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Scanning and Transmission Electron Microscopy Study of Antibody-Dependent Lymphocyte-Mediated Cytotoxicity on Measles Virus-Infected Cells

Rentier B, Wallen W C

Infectious Diseases Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205

Abstract : The structural events related to antibody-dependent lymphocyte-mediated cytotoxicity (ADLC) have been studied on measles virus-infected cells using human peripheral blood lymphocytes (PBL) and anti-measles virus serum. The first event in ADLC was a recognition process occurring within 15 min after contact between the infected cells and lymphocytes. Plasma membrane and microvilli of adsorbed PBL were specifically attached to virus-induced ridges over nucleocapsids and to viral buds. After 30 min, a fraction of adsorbed PBL (K cells) changed shape and extended long filipodia toward the target cells which, in turn, showed long villi contacting the PBL. At 4 h, when cytotoxicity as measured by chromium release was maximum, K cells had flattened and numerous blebs and ruffles formed on their surface. The K-cell alterations varied in intensity with the type of measles-infected target cell, but frequently the K cells appeared irreversibly damaged. T- and non-T-cell fractions were separated, and in situ erythrocyte rosettes were used as markers for subpopulations which were easily recognized by scanning electron microscopy. Most of the cytotoxic K cells were identified as non-T cells carrying Fc receptors for immunoglobulin G. However, a small subpopulation of cells bearing both sheep erythrocyte and Fc receptors was also found to be involved in ADLC by chromium release assay as well as by electron microscopy. Some of these interacting T cells extended a long uropod on the target cell, but their intracellular structure remained unaltered through ADLC, in contrast with the other T cells and the non-T killer cells. This suggests that perhaps some T killer cells might remain functional after the cytotoxic interaction with a target cell.

Cells infected with paramyxoviruses display virus-induced morphological changes in their plasma membrane. Studies by transmission electron microscopy (TEM) have shown that nu-cleocapsids align beneath cell membrane areas which are covered with projections (8, 12-15). During measles virus infection of a cell monolayer, immunolabeling techniques combined with thin-sectioning, surface replication, and scanning electron microscopy (SEM) have demonstrated that these virus-modified areas of the host membrane (ridges) contain viral antigen and hemadsorbing sites (12, 13, 31). In the absence of complement, anti-measles antibodies produce a redistribution of the cell membrane components and clearing of antigen-antibody complexes (21, 22).

Antibodies also mediate a cellular immune response, the antibody-dependent lymphocyte cytotoxicity (ADLC) (27). This hypersensitive cell-killing activity, which is known to also operate against virus-infected cells in vivo (17,18), can readily take place during viral infection because it requires the presence of nonsensitized Fc receptor-bearing leukocytes and specific antibodies (27, 28, 34). Hence, it appears to be an important mechanism of immune response to viral infection. It might be very effective in measles virus infection since circulating antiviral antibodies remain present for a lifetime after primary infection and since anti-measles virus antibody levels are extremely elevated in the serum and cerebrospinal fluid of patients with subacute sclerosing panencephalitis. In ADLC, the primary effector cells have been described as nonadherent and nonphagocytic lymphocytes (36), carrying receptors for the Fc portion of immunoglobulins (K cells) (28, 34).

We have studied the attack of two cell lines infected with measles virus (target cells) by peripheral blood lymphocytes (PBL) in the presence of anti-measles virus serum. We have characterized the sites of recognition between PBL and target cells and observed the interactions between effector cells and target cells using TEM and SEM. These observations were correlated with cytotoxicity measurements by the ^{51}Cr release assay. Differential labeling allowed us to determine which PBL subpopulations were involved in this cytolytic process. (Parts of this work were presented at the Fourth International Congress for Virology, The Hague, 1978, and at the 79th Annual Meeting of the American Society for Microbiology, Los Angeles, Calif, and **Honolulu,** Hawaii, 1979.)

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Materials and methods

Cells and viruses.

Vero cells, a continuous line of African green monkey kidney cells, were grown in 75 cm^2 plastic flasks and on 13-mm glass or plastic cover slips and inoculated at confluency with the Edmonston B strain of measles virus as described previously (31). Experiments were performed when giant cells were scattered over the cultures.
MA160 cells (Microbiological Associates, Walkers-ville, Md.), a human prostate cell line, were grown in the same manner as the Vero cells. MA72046 cells derived from MA 160 ceils and persistently infected with the Mantooth strain of SSPE virus (14) were employed when 90% of the cells had measles virus-associated membrane antigen, as detected by immunofluorescence.

PBL purification

Lymphocytes were obtained from measles virus seropositive and seronegative human donors and separated from heparinized peripheral blood by Ficoll-Hypaque gradient centrifugation (5). The mononuclear cells were collected, washed twice in phosphate-buffered saline (PBS, pH 7.2), and suspended in RPMI-1640 medium (Microbiological Associates) supplemented with 10% fetal calf serum and 1% L-glutamine. Fetal calf serum used in these studies was adsorbed three times with fresh sheep erythrocytes.

Separation of PBL subpopulations by resetting techniques

Subpopulations of T and non-T lymphocytes were obtained by separating sheep erythrocytes (SE) rosetting cells (T cells) from nonrosetting cells using Ficoll-Hypaque density gradient centrifu-gation. Equal volumes of a 2% washed sheep erythrocyte suspension and PBL at 5 X 10^7 cells per ml were mixed thoroughly, incubated at 37° C for 5 min, and then at 4°C for 90 min. The SE-PBL mixture was then placed on Ficoll-Hypaque gradient and centrifuged at 60 X *g* for 30 min. A band of nonrosetting cells was collected, and rosettes were harvested from the pellet and placed in separate tubes. The SE-PBL rosettes were dissociated by incubation at 37° C for 20 min followed by treatment with distilled water for 30 s. Dissociated rosettes were then mixed with an equal volume of 2x Eagle minimum essential medium (Grand Island Biological Co., Grand Island, N.Y.), and the cells were washed twice with RPMI-1640 medium. The above-described SE rosetting procedure was repeated once with the SE rosetting subpopulation to obtain the final cell fractions.
The SE nonrosetting population was further treated to isolate the Fc receptor-bearing cells. The SE non-rosetting cells were placed in a tissue culture A flask (Corning Glass Co., Corning, N.Y.) and allowed to incubate at 37°C for 45 min. The nonadherent cells were gently removed and placed in a test tube. Chicken erythrocytes (CE) were incubated for 30 min at 37°C with a subagglutinating dilution of rabbit anti-CE immunoglobulin G (IgG) purified by Sephadex G200 filtration and diethylaminoethyl (DEAE)-cellulose chromatography. The sensitized cells (CE-AB) were washed twice and suspended to 2% in RPMI-1640 medium. Equal volumes of nonadherent, SE rosette-negative PBL and CE-AB were mixed and incubated at 37 °C for 45 min to allow rosetting. The mixture was then placed on a Ficoll-Hypaque gradient and centri-fuged at 60 X *g* for 30 min to separate rosetting (Fc receptor-bearing) cells from nonrosetting cells. The CE-AB rosetting cells were harvested from the pellet, and the CE were removed by a second distilled water treatment. The CE-AB rosette-positive PBL were washed twice in RPMI-1640 medium.

