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MHC-Specific Cytotoxic T Lymphocyte Killing of Dissociated Sympathetic Neuronal Cultures

PAMELA T. MANNING, PhD, EUGENE M. JOHNSON, Jr., PhD, CHRISTINE L. WILCOX, PhD, MARGARET A. PALMATIER, PhD and JOHN H. RUSSELL, PhD

From the Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri

Experiments were conducted to determine whether neurons in culture can serve as targets for immunologic attack mediated by major histocompatibility complex (MHC)-specific cytotoxic T lymphocytes (CTLs) which recognize Class I antigens. Allogeneic C3H/He primary neuronal cultures were quickly destroyed after CTL addition, while syngeneic C57BL/6J neurons were not lysed. Alterations in the distribution of chromatin were the first ultrastructural changes that occurred, followed by loss of nuclear morphology, cytosolic changes, and eventually fragmentation of both the nucleus and cytosol. With Campenot chambers, it was possible to separate the membrane and nuclear lesions. CTLs exposed to neurites, but separated from the cell body by the chamber barrier, caused degeneration of neurites but did not cause lysis and cell death. Neuronal lysis mediated by antibody and complement appeared to be distinct from CTL-mediated lysis. These experiments demonstrate that neurons in culture are targets for MHC-specific CTLs, and therefore probably express functional levels of Class I antigens. The signal for killing by CTLs is not retrogradely transported from the neurite to the cell body, and morphologic events following CTL–neuron interaction resemble those that occur in dividing tumor target cell populations. (Am J Pathol 1987, 128:395–409)

THE INTERACTION between the cytotoxic lymphocyte (CTL) and its antigen-bearing target cell provides an interesting model for cell–cell interaction and communication. Lysis of the target cell requires recognition between the antigen receptor on the CTL and a Class I major histocompatibility (MHC) antigen alone or in combination with a foreign or modifying antigen (eg, virus) on the surface of the target cell. This recognition cannot be inhibited by soluble antigen preparations or by membrane fragments. Once recognition is established, the CTL initiates a lytic process that has been characterized by several biochemical and morphologic criteria with the use of a number of different tumor cell types as targets.[1]

A unique feature of the lytic process initiated by the CTL in response to antigen-bearing target cells is an event that quickly affects the entire target cell. When target cells are attacked by CTLs, the entire cell, including its nucleus, is rapidly destroyed. The terminal event has been described as zeoisis or boiling of the cytosol.[2–4] The speed (within 5–10 minutes with some targets) and morphologic similarities to random, "natural" cell death within populations of cells has led a number of investigators to suggest that the CTL-initiated lytic process cannot be ascribed simply to a membrane lesion at the point of contact. Rather, it may involve a signal from the CTL to the target which initiates an autolytic destruction by the target itself.[3,5,6]

As mentioned above, the Class I MHC antigens are cell surface antigens that are critical to various CTL-mediated immune responses, including graft rejection and elimination of virally infected and abnormal cells. Although most cell types express Class I surface

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Address reprint requests to Pamela T. Manning, Monsanto Co., Department of Biological Sciences, AA3G, 700 Chesterfield Village Parkway, Chesterfield, MO 63198.

antigens, peripheral and central neurons, as well as certain tumors of neuronal origin, do not have MHC antigens or express them at levels too low to be detected by immunohistochemical methods.[7-11] However, recent evidence indicates that Class I expression can be induced on central neurons both *in vivo* and *in vitro* by gamma interferon.[12] In addition, cells with MHC expression below the threshold of immunohistochemical techniques have been shown to be recognized and lysed by CTLs.[13] We have therefore used cloned, MHC-specific murine CTLs and dissociated, sympathetic neurons from murine superior cervical ganglia (SCG) to determine whether cultured neurons express MHC antigens and whether they can serve as targets for immunologic attack under noninduced conditions.

In addition, we have compared the morphologic changes in the postmitotic neuronal targets with a unique nuclear lesion previously described in tumor cell targets.[14] This nuclear lesion does not occur in hypotonic shock or complement-mediated lysis and is characterized biochemically by sensitivity of the nuclear membrane to non-ionic detergent,[6] by fragmentation of the target cell genome,[15,16] and morphologically by the loss of the double membrane and its pore structure.[14] These nuclear changes have the same physiologic requirements (temperature and ions) as the initiation of the plasma membrane lesion, and both lesions appear to be initiated at the same time.[6] Thus, it remains unclear whether the plasma membrane and nuclear lesions are secondary end points of a single primary lesion or end points of distinct primary lesions. Because the two lesions were not separable by physiologic techniques, we have used the neuronal cultures to separate anatomically these two processes within the same target cell. The effect of changing the point of CTL attack on the plasma membrane with respect to the cell nucleus was examined by means of Campenot chambers, which allow a physical separation between the cell bodies and their neuritic processes.

Materials and Methods

Animals

C57BL/6J (H-2^b), C3H/He (H-2^k), and DBA/2 (H-2^d) mice were purchased from the Jackson Laboratory (Bar Harbor, Me).

