FUNCTIONAL ANATOMY OF THE LYMPHOCYTE IN IMMUNOLOGICAL REACTIONS IN VITRO

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Behavioral characteristics of viable lymphocytes in vitro may be pertinent to the functions of these cells in vivo. Lewis (1) demonstrated the distinctive locomotion of the lymphocyte, a characteristic which has proven of value in the identification of immature cells in certain malignant and immunologic states (2). The lymphocyte's propensity to migrate over the surface of another cell and to enter its cytoplasm on occasion has been described by Sharp and Burwell (3), and Humble et al. (4) who termed these activities peripolesis and emperipolesis respectively.

In recent years, knowledge of the role of the lymphocyte in immunological reactions has been supplemented by demonstrations of the cell's ability to respond in vitro to specific and nonspecific antigens. Although this responsiveness is generally of the secondary type, blastogenesis also results when leukocytes from two unrelated individuals are cultured together (5). In early studies of this mixed leukocyte reaction it was found, in agreement with observations of others (6), that if the leukocytes from unrelated donors were separated by a filter membrane with 0.4 μ pores, blastogenesis was prevented. This suggested that cell contact might be essential for the initiation and propagation of the mixed leukocyte reaction. Further studies of this reaction employing time-lapse cinephotomicrography demonstrated that during interaction the viable lymphocyte possesses an anatomically distinct cytoplasmic process with which it "attaches" to other cells, debris, or the surface of the culture flask (7). The prominence of this process, hereinafter termed the uropod (Greek: *oura*, tail and *podos*, foot, stalk), suggests that a unique advantage, perhaps essential for certain lymphocyte functions, may be provided by this anatomic arrangement.

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Methods

Methods for obtaining and preparing blood leukocytes for mixed leukocyte cultures have been described previously (7). In the present experiments venous blood specimens from two unrelated individuals were collected in sterile plastic syringes, and leukocyte-rich plasma was obtained by sedimentation of the erythrocytes. No attempt was made to obtain "pure" lymphocytes. After determining total leukocyte counts on each specimen, 3.0 ml cultures were made consisting of 1.0×10^6 leukocytes from each donor, 20% plasma (10% from each donor) and 80% TC 109. Cells were cultured in vertical 16×150 mm screw-capped tubes (Bellco Glass, Inc., Vineland, New Jersey). At irregular intervals during 7 days, cultures were sacrificed and aliquots of the cells were transferred by gentle pipetting to clean, prewarmed 43×50 mm No. 2 cover slips. Cell suspensions were sealed beneath 22×40 mm cover slips with a 2:1 mixture of paraffin and petrolatum. In some experiments cultures were placed in modified Carrel flasks to provide constant observation without disturbing cell activities. Cells were observed and photographed in a 37°C incubator with a Zeiss Plankton inverted phase-contrast microscope with attached camera.

For radioautographic studies, 2.0 μc thymidine-H^3 (specific activity 12.8 c/mM) was added to each culture on the 6th day for the last 6 hr of culture. Cells were washed three times in normal saline and dried films were prepared on microscope slides. These were coated with liquid photographic emulsion (Kodak Nuclear Track Emulsion Type NTB3) and stored in the dark at 4°C for 1 wk. The film was then developed and the cells were stained with Wright and Giemsa stains.

OBSERVATIONS

The motile lymphocyte in suspension cultures is readily recognized by its irregular "pear" configuration (Fig. 1). This configuration is most conspicuous as the lymphocyte migrates, suggesting, as emphasized by DeBruyn (8), that the cell has "polarity." The pseudopods appear and disappear at the anterior end of the cell but the "tail" or uropod (*vide supra*) tends to remain prominent and relatively constant at the rear. Even when the uropod shortens momentarily as the lymphocyte becomes more spherical, it appears that the same area of the cytoplasm becomes the uropod when the cell resumes motility again.