^{51}Cr release assay

The ^{51}Cr release assay was performed as previously described (26, 40). Briefly, uninfected and measles virus-infected Vero or MA 160 and MA72046 (see Cells and Viruses above) were used as target cells. The target cells were incubated with 10 ml of Dulbecco PBS containing 150 μCi of $Na_2{}^{51}CrO_4$ (specific activity, >350 mCi/mM, Amersham Corp., Arlington Heights, I11.) for 1 h at 37°C.

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The cells were washed 4 times, removed by gentle trypsinization and scraping, and resuspended as dissociated cells at 5 X 10^4 viable cells per ml in RPMI-1640 medium supplemented with 5% absorbed fetal calf serum and 1% L-glutamine. The target cell suspension was then placed into round-bottom microtiter test plates (Linbro Co., Waltham, Mass.) in 0.1-ml volumes. Lymphocyte populations were suspended at 5 X 10^6 cells per ml and added in 0.1-ml volumes. Serum was added to appropriate wells in 0.05-ml volumes. The positive serum had a titer of 1:160 by hemagglutination inhibition assay (35) and 1:1,280 by ADLC assay and was used at dilutions of 1:80 and 1:320, while the negative serum was used at 1:40. The cells were incubated at 37°C for 4 h. Then supernatant fluid was harvested with a Skatron automatic supernatant fluid collector (Flow Laboratories, Rockville, Md.), and the radioactivity in the fluid was detected with a gamma counter. The total amount of ^{51}Cr available per test was determined by placing 0.1 ml of labeled target cells into glass tubes (10 by 75 mm) in triplicate and adding 3 ml of distilled water to disrupt the cells. The level of cytotoxicity was determined from the mean of triplicate values for each test as follows:

% Cytotoxicity

$$= \frac{\mathrm{CPM}(T + L + S) - \mathrm{CPM}(T + S)}{\mathrm{CPM(total)} - \mathrm{CPM}(T)} \times 100,$$

where T = target cells, L = lymphocytes, S = serum, and CPM = counts per minute.

SEM and TEM

Target cells grown on coverslips were washed twice with MEM. Lymphocytes at 2 X 10^7 cells per ml were placed onto the cover slips in 0.2-ml volumes along with 0.2 ml of serum (with or without antibody to measles virus) diluted to 1:80. For in situ rosetting experiments, the cells were then kept at 37°C for 4 h and then thoroughly washed with MEM to remove noninteracting PBL. A 2% suspension of SE or CE-AB or both was added in 1-ml volumes to the cultures after the 4-h incubation. In situ SE rosetting was performed at 37°C for 5 min and then at 4°C for 90 min. In situ CE-AB rosetting was achieved in 40 min at 37°C. For double rosetting with SE and CE-AB, the cells were incubated at 37°C for 5 min and then placed at room temperature for 2 h. At the end of the appropriate time periods, each preparation was washed four times with MEM and then fixed in glu-taraldehyde *(2%* in cacodylate buffer 0.1 M, pH 7.2) at room temperature for 1 h. The preparations were washed in cacodylate buffer, postfixed in *1%* OsO_1, processed for SEM or TEM as previously described (31), and observed with an ETEC Autoscan scanning electron microscope and with a Philips 201 transmission electron microscope.

Results

Structural observations during ADLC with unseparated PBL. (i) Early interaction: recognition step

Approximately 15 min after anti-measles antibody and PBL had been added to the culture medium, many PBL were adsorbed to the infected monolayer and could not be detached by repeated washings. This observation was made with both target cell lines studied. As observed by SEM, all adsorbed PBL retained a spherical shape and were covered with short microvilli. TEM examinations of thin sections showed a specific attachment of most PBL to modified areas of target cell membrane under which viral nucleocapsids were aligned (Fig. 1). Areas of contact were large expanses of PBL membrane or short PBL microvilli. In some cases, no clear relationship between PBL adsorbing sites and the presence of nucleocapsids under the membrane of target cells could be detected. At this time, no significant cytotoxicity was detected by ^{r_1}Cr release.

After 30 min, ca. one-third of the adsorbed PBL were extending long filopodia onto the target cell membrane. The target cell itself also extended long villi towards the lymphocyte.

(ii) Late interaction: cytotoxicity

After 4 h, ca. 50% of the adsorbed PBL were strongly interacting with the target cell membrane. Many persistently infected cells (MA72046) were rounding up and retracting from the glass or plastic substrate to which they were still attached by long processes (Fig. 2). These cells and the PBL were often intensely damaged: their cytoplasm became less dense and organelles were destroyed. The cell

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membranes formed giant protrusions and blebs and were often disrupted. At this time, the ADLC, as measured by ^{51}Cr release, was near maximum and subsequently remained approximately the same as compared to controls (Fig. 3).
The membrane of Vero syncytia appeared more resistant to ADLC after 4 h than the membrane of persistently infected cells, and the shape and structure of PBL interacting with these giant cells changed dramatically. Cytotoxicity levels remained very low on Vero cells. PBL reacting with target cells were covered with membrane blebs and ruffles and extended their filopodia over a large area of the target cell membrane (Fig. 4 and 5). Some PBL extended a long uropod on the giant cell surface (Fig. 4). Contact between PBL membrane and ridges over viral nucleocapsids was frequent, and many virions were adsorbed on the PBL or even engulfed by them (Fig. 6). Giant cell villi containing nucleocapsids were also seen to converge toward PBL villi (Fig. 6, inset). Part of the target cell containing nucleocapsids often invaded the PBL, resulting in an intricate mixture of both cells (Fig. 5). In these instances, however, membrane fusion was not clearly observed between target cells and PBL.
Half of the adsorbed PBL population was not interacting with the target cell surface. These PBL had a spherical shape, short microvilli, and normal intracellular structure as seen after 15 min of ADLC. PBL were sometimes completely engulfed in the Vero syncytium (Fig. 7). TEM revealed that many nonreacting PBL were also attached to areas rich in membrane-bound nu-cleocapsids.
The distribution of PBL adsorbed on measles virus-infected Vero cells was homogenous and contrasted with the localized and clustered distribution of monkey erythrocytes during hemadsorption (31). In addition, there was no patch or ring formation of PBL on the target cell surface, in contrast with the distribution of viral antigens on giant cells after anti-measles virus antibody treatment (21).

