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Electron microscopic study of contactual interaction of sensitized lymphocytes with homologous target cells

Tetsuya Mannami*

*Okayama University,

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Tetsuya Mannami

Abstract

When the lymph node cells sensitized by Ehrlich ascites tumor were mixed and cultured with JTC-II cells derived from Ehrlich ascites tumor, the interaction of the two cell groups exhibited a contactual phenomenon accompanied by the destruction of JTC-II cells. These two cell groups in contact were fixed with OsO₄ solution and the ultra-thin sections were observed in the electron microscope. As a result the following findings were obtained. In the interaction where lymph node cells become attached to JTC-II cells, resulting in the destruction of JTC-II cells, lymph node cells were also destroyed. Effector cells seem to be a kind of cells in the lymph nodes, and from their morphological characteristics they are considered to be lymphocytes. Electron microscopic observations of the surface of contact revealed the following: some cells are adhered to one another at the surfaces of the cell membranes that run in parallel; some are in contact by means of filamentous projection of lymphocytes; the cell membranes of the two cells form interdigitation; and both surfaces of two cell membranes are disrupted at the point of contact and the cytoplasm of the two cells appears to be directly connected with one another.

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**ELECTRON MICROSCOPIC STUDY OF CONTACTUAL
INTERACTION OF SENSITIZED LYMPHOCYTES
WITH HOMOLOGOUS TARGET CELLS**

Tetsuya MANNAMI

*Department of Surgery, Okayama University Medical School, Okayama, Japan
(Director: Prof. Sanae Tanaka)*

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It is said that transplantation immunity and cancer immunity are mediated by lymphoid cells, but it is not definitely known which cells correspond to the effector cells. In the observations of the direct effect of lymphoid cells on target cells *in vitro*, it has been demonstrated that the lymphoid cells adhere and aggregate on the target cell and ultimately destroy the latter (1—18). HARA (13, 19, 20) and SATOH (16—18) of our laboratory have studied such interaction by counting the number of nuclei as well as by the phase-contrast microscopic observations. As a result it has been found that the lymph node cells of the lymphoid system correspond to the effector cells. They have suggested that there is a considerably high specificity in the interaction between lymphoid cells and target cells, and that this reaction *in vitro* represents one of the immunological reactions of the host *in vivo*.

The objective of the present experiment was to study the state of the contactual surface between the sensitized lymphoid cells and target cells as well as to find out which cells are effector cells.

MATERIALS AND METHODS

Animals: The animals used were inbred AKR mice procured from the Mouse Colony of Okayama University and fed on solid feed, MF of Oriental Yeast Company.

Sensitization of mice: These mice received subcutaneous injection on the back between the scapulas of approximately 5×10^6 Ehrlich ascites tumor cells.

Tissue culture medium: We used YLE medium containing 20% bovine serum inactivated at 56°C for 30 minutes without addition of antibiotics.

Tissue culture cells: JTC-11 strain, derived from Ehrlich ascites tumor cells and maintained at the Cancer Institute of Okayama University Medical School, were cultured in the YLE medium mentioned above. These cells are transplantable to mice.

Lymph node cell suspension: Ten days after sensitization mice were sacrificed, and axillary and cervical lymph nodes were extirpated under aseptic conditions. These lymph nodes were cut into small pieces with ophthalmic scissors in cold Hanks solution and passed through 80 mesh filter. The filtrate was washed three times with Hanks solution by centrifugation at 1500 rpm for 5 minutes and then suspended in the culture medium. The cell suspensions of lymph node cells prepared in similar manner from normal mice served as controls.

Culture methods: 1) Both normal and sensitized lymph node cells (about 1×10^5 cells each) were suspended in the YLE medium mentioned above and cultured separately in test tubes at 37°C for 24–48 hours. 2) JTC-11 cells (cir. 1×10^4 cells) suspended in the same medium were cultured in test tubes at 37°C for 48 hours. These cells become attached on the glass wall of the culture vessel and proliferate. 3) Mixed culture of lymph node cells and JTC-11 cells. To the JTC-11 cells proliferating on the vessel wall in monolayer form, either normal or sensitized lymph node cells were added and cultured at 37°C for 24–48 hours. (For the phase-contrast microscopic observations some mixed cultures were conducted in Td-15 bottles. The ratio of lymph node cells to target cells was 50–100:1.

Electron microscopic observations: As the fixative 1% OsO₄ solution buffered according to MILLONIG (21) containing 5.4% sucrose (22) was used. 1) Normal and sensitized lymph node cells were each centrifuged at 1000 rpm for 5 minutes and the pellets thus obtained were fixed in the fixative at 4°C for 10–30 minutes. 2) In the case of JTC-11 cells, after decanting the medium the cells were washed three times with MILLONIG's phosphate buffer solution (pH 7.4) and fixed. After fixation the cells attached on the vessel wall were gently scraped off with a rubber cleaner and pellets were prepared by centrifugation at 1000 rpm for 5 minutes. 3) In the case of mixed culture, both normal and sensitized, after decanting the medium the cells were washed with the buffer solution as above and fixed at 4°C for 10–30 minutes. Next, by removing the cells from the vessel wall with a rubber cleaner, they were subjected to a mild centrifugation for 5 minutes.

