Scanning and Transmission Electron Microscopic Studies of Complement-Mediated Lysis and Antibody-Dependent Cell-Mediated Cytolysis of Herpes Simplex Virus-Infected Human Fibroblasts

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The morphologic aspects of complement-mediated and antibody-dependent cell-mediated cytolysis (ADCC) of human fibroblasts (HuFs) infected by herpes simplex virus (HSV) is described. Human antiviral antibody (antiHSV) was shown by transmission and scanning electron microscopy (TEM and SEM) to cause the deposition of an amorphous material over the surface of infected cells and virus particles. Associated with antiHSV treatment, the HuFs underwent endocytosis, with the appearance of pinocytotic vesicles immediately beneath the plasma membrane. The addition of complement resulted in lysis of the infected HuFs and massive dilatation of the perinuclear cisternae, but the virus particles associated with the cell surface did not appear lysed. Instead, an additional deposit was noted on the enveloped particles after the addition of complement (C). Human peripheral blood mononuclear leukocytes (MNLs) also lysed the antibody-coated, infected HuFs. Lymphocytes formed broad-based areas of attachment to the antiHSV-treated cells. Beneath these areas of contact occurred focal cytoplasmic changes that preceded cell lysis. Monocytes showed multiple points of binding and sent cytoplasmic projections over the surface of the infected HuFs. Virus particles and segments of target cell cytoplasm were gathered into vacuoles of the monocyte. In accord with the above morphologic findings, the relative roles that antibody, C, and leukocytes may play in human viral diseases is discussed. (Am J Pathol 1980, 100:663-682)

AFTER HERPES SIMPLEX virus (HSV) infection, human cells express viral antigens on their surface [1] and release infectious virus.[2] In response to the viral invasion, leukocytes [3,4] and complement-reactive antibodies [4] accumulate within the lesions of patients with herpetic infections. These elements of the immune system are thought to act locally in the removal of virus and HSV-infected cells.[1,2]

Numerous *in vitro* studies have been conducted on the role antibody, complement (C), and leukocytes play in the removal of virus and infected cells. Infectivity studies have shown that antibody and C can neutralize virus by lysis,[5-8] aggregation,[9] or coating the viral surface with immunoglobulin and C.[10-12] HSV-infected human cells can be destroyed by two immunologically specific mechanisms: antibody-dependent, C-mediated lysis and antibody-dependent, cell-mediated cytotoxicity (ADCC).[13]

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Although much is known concerning the specificity and stoichiometry of these immune reactions, little information is available on the morphologic aspects of these anti-HSV defense mechanisms. Such data could be useful in the morphologic evaluation of herpetic lesions. The purpose of this study was to describe those ultrastructural events that take place when HSV-infected human fibroblasts (HuFs) interact with elements of the immune system. Specifically, we determined those alterations of virus and cells that take place when HSV-infected HuFs undergo C-lysis and ADCC.

Materials and Methods

Virus and Cells

Type 1 HSV, strain CHR-HSV-3, was passaged in human embryonic skin fibroblasts [14] and was assayed as plaque-forming units (PFUs) on primary rabbit kidney cells.[15] HuFs were grown and all virus dilutions were done in Eagle's minimal essential medium (MEM) containing 10% (vol/vol) fetal bovine serum, penicillin G (100 U/ml), and streptomycin (50 μg/ml) (EMFBS).

Serums

Human HSV immune serum (anti-HSV) was collected from a patient with recurrent herpes labialis. The heat-inactivated (56C, 30 minutes) serum had a neutralization titer of 1:256.[16] Nonimmune serum, obtained from a patient with no history of HSV infection, was without neutralizing activity when tested at a 1:2 dilution. Where indicated, whole, fresh guinea pig serum, without detectable anti-HSV activity, was used as the source of C, and heat-inactivated guinea pig serum (HIC) was used as a control serum.

Human Mononuclear Leukocytes

Peripheral venous blood from human volunteers was collected in plastic tubes containing sodium heparin (10 U/ml blood) (Upjohn, Kalamazoo, Mich). Human mononuclear leukocytes (MNLs) were isolated on a Ficoll-Hypaque gradient,[17] washed twice in MEM, resuspended to 5×10^6 cells/ml in EMFBS, and used in the experiments.

C-Lysis Assay

HuFs were grown to confluence ($10^{4.0}$ cells/well) in microtiter dishes (Model 1SFB-96, Linbro, Hamden, Conn). Each well was incubated with 0.1 ml EMFBS containing $10^{5.5}$ PFUs of HSV and 1.0 μCi $Na_2\ ^{51}Cr_2O_7$ (specific activity 237.4 mCi/mg, New England Nuclear, Boston, Mass). After 11 hours of incubation, the cells were washed three times with EMFBS, and to each well was added 0.05 ml of the heat-inactivated immune or nonimmune human serums at the dilution indicated. After a 1-hour incubation period, the cells were washed four times with EMFBS, and then were added 0.1-ml amounts of EMFBS containing C or HIC at 1:8 dilution in EMFBS. At intervals thereafter, supernatants were removed and were assayed for radioactivity (Auto-gamma Spectrophotometer, Model 314 EX, Packard Instrument Co., LaGrange, Ill). The cells remaining attached to the plastic dish were solubilized with 5.0 M NaOH and then were assayed for radioactivity. The percentage of ^{51}Cr released for each well was calculated according to the conservative formula: % ^{51}Cr release=S/S+C, where S = counts per minute (cpm) in the supernatant, and C = cpm associated with the cell monolayer. The values shown in the

figures represent the mean of triplicate determinations ± 1 SD where indicated. Experiments were repeated at least twice, and representative data are presented.

ADCC Assay

HuF were ^{51}Cr-labeled, infected, and treated with anti-HSV or nonimmune human serum. After being washed four times with the medium, the wells were incubated with 0.1 ml EMFBS containing 5×10^5 MNLs (effector:target ratio = 50:1). At intervals, the supernatants and cells were harvested and assayed for radioactivity, and the ^{51}Cr release was determined as described above.

Morphologic Methods

In a series of experiments paralleling the C-lysis and ADCC assays, fibroblasts were prepared for transmission and scanning electron microscopic (TEM and SEM) studies. The cells were fixed for TEM at 4C for 2 hours in 0.05 M sodium cacodylate-buffered (pH 7.4) 4% (wt/vol) glutaraldehyde and postfixed for 30 minutes in 0.2 M cacodylate-buffered (pH 7.4) 1% (vol/vol) OsO_4. After *en bloc* staining with 0.02 M Veronal acetate-buffered (pH 7.2) 0.5% (wt/vol) uranyl acetate, the cells were dehydrated in increasing ethanol concentrations. Monolayers were detached from the plastic dish with propylene oxide and processed as previously described.[18] We examined representative fields, using either a Hitachi HS-8 or HU-11E electron microscope at 50 and 75 kV, respectively.

Monolayers selected for SEM study were grown on sterilized glass coverslips and fixed for 48 hours at 37C in 0.05 M sodium cacodylate-buffered (pH 7.4) 2% (wt/vol) glutaraldehyde adjusted to 300 mOsm with sucrose. The HuFs were postfixed in OsO_4 and dehydrated in ethanol. The cells were dried at the critical point in a Samdri PVT-3 device (Tousimis Research Corp., Rockville, Md) and were coated with platinum-paladium. Examinations of the monolayers were done with model 1000 ETEC scanning electron microscope at 30 kV.