Many lymphocytes in these cultures appear spherical and nonmotile, a fact that may be related to the recognized heterogeneity of any lymphocyte population. Particularly noticeable however, are the many lymphocytes that have assumed the elongated "pear shape" and are either motile or are attached to the glass or a small amount of debris at the end of the uropod. Although these attached cells have the same configuration as the motile lymphocyte and pseudopods are constantly changing shape distally, the end of the uropod remains fixed. This is especially evident in cultures observed in Carrel flasks in which, with slight agitation of the culture flask, the main body of the lymphocyte can be seen to move to and fro in the medium whereas the uropod remains fixed to the glass.

As the culture becomes older and debris accumulates, lymphocytes can be seen around the periphery of masses of debris with the pseudopods moving distally and the uropod projecting among the accumulations of debris (Fig. 2).

The point of contact of the uropod is changed frequently suggesting that a function of the pseudopods is to position the uropod properly. Occasionally as a cell migrates away from an area of debris the end of the uropod appears "stringy" and may have particles adherent to it (Fig. 3).

Certain macrophages appear to be particularly attractive to lymphocytes and time-lapse sequences have shown that the motile lymphocyte approaches the macrophage, contacts its surface with the pseudopods briefly demonstrating peripolesis, and then maneuvers into a position that permits the uropod to contact the surface of the macrophage. In some instances the uropod appears actually to be "backed up" to contact the macrophage. During this macrophage-lymphocyte interaction, which may continue for hours, the lymphocyte appears to vary its position with respect to the macrophage so that the uropod contacts different areas of the macrophage's surface (Fig. 4). Furthermore, as lymphocytes become larger in the course of "blast" transformation and acquire visible vacuoles and granules, these organelles tend to be concentrated in the uropod and the nucleus tends to remain in the area of the pseudopods. During brief periods when the cell becomes more spherical in shape the visible cytoplasmic bodies stream bilaterally around the nucleus.

As cell debris becomes increasingly abundant, macrophages tend to collect in groups. One sees clusters of macrophages surrounded by lymphocytes which are arranged radially with elongated uropods attached to the central cells. In many instances the lymphocytes so outnumber the several macrophages in the center that the group resembles a cluster of grapes (Fig. 5). These clusters are the viable representation of the central macrophage with satellite lymphocytes, the "rosette", that is commonly seen in fixed preparations from mixed leukocyte and antigen-stimulated cultures. Transformed lymphoblasts, when they begin to appear, are most readily found in these clusters of interacting lymphocytes, macrophages and debris. Further evidence for this observation is provided by radioautographic studies which show marked thymidine incorporation and therefore DNA synthesis in lymphocytes that comprise the rosettes (Fig. 6).

With the appearance of transformed lymphoblasts, it is not uncommon to find lymphocytes interacting with lymphoblasts and the point of contact between the two cells is again at the ends of the uropods (Fig. 7). During this type of interaction the pseudopods may move the main body of either cell to and fro so that the uropods may make end-to-end or side-to-side contact but contact is still limited to the area at the ends of the uropods. Not infrequently several small lymphocytes interact with the uropod of a large lymphoblast (Fig. 8). As one observes the point of contact and is able to keep the area in focus, it appears that there are threadlike connections between the ends of the uropods. In some instances the connections appear sufficiently wide to suggest a continuous cytoplasmic connection between the interacting cells (Fig. 7).

Mitotic activity can be observed most readily during the 5th to the 7th day

in cultures that remain undisturbed in Carrel flasks. Lymphoblasts that are about to undergo mitosis become spherical and motionless. The nucleus becomes indistinct and chromosomes are clearly discernible during metaphase. At this time the metaphase plate appears to rotate through different planes in the center of the cell. The period from the onset of anaphase until the completion of cytokinesis occupies approximately 3 to 5 min. After completion of cytokinesis, the daughter cells remain immobile and symmetrical for a variable period of 15 to 90 min (Fig. 9). Then pseudopods appear and the cells rapidly elongate into the characteristic pear shape. At this stage it is noteworthy that the uropod develops in the area of the last point of contact between the daughter cells (Fig. 10). Frequently one daughter cell precedes the other in becoming motile and, as they both assume motility, a thin, threadlike remnant of the spindle bridge connects the two uropods. In suspension cultures, in contrast to tissue explants or cells in more solid medium, the daughter cells seem to undergo rather vigorous movements and migrations over a prolonged period before this connection is finally severed.