These five cell specimens were dehydrated gently in graded alcohol for 10 minutes each, and embedded in Epon according to LUFT (23). Respective controls were similarly prepared.

Ultra-thin sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome, and subjected to electron staining with aqueous solution of uranyl acetate for 30 minutes. The thin sections were observed in HU-11 and JEM-7 ultramicroscopes. For the phase-contrast microscopic observations, the cells cultured in Td-15 bottles were observed with Nikon-M phase contrast microscope and cinematographed with EFMD cinematographic apparatus.

RESULTS

In the case of normal lymph node cells the cell pictures of the thin

sections resembled closely to those of lymphoid cells described in the available literature (24—28). As for sensitized lymph node cells, the cell pictures were practically the same as those of normal lymph node cells, but there were observed an increase in the cells corresponding to plasma cells and lymphogonia (24, 39).

The electron micrographs of normal JTC-11 cells revealed the following: The cells are of a large body with villi or pseudopodial projections, and the cytoplasm contains numerous vacuoles of varying sizes which are mostly located in the cell center. Mitochondria are of a round or irregular-rod shape having cristae, and they are observed relatively numerous. A few endoplasmic reticula of rough surface are seen, but no lamellar structure. There are often found electron dense particles in the cytoplasm, what appear to be lipid droplets. Occasionally, some cells reveal electron dense packed granular bodies. The nucleus is of an irregular shape and a portion of chromatins is condensed around the nuclear membrane.

The phase-contrast micrographs of aggregated, sensitized lymphoid cells and JTC-11 cells are illustrated in Fig. 1. When a large number of lymph node cells are added, even after 24-hour culture there can be observed many aggregations on the target cells.

In the case of the mixed culture of normal lymph node cells with JTC-11 cells, although we can see the attachment of the two groups of cells, in the procedures leading to the Epon embedding, especially at the first washing stage, both cell groups become detached from one another. For this reason, it was impossible to observe the contact of these two cell groups by the electron microscopy.

In the mixed culture of sensitized lymph node cells with JTC-11 cells, the contact between these two cell groups is maintained even after the washing with the buffer solution. These cells were scraped off from the vessel wall with a rubber cleaner after washing and fixing. The centrifugation was gently carried out as only to sediment the cells. Even after this procedure the contact between the two cell groups remained intact. When observed by the electron microscopy, there could be seen adhesion of the two cell membranes in various ways as well as a discontinuity of the membranes at the site of this contact. Namely, a contact between two membranes running approximately parallel (Figs. 2, 7, 11, 13); the contact made between long, slender filamentous projections of JTC-11 cell and lymph node cells (Figs. 3, 4); the contact achieved by the interdigitation of both cell membranes (Figs. 2, 5, 6); the contact being made by the projections of lymph node cell penetrating into JTC-11 cells (Fig. 8); and the membranes of the two cells showing discontinuity at the point of