The number of MNLs that attached to the HSV-infected cells was determined: HuFs were grown to confluence in 16-mm plastic wells (Model 76-033-05, Linbro) and inoculated with $10^{6.0}$ PFUs HSV in 0.5 ml EMFBS. After 11 hours the cells were washed with MEM (×3), and to each well as added 0.4 ml immune or nonimmune human serum (1:8 dilution). Following a 1-hour incubation, the HuFs were washed with MEM (×3), and to each well was added 0.2 ml of EMFBS containing $1 \times 10^{6.0}$ MNLs. After 3 hours the wells were washed (×3) to remove the unattached leukocytes, and 0.4 ml of trypsin (0.25 wt/vol% in Puck's saline A) was added for 15 minutes at 37C. The trypsinized cells were centrifuged at 320g for 10 minutes and resuspended in 0.1 ml MEM. Amounts of the cell suspensions were placed on glass slides, air-dried, and stained with hematoxylin and eosin. The number of MNLs per 1000 HuFs was determined by the use of a light microscope (×400). The results were expressed as the mean of triplicate determinations ± 1 SD.

Results

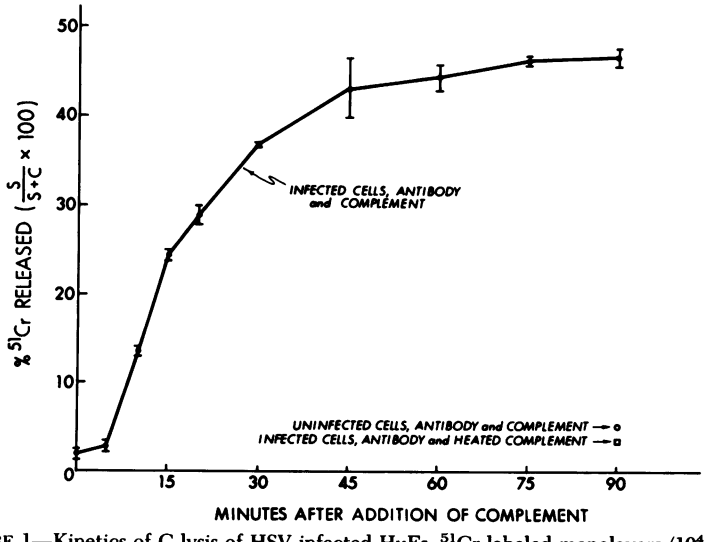
Kinetic Studies of Immune Lysis

The purpose of this study was to define those morphological alterations that take place when HSV-infected human HuF undergo C-lysis and ADCC. Experiments first established how soon after adding the immune reactants that these modes of immune lysis occurred in our *in vitro* cultures.

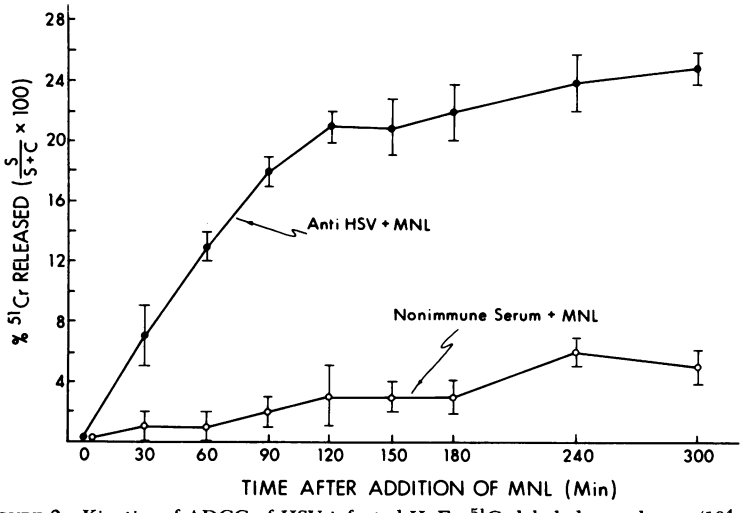
Immune lysis experiments were begun 11 hours after infection because

at this time preliminary studies had determined virus synthesis and release was occurring at a logarithmic rate in the HSV-infected HuFs. As assessed by ^{51}Cr release, maximal C-lysis of HSV-infected HuFs was achieved with anti-HSV and C at dilutions of 1:2 and 1:8, respectively (not shown). With these optimal concentrations, the time course of C-lysis of HSV-infected HuFs is shown in Text-figure 1. Not until 10 minutes after addition of C was a significant amount of ^{51}Cr released from the anti-HSV–treated cells. Thereafter the quantity of ^{51}Cr in the medium overlay increased steadily and reached a plateau after 60 minutes of incubation. Neither uninfected cells treated with anti-HSV and C nor infected cells treated with anti-HSV and HIC showed significant levels of lysis over the time period studied.

Maximal ADCC of HSV-infected HuFs was achieved with a 1:8 dilution of anti-HSV and MNLs at an E:T ratio of 1:50. With these optimal conditions, the time course of ADCC lysis is shown in Text-figure 2. Significant ^{51}Cr was released from the anti-HSV–treated cells 30 minutes after the addition of MNLs, and after a steady increase a plateau was reached at 4 hours. Infected HuFs treated with nonimmune serum and MNLs released significantly smaller amounts of ^{51}Cr. Thus we determined that the anti-HSV–treated infected HuFs were maximally lysed by C and



TEXT-FIGURE 1—Kinetics of C-lysis of HSV-infected HuFs. ^{51}Cr-labeled monolayers (10^4 cells/well) were incubated with EMFBS alone or medium containing 10^5 PFU of HSV for 11 hours. After being washed, the cells were exposed to anti-HSV for 1 hour, and then C or HIC was added. At various times after C addition, the percent of age of ^{51}Cr released was determined. Each point represents the mean of triplicate determinations and vertical lines 1 SD.



TEXT-FIGURE 2—Kinetics of ADCC of HSV-infected HuFs. ^{51}Cr-labeled monolayers (10^4 cells/well) were incubated with EMFBS containing 10^5 PFU of HSV for 11 hours, and then the cells were exposed to anti-HSV or nonimmune human serum for 1 hour. After being washed, HuFs were incubated with 5×10^5 human MNLs, and at intervals replicate monolayers were assessed for the percentage of ^{51}Cr released. Each point represents the mean of triplicate determinations and vertical lines 1 SD.

MNLs after 1 and 4 hours of incubation, respectively. Experiments next defined those ultrastructural changes that occurred after virus infection and immunolysis.

Scanning Electron Microscopic Studies

Effects of the Infection

Uninfected HuFs were flat, elongated, and tightly interdigitated cells (Figure 1). Numerous microvilli were dispersed over the surface, and narrow ridges were seen traversing the long axis of the cells. After HSV infection, the HuFs contracted and partially separated from one another, yet maintained their contacts to the glass substratum via fingerlike cell processes (Figure 2). The surface of infected HuFs was remarkably smooth and had few microvilli. Prominent, however, on the cell surface were multiple spherical profiles (inset, Figure 2) identified as herpes virus particles by TEM (see below).

Effects of Anti-HSV

Although by light microscopy infected cells treated with anti-HSV were indistinguishable from those incubated with nonimmune serum, a difference was noted by SEM. Specifically, infected cells treated with an-

tiviral antibody developed an amorphous deposit on their plasma membranes (Figure 3). The deposit was often aggregated on certain areas of the cell surface and partially obscured cell processes and viral particles.

Effects of Anti-HSV Plus C

When C was added to infected monolayers treated with anti-HSV, marked surface alterations were evident (Figure 4). The cells were rounded and had irregular cytoplasmic borders. The surface became granular, "moth-eaten," and littered with debris that further obscured cell processes and virus particles.