CTL Clones

Two lines of cloned murine cytotoxic T lymphocytes (CTL-1 and CTL-3) were used. Clones were derived by limiting dilution of secondary mixed lymphocyte cultures (C57BL/6J anti-DBA/2) as originally described by Glasebrook et al.[17] Both express the cell differentiation antigens Thy 1.2 and Lyt 2.2. The lytic and proliferative specificities have been mapped with the use of recombinant inbred strains of mice[18] to the following Class I MHC antigens: CTL-1, H-2^d; CTL-3, H-2^k.Clones were maintained in RPMI 1640 medium supplemented with 5% fetal bovine serum, 2 mM glutamine, 5×10^{-5} M β-mercaptoethanol, 2 mM sodium pyruvate, additional amino acids, and 20–40% conditioned medium from secondary mixed lymphocyte cultures.[19] Clones were restimulated weekly with irradiated DBA/2 spleen cells. All tissue culture reagents used for CTL clones, as well as for neuronal cultures, were obtained from GIBCO (Grand Island, NY) unless otherwise specified.

Neuronal Cultures

Primary cultures of dissociated sympathetic neurons, prepared from SCG of 2–4-day-old, inbred mice (strains described above), were used as target cells for CTL killing. Cultures were prepared essentially as described by Wakshull et al.[20] SCG were removed aseptically and collected into Hanks' Ca^{2+}, Mg^{2+}-free balanced salt solution (HBSS). Ganglia were decapsulated and enzymatically dissociated with 0.25% collagenase (4194 CLS, Worthington Diagnostics, Freehold, NJ), followed by 0.25% trypsin, each for 20 minutes at 37 C. The neurons were washed twice with Hanks' and twice with serum-containing medium used to maintain the cultures. A single cell suspension was then obtained by mechanically dissociating the tissue by trituration through a small-bore glass pipet. The cell suspension was filtered through nylon mesh (15-μ pore size, Nitex HC3-15, Tetko, Rolling Meadows, Ill) for removal of debris and nondissociated cells. Cells were plated into collagen-coated, 16-mm wells, 16-mm aclar dishes for electron microscopy, or 35-mm plastic dishes for Campenot chambers (see below) and grown in Eagle's minimal essential medium with Earle's salts (EMEM) containing 10% fetal bovine serum (GIBCO, #309), 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 20–5000 ng/ml 2.5S nerve growth factor (NGF). The 2.5S NGF was purified from male mouse submaxillary glands with minor modifications according to the method of Bocchini and Angeletti.[21] Nonneuronal, dividing cells (>95%) were eliminated by including 10 μM 5-fluorodeoxyuridine and 10 μM uridine (Sigma Chemical Co., St. Louis, Mo) in the medium for the first week. Neurons were fed every 3 days and were used 2–3 weeks after plating.

Cytotoxicity Assays

Various numbers of CTL effector cells (H-2^b anti-H-2^k or anti-H-2^d) were washed, centrifuged through a Ficoll-Hypaque gradient and added to 2–3-week-old neuronal cultures. Cultures were observed for morphologic changes (neurite graininess and degeneration, changes in neuronal soma, and apparent cell death) at various times following addition of effector cells by means of phase-contrast microscopy. Alternatively, to compare the changes mediated by cytotoxic T cells with those produced by antibody and complement, we added a 1/40 dilution of a rabbit anti-mouse (generated against mouse myeloma 167) antiserum (anti-167) and a 1/20 dilution of guinea pig serum (source of complement) to the neuronal cultures.

Campenot Chambers

Cultures of dissociated SCG neurons were also grown in compartmentalized chambers (35-mm collagen-coated, plastic dishes into which a Teflon chamber is placed and secured with silicone grease) as described by Campenot.[22] SCG were dissociated as described above. Prior to plating, cells were centrifuged at 500*g* for 10 minutes and resuspended in complete medium (described above) containing 0.25% Methocel (A4M premium, Dow Chemical Co., Midland, Mich). Methocel prevents diffusion of components or leakage between the center and side compartments of the chambers. Cells (equivalent of 0.5 ganglion/chamber) were plated into the center compartments of the chambers and were fed every 3 days with Methocel-containing medium. Nonneuronal cells were eliminated from the cultures as described above. Within 2 weeks, neurites had extended from the cell bodies, had grown underneath the silicone-greased side barriers, and were present in both side compartments of the chambers. CTL effector cells could then be added either to the cell bodies or to the neurites.

Electron Microscopy

Neuronal cultures were processed for scanning and electron microscopy at various times after addition of CTLs. Cultures were rinsed with serum-free medium and fixed with 2.5% glutaraldehyde/0.1 M cacodylate buffer, pH 7.2, for 1 hour. Cultures were then rinsed with 0.2 M cacodylate buffer, pH 7.2, and postfixed with 1% aqueous osmium tetroxide for 1 hour. For scanning electron microscopy, cultures were rinsed with distilled water, dehydrated through graded ethanol solutions, and dried in a critical-point-drying chamber. Samples were coated with 125 Å gold and examined with a scanning electron microscope. For transmission electron microscopy, samples were stained with 1% aqueous uranyl acetate for 1 hour following the osmium tetroxide step and were dehydrated through graded ethanol solutions. Samples were embedded in Polybed 812 by incubating sequentially in 2:1, 1:1 and 1:2 mixtures of 100% ethanol and Polybed 812 (Polysciences, Inc., Warrington, Pa) for 30 minutes each and in 100% Polybed 812 for 1 hour. Fresh Polybed was then added and polymerized at 60 C overnight. Thin sections were stained with lead citrate and examined with an electron microscope.