DISCUSSION

The term "uropod" is currently used in Biology to denote the foot processes on the abdomen of certain crustaceans, but the word seems equally or better suited to describe the cytoplasmic appendage that is in one instance the "tail" of the migrating lymphocyte and in another instance the "stalk" by means of which the lymphocyte interacts with debris and other cells in culture. Interaction by means of the uropod may be conveniently termed "uropodapsis" (Greek: *hapsis*, joining, juncture).

From a functional standpoint it appears that the uropod is a specialized area of the cytoplasm which is quite different from the cell's pseudopods. The pseudopods are located at the anterior end of the motile cell and appear to provide locomotion until the lymphocyte interacts. During interaction with other cells or debris the pseudopods appear to function in positioning the uropod.

It is impossible from these observations to determine whether the uropod always develops at a particular area of the cytoplasm or membrane as the lymphocyte changes from an inactive, spherical shape to the elongated pear shape. However, time lapse sequences show clearly that the uropod is both the "tail" and the interacting cytoplasmic appendage of the active lymphocyte.

The fingerlike connections which are discernible by light microscopy between the uropods during uropodapsis have been noted in electron microscopic examination (7), but more detailed studies will be required to determine whether or not these actually constitute cytoplasmic bridges. Connections of this sort would certainly provide for direct transfer of nucleic acids or other materials important in an immunologic reaction.

The observations of the behavior of daughter cells following mitosis seem especially pertinent. Polarity is established as soon as the daughter cells become motile and the uropods form at the last point of connection between the two cells. This suggests that internal relationships and forces established at this time persist or can be reestablished during locomotion and interaction, perhaps to provide a physicobiologic advantage essential for lymphocyte function. Although the lymphocyte may "rest" in a spherical posture for prolonged periods, when it resumes motility and interacts, it assumes a configuration that was established at the time of mitosis.

The frequency of lymphocyte-macrophage interaction in immunologic reactions in vitro is impressive. Studies by Fishman and Adler (9), and Askonas and Rhodes (10) have demonstrated that RNA extracted from macrophages is capable of stimulating antibody response in vivo. The behavior of cells in the present in vitro studies suggest that the lymphocyte may acquire stimulatory macrophage material via the uropod during lymphocyte-macrophage interaction. Contrary to the results of Harris (11), our studies suggest that the lymphocytes that undergo transformation are those that have been interacting with either macrophages or lymphoblasts. Fig. 6 is a radioautographic demonstration of a typical "rosette" consisting of a macrophage surrounded by lymphocytes. All of the satellite lymphocytes had incorporated thymidine-H^3 indicating DNA production.

In control, unstimulated cultures lymphocyte-macrophage interactions seldom occur and appear to be brief in duration (7). The fact that interactions occur at all in control cultures suggests that this may be a normal event that results in lymphocyte transformation only when the macrophage has phagocytized antigen under appropriate conditions or has been exposed to stimulatory material.

Lymphocyte-lymphoblast interaction is a particularly noteworthy feature of these cultures in that it may have important implications in the kinetics of an immunologic reaction. If the lymphoblast and the "stimulated" lymphocyte, i.e. one that is in the process of transformation to a lymphoblast, are each capable of interacting with other lymphocytes to stimulate them to transform, a mechanism is provided whereby large numbers of committed cells can be mustered in a relatively short period of time. It would imply that the production of committed cells may be through vertical and horizontal processes: vertically by mitosis of transformed cells and horizontally by interaction of transformed or stimulated cells with other unstimulated lymphocytes. These simultaneous processes could explain the findings, especially in primary immune reactions, of greater numbers of lymph node cells labeled with thymidine-H^3 than could be accounted for on the basis of mitotic activity alone (12). It is also evidence that if the macrophage is critical in initiating a particular immunologic reaction, few of these cells need be available for the reaction to begin.