Transmission Electron Microscopic Studies

Effects of the Infection

The normal HuFs (not shown) were spindle-shaped cells with microvilli occasionally seen on cross-section. The cytoplasm of the uninfected HuFs was densely packed with numerous organelles. The Golgi apparatus, endoplasmic reticulum, and mitochondria were readily evident. The nuclei contained chromatin material distributed in a diffuse pattern, and typically one or more nucleoli were present.

In contrast to the normal HuFs, the nuclear chromatin of the HSV-infected HuFs often was clumped at the periphery of the nuclear membrane, and nucleoli were rarely seen (Figures 5–7). Numerous immature virus particles were prominent within the nucleus, and occasionally these structures were seen free in the cytoplasm or within the endoplasmic reticulum. Virus particles that were noted on the surface of infected cells by SEM (Figure 2) were also seen in cross-section by TEM (Figure 7). Typical of all mature HSV particles, the dense nucleocapsid was enclosed by the envelope, which had a smooth surface and trilaminar, membranous structure.

Effects of Anti-HSV

Treatment of the infected HuFs with antiviral serum did not alter nuclear or cytoplasmic structures (Figures 8 and 9) but did result in changes at the periphery of the cell. Following incubation with anti-HSV, numerous smooth membrane vesicles appeared immediately below the plasma membrane (inset, Figure 8). HSV particles that were attached to the exterior of the cell acquired an appreciable deposition of osmiophilic, amorphous material that coated the surface of the viral envelopes (Figure 10).

Effects of Anti-HSV plus C

Infected cells incubated with anti-HSV and C underwent considerable ultrastructural changes (Figures 11, 12, and 13). Mitochondria and endoplasmic reticulum were markedly swollen. Of particular note, the inner and outer nuclear membranes underwent an irregular separation, resulting in ballooning dilatation of the perinuclear cisterna (Figures 11 and 12). Virus particles associated with the surface of the C-treated necrotic cells (Figure 13) were not lysed, but their envelopes were more thickly coated with an osmiophilic, amorphorus material than those exposed to anti-HSV alone (Figure 10).

Effects of Anti-HSV Plus MNLs

When MNLs were added to the infected HuFs, leukocytes attached to both the nonimmune serum-treated (236 ± 22 MNLs/1000 HuFs) and the anti-HSV–treated (2122 ± 385 MNLs/1000 HuFs) monolayers. The MNLs that bound to HuFs incubated with nonimmune serum showed few points of attachment and did not induce cytotoxic changes in the target cells over the time period studied. Antiviral antibody caused not only a significant ($P < 0.01$) ninefold increase in the number of MNLs bound to the HuFs but also resulted in a different morphologic interaction between infected cells and effector leukocytes.

The interaction between human MNLs and anti-HSV–treated infected HuFs depended on the type of leukocyte bound. Monocytes formed multiple points of attachment via fingerlike cytoplasmic projections (Figures 14 and 15). These monocytic extensions probed the surface of the HuFs to various depths and often dissected their way between infected cells. Clusters of HSV particles appeared to be gathered by these leukocytic projections and incorporated into heterophagosomes of the monocytes (Figure 14). Frequently the monocytic extensions formed tight interdigitations around cytoplasmic projections of the HuFs, and pieces of the infected cell's cytoplasm appeared to be lying in vacuoles of the monocyte (Figure 15). Despite this rather extensive contact with the anti-HSV–treated HuFs, the monocytes did not induce cytotoxic alterations in the fibroblasts over the time period studied.

Lymphocytes interacted with the anti-HSV–treated HuFs in a different fashion and formed broad-based, curvilinear points of attachment (Figures 16 and 17). At sites of apposition the contour of the lymphocyte changed and undulated with the surface of the HuF (Figure 16). Points of membrane fusion between the effector and targets could not be identified, and neither were plasma membrane breaks noted. Immediately beneath the

site of lymphocyte attachment, however, cytotoxic changes were evident within the cytoplasm of the HuF (Figure 17). These consisted of focal swelling of the endoplasmic reticulum (ER) with fragmentation of intracytoplasmic membranous profiles and an increased number of lipid-laden vacuoles. Adjacent to foci of injury in the HuF, other segments of ER often appeared pushed aside and compressed, assuming a stacked configuration. Following this regional lesion, the entire cell underwent lysis and resembled those treated with anti-HSV plus C. Massive dilatation of the perinuclear cisternae, however, was not noted in ADCC-lysed HuFs.

Discussion

The results show that distinct morphologic alterations occur when HSV-infected HuFs undergo ADCC or C-lysis. By examining the sequential effects of incubating infected cells with anti-HSV and C or human MNLs, we found ultrastructural changes induced by each of these immune reactants.

Anti-HSV caused the deposition of an amorphous material on the surface of the cultured infected cells and virus particles (Figures 3 and 10). Uninfected cells treated with immune serum had no depositions on their plasma membranes, and neither did infected HuFs incubated with nonimmune human serums. Thus, we have interpreted the amorphous material to represent antiviral antibody attached to the surface of these structures. In support of this interpretation, previous competitive-binding studies have shown, with the use of fixed HSV-infected cells and ferritin-labeled antibody, that the surface-bound material represents anti-HSV immunoglobulin.[19]

Antiviral antibody alone had no discernible effect on the organelles or the nuclei of the infected cells but altered only surface-related structures. This result is in accord with the currently accepted concept that antibody does not cross intact cell membranes. The surface-related changes, however, are potentially important. Anti-HSV is known to prevent the extracellular spread of virus.[20] Our SEM and TEM findings suggest that antiviral antibody prevents dissemination by coating the surface of the particle with immunoglobin as HSV emerges to the exterior of the cell. This antibody deposit on the viral envelope could neutralize the virus and thereby prohibit dissemination.

Less certain is the significance of the pinocytotic vesicles that appeared beneath the plasma membrane of HSV-infected HuFs treated with antiviral antibody (Figure 8). Similar vesicles have been noted in cultured malignant cells incubated with antitumor cell antibody.[21] Perhaps this pinocytotic process represents the efforts of the cell to remove antibody-

coated segments of plasma membrane. Such a process might be an effective mechanism by which the humoral immune system could moniter the surface of tissues and, in conjunction with the cell, remove undesirable portions of the plasma membrane without destroying the entire cell. In certain viral infections, this mechanism could effectively suppress the egress of particles from the cell, especially those RNA viruses that acquire an envelope by budding through the plasma membrane. Perhaps this is the mechanism by which antiinfluenza antibody inhibits the release of influenza virus from infected cells.[22]

In herpetic infections, however, removing viral components from the cell surface would not be expected to inhibit the release of infectious particles, since HSV acquires its envelope mainly from the nuclear membrane.[23] Excision of herpetic antigens from the plasma membrane instead might protect HSV-infected cells from destruction by elements of the immune system and permit a latent or persistent infection in an immunocompetent host. In support of this hypothesis, Joseph and Oldstone have published data showing that antimeasles antibody modulates the surface of rubeola-infected cells and protects them from C-lysis.[24] Furthermore, Stephens has presented experimental evidence to suggest that anti-HSV antibody is important in maintaining an *in vivo* latent ganglionic infection.[25] Whether anti-HSV–induced endocytosis is the mechanism that permits latency in neural tissue is unknown and deserves further study.