Results

Lysis of Neuronal Cultures by CTLs

Initial experiments were conducted to determine whether sympathetic neurons in culture could serve as targets for immunologic attack mediated by cytotoxic lymphocytes that recognize Class I MHC antigens and thus whether the neurons express functionally significant levels of MHC antigens. Neurons have several advantages as targets: 1) they are large cells with anatomically distinct membrane regions, the neurites, from the cell body and nucleus, and 2) they are postmitotic cells, in contrast to cell types which are normally used as targets.[2,3,16] Primary cultures of C3H/He (H-2^k) or C57BL/6J (H-2^b) sympathetic neurons, grown in the presence of varying concentrations of NGF for 2–3 weeks, were used as targets for CTL-3 (H-2^b anti-H-2^k). Initially, attempts were made to quantify cytotoxicity by labeling the neuronal targets with either ^{51}Cr or ^{111}In, as has been done using more conventional target cells. However, these isotopes were either released at a high spontaneous rate or were toxic, causing neuronal cell death within 24 hours. Therefore, killing was monitored morphologically with phase-contrast, scanning, and transmission electron microscopy. Because the morphologic changes that occur during CTL-mediated lysis are unique and can, in fact, distinguish CTL-mediated lysis from other forms of cell death which are not distinguishable by most of the standard isotopic release assays,[3,5,14] this assessment of killing was not a disadvantage, but rather provided a greater amount of information than isotopic analysis.

Within 2 hours after addition of high effector concentrations (1.5×10^5 CTL-3/16-mm well) near complete destruction of C3H/He neurons (H-2^k) occurred; this demonstrated the presence of Class I antigens of the MHC on the surface of these cultured