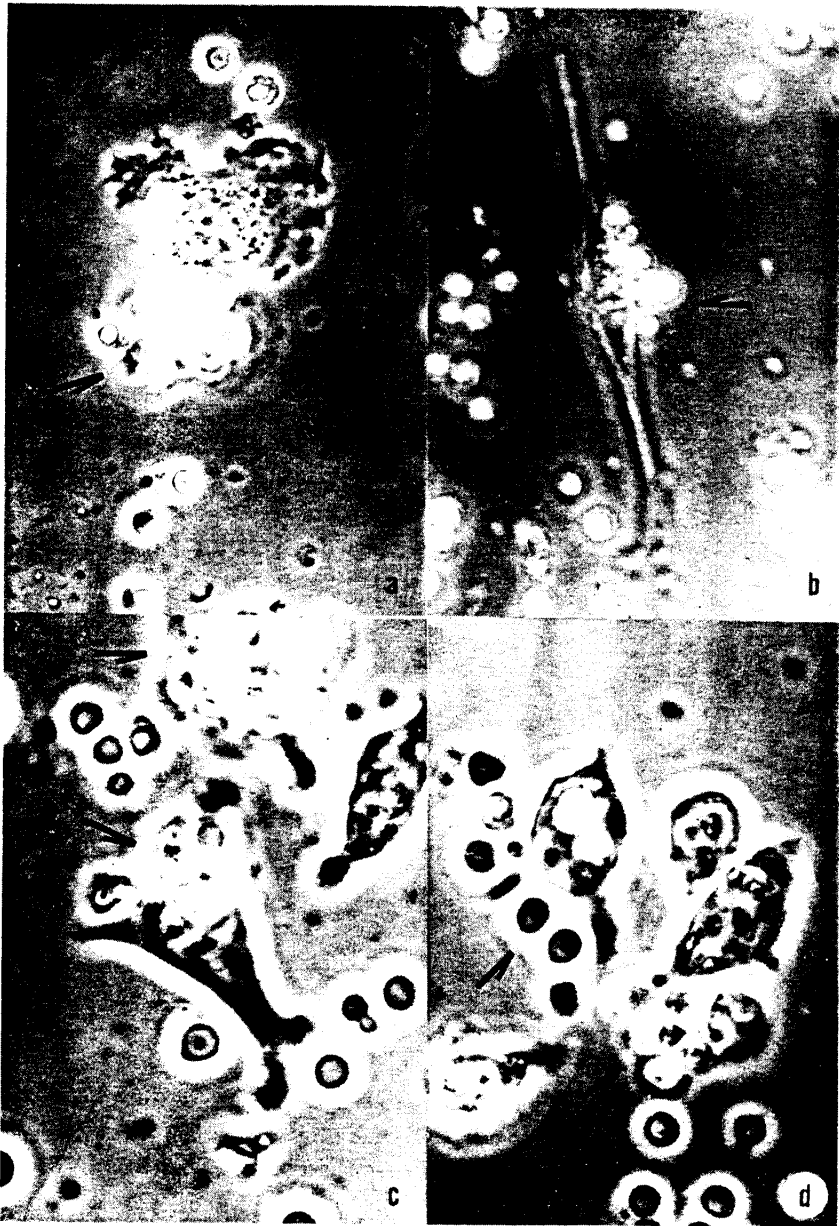


Fig. 1 Phase contrast microscopy. Arrows show the aggregation between lymphocytes and JTC-11 cell).

(SL: sensitized lymphocyte, TC: JTC-11 Cell)