Obviously caution and restraint must be exercised in extrapolating events in vitro to cell functions in vivo, and one may question the relevance of the present observations to lymphocyte function. Clustering of plasmacytes around macrophages has been noted in studies of fresh, viable lymph node cells, suggesting that plasmacyte-macrophage interaction does occur in vivo (13). The common association of lymphocytes with macrophages in fixed preparations led to the concept that the macrophage may serve a nutritive function for the lymphocyte (14). Assuming the uropod to be a functional appendage in vivo, it may be difficult to demonstrate with routine methods because as the cell dies during fixation it acquires a spherical shape. Furthermore, in vivo the space limitations may be such that the lymphocyte rarely expands into the elongated pear shape with extended uropod. However the prominence of the uropod in leukocyte cultures is striking and suggests at least that interaction in vivo may occur at a particular area of the membrane or cytoplasm. In other words it implies a special site on the membrane, an "interaction site," that provides for contact whether the cell is elongated or spherical in configuration.

SUMMARY

The motile lymphocyte in vitro has a prominent "tail" that becomes a means of "attachment" to other cells and debris during interaction. The term "uropod" is proposed to designate this specialized cytoplasmic projection which appears totally different, anatomically and functionally, from the pseudopods. Observations of lymphoblasts during mitosis indicate that the uropod is formed immediately following mitosis at the point of final cytoplasmic connection between daughter cells, a fact that may prove significant as lymphocyte function is better understood.

In the mixed leukocyte reaction the lymphocyte interacts with macrophages, cell debris, and lymphoblasts via the uropod, suggesting that stimulatory material may be acquired through this specialized appendage. Lymphoblast-lymphocyte interaction is noteworthy and implies that immunologically committed cells may be mustered through horizontal as well as vertical processes: horizontally by lymphoblast-lymphocyte interaction and vertically by mitosis of transformed lymphoblasts. The possible relevance of these in vitro observations to lymphocyte functions in vivo is discussed.

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EXPLANATION OF PLATES

All magnifications are approximately 200.