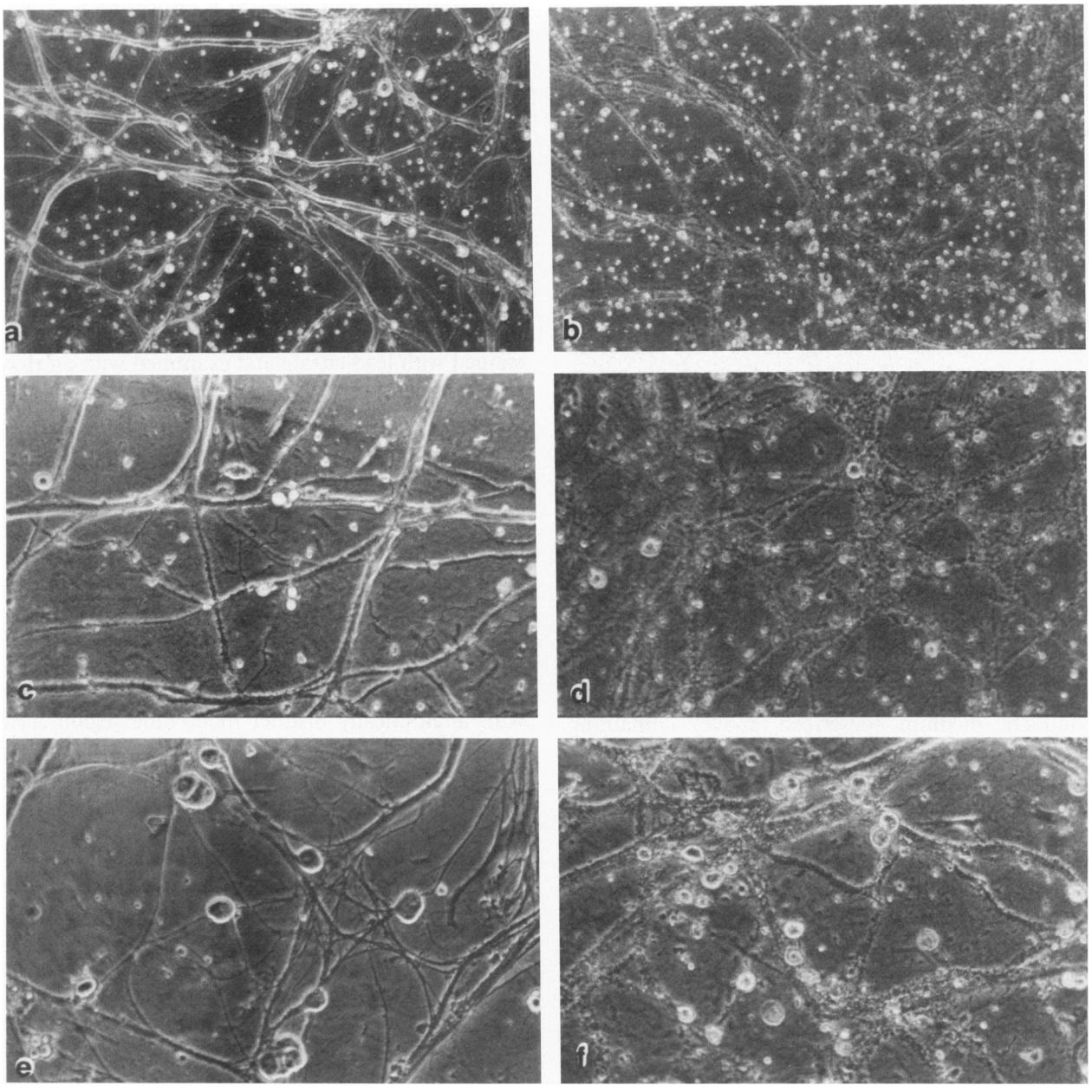


Figure 1—Phase-contrast micrographs of mouse SCG neurons cultured with MHC-specific CTLs. Cloned CTL-3 were added to 2–3-week-old cultures of dissociated sympathetic neurons. Two hours after the addition of a high-effector number of CTL-3 (1.5×10^5), syngeneic C57BL/6 neurons appeared normal (**A**), while allogeneic C3H/He neurons were lysed (**B** and **D**). CTL-mediated lysis occurred more slowly upon addition of lower CTL-3 (7.5×10^4) effector numbers (**C**, **E**, and **F**). The neurites of C3H/He neurons appeared grainy 2 hours after CTL-3 addition (**C**). Neuritic degeneration as well as loss of cell bodies occurred at longer times (7 hours) after CTL-3 addition to C3H/He neurons (**F**), whereas no apparent changes occurred in C57BL/6 neurons (**E**). (**A** and **B**, ×80; **C–F**, ×160)

neurons, as well as their suitability as targets for immunologic attack by cytotoxic T lymphocytes. The first observable change that occurred following CTL addition was a grainy appearance of the neurites (Figure 1C). Degeneration of the entire neuritic network, as well as loss of the majority of phase-bright neuronal cell bodies, followed (Figure 1B and D). In contrast, addition of CTL-3 to syngeneic cultures of C57BL/6J neurons ($H\text{-}2^b$) did not cause any observable morphologic alterations either at low or high effector concentrations (Figure 1A and E), although contact between CTL and neurons appeared to occur. At lower effector numbers (7.5×10^4 CTL-3), degeneration and cell death of C3H/He neurons occurred, but more slowly. Seven hours after the addition of low numbers of CTLs, much neurite degeneration had