^{51}Cr release from target cells during ADLC by lymphocyte subpopulations

Lymphocytes from measles virus seropositive and seronegative donors were employed as whole or fractionated populations in the ADLC-^{51}Cr release assay. The double SE rosette separation technique yielded a highly enriched fraction of T cells (94.0 ± 1.6% SE rosette-positive cells) which also contained 3.8 ± 0.6% Fc receptor-positive cells. The non-T-cell fractions contained 78.5 ± 7.8% C_3 receptor-positive cells, 54.3 ± 4.8% surface immunoglobulin-positive cells and 60.0 ± 3.7% Fc receptor-positive cells. There was a slight contamination by 2.2 ± 0.6% T cells (SE receptor positive) also in this fraction.
Spontaneous lymphocyte cytotoxicity by each cell population without additional serum was also tested. The results are presented as mean specific cytotoxic activity against the measles-infected target cell minus the activity against the uninfected cell line. The whole lymphocyte population from three seropositive donors gave 26.1 to 31.3% cytotoxicity, whereas the two seronegative donors gave 27.1 and 44.3% cytotoxicity in the presence of the positive serum at a dilution of 1:80 (Table 1).

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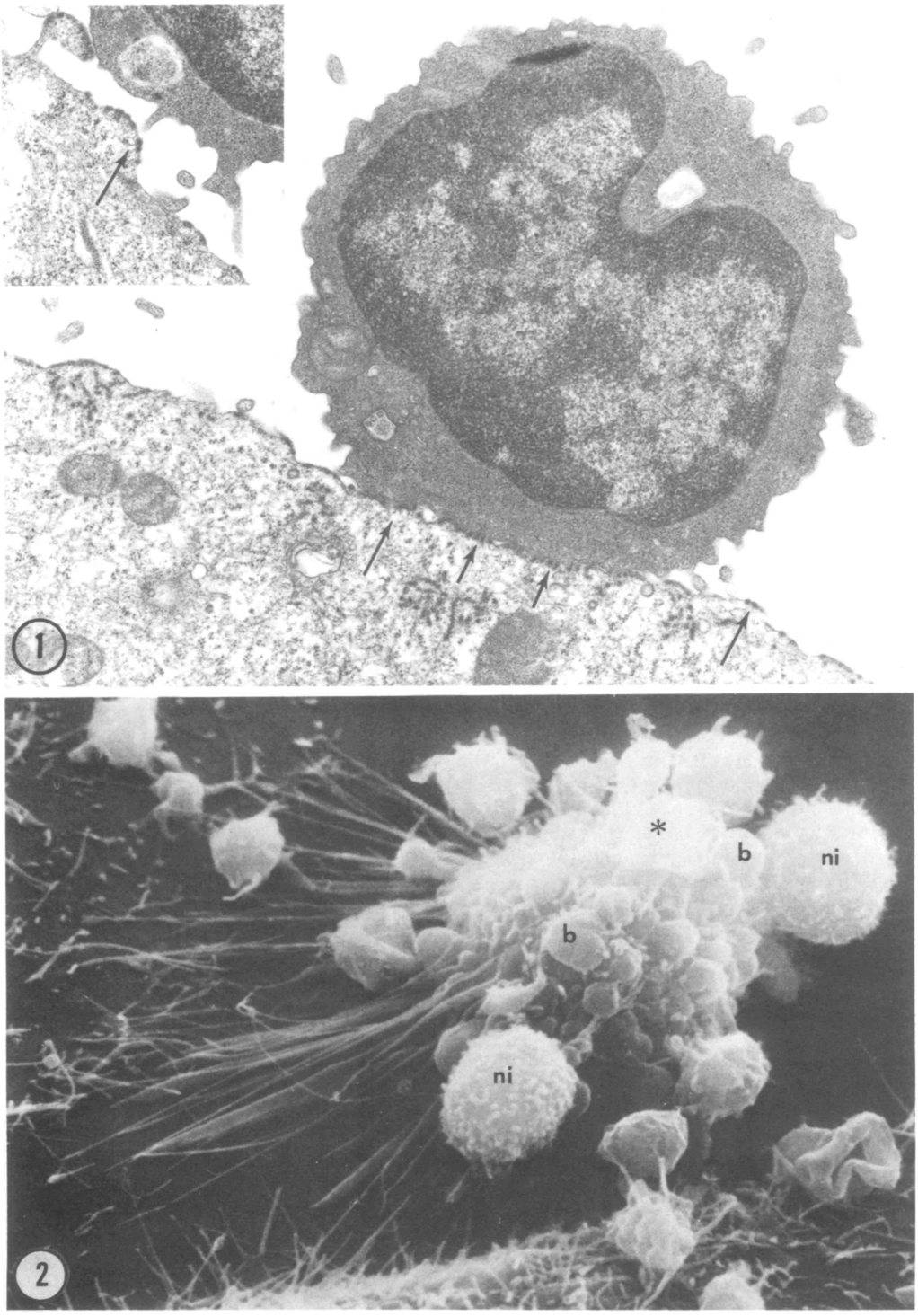

FIG. 1. Thin section through a PBL adsorbed on a measles virus-infected target cell after 30 min at 37°C in the presence of anti-measles virus antibody. The PBL is attached to areas of the target cell membrane where nucleocapsids are aligned (arrows). Inset: PBL attached by its microvilli to an area of the target cell membrane where viral nucleocapsids are less densely packed. X 22,500.