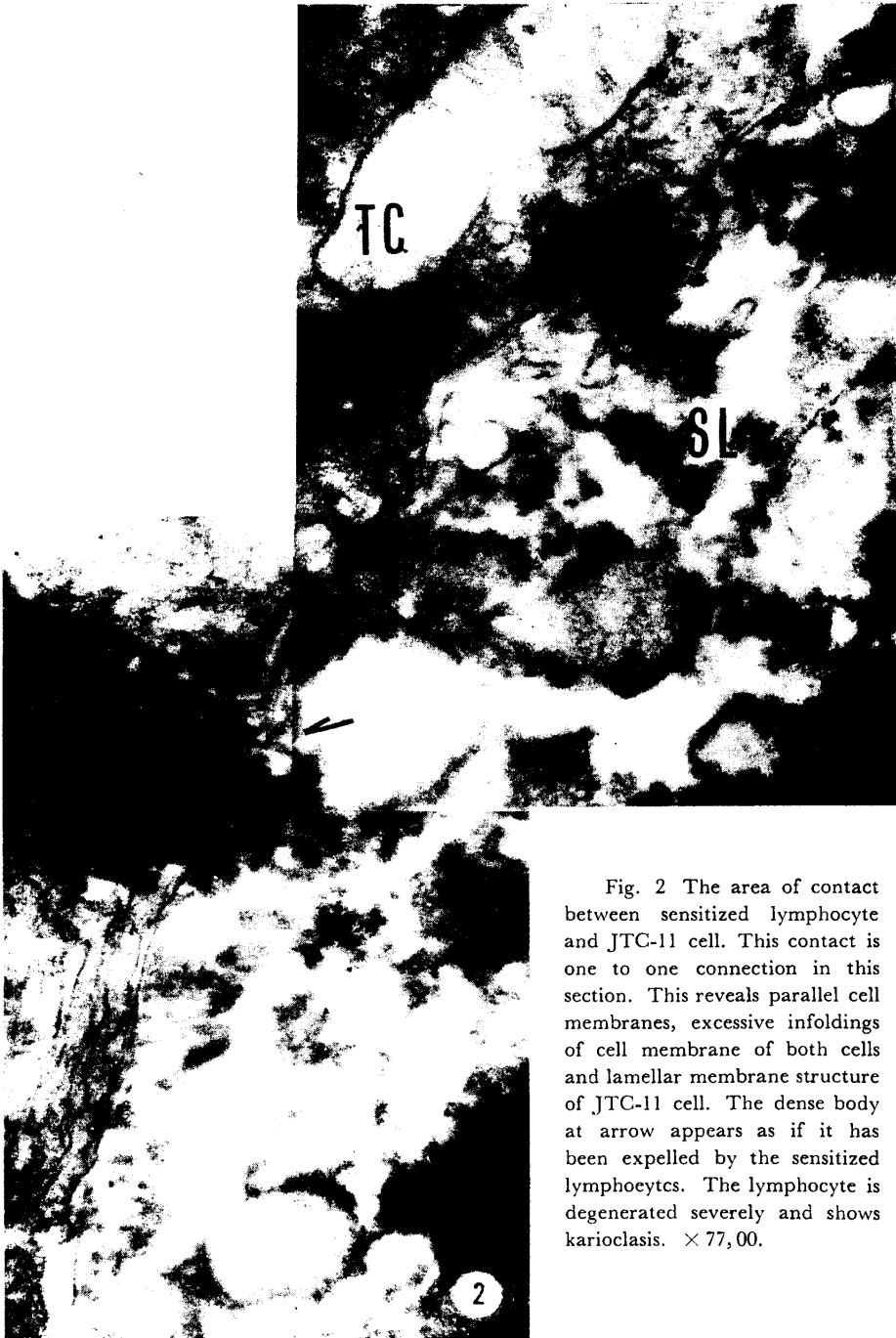


Fig. 2 The area of contact between sensitized lymphocyte and JTC-11 cell. This contact is one to one connection in this section. This reveals parallel cell membranes, excessive infoldings of cell membrane of both cells and lamellar membrane structure of JTC-11 cell. The dense body at arrow appears as if it has been expelled by the sensitized lymphocytes. The lymphocyte is degenerated severely and shows karyoclasia. $\times 77,00$.

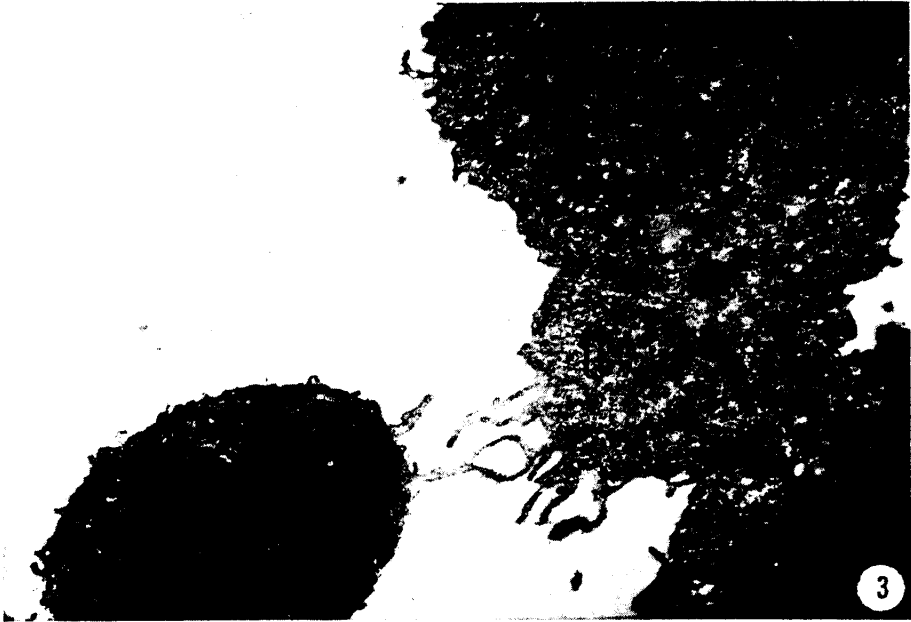


Fig. 3 The area of contact between a sensitized lymphocyte and a JTC-11 cell. Contact is made by slender, long cytoplasmic processes of JTC-11 cell. $\times 10,000$.

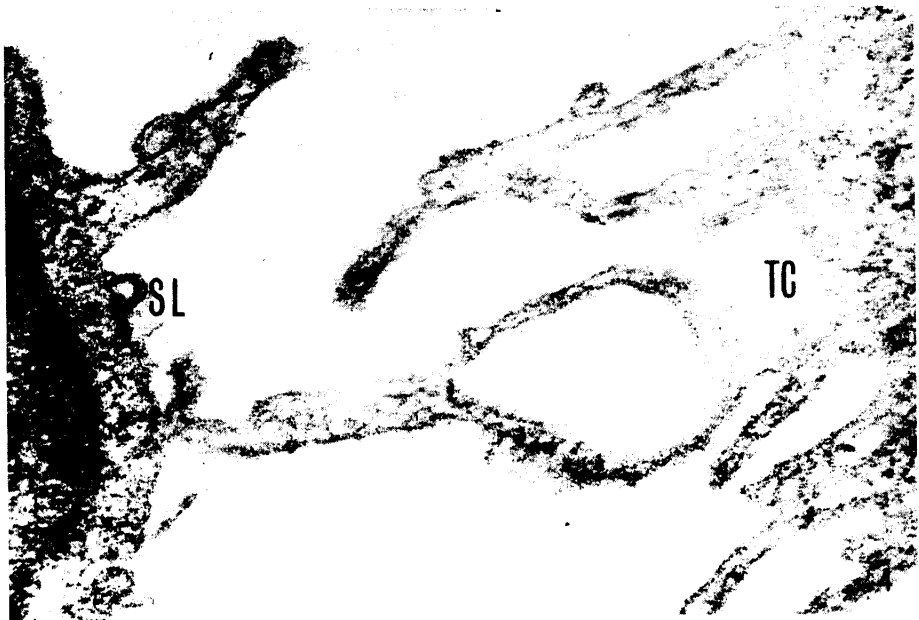


Fig. 4 Higher magnification of Fig. 3. $\times 46,200$.