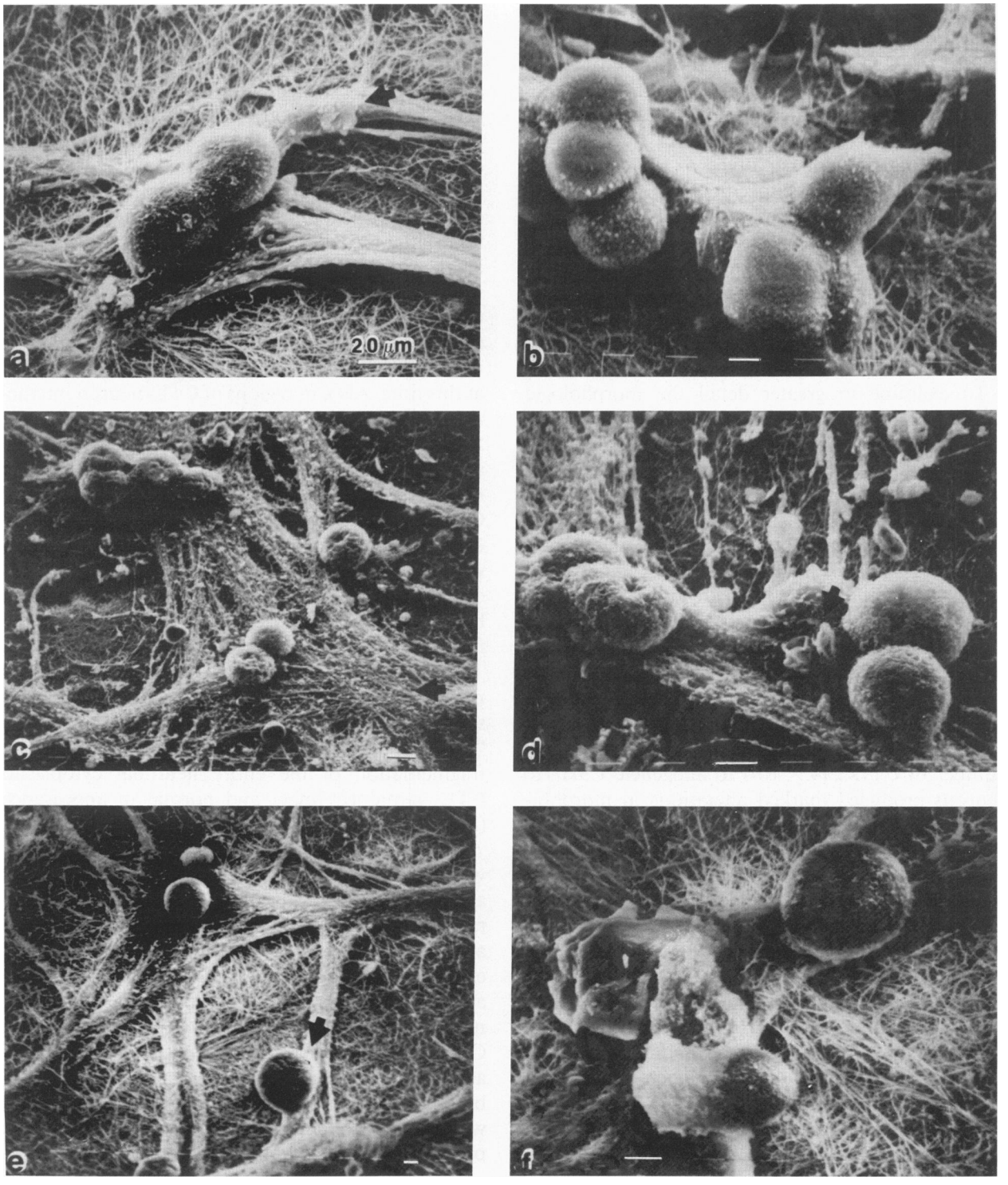


Figure 2—Scanning electron micrographs of mouse SCG neuronal cultures cocultured with 4×10^4 CTL-3 or after addition of antibody and complement. **A**—Thirty-two hours after addition of CTL to C57BL/6 neurons. Note the association of CTLs (*arrow*) with neuronal processes. **B**—Untreated control C3H/He neurons. **C** and **D**—Eighteen hours after CTL addition to C3H/He neurons. Note abnormal grainy neurites (**C**, *arrow*), distortion of cell bodies, and close association of CTLs with neuronal soma (**D**, *arrow*). **E** and **F**—One hour after addition of antibody and complement to C3H/He neurons. *Arrow* denotes a swollen neuronal cell body (**E**). (Bars = 20 μ)

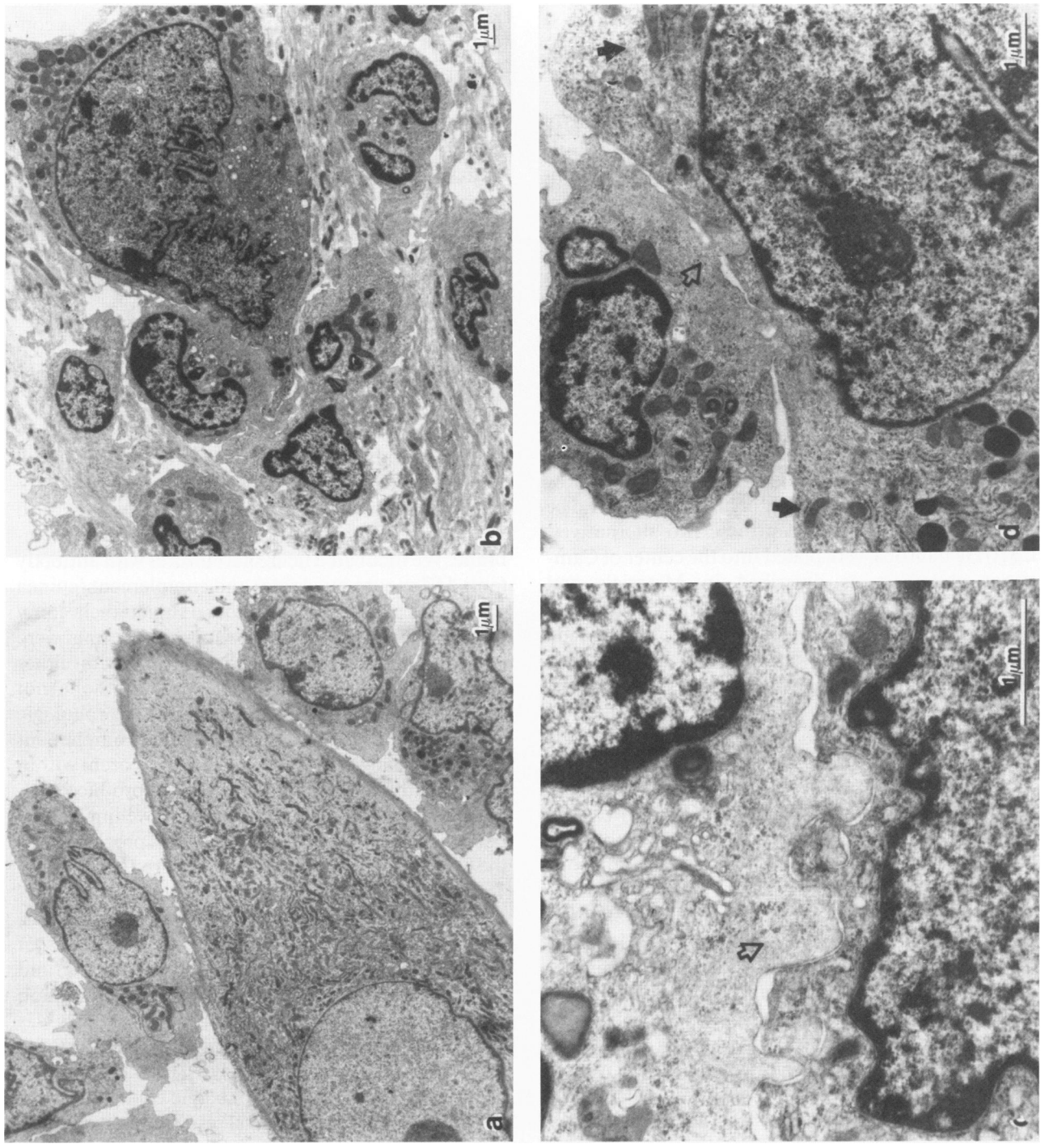
occurred, yet many phase bright cell bodies remained (Figure 1F). Many, but not all, of these died within a 48-hour period (not shown). Increasing the NGF concentration from 20 ng/ml to 5 μg/ml did not protect against the CTL killing process (not shown), which demonstrates that the factor upon which the sympathetic neuron is dependent for survival cannot protect the neuron from immunologic attack by CTLs. Finally, killing was not haplotype or CTL-3 specific. Addition of CTL-1 (lytic specificity, H-2^d) to cultures of DBA/2 (H-2^d) neurons resulted in destruction, while no destruction occurred after addition of CTL-1 to C3H/He (H-2^k) neurons (not shown). Similar results were obtained in six different experiments using CTL-3 and in two experiments using CTL-1, each consisting of five or more independent neuronal cultures.