*FIG. 2. Killing of persistently infected (MA72046) cell after 4 h at 37° C in the presence of PBL and anti-measles virus antibody. The target cell is covered with blebs (b) and is rounding up. Long retraction processes are still attached to the glass substrate, ni, Noninteracting PBL; *, structure which might be the remains of an interacting PBL. x6,000.*

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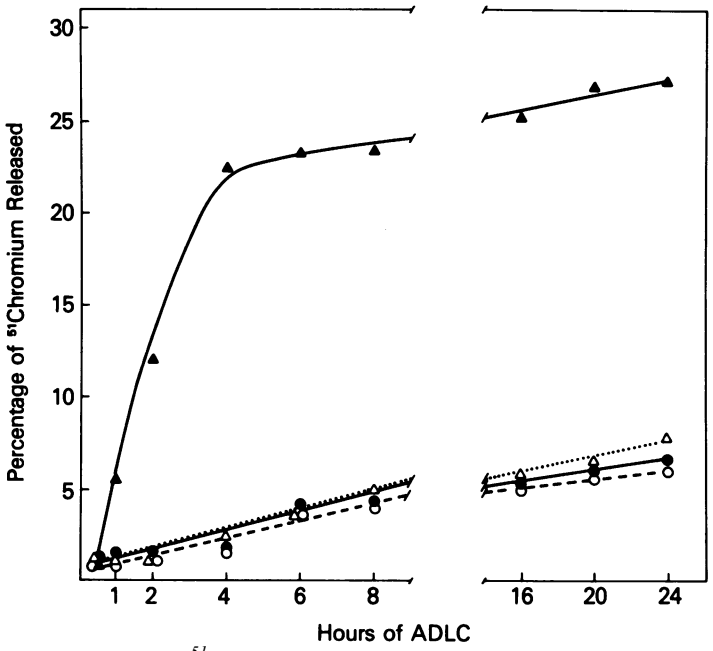

FIG. 3. Kinetics of ^{51}Cr release at 37°Cfrom MA72046-infected cells (Δ) and from MA160 control cells (O) in the presence of PBL and positive (------) or negative (- - ------) anti-measles virus serum.

The activity dropped significantly when a higher dilution (1:320) of serum was employed (9.6 to 17.8% for seropositive donors and 16.3 and 19.6% for the seronegative donors). No significant cytotoxicity was seen in the presence of the negative serum nor were the donor lymphocytes from either seropositive or seronegative individuals spontaneously cytotoxic.
The enriched non-T-cell fraction gave cytotoxic activity ranging from 18.3 to 42.9% for the seropositive donors and 35.3 and 51.1% for the seronegative donors with the low dilution of positive serum (1:80). No significant activity was seen with the non-T cells against either target cell when negative serum was employed nor was there any spontaneous cytotoxic activity with this fraction. When the T-cell fraction of lymphocytes was employed, we found one (9.4%) of three seropositive donors and both (9.6 and 11.3%) seronegative donors with highly significant ($P < 0.005$) levels of cytotoxic activity in the presence of positive serum. Two of the three seropositive donors had significant ($P < 0.001$) activity but at a much lower level (5.8 and 4.9%). This T-cell fraction did not have any cytotoxic activity in the presence of negative serum nor were they spontaneously active against either of the target cells.

Structural observations during ADLC with purified PBL populations

In situ erythrocyte rosetting was performed to identify by surface markers subpopulations of PBL interacting and not interacting with the target cell surface. At 4 h, all PBL which rosetted with CE-AB and were attached to target cells had undergone structural changes (Fig. 8), although not all interacting PBL could be labeled by this technique. In contrast, almost all PBL forming SE rosettes remained morphologically unaltered on the target cell (Fig. 9), but all noninteracting cells could not be rerosetted. However, a few of them developed one long uropod carrying the receptors for SE (Fig. 10a). This uropod sometimes created a large fold in the target cell and penetrated deeply into it (Fig. 10b).
Finally, a fraction of the T cells purified by SE rosetting was submitted to a CE-AB roset-ting separation to obtain a subpopulation of T cells bearing Fc receptors (TFc^+). Purified TFc^+ cells were then observed by SEM after 4 h in the presence of antibody. Most of these cells showed uropodial extension and were labeled with both SE and CE-AB after double rosetting in situ (Fig. 11).

Discussion

In the present study, structural changes occurring in PBL and in target cells during ADLC on measles

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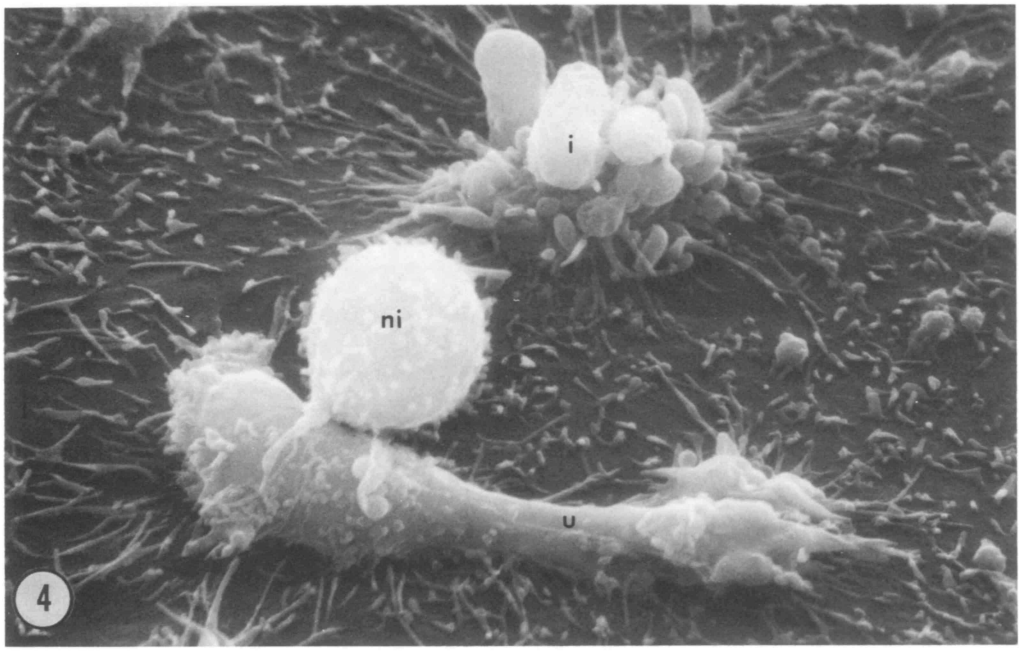
virus-infected cells were analyzed by TEM and SEM. SEM combined with erythrocyte labeling allowed us to identify lymphocyte subpopulations intimately interacting with target cells and having a cytotoxic activity demonstrated by biological assay (chromium release).