The addition of C caused the antibody-treated HuFs to undergo the typical changes of colloid osmotic lysis.[26] Interestingly, virus particles associated with the exterior of the cells did not appear lysed (Figure 13); instead, C caused additional material to accumulate on the surface of enveloped particles treated with antibody. This additional material deposited on the HSV envelope was interpreted to represent C components binding to the viral surface, as has been reported with other viruses.[7,8] Although C has been reported to lyse laryngotracheobronchitis,[7] RNA tumor,[5] and equine arteritis [6] viruses, not all virions may be subject to this form of immune destruction. Infectivity studies with HSV,[12] Newcastle's disease,[11] and polyoma [8] viruses have indicated that C-mediated neutralization occurs by the early-acting C components. These nonlytic components of C are thought to neutralize virus by coating the viral surface with C. Our morphologic results with HSV lend support to this premise.

When the HSV-infected HuFs underwent C-lysis, the inner and outer nuclear membranes underwent massive separation (Figure 11). Although a mild degree of dilatation of the perinuclear cisternae occurred in ADCC-lysed infected cells, marked ballooning dilatation was observed only in the C-lysed infected cells. Because the rough endoplasmic reticulum is contin-

uous with the perinuclear cisterna,[27] once a cell loses volume control, both compartments have been shown to undergo dilatation.[26] Why there was such accentuated perinuclear swelling of HSV-infected cells that underwent C-lysis is unclear. HSV infection is known to alter the intramembranous structure of the nuclear membranes by the insertion of viral antigens.[28] Perhaps this process makes the nuclear membranes more susceptible to dilatation once the cell undergoes rapid osmotic lysis. Alternatively, antibody and complement could have diffused into the perinuclear cisterna, injured the nuclear membrane, and caused massive focal dilatation. After HSV infection, viral-induced tubular channels appear in cells that connect the perinuclear cisterna to the extracellular space.[23] These channels are thought to be the means by which HSV is released from the cell. Antibody and C could have gained access to the nuclear membranes via these connections, induced lesions, and caused massive perinuclear cisternal dilatation. Further studies are needed.

Lysis induced by human MNLs was different morphologically from that produced by C. The ultrastructural alterations observed in the infected cell undergoing ADCC also varied according to the type of effector cell involved. Lymphocytes formed broad-based regions of attachment on the antibody-treated, infected HuFs (Figure 17). At points of apposition, the cytoplasm of the fibroblast showed severe focal alterations consisting of swollen, fragmented endoplasmic reticulum and an increased number of lipid-laden vacuoles. The above changes have not been described for virus-infected human target cells undergoing lymphocyte-mediated ADCC; however, similar alterations have been noted in other forms of lymphocyte-mediated cytotoxicity.[29–31] The lymphocyte is thought to induce membrane changes at points of contact with the targets. Although others have published photographs suggesting membrane breaks[32] or points of fusion between effectors and targets,[33] no such lesions were observed in our study.

Once in contact with the antibody-treated HuF, the human monocyte displayed an entirely different mode of behavior. The monocyte sent cytoplasmic processes over the surface of the target cells and gathered virus particles within the leukocyte's cytoplasm. Segments of HuF plasma membrane were encased within vacuoles of the monocyte. Similar changes have been observed to occur when immune murine peritoneal macrophages lyse L-cells *in vitro*[34] and *in vivo*.[35] From SEM studies of ADCC, the human monocyte appears to pull at the surface of antibody-coated target and tear the cell apart.[36] Our TEM observations of human monocytes are compatible with this mechanism of antiviral defense. Human lymphocytes,[37] polymorphonuclear leukocytes,[38] and monocytes[39]

have been shown to cause ADCC of HSV-infected cells *in vitro*. The rate at which each type of leukocyte lyses a target cell is different,[37–39] and monocytes cause ADCC at the slowest rate.[39] If monocytes destroy cells by pinching away at the target's cytoplasm, one might expect their rate of lysis to be slow. Theoretically, the target could regenerate membrane to replenish lost plasmalemma, and only after the target cell was incapable of keeping up with the monocyte's attack would lysis result. HSV has been shown by us to abortively infect human monocytes [18] and prevent them from responding to chemotactic stimuli.[40] Although the virus may exert these effects on the human monocyte, the present report indicates monocytes are still capable of carrying out important functions in an infected environment: phagocytosis of HSV and ADCC.

In summary, the studies have shown that distinctive alterations occur when infected human fibroblasts are killed by the elements of the immune system. Cells lysed by C can be distinguished from those attacked by human lymphocytes or monocytes. Although all of these reactions could be occurring *in vivo*, little is known concerning which immune reactions are taking place within human herpetic lesions. From the information gathered in this report, it may be possible for us to examine patients' lesions morphologically and determine which of these modes of antiviral defense occur *in vivo*.