To examine in greater detail the morphologic changes that occurred following the addition of CTL-3 to both C3H/He (H-2^k) and C57BL/6J (H-2^b) neuronal cultures, scanning electron microscopy was performed. Untreated, control C3H/He neurons are shown in Figure 2B. The fibrous appearing network to which the neurons are attached is the collagen substratum. A cluster of rounded cell bodies with smooth processes extending from them are visible. No apparent morphologic changes occurred after the addition of CTL-3 to syngeneic C57BL/6J neurons, even after coculture for up to 32 hours. Close association of CTLs (small, irregularly shaped structures, denoted by arrow) and of the neuronal soma and processes was apparent (Figure 2A). In contrast, addition of a low number of CTL-3 (4×10^4) to allogeneic C3H/He neurons produced marked alterations in morphology. The neuritic surfaces were irregular and grainy and appeared to be disintegrating with the loss of small amounts of cytoplasm (Figure 2C, arrow). In addition, the majority of the neuronal cell bodies were distorted by pronounced indentations. Two CTLs can be seen in close association with what appear to be normal cell bodies (Figure 2D, arrow) but which possess degenerating neurites. This suggests that neurite degeneration may precede gross alterations in the cell soma and in cell death and that the membrane and nuclear lesions may be separable when one is using the neuron as a target cell.

CTL–neuronal target cell interactions were also examined at the ultrastructural level to define more precisely the morphologic changes that occur during this interaction which lead to target cell death. Early changes in the lytic process following addition of CTL-3 to neuronal targets are shown in Figure 3. No change was apparent following addition of CTL-3 to syngeneic C57BL/6J neurons, although a number of CTLs were in close contact with the neuronal soma (Figure 3A). Of note is the regular shape of the nucleus, having only shallow indentations and a central location. Chromatin is finely dispersed and exhibits no clumping near the nuclear membrane. However, 2 hours after the addition of CTL-3 to allogeneic C3H/He neurons, marked nuclear changes, irregular shape with deep indentations and condensation of chromatin, especially near the nuclear membrane, occurred (Figure 3B–D). Cytosolic components, including mitochondria (Figure 3D, solid arrows) were normal at this time. Also, in regions of CTL–neuron interaction, several CTL inserted cytoplasmic projections or "uropods"[4] into neurons near the nucleus (Figure 3C and D; open arrows). Changes in the C3H/He target neurons late in the killing process (3–4 hours after CTL addition) involved both the nucleus and cytosol. Nuclear changes including an eccentric location within the cell were more pronounced and numerous cytoplasmic vacuoles and heterogeneous dense bodies were present (Figure 4A). A progression of the final stages of target cell lysis is shown in Figure 4B–D. The nucleus and small pieces of cytosol at the cell membrane began to fragment, or, as has been described previously for other target types,[2,3,5,14] to "boil" (Figure 4B). Cell lysis resulted from zeosis or fragmentation of the entire neuronal cytoplasm. CTLs remained intact and normal in appearance (Figure 4C and D).

The early and late nuclear changes that occur within neuronal targets after CTL addition are shown at higher magnification in Figure 5. CTLs with normal appearing nuclei and nuclear pores (Figure 5A, arrow) were present in the same culture as neurons exhibiting late nuclear changes (Figure 5D); thus, fixation artifact is not a possible explanation for neuronal abnormalities. At early times after addition of CTLs, both mitochondria and nuclear pores (open arrow) appeared normal, although the chromatin began to condense and some membrane irregularity was evident (Figure 5B). In contrast, late in the lytic process, the nucleus was irregular in shape, the chro-

Figure 3—Electron micrographs of CTLs and neurons showing early changes in the lytic process. **A**—C57BL/6 neurons 2 hours after addition of 1.5×10^5 CTL-3. Note the absence of cytoplasmic projections of CTLs into a non-target neuron. **B–D**—C3H/He neurons 2 hours after addition of 1.5×10^5 CTL-3. Note cytoplasmic projections of CTLs into neuronal targets (*open arrows*, **C** and **D**) and the normal appearance of mitochondria (*solid arrow*, **D**). (Bars = 1 μ)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matin was clumped and no apparent nuclear pores were visible. Also, the mitochondria were swollen and irregularly shaped (Figure 5D).