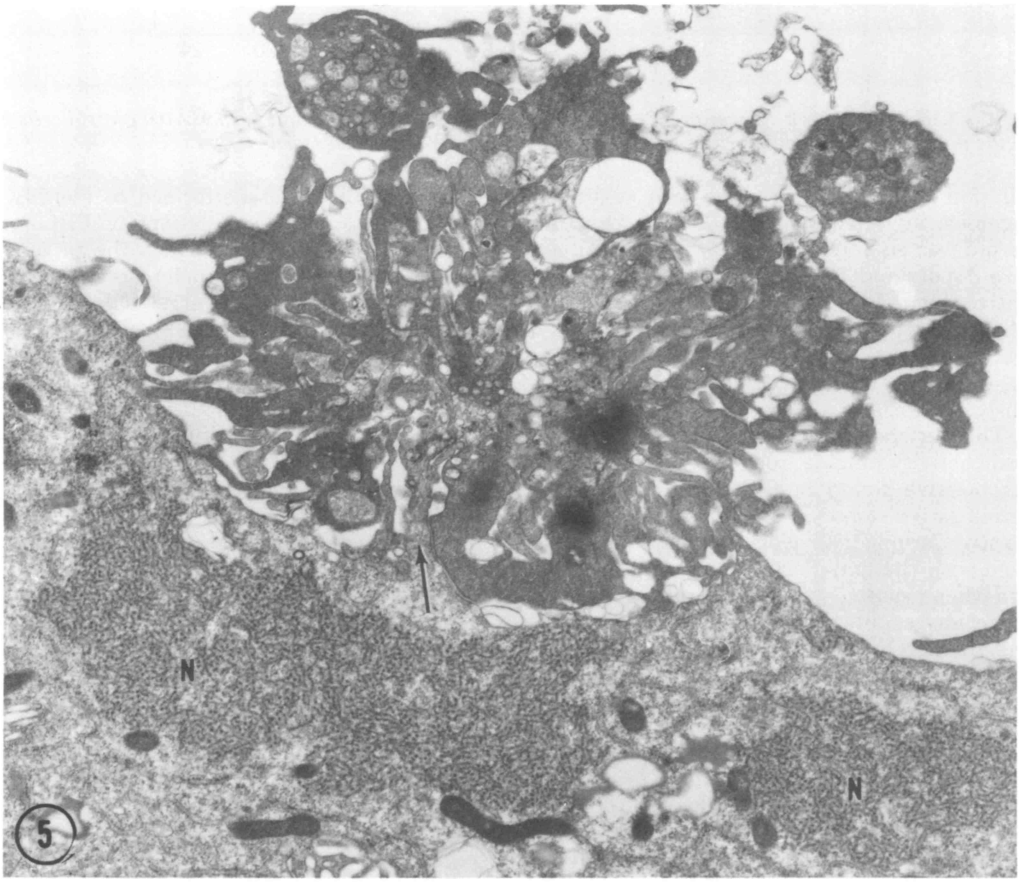


FIG. 4. *Scanning electron micrograph of a noninteracting PBL (ni) and of two types of interacting PBL (i) on the surface of a measles virus-infected Vero syncytium after 4 h of ADLC. The appearance of the interacting PBL at the top is the most frequent one. The PBL at the bottom extends a long uropod (u) on the target cell membrane. The latter type of interaction is rarely seen,* x *7,500.*
FIG. 5. *Maximal interaction between a PBL with a measles virus-infected Vero syncytium after 4 h at 37° C with anti-measles virus antibody. The target cell membrane is slightly depressed by the PBL and*

is lying over a large cytoplasmic inclusion of viral nucleocapsids (N). The cytoplasm of the target cell has a long extension penetrating deeply into a PBL membrane invagination (arrow). The PBL shows vacuolation and increased density, while the cytoplasm of the Vero cell appears lighter and contains healthy organelles. X 17,000.

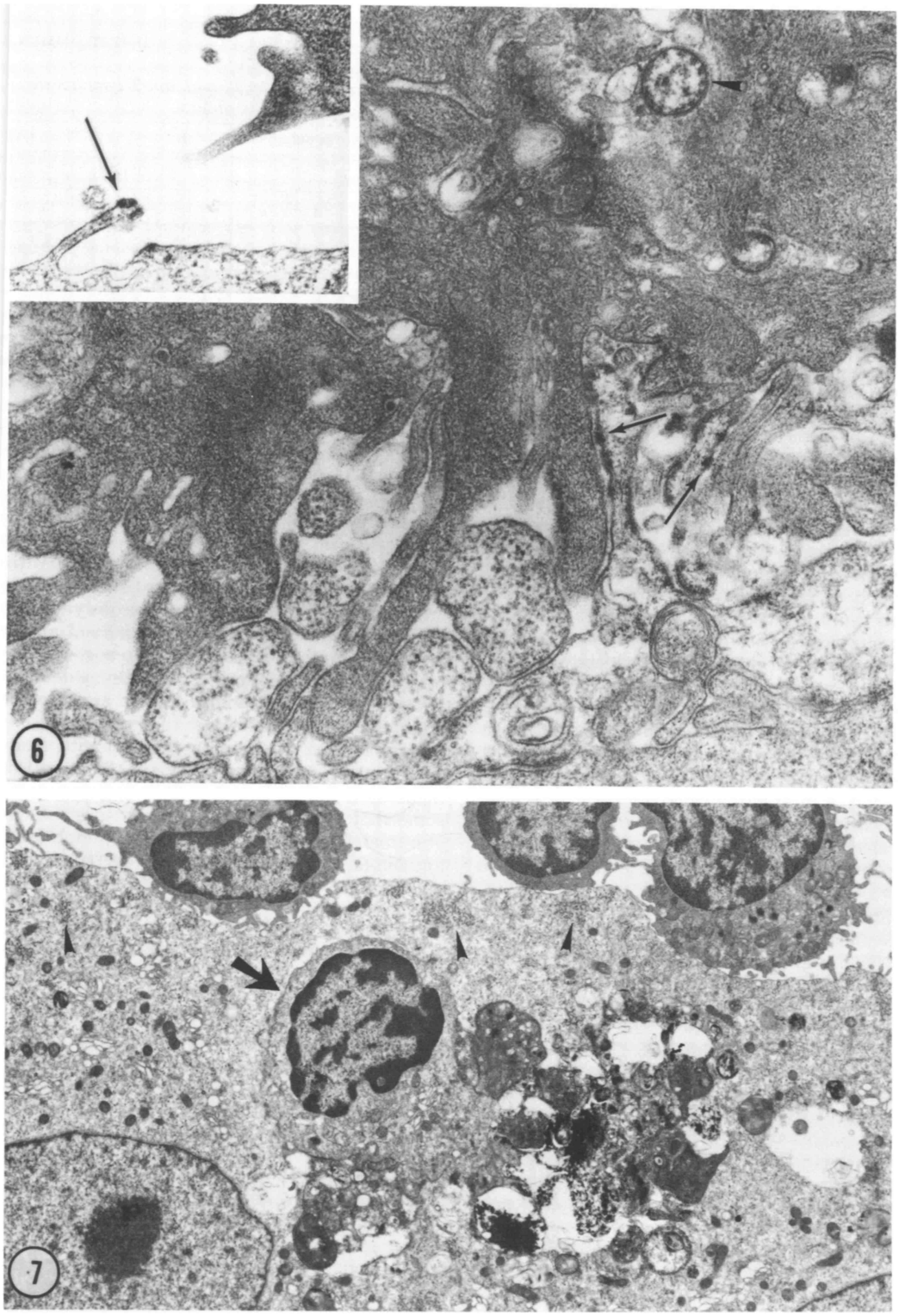


FIG. 6. *Area of strong interaction between PBL and Vero cell infected with measles virus after 4 h of ADLC. Long cytoplasmic processes from both cells interweave at contact area. Vero cell extensions contain viral nucleocapsids (arrow). A complete virion (arrowhead) is engulfed in the PBL. Inset shows a microvillus containing viral nucleocapsids and pointing toward a PBL microvillus. x35,000.*