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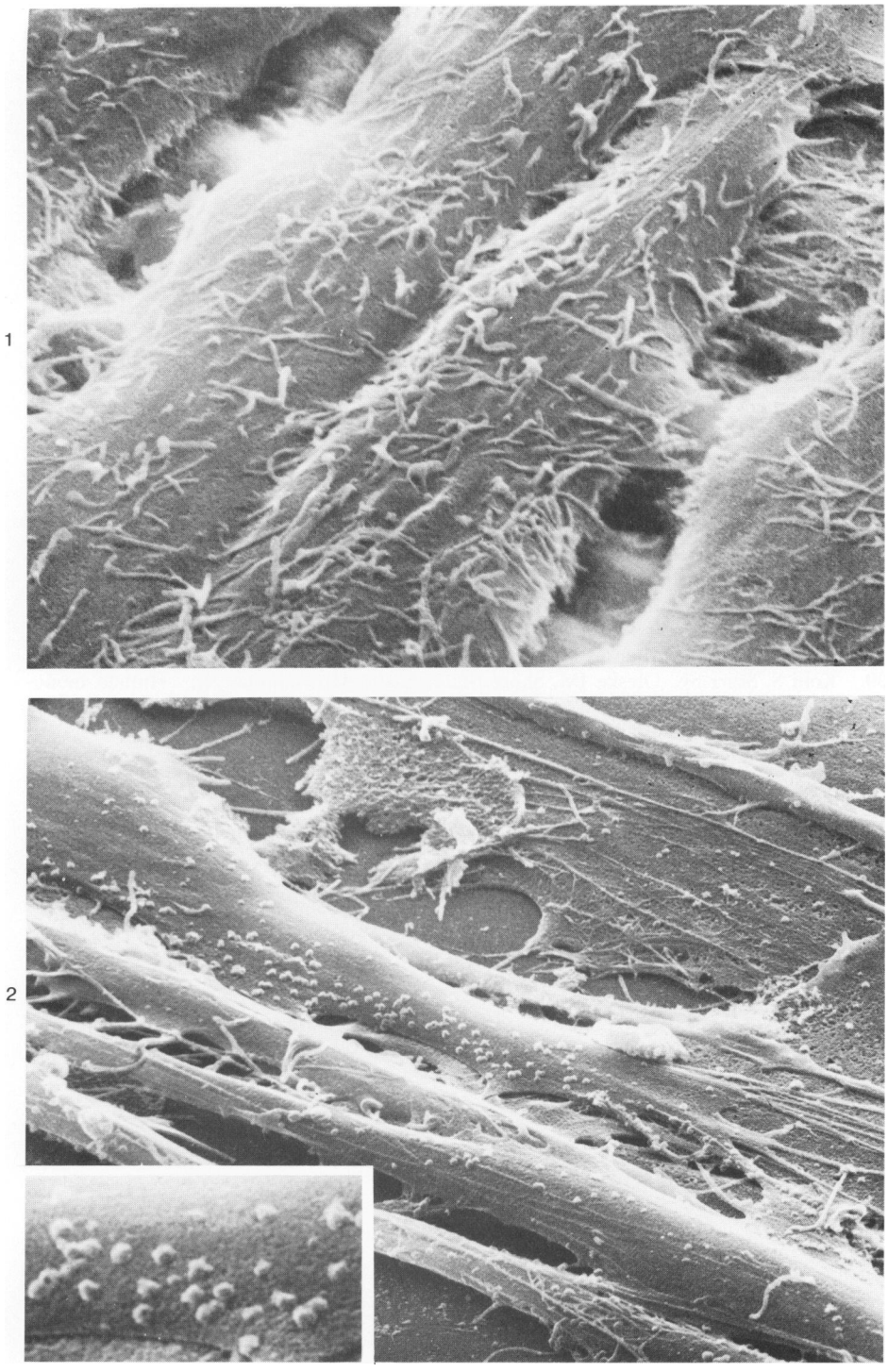
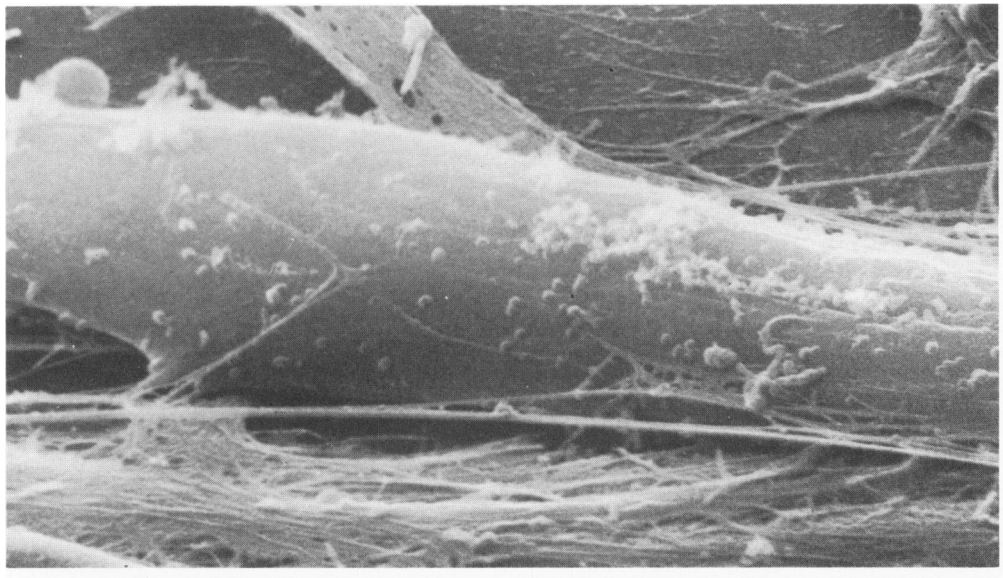


Figures 1 and 2—A scanning electron micrograph of a normal HuF demonstrates the presence of numerous microvilli projecting from the cell (**Figure 1**). (×6100) Eleven hours after incubation with HSV, the HuFs are contracted and have a paucity of microvilli (**Figure 2**). (×4400) Many spherical virus particles protrude from the cell surface. (**Inset**, ×14,300)



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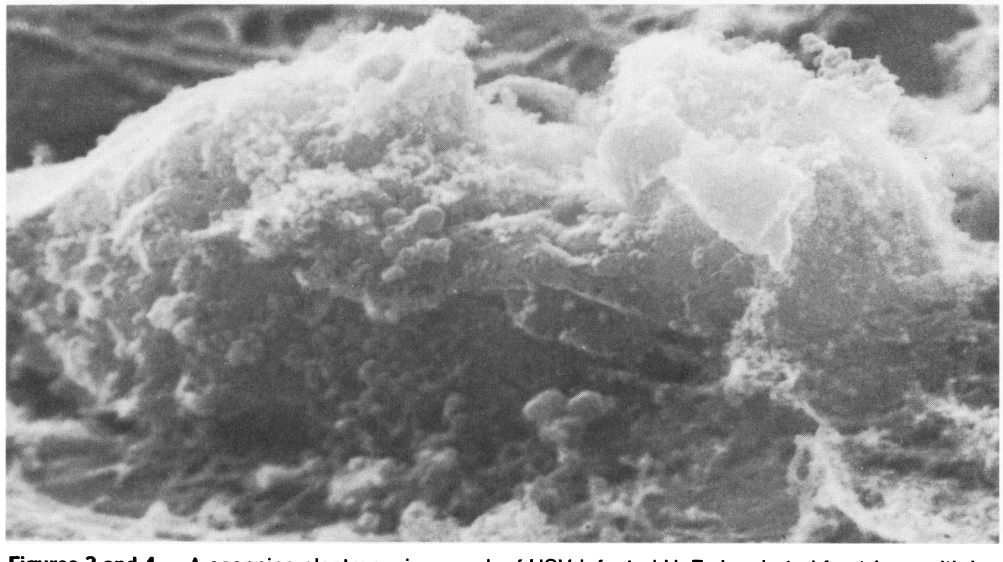

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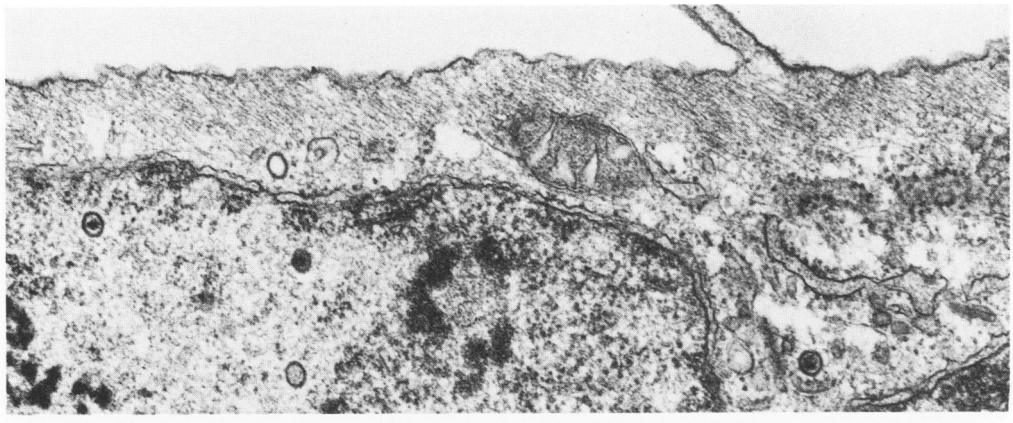
Figures 3 and 4 —A scanning electron micrograph of HSV-infected HuFs incubated for 1 hour with human anti-HSV and HIC shows few virus particles and the focal accumulation of amorphous debris on the cell surface (**Figure 3**). (×8600) Infected fibroblasts incubated with anti-HSV and active complement were rounded, fragmented, and littered with surface debris (**Figure 4**). (×7800)