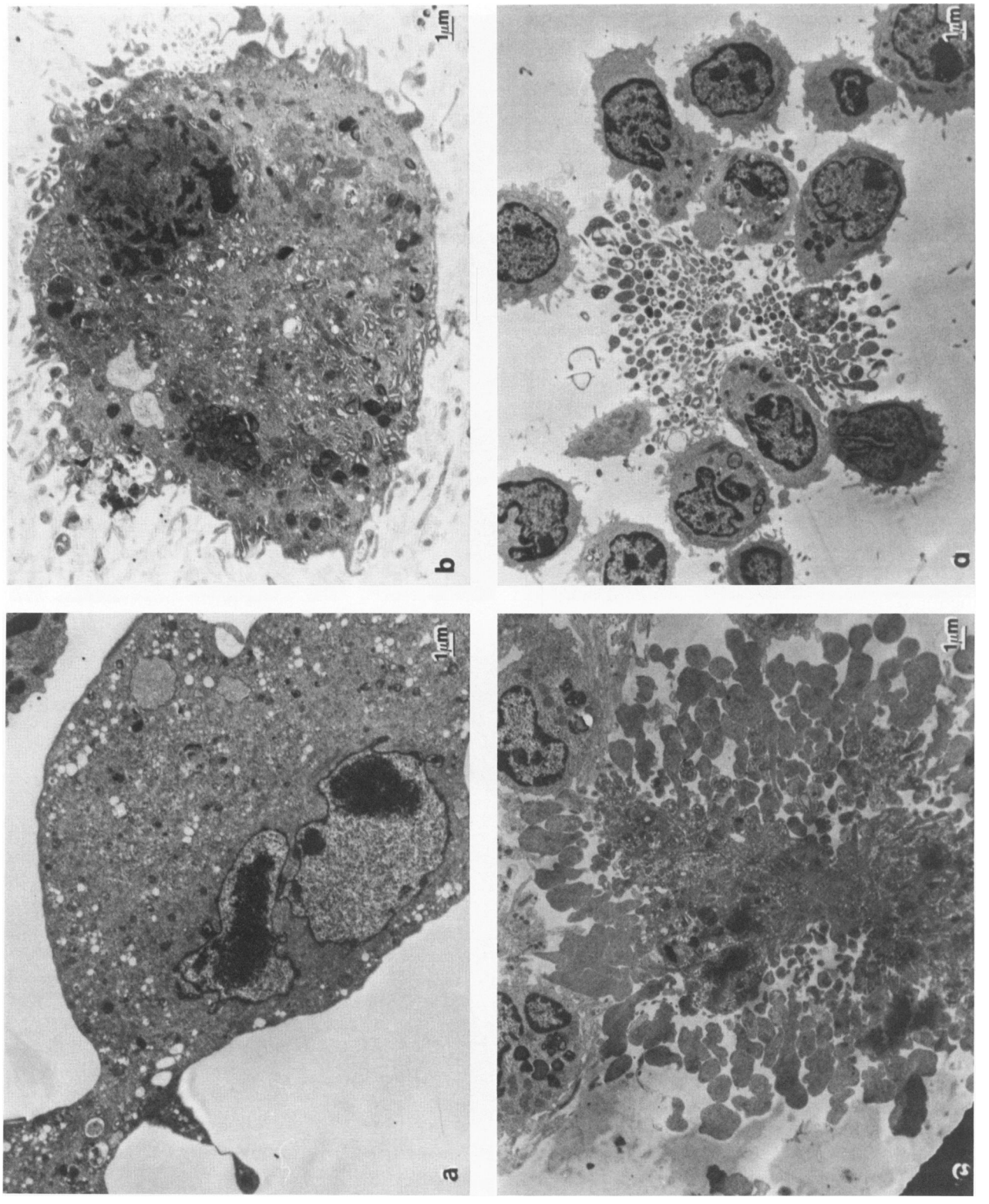
Separation of Membrane and Nuclear Lesions

We observed that when lower numbers (7×10^4) of CTLs were added to neuronal cultures, the killing process occurred more slowly than when high numbers (1.5×10^5) of effectors were added. In fact, under these conditions, some of the cell bodies were spared, even though the neuritic network had degenerated. Two alternatives exist to explain this finding. One possibility is that the signal for killing has a limited range and is thus not retrogradely transported from the neurite to the cell body. Therefore, with a low number of effectors, the probability of a direct hit on or near the cell body, compared with the neuritic network, is low. A second explanation might be that the number of actual hits per cell is important, irrespective of the location of the interaction. Thus with low effector numbers, the number of hits per cell would be low. To distinguish between these two alternatives, C3H/He neurons were plated into the center of Campenot chambers as described in Materials and Methods. Neurites grew under the barrier (black area, Figure 6B) which separated the center and side chambers and into the two side chambers where they were physically separated from the neuronal cell bodies. Two hours after the addition of high numbers of CTL-3 to the right side of the chamber (6×10^5/side chamber), CTLs had begun to settle in the vicinity of the neurites; however, both neurites and cell bodies appeared normal (Figure 6B). The slower time course of killing in these experiments is probably due to the increased viscosity of the culture medium containing Methocel, which is used to prevent diffusion between chamber compartments. Ten hours after the addition of CTLs to the side chamber, CTLs completely decorated the neurites, which appeared grainy and partially degenerated (Figure 6D). However, both the cell bodies in the center chamber and the neurites extending into the left chamber to which CTLs were not added were normal (Figure 6C and D). Twenty-six hours after CTL addition, complete degeneration of neurites in the right side of the chamber had occurred (Figure 6F). Again, there were no apparent morphologic alterations in either the cell bodies within the center chamber (Figure 6F) or the neurites in the left side of the chamber (Figure 6E). As a control, CTLs were added directly to the cell bodies in a center chamber. Complete destruction of neurites and cell bodies occurred within 10 hours (Figure 6A). In another experiment, when CTLs were added to both sides of the chamber, neurites degenerated in both sides, but the cell bodies in the center chambers appeared normal (not shown). These results suggest that the signal for complete cell destruction is not retrogradely transported, but, rather, has a short intracellular range and that the membrane lesion is separate from the nuclear lesion.

