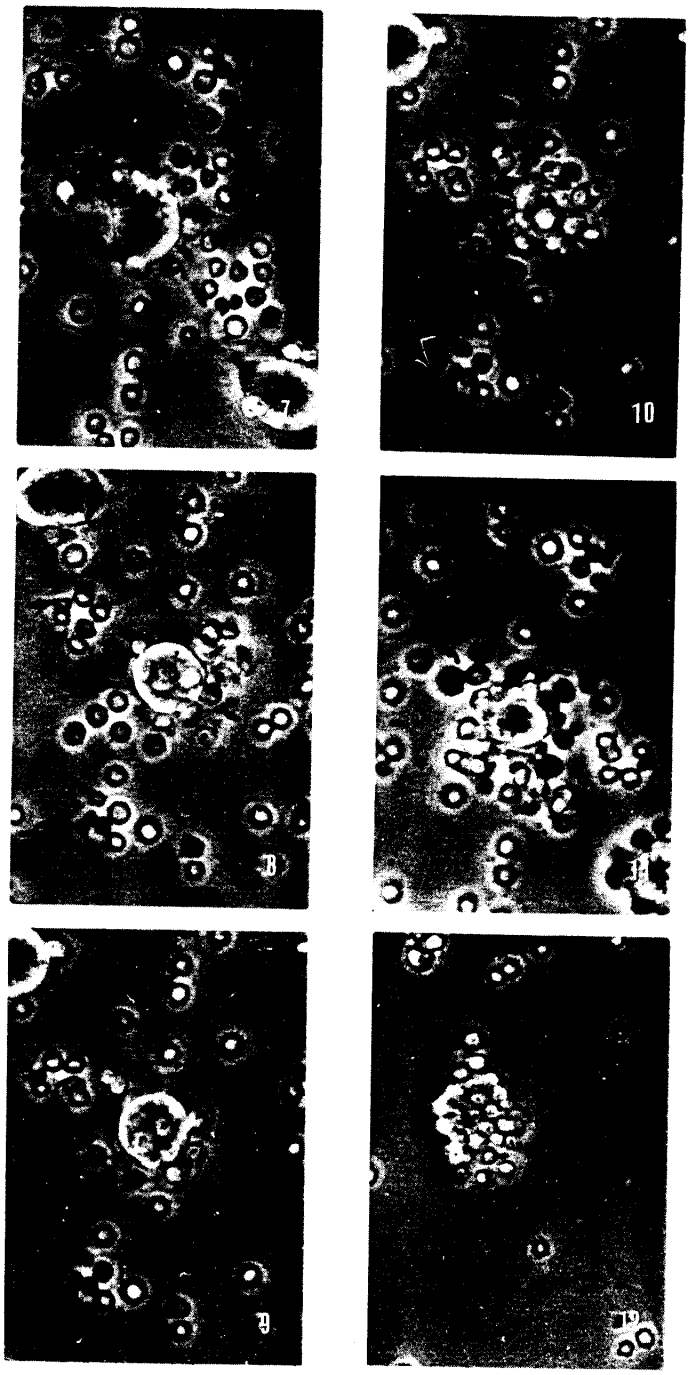
Normal mouse lymph node cells treated with PHA inhibited the growth of JTC-11 cells. Compared with normal mouse lymph node cells without PHA, these PHA-treated cells clearly inhibited the growth of the target cells, that is, by culture hour 24, the growth of 80% target cells, and by 48 hours 76% of target cells. Such PHA-treated lymph node cells also inhibited the growth of A-cells derived from C3H mouse mammary cancer. In this instance, the growth rate of the target cells was 73% by culture hour 24 and 73% by 48 hours, as proven by the ratio of test cells

TABLE 6. NON-SPECIFICITY OF ANTI-GROWTH EFFECT OF NORMAL LYMPH NODE CELLS BY PHA

Target cell	Concentration of PHA (% v/v)	Mean No.* of surviving target cells after culture (Mean ± S. E.)		Percent** of target cells surviving
		24 hr.	48 hr.	
A-Strain cell	2.0	1.30 ± 0.14	2.42 ± 0.14	73
	0	1.77 ± 0.14	3.31 ± 0.10	
Hela-S_3	2.0	2.50 ± 0.17	3.56 ± 0.32	87
	0	2.92 ± 0.10	4.08 ± 0.44	
JTC-11 cell	2.0	1.45 ± 0.20	3.37 ± 0.17	76
	0	1.81 ± 0.17	4.43 ± 0.26	

* $\times 10^4$

** $\frac{\text{No. of target cells surviving after culture with PHA mixed nLy.}}{\text{No. of target cells surviving after culture with nLy.}} \times 100$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to control. In addition, such PHA-treated cells inhibited the growth of HeLa-S_3 cells, a heterogenous cell line derived from human uterine cancer. Each of these groups showed a significant difference ($P<0.01$) from respective controls (Table 6).