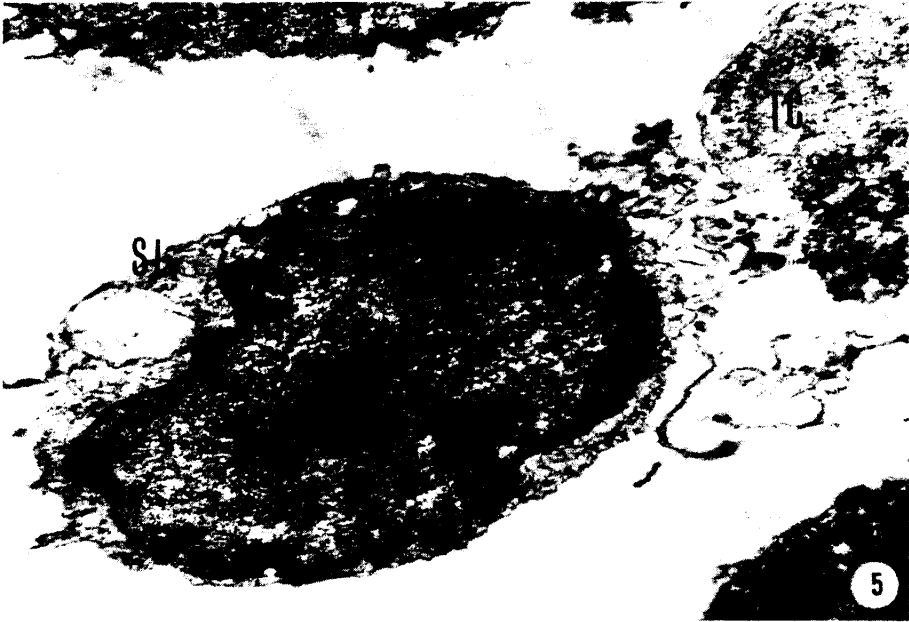


Fig. 5 The area of contact between a sensitized lymphocyte and a JTC-11 cell. There is seen interdigitation of cytoplasmic process of both cells and complex imbricating of cell membrane of JTC-11. $\times 11,600$.



Fig. 6 Higher magnification of Fig. 5. $\times 29,100$.