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FIG. 7. *PBL completely engulfed (arrow) in a Vero syncytium induced by measles virus, after 4 h at 37°C with anti-measles virus antibody. Intracellular debris on the right of the invading lymphocyte could be degraded Vero cell cytoplasm. Arrowheads point to viral inclusions. X8,000.*

TABLE 1. *Percent specific ADLC against measles virus-infected target cells by T- and non-T lymphocyte subpopulations from seropositive and seronegative lymphocyte donors*

% Cytotoxicity by lymphocyte populations															
Donor lympho-cytes	Whole PBL					Non-T cell					Tcell				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Seropositive															
Positive serum															
1:80	**26.1**	**29.8**	**31.3**			**18.3**	**42.9**	**20.4**			**5.8**	**4.9**	**9.4**		
1:320	**9.6**	**13.6**	**17.8**			**11.4**	**17.4**	**9.3**			**4.2**	**2.4**	**4.8**		
Negative serum	**2.0**	**1.4**	**0**			**2.2**	**1.3**	**0.9**			**1.4**	**1.1**	**1.0**		
No serum	**0.4**	**0**	**2.0**			**1.2**	**0.4**	**0.5**			**0.6**	**1.3**	**1.6**		
Seronegative															
Positive serum															
1:80				**44.3**	**27.1**				**51.1**	**35.3**				**9.6**	**11.3**
1:320				**19.6**	**16.3**				**30.2**	**22.4**				**5.2**	**5.0**
Negative serum				**1.7**	**2.1**				**2.1**	**1.4**				**2.3**	**2.1**
No serum				**0.3**	**0.7**				**0.6**	**0.9**				**1.7**	**1.9**

[a] *Lymphocytes were employed from three donors who were seropositive for measles virus antibody (a, b, and c) and from two donors who were seronegative for measles virus antibody (d and e).*

Two types of adsorbed lymphocytes which resisted vigorous washing could be easily distinguished by morphological criteria. Approximately half of the adhering PBL cells had retained their native shape and surface appearance. The other half had undergone dramatic alteration after interaction with the target cells. There was a good correlation between the proportion of interacting cells and the ^{51}Cr release cytotoxicity assay throughout this study. From these observations, we assume that the interacting PBL correspond to the antibody-dependent killer cell population (K cells).

The ADLC activity is mediated by lymphocytes bearing Fc receptors for IgG (28). In our study, the interacting PBL could indeed be labeled in situ by CE-AB. Noninteracting lymphocytes were generally labeled with SE, confirming at first the general opinion that T lymphocytes are not effective in ADLC (6, 25, 34, 39). The sites of recognition for the K cells on the target membrane were clearly associated with the surface viral antigens organized over nucleocapsids and were already detected at the end of the 15-min prolytic phase described by others (33). Although adsorption is an absolute prerequisite for cytotoxicity (33, 42), PBL can adhere to the target cells without exerting their cell-killing activity (19). Indeed, PBL noninteracting with the target cells were also found adsorbed on areas of the membrane over numerous viral nu-cleocapsids. These PBL could be part of a natural killer cell population which recognized altered cell surface structures (29) but, as demonstrated with the chromium release assay, were unable to kill this particular target cell.

Lymphocytes interacting strongly with measles virus-infected target cells formed ruffles, blebs, and long filopodia. This appearance is somewhat different from the "fried-egg" shape of human lymphocytes adhering to immobilized antigen-antibody complexes (1). The "fried-egg" appearance seems to be caused by the adhesion to an immobilized substrate, like polylysine-coated glass (B. Rentier and J. M. Seigneurin, unpublished data). However, it is not induced by adsorption of lymphocytes onto lipid bilayers (20). These differences are thus related to the fluidity of the target.

The detection in purified T-lymphocyte populations of cells carrying Fc receptors for IgG (7, 10, 11, 16, 36, 38, 41) suggested that this small subpopulation could be active in ADLC. Measurements of ^{51}Cr release by MA72046 (measles-infected) cells in the presence of anti-measles serum and separated T cells indicated that these TFc^{+} cells accounted for more than one-fifth of the total cytotoxic activity exerted by the whole PBL population.

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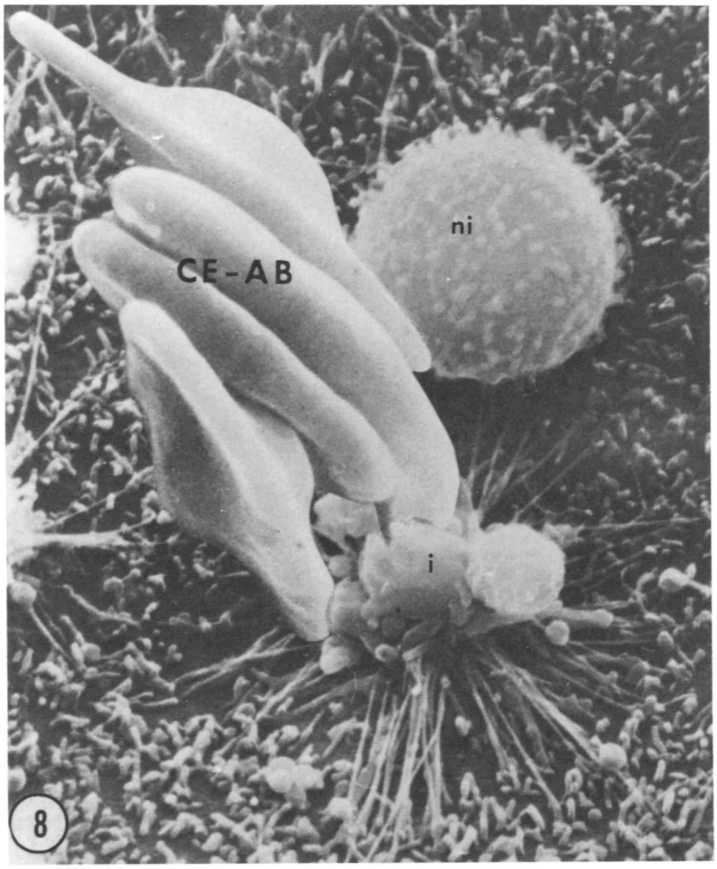