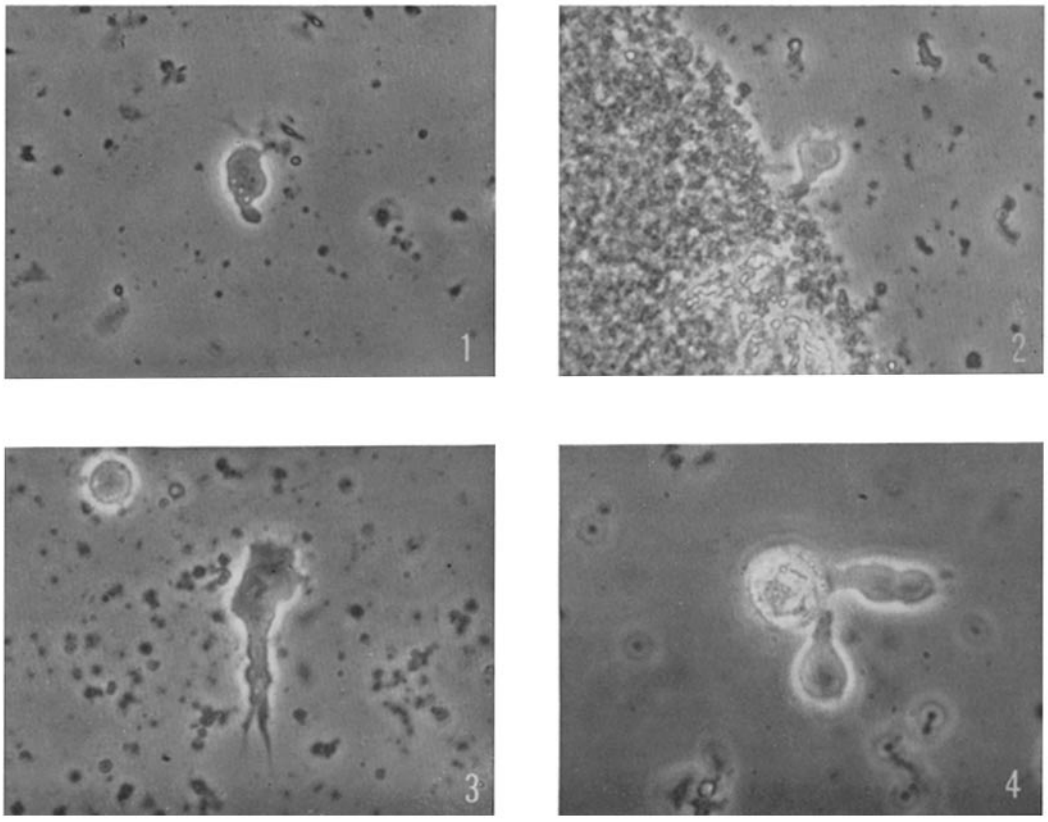
PLATE 84

FIG. 1. The motile lymphocyte in suspension culture is readily recognized by its elongated "pear shape." This lymphocyte was migrating in the direction of the top of the photograph. The pseudopods are visible anteriorly and the uropod (see text) is prominent posteriorly.

FIG. 2. Lymphocytes contact particles of cell debris with the uropod. The pear shape is maintained. The pseudopods appear to function in positioning the uropod as it contacts different areas.

FIG. 3. As a lymphoblast migrates away from an area of cellular debris the end of the uropod often appears "stringy" and "sticky" with particles adherent.

FIG. 4. Uropodapsis (see text) involving two lymphocytes and a macrophage. The points at which the uropods contact the macrophage membrane are constantly changing during this prolonged type of interaction.



(McFarland et al.: Lymphocyte in immunological reactions)

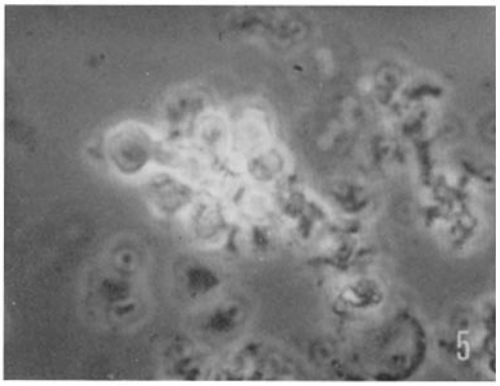
PLATE 85

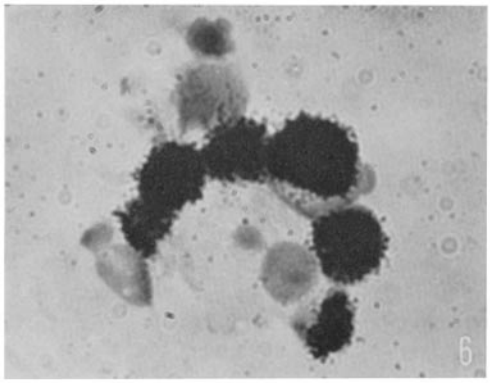
FIG. 5. Certain macrophages and groups of macrophages appear to be particularly attractive to lymphocytes and result in clumps of interacting cells of both sorts resembling clusters of grapes. Although the depth of focus of this photograph shows the uropod on only one lymphocyte, each lymphocyte in the group had projected its uropod to contact the central macrophages. This type of aggregation is the viable counterpart of the "rosettes" seen in fixed preparations consisting of macrophages surrounded by satellite lymphocytes.