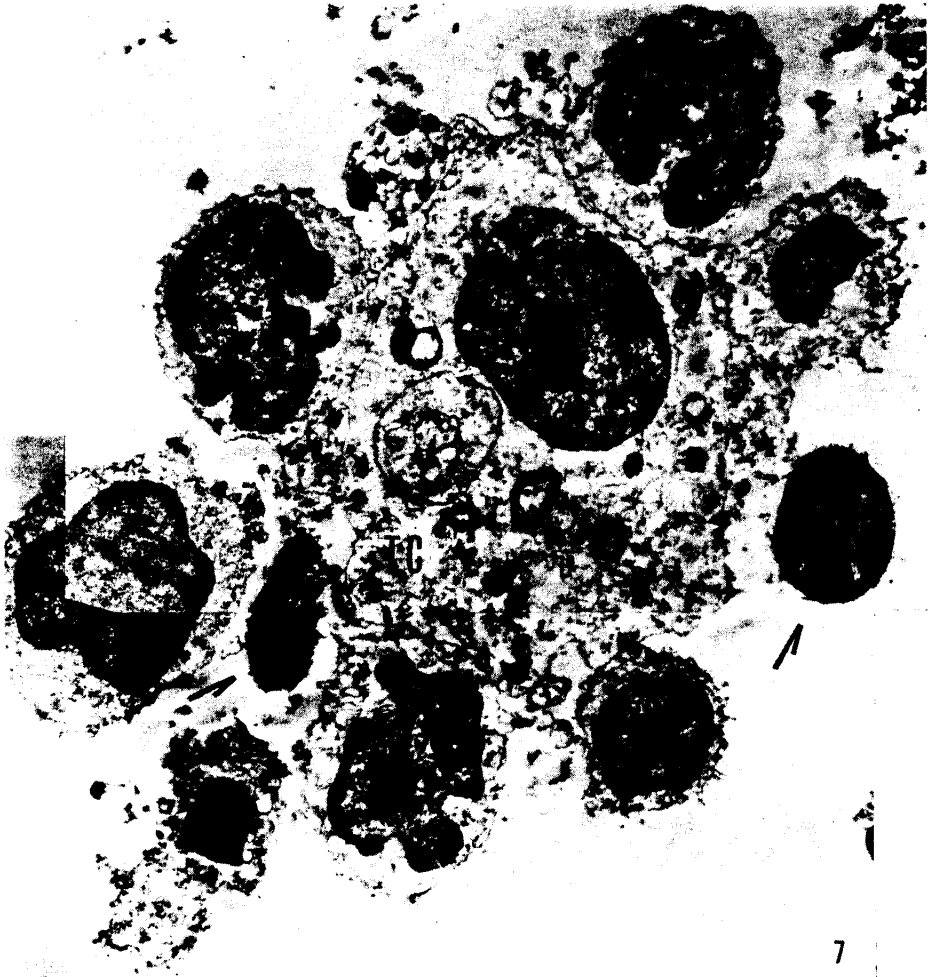


Fig. 7 The area of contact between sensitized lymphocytes and JTC-11 cell. Seven lymphocytes are in contact with JTC-11 cell. Two lymphocytes (arrows) show degeneration. Nucleus of condensed chromatin and destroyed cytoplasm are observed. JTC-11 cell is degenerating. Its nucleus is oval. Cytoplasm contains many lipid droplets and undifferentiated bodies. $\times 5,200$.

contact (Figs. 7, 9, 10, 12). The discontinuity of the membranes at the site of contact suggests a direct communication of the cytoplasm of the two cells. Incidentally, we could not observe what SHELTON *et al.* call "emperipolesis" (30).

The morphology of lymph node cell that became attached to target cell; those lymph node cells that came in contact with JTC-11 cells had cellular structures practically identical with those of controls, though they



Fig. 8 Higher magnification of Fig. 7 (lower right). Cytoplasmic process of lymphocyte invades into JTC-11 cell (arrow). Numerous smooth surfaced vesicles are scattered through the cytoplasm of lymphocyte. $\times 29,100$.



Fig. 9 Another section of Fig. 8. There is breach in continuity of cell membrane of both types of cell (arrow) and reveals cytoplasmic fusion. $\times 23,700$.

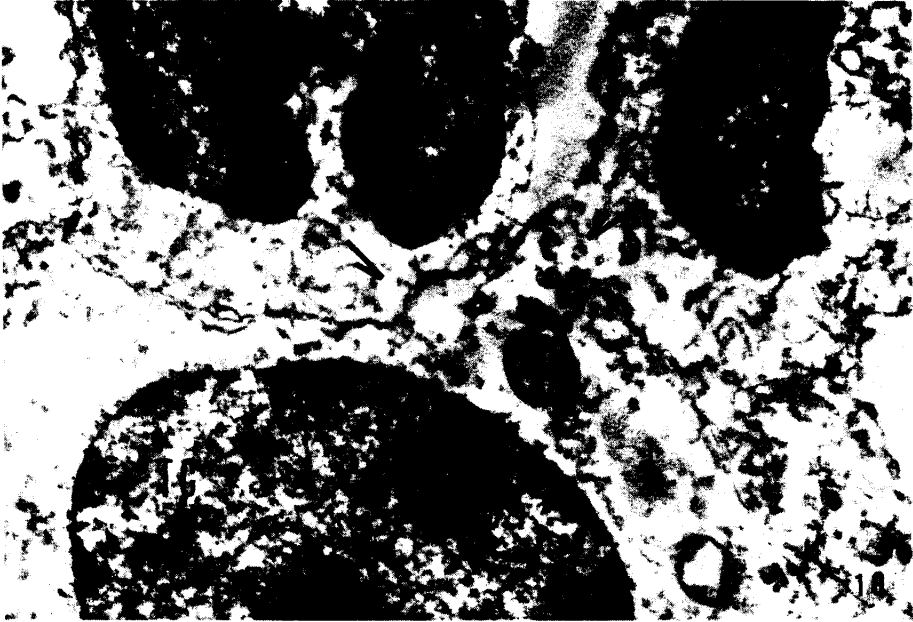


Fig. 10 Higher magnification of Fig. 7 (upper right). There is seen cytoplasmic fusion of both types of cell (arrows). $\times 12,900$.



Fig. 11 Another section of Fig. 10. There is a continuity of cell membrane. $\times 12,500$.