FIG. 8. *Scanning electron micrograph of an interacting PBL (i), recognized by the blebs and long extensions as in Fig. 4, on a Vero syncytium after 4 h at 37° C with anti-measles virus serum and addition of CE-AB. The elongated shape with central swelling and the size are characteristic of CE. CE-AB rosetting demonstrates the presence of Fc receptors on interacting PBL. One noninteracting PBL is unlabeled (ni). Xll,500.*

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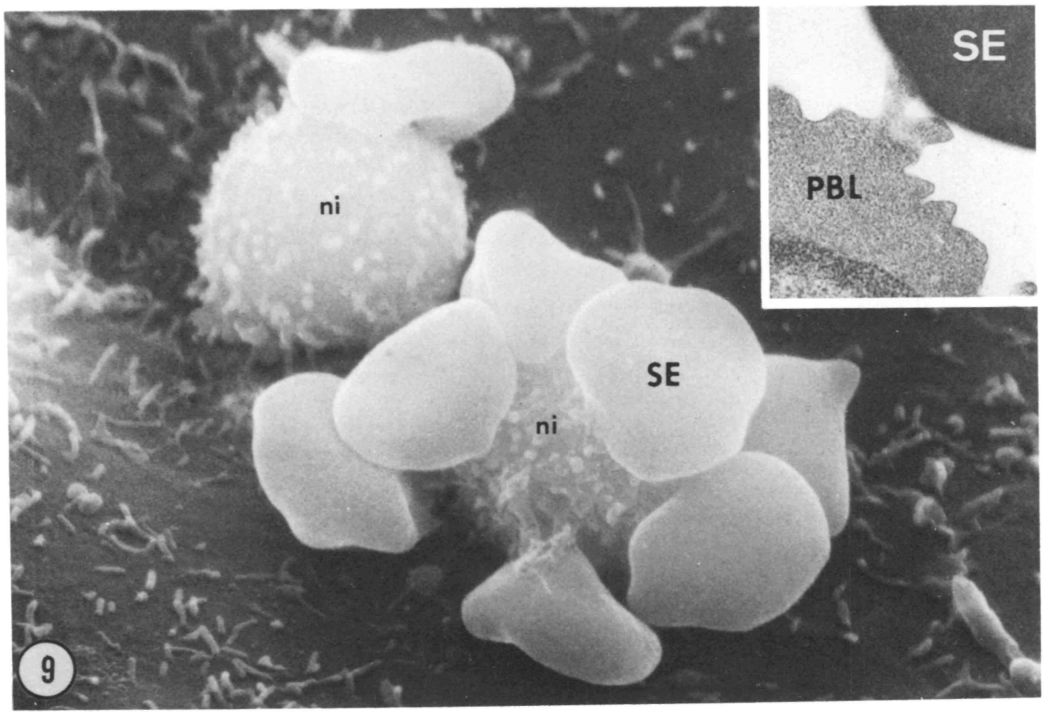

FIG. 9. *Scanning electron micrograph of two noninteracting PBL (ni) on a Vero syncytium after 4 h at ST with anti-measles virus serum and addition of SE. SE are spherical and smaller than CE. One PBL is the center of a complete rosette of at least 7 SE; the other PBL has adsorbed only one SE. Inset: thin section reveals PBL microvilli attached to SE.* x *11,500; inset, X35,000.*

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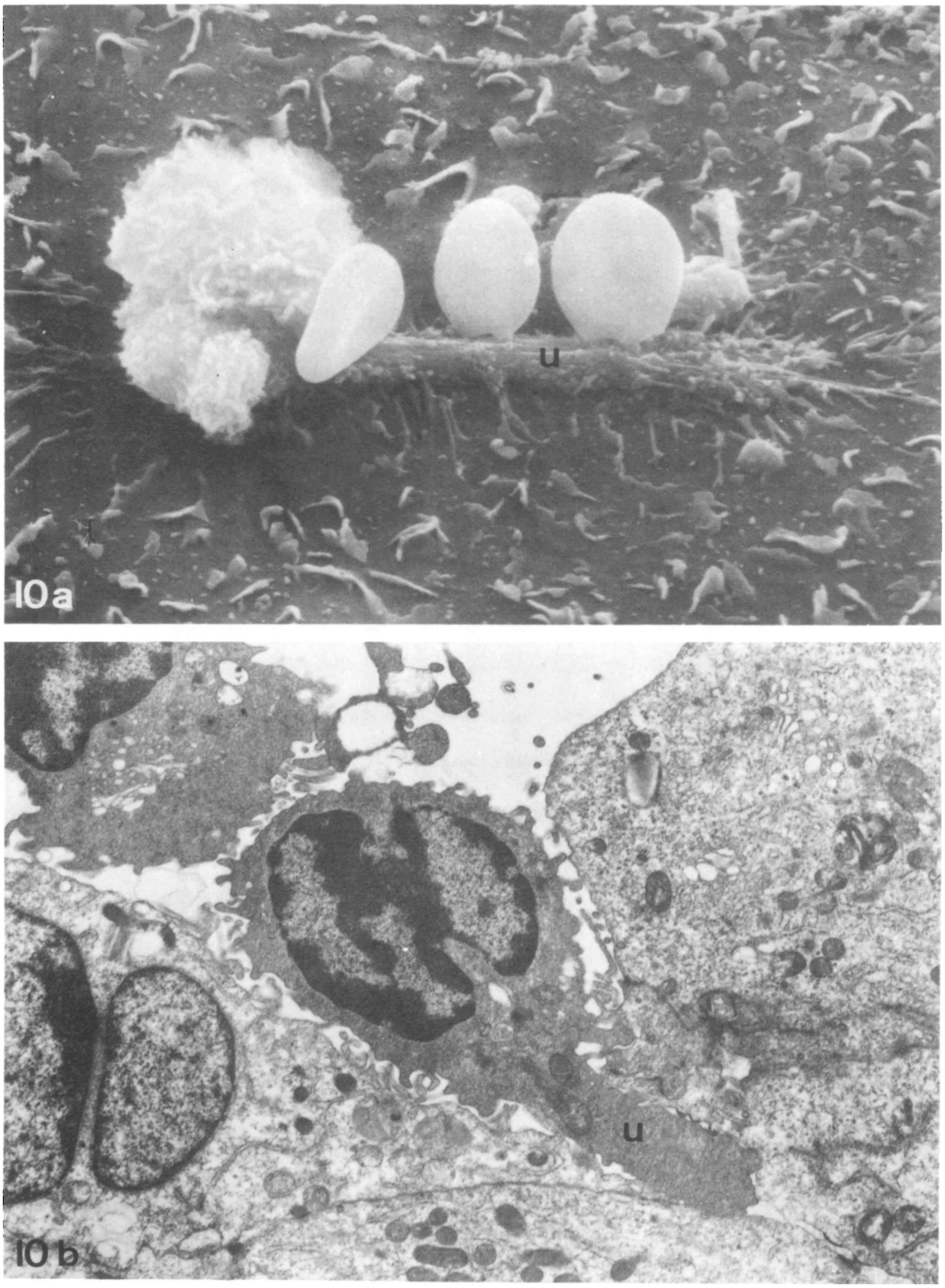