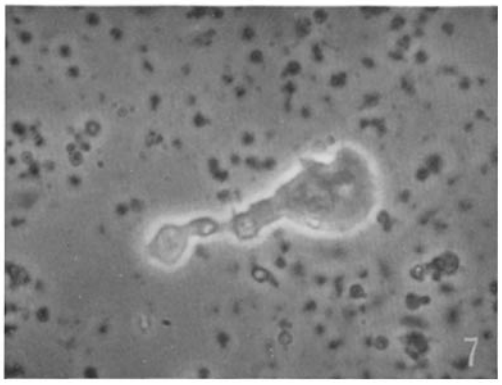
FIG. 6. This radioautograph is the result of thymidine-H^3 incorporation during the last 6 hr of culture. It shows a typical "rosette" consisting of a macrophage with satellite lymphocytes and lymphoblasts. The heavy incorporation by the satellite cells is evidence in support of the morphologic impression that transformed cells are those that have interacted with macrophages or lymphoblasts.

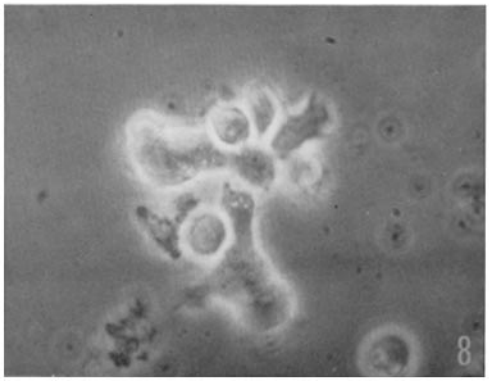
FIG. 7. Lymphocyte-lymphoblast uropodapsis is quite common and has important implications in the kinetics of an immunologic reaction. If a stimulated or transformed cell is capable of stimulating other lymphocytes, a mechanism is provided for the rapid production of committed cells. In this particular scene it appears that a cytoplasmic bridge connects the two uropods.

FIG. 8. Another example of lymphocyte-lymphoblast interaction. In this photograph uropodapsis involves four lymphocytes (upper right) and one of two large daughter lymphoblasts that had recently divided and were still attached to each other.







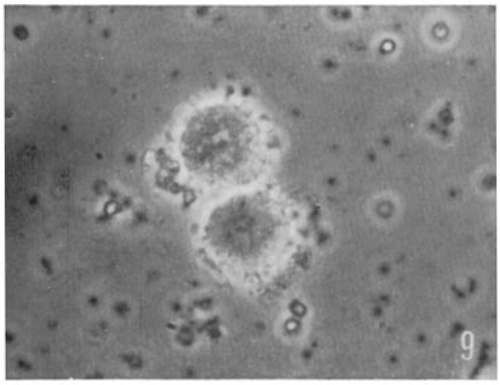


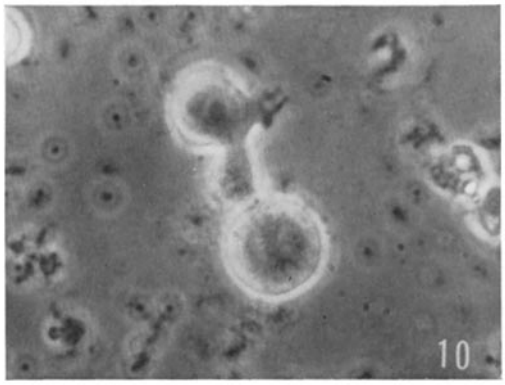
(McFarland et al.: Lymphocyte in immunological reactions)

PLATE 86

FIG. 9. Daughter lymphoblasts remain relatively motionless for a prolonged period after cytokinesis.

FIG. 10. One daughter cell may precede the other in resuming motility following mitosis. This photograph demonstrates that the functional anatomy of the lymphocyte is established at the moment that a daughter cell resumes motility. The pseudopods and nucleus assume an anterior, distal position whereas the uropod is formed in the area of the last cytoplasmic connection between daughter cells.





(McFarland et al.: Lymphocyte in immunological reactions)