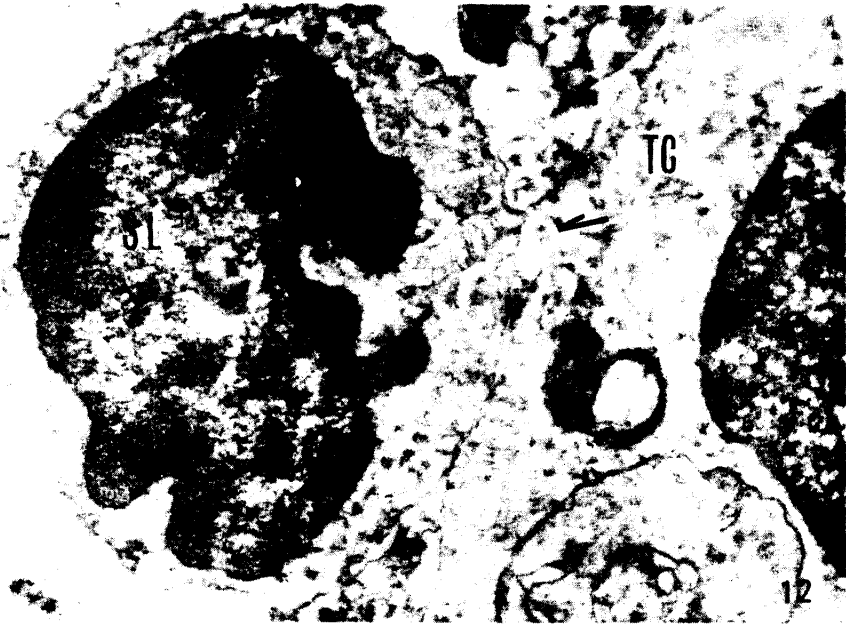


Fig. 12 Higher magnification of Fig. 7 (left), showing cytoplasmic fusion of both types of cell (arrow). $\times 12,300$.

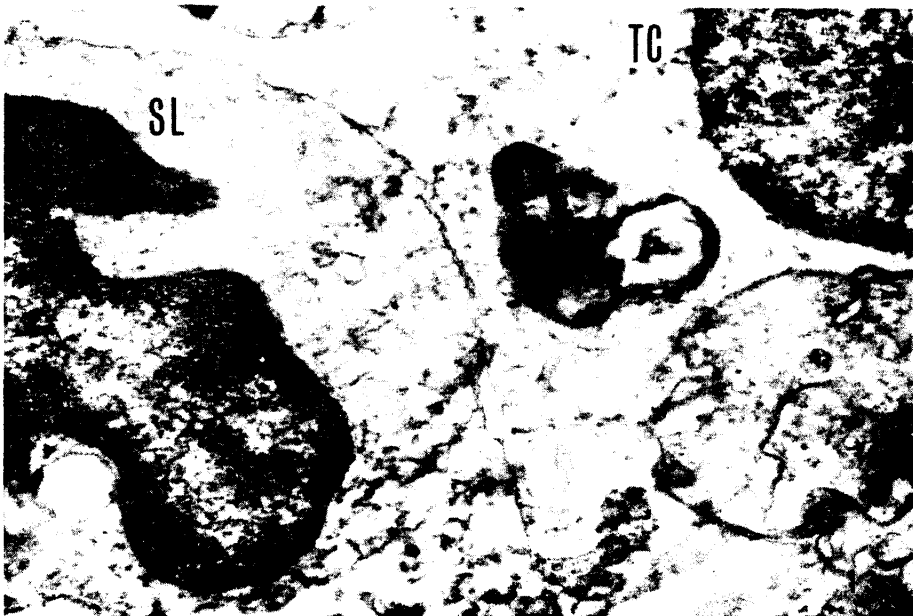


Fig. 13 Another section of Fig. 12. There is continuity of paralleled cell membranes of both types of cell. $\times 16,000$.

showed a slight degeneration (Figs. 3, 5, 7). Characteristic features of such lymph node cells were their large nucleus and narrow cytoplasm with less organelles in it. Most of the nuclei showed deep indentation and chromatin were fine and aggregated on the nuclear membrane. The cell membrane had irregular pseudopodial projections and the cytoplasm appeared clear. Mitochondria had cristae and were swollen. The cell had vacuoles and smooth surfaced vesicles, and Golgi apparatus was incompletely developed, revealing hardly any endoplasmic reticulum. Small granules what appeared to be ribosomes formed a small rosette in a certain portion of the cytoplasm. Such findings coincide well with those of lymphocytes as reported in the literature (Figs. 31—33), and these are the cells numerously observed in lymph node mentioned in the foregoing.

In the case of the lymph node cells that aggregated onto target cell the cellular structures became indistinct and they showed destruction of the cell membrane and karioclasia (Fig. 7). Blast cell and plasma cell did not make their appearance in this interaction. As for the morphology of JTC-11 cell on which lymph node cells became attached, their cellular structures are better preserved than the latter and change of the cytoplasm is more marked than that of the nucleus (Fig. 7).

DISCUSSION

Many reports indicate that homograft and tumor immunity is mediated by mononuclear cells of host origin, probably lymphocytes. WINN (34), and WIENER and coworkers (35) observed that lymphoid cells *in vivo* interact directly against grafted tissues. Namely, in the quantitative study of the immunologic activity of sensitized lymphoid cells on homologous tumor cells, WINN (34) injected the mixture of lymphoid cells sensitized by homologous tumor cells and ascites tumor cells of donor subcutaneously into homologous recipient mice. By removing and weighing tumors formed in the recipients after a certain time, he found that the sensitized lymphoid cells inhibited the tumor growth and that there was an obvious relationship between the inhibition of tumor growth and the number of the lymphoid cells. However, when such sensitized lymphoid cells and tumor cells were transplanted to the same animal but at different sites, there was observed only a trivial inhibition of tumor growth. Hence, he suggests that lymphoid cells act directly on the tumor and contact or a close association between the lymphoid cell and tumor cell is necessary for tumor growth inhibition.