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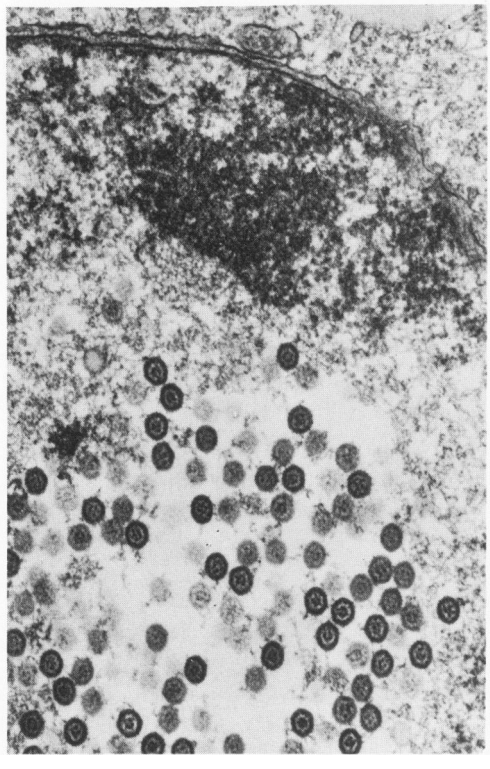

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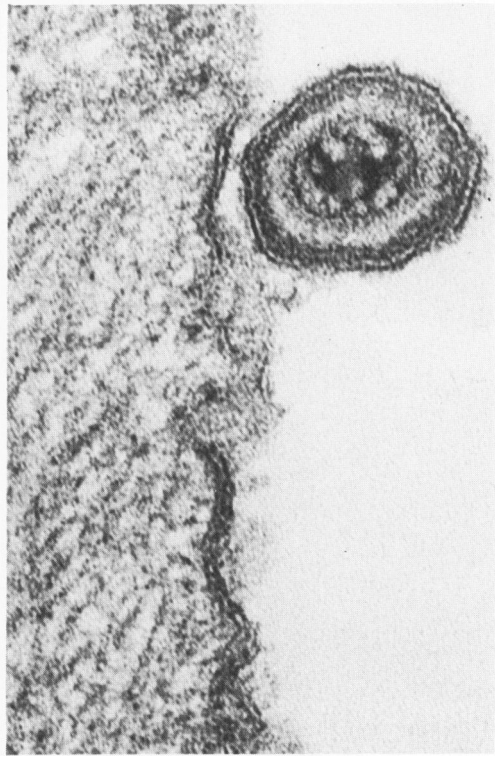

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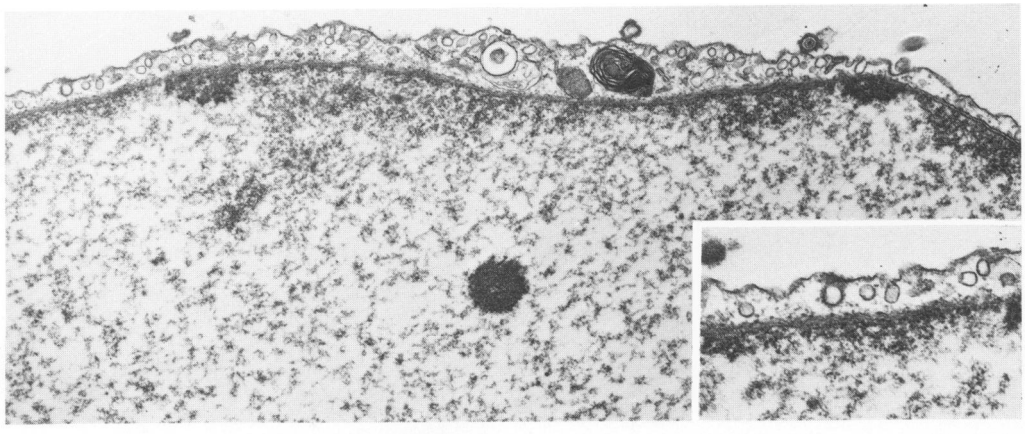
Figures 5–7 —Transmission electron micrographs of HSV-infected HuFs incubated for 1 hour with nonimmune human serum show normal-appearing mitochondria, endoplasmic reticulum, and nuclear membranes (**Figure 5**). (×40,000) Numerous immature virus particles can be seen within the nucleus (**Figure 6**). (×40,000) A mature herpes virus particle, composed of a dense nucleocapsid and a smooth-surfaced envelope, is present on the plasma membrane of an infected cell (**Figure 7**). (×200,000)



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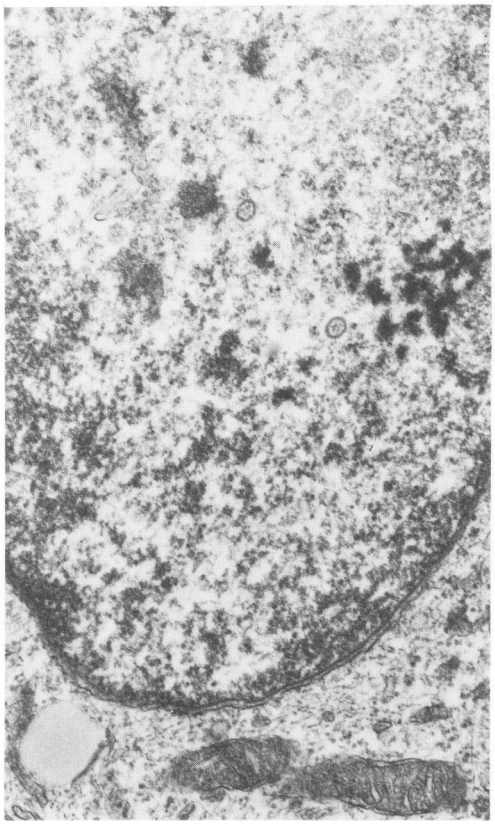

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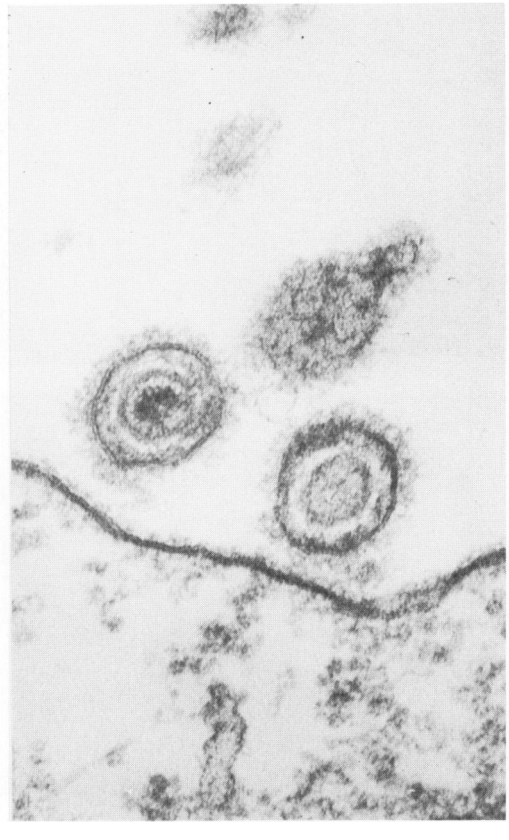