DISCUSSION

Recently, there are many studies being carried out concerning the intercellular reaction between sensitized lymphoid cells and antigenic cells by tissue culture method, and these studies are applied to allogeneic transplantation immunity and cancer immunity. In 1943 allogeneic transplantation of mouse sarcoma was attempted by HARRIS *et al.* (12) followed by attempts at homologous skin transplantation by BILLINGHAM and SPARROW (13), WEAVER (14) and SCOTHONE and NAGY (15). By these studies, however, no destruction of antigenic cells by lymphoid cells has been recognized. More recently, MERRILL *et al.* (16) reported of the *in vitro* cytotoxic effect of peritoneal exudative cells of rabbit on the target cells, and GOVEARTS (17) about similar cytotoxic effect of dog thoracic duct lymphocytes. ROSENAU and MOON (15), after sensitizing BALB/c mouse with L cells (derived from C3H mouse) took out lymphoid cells from the spleen of the sensitized animal and conducted tissue cultures of these cells mixed with L cells in the ratio of 20 : 1 without any complement or antiserum. As a result they found that by culture hour 18-40 these lymphoid cells became attached and aggregated onto L cells, leading the latter to destruction. It has also been shown that using tumor cells as the target cells lymphoid cells from sensitized animal inhibited the growth of the target antigenic cells. Namely, HANAOKA *et al.* (19) injected SCl cells (a lymphatic leukemic cell line derived from S mouse) subcutaneously into the axilla of A/Jax mouse, and found that regional lymph node cells from such a sensitized mouse showed anti-growth effect.

HARA (1) studied the effects of regional lymph node cells and spleen cells from Cb mice after being sensitized with Ehrlich ascites tumor cells derived from hybrid mouse, and SATOH (2) also observed time-lapse effects of regional lymph node cells from C3H mouse sensitized with A cells derived from C3H mouse mammary cancer. As a result it was shown that

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Photoplates show JTC-11 cells in the mixed cell culture with PHA treated normal lymph node cells.

7 : At culture hour 3
8 : At culture hour 8
9 : At culture hour 10
10 : At culture hour 20
11 : At culture hour 28
12 : The other cell at culture hour 20

such regional lymph node cells had the strongest inhibitory effect around 10-14 days after sensitization. As the same time it was reported that for sensitized lymph node cells to act more effectively they had to adhere and aggregate to antigenic cells.

As to effector cell, however, opinions are divided: Namely, GRANGER *et al.* (20, 21) consider it to be sensitized peritoneal macrophage, and SHIRAI (22) claims it to be the effect of peritoneal macrophage of the sensitized rat. By the electron microscope observations MANNAMI (23) has demonstrated the effector cell to be lymphocytes. Further such inhibitory effect is found not only in the lymph node cells but also in those lymphocytes of spleen, thoracic duct and peripheral blood of the sensitized animal. As is obvious from all these studies, inactivated serum is used in the culture medium, which contains no complement in every experiment, and there can be seen no fundamental difference in the mechanisms of transplantation immunity and cancer immunity. In addition, there is clearly specificity in the reaction, as demonstrated by BRONDZ (24), ROSENAU and MOON (25), and SATOH (3, 4). Hence such an *in vitro* reaction seems to represent *in vivo* immune phenomenon. However, the mechanism of cellular interaction leading to cytolysis of the target cells is not analyzed enough. But it is the common fact in many experimental systems described previously that the sensitized lymphoid cells adhere and aggregate on to the target cells.