In the light and electron microscopic studies on skin homografts,

WIENER and coworkers (35) observed that basophilic mononuclear cells infiltrated into dermis and epidermis, and graft rejection directly paralleled with the extent to which the mononuclear cells invaded the epidermis. These mononuclear cells proved to be lymphocytes by electron microscopy, and the apposing cell surfaces between the invading mononuclear cells and epidermal cells were in contact and they showed numerous cytoplasmic projections and inholdings. Often the apposing surfaces became indistinct, suggesting the disruption of the cell membrane.

In vitro system, on the other hand, ROSENAU and MOON (1) observed the similar action of lymphoid cells. Namely, when the lymphoid cells from homologous mice sensitized by L cells were mixed with L cells and cultured, the sensitized lymphoid cells clustered around the target cells in increasing numbers and came into direct contact with these cells, and this was followed by marked and progressive cytopathic changes of the target cells, finally resulting in the destruction of the cells.

Later intensive investigations in this field were carried out by KOPROWSKI and FERNANDES (2), HANAOKA and NOTAKE (3), ROSENAU (4), TAYLER and CULLING (5, 12), BERG and KALLEN (6), WILSON (7) (11), ROSENAU and MOON (8, 15), BRONDZ (9), VANIO and KOSKIMIS (10), HARA (13), ROSENAU and MORTON (14), and SATOH (16—18). These studies demonstrated that lymphoid cells from lymph nodes, spleen, and thoracic duct of sensitized animals aggregate, adhere and destroy homologous or isologous target cells. In contrast, while normal lymphoid cells do adhere to the target cells, they do not bring about the destruction of the latter. In such instances, an intimate contact between lymphoid cells and target cells is necessary for the cell destruction, but humoral antibody or complement is not required. Further, the lymphoid cells that enter into this reaction also die simultaneously.

This contact has been proven to be immunologically specific (8, 17). The contact between the lymphoid cell and the target cell is called "immune adherent cell lysis phenomenon" (18) or "contactual agglutination" (2).

The investigators mentioned in the foregoing have all observed the adherence, aggregation and cell destruction in the interaction of the two cells, but there is no report dealing with the fusion of cytoplasm. However, in our electron microscopic observations we find a discontinuity of the cell membranes at the point of contact and the cytoplasm of both cells is communicating with one another. In Fig. 7 a target cell is surrounded by 7 lymphoid cells. Of these seven lymphoid cells, two are of naked nuclei, and of the rest five, three show the fusion of the cytoplasm at the

site of contact. Looking at this cell group with other thin sections, the remaining two cells also show similar cytoplasmic fusion at the point of the cell contact. In Fig. 2 there can be seen a discontinuity of the cell membranes at the portion which has complex interdigitation and this part has become indistinct. From this finding it seems that, if we can prepare serial sections of the cells in the process of destruction, the fusion of cytoplasm of the two cells perhaps plays a role in the destruction of the target cell. Similar findings were obtained *in vivo* by WIENER *et al.* (35), and by JOURNEY and AMOS (36) with the histiocyte response to ascites tumor by electron microscopy.

When animal receives antigenic challenge, there occurs an increase in large pyronophilic cells in the regional lymph nodes (37—45). Moreover, this cell proliferates only in the lymph nodes and never appears at the site of antigen injection. When observed by electron microscopy, this cell has a large cytoplasm and always ribosomes in a rosette formation, but with undeveloped endoplasmic reticulum with a round nucleus. This is the cell of the lymphatic cell series and as an immunologically competent cell, various names are given to it (29, 41, 46, 47). There still remains a problem how this large pyronophilic cell is associated with the direct reaction between lymphoid cell *in vitro*, the investigation of which had its beginning with ROSENAU and MOON. However, the electron micrographs of the present experiment reveal that this cell is located in the lymph nodes but it is not the cell in contact. Since those cells in contact with the target cells are lymphocytes, the large pyronophilic cell is not directly involved in this interaction.

SUMMARY

When the lymph node cells sensitized by Ehrlich ascites tumor were mixed and cultured with JTC-11 cells derived from Ehrlich ascites tumor, the interaction of the two cell groups exhibited a contactual phenomenon accompanied by the destruction of JTC-11 cells. These two cell groups in contact were fixed with OsO₄ solution and the ultra-thin sections were observed in the electron microscope. As a result the following findings were obtained.

In the interaction where lymph node cells become attached to JTC-11 cells, resulting in the destruction of JTC-11 cells, lymphnode cells were also destroyed.

Effector cells seem to be a kind of cells in the lymph nodes, and from their morphological characteristics they are considered to be lymphocytes.

Electron microscopic observations of the surface of contact revealed the following: some cells are adhered to one another at the surfaces of the cell membranes that run in parallel; some are in contact by means of filamentous projection of lymphocytes; the cell membranes of the two cells form interdigitation; and both surfaces of two cell membranes are disrupted at the point of contact and the cytoplasm of the two cells appears to be directly connected with one another.

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