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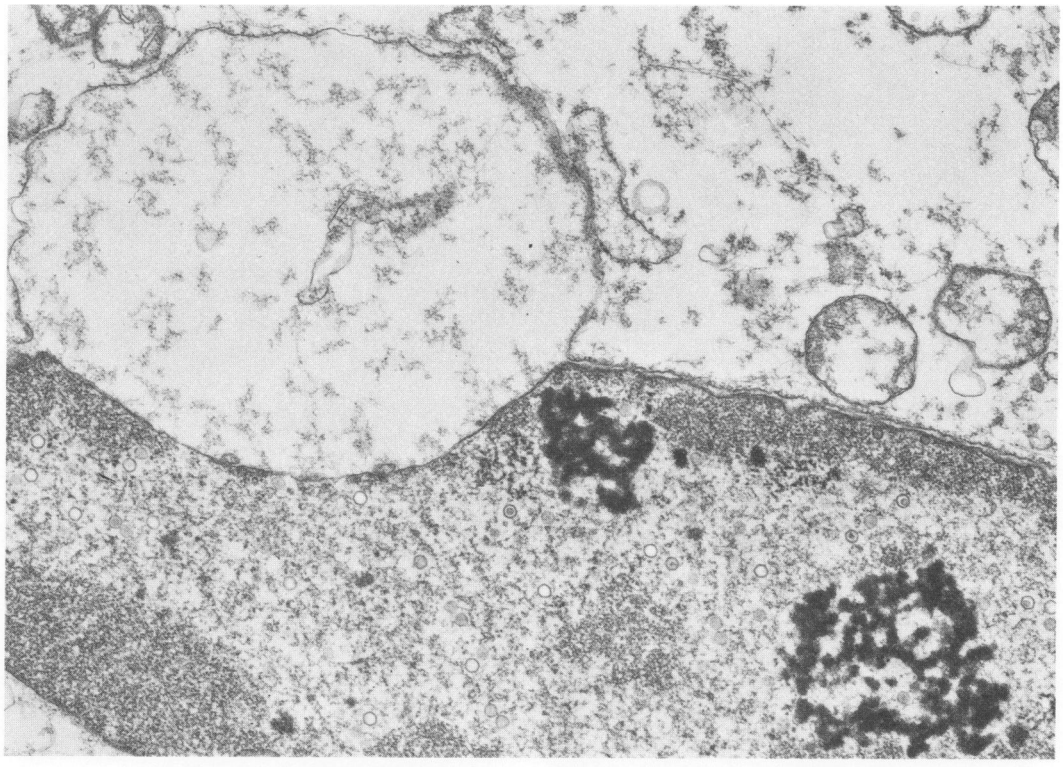
Figures 8–10 —Transmission electron micrographs of HSV-infected HuFs incubated for 1 hour with anti-HSV and HIC show cells with many pinocytotic vesicles present immediately below the plasma membrane **(Figure 8)**. (×14,000; **inset**, ×22,000) Although the cytoplasmic organelles and nuclear structures appear unaltered by the anti-HSV treatment **(Figure 9)**. (×25,000), the mature virus particle seen on the surface of these cells have envelopes coated with an amorphous, electron-dense deposit **(Figure 10)**. (×100,000)

11



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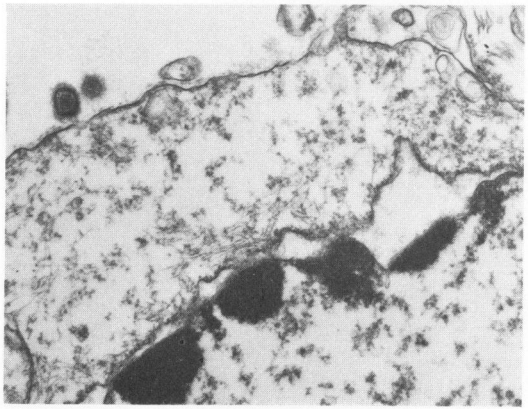

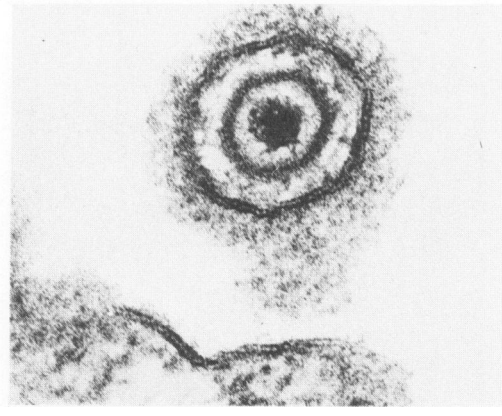

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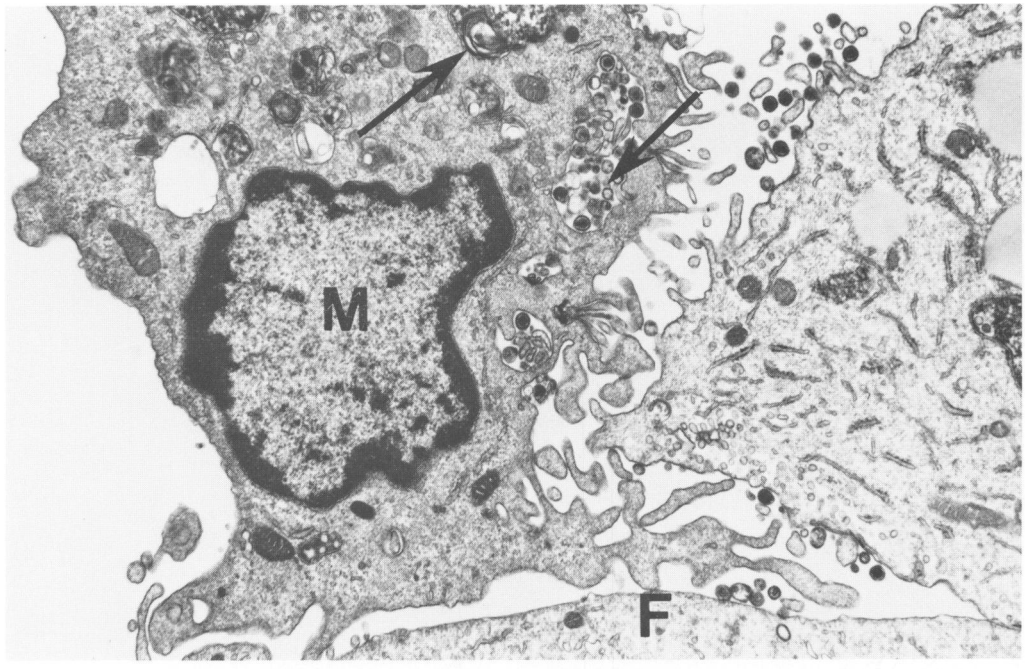
Figures 11–13—Transmission electron micrographs of HSV-infected HuFs incubated for 1 hour with anti-HSV and active C show ballooning dilatation of endoplasmic reticulum, perinuclear cisternae, and mitochondria. (**Figure 11**, ×17,000; **Figure 12**, ×20,000) Virus particles outside these necrotic cells (**Figure 13**) (×140,000) were more thickly coated with the amorphous, electron-dense deposits than those noted in Figure 10.