On the other hand, HOLM *et al.* (26) demonstrated that in the tissue cultures of peripheral blood lymphocytes mixed with primary culture cells of human embryonic kidney or rat kidney supplemented with phytohemagglutinin (PHA), an extract from *Phaseolus vulgaris*, the lymphocytes attached to the target cell and let the latter to its destruction, and ROSENAU (27) observed normal mouse lymphocytes added L-polylysene HCl attached and aggregated on homologous cells. Further, according to the studies by MÖLLER (7, 8), HELLSTRÖM *et al.* (5, 6) in the mixed tissue cultures of two groups of cells having different histocompatibility antigens with addition of PHA, there occure cell degeneration and growth inhibition, the phenomenon of which is called "allogeneic inhibition" or "F_1 hybrid effect". Therefore, it has been clarified that, when the cells adhere and aggregate to the target cells by such a non-immunological method where the H-2 antigens differ, the target cells are destroyed. By the experimental systems of the author there are two effective ways of the cells acting on target cells: One is the effect of sensitized lymph node cells and the other is the effect of normal lymph node cells with addition of PHA each of which inhibits the growth of the target cells. Especially

in the latter case, it is demonstrated that not only the addition of PHA to the culture medium of the mixed tissue cultures but also a pretreatment of lymph node cells would impart anti-growth effect.

The phase contrast microscope observations reveal that like intercellular reaction of the sensitized lymph node cells and antigenic cells, the reaction is observed in two steps, at first lymph node cells adhere and aggregate to the target cells and then the destruction of the latter takes place. However, differing from the reaction observable with the sensitized lymph node cells, the phenomenon like adherence, aggregation and destruction occur at earlier stage of culture. However, as stated by MÖLLER and HELLSTRÖM, between syngenic cells there occurs adherence, no destruction ensues, but with F_1 cells target cells that are semi-syngenic parents are destroyed, and in the present experiment normal C57BL mouse lymph node cells in the presence of PHA aggregate and destroy JTC-11 cells (derived from hybrid mouse), A cells (derived from C3H mouse), and HeLa-S_3 cells (derived from human). These observations indicate that the effect of PHA-treated lymphocytes may be said to have no specificity at all. Judging from the effect of PHA-treated lymphocytes, it may be assumed that, when cells with different surface structure became adhered and aggregated with one another, the lymphocytes are destroyed together with target cells. One other words, the destruction itself of the target cell should be considered as a secondary, non-specific phenomenon. It seems that PHA imparts a certain non-specific adhesiveness like glue to normal lymphocyte. Consequently it is considered that the specific aggregation of lymphocytes to the antigenic cells is really an immune reaction but the subsequent destruction is a non-specific phenomenon induced by a secondary, allogeneic inhibition-like mechanism.

These observations indicate that the effect of sensitized regional lymph node cells on the antigenic cell cannot very well be explained simply by non-specific allogeneic inhibition, and a consideration should be given to a possiblity of a specific surface receptor that makes the adherence to target cell possible.

SUMMARY

Under *in vivo* conditions JTC-11 cells derived from Ehrlich ascites tumor are led to destruction by lymph node cells by two processes. The one is the interaction of lymph node cells of the C57BL (♀) mouse sensitized with Ehrlich ascites tumor cells, and the other is the interaction of normal C57BL (♀) mouse lymph node cells treated with PHA-M. In

these two reaction systems the following differences have become clear.

The regional lymph node cells from the C57BL (♀) mouse sensitized with Ehrlich ascites tumor cells show a marked inhibitory effect on the growth of JTC-11 cells by 10 days after sensitization.

In the observations under the phase contrast microscope these lymph node cells tend to adhere around the antigenic cells by culture hour 5-6, and by culture hour 24-48 they lead the latter to undergo cytolysis.

The normal lymph node cells of C57BL (♀) mouse treated with PHA show anti-growth effect of JTC-11 cells. PHA-M used proves to be effective in the concentration of 2% (v/v). Likewise after such normal lymph node cells are previously treated with 2% PHA-M for 12 hours, they also inhibit the growth of JTC-11 cells when two cell groups are cultured together.

In such intercellular reaction between the two cell groups there is no specificity. By observations under the phase contract microscopy, by culture hour 2-3 the adherence and aggregation of lymph node cells begin to occur, and by 18-24 hours of culture the target cells are led to undergo cytolysis. In this instance, lymph node cells are prone to adhere and aggregate on one side of the target cell.

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