FIG. 10. *Appearance in SEM and TEM of T lymphocytes extending uropod (u) on target cell (a) The cell body of the lymphocyte remains spherical and shows slight ruffling, the uropod interacts with the target cell. The thymic origin of this cell is demonstrated by labeling with SE, which are all attached to the uropod. (b) TEM reveals that an uropod forming PBL penetrates deeply between two measles virus-infected Vero cells. Lymphocyte organelles are present in the uropod. X9,500.*

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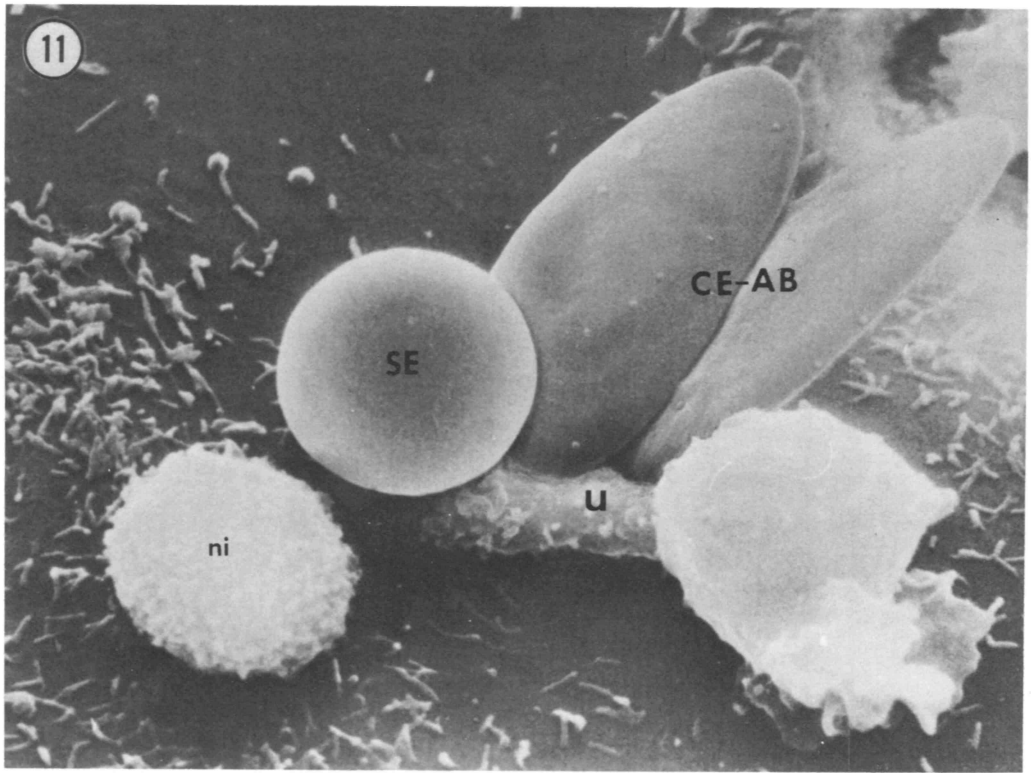

FIG. 11. *Scanning electron micrograph of an uropod-forming PBL after 4 h at ST with anti-measles virus serum and addition of a mixture of SE and CEAB. Adsorption of one SE and 2 CEAB demonstrates that this uropod-forming PBL is a T cell carrying Fc receptors. Both SE and CEAB adsorb on the uropod (u). One noninteracting PBL (ni) shows spherical shape and short microvilli. X 12,000.*

This is a surprisingly high ratio since the Fc receptor-bearing cells represented only ca. 4% of the total T-cell population. It might be explained by the fact that some TFc^+ cells may be recycled and perform several times successively their ADLC activity, while non-T killer cells (K cells) could not. This purely speculative assumption is supported by the visual appearance of interacting cells. Non-T killer cells strongly interacting with the target cells appear to be irreversibly altered, while TFc^+ cells undergo limited deformation during interaction, except for a uropod extension.

Uropod formation by lymphocytes has long been observed and associated with motility (9, 23). It has also been described during adhesion to macrophages (30), stimulation by mitogens (3), by anti-IgG antibody (37) or by antigen-antibody complexes (4, 1), and even in unstimulated lymphocyte populations (24). Biberfeld and co-workers (4) have shown that contact between the effector cell and target cell triggers the activation of the killer cell and stimulates the formation of a long uropod which causes detachment and lysis of the target cell. However, in our system, uropod formation was infrequent and restricted to TFc^+ cells. The T-cell identity of the uropod-forming lymphocyte has been previously suggested (32) and.was confirmed here by consistent in situ resetting with SE. Interestingly, the adsorption sites for SE have migrated towards the uropod on the lymphocyte surface. In addition, double in situ rosetting with SE and CE-AB clearly demonstrated that the uropod-forming cells corresponded to the TFc^+ subpop-ulation.

In conclusion, a correlation between electron microscopic observations and biological (chromium release) assay has demonstrated the presence of two distinct subpopulations of PBL interacting with measles virus-infected target cells and producing a cytotoxic attack directed by antibody. One population consists of a group of non-T cells, and the other consists of a small group of T cells, both carrying Fc receptors for IgG. Each group displays a distinct type of morphological interaction with the target cell, the non-T cells undergoing dramatic deformation and extending filopodia, and the T cells forming a large uropod. Neither shows any evidence of membrane fusion with the target cell, but both have cytotoxic activity.

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