Lysis of Neurons With Antibody and Complement

It has been shown by using tumor cells as targets that the morphologic events following CTL-target cell interaction differ markedly from those following incubation with antibody and complement,[3,23,24] in which the primary lesion occurs at the plasma membrane. We incubated neuronal cultures with antibody (anti-167 mouse myeloma) and complement (guinea pig serum) to determine whether a similar dichotomy of events occurred when postmitotic neurons were used as targets. Incubation of neurons with either complement alone (Figure 7A) or antibody alone (not shown) did not cause any apparent morphologic changes. Incubation with antibody and complement produced changes which were readily apparent within 1 hour and which differed from those produced following CTL addition. Pronounced swelling of the neuronal cell bodies and the neuritic processes was the first observable changes that occurred (Figure 7B). Two hours after addition of antibody and complement, most of the cell bodies had undergone lysis. Neurite degeneration was apparent only after most of the cell bodies were no longer present (Figure 7C) and was virtually complete after three hours of incubation (Figure 7D). Swelling of the neuronal cell bodies was also evident by scanning electron microscopy (Figure 2E) and appeared to result in lysis and neuronal cell death (Figure 2F). The morphologic changes observable at the ultrastructural level resulting from incubation with antibody and complement also revealed

Figure 4—Electron micrographs showing late changes in the CTL lytic process. **A**—C3H/He neurons 3–4 hours after CTL-3 addition. Note the irregular shape of the nucleus, condensed chromatin, and numerous cytoplasmic vacuoles. **B–D**—Progression of final stages of neuronal cell lysis resulting in zeosis or fragmentation of the neuronal nucleus and cytoplasm. (Bars = 1 μ)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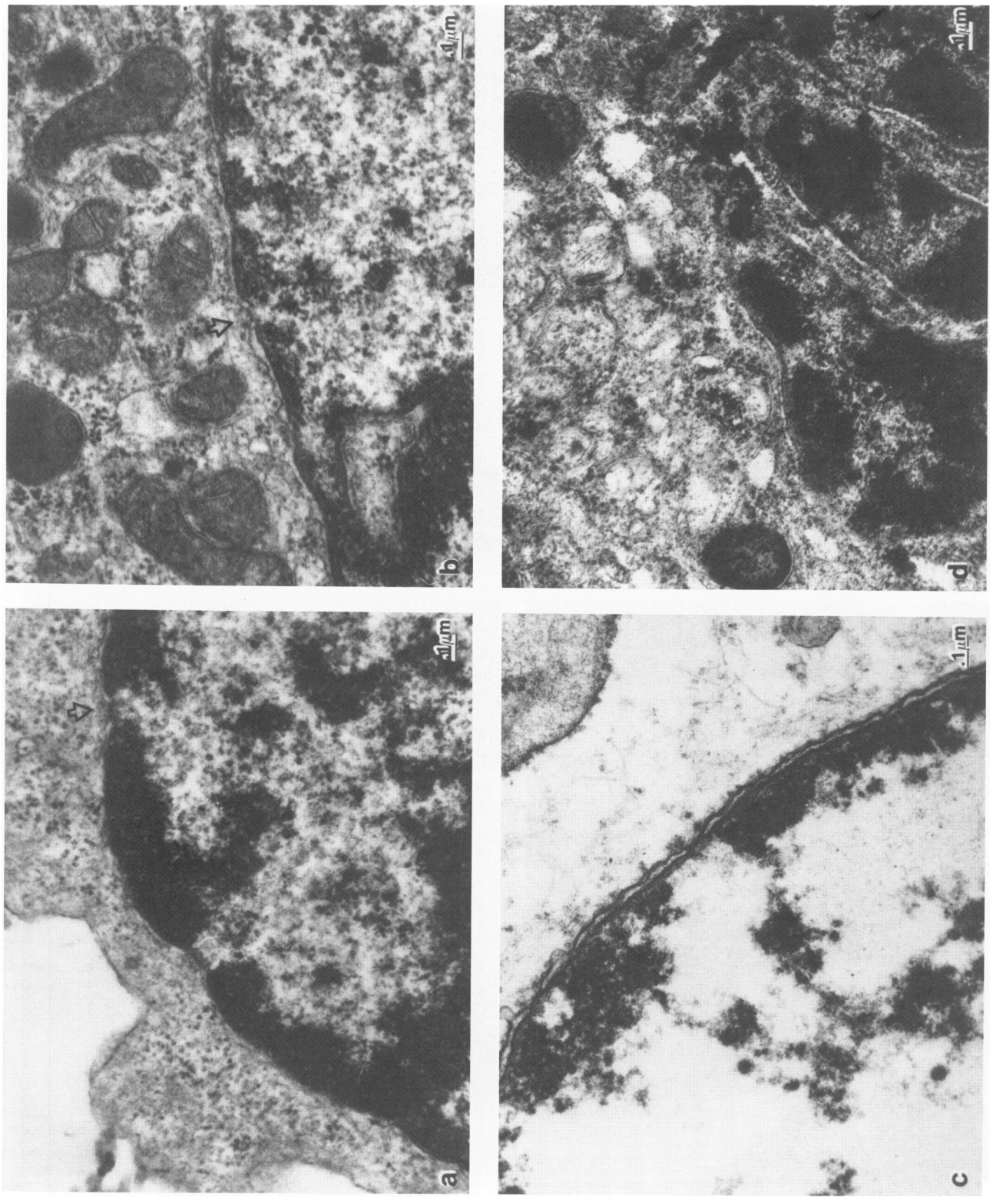
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