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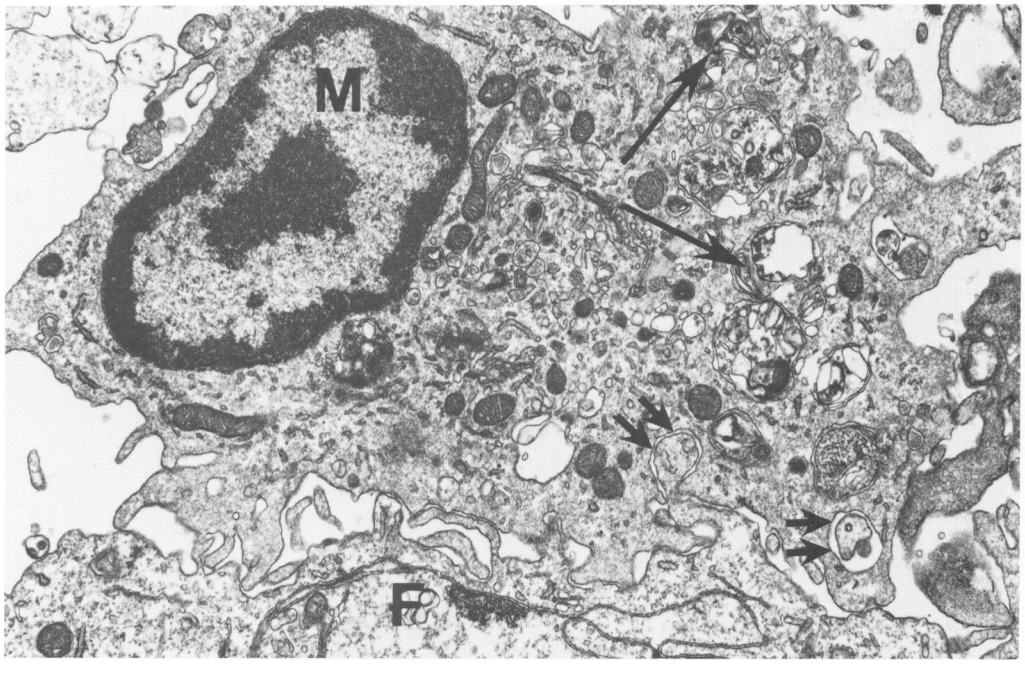

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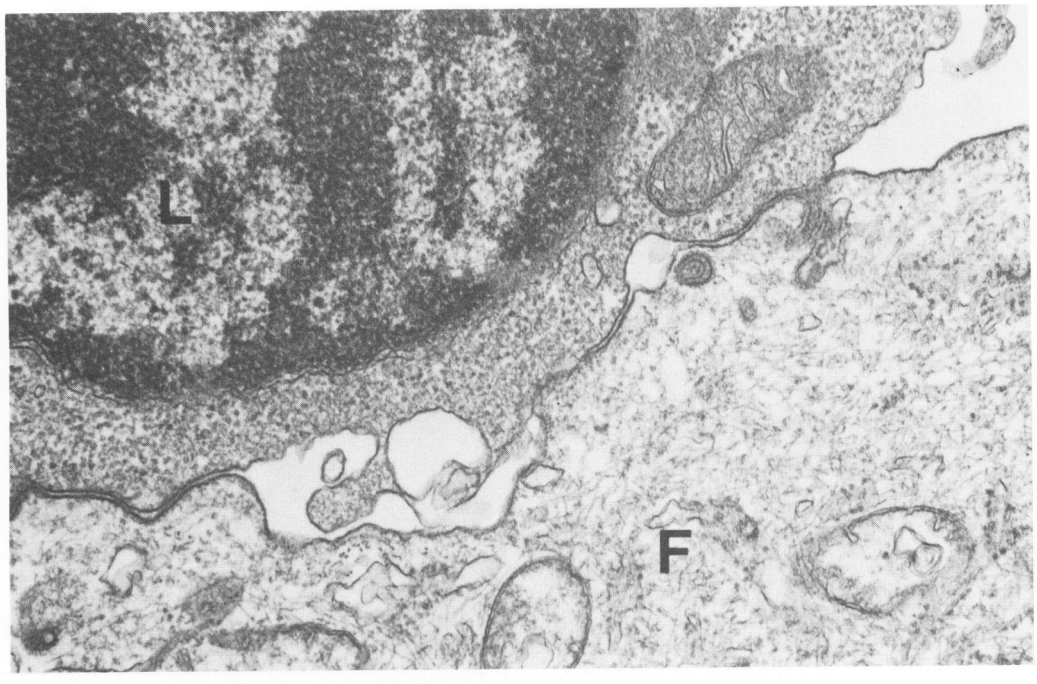
Figures 14–15 —Transmission electron micrographs of HSV-infected HuFs, incubated for 4 hours with anti-HSV and human MNLs, show monocytes (*M*) adjacent to fibroblasts (*F*). The leukocytes contain phagocytized virus particles and membranous debris within their heterophagosomes (*arrows*). Certain monocytes (**Figure 14**) (×18,000) have fingerlike projections dissecting the fibroblasts and probing toward the infected cell surface. Other monocytes (**Figure 15**) (×13,000) show a high degree of interdigitation of their projections with processes of the infected fibroblasts. In areas, cytoplasmic processes of the HuFs are pinched off by monocyte projections, and segments of the fibroblast are within vacuoles of the monocyte (*double arrows*).

16



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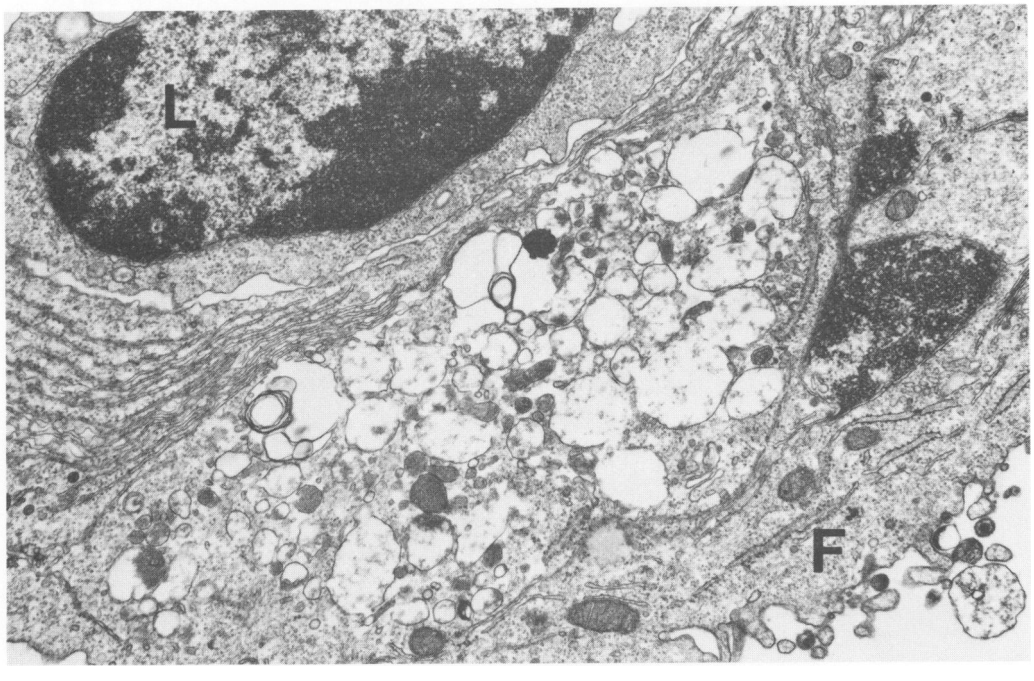

Figures 16–17 —Transmission electron micrographs of HSV-infected HuFs incubated for 4 hours with anti-HSV and human MNLs demonstrate lymphocytes (*L*) adherent to fibroblasts (*F*). Broad-based, parallel, curvilinear attachments are evident between the lymphocytes and the infected target cells. Mild mitochondrial swelling is seen (**Figure 16**). (×102,000) A more advanced degree of cytotoxicity was also found (**Figure 17**). (×13,000) At points of lymphocyte attachment the HuFs show a localized, massive swelling of the endoplasmic reticulum and fragmentation of cytoplasmic membranous structures. The perinuclear cisternae of the HuFs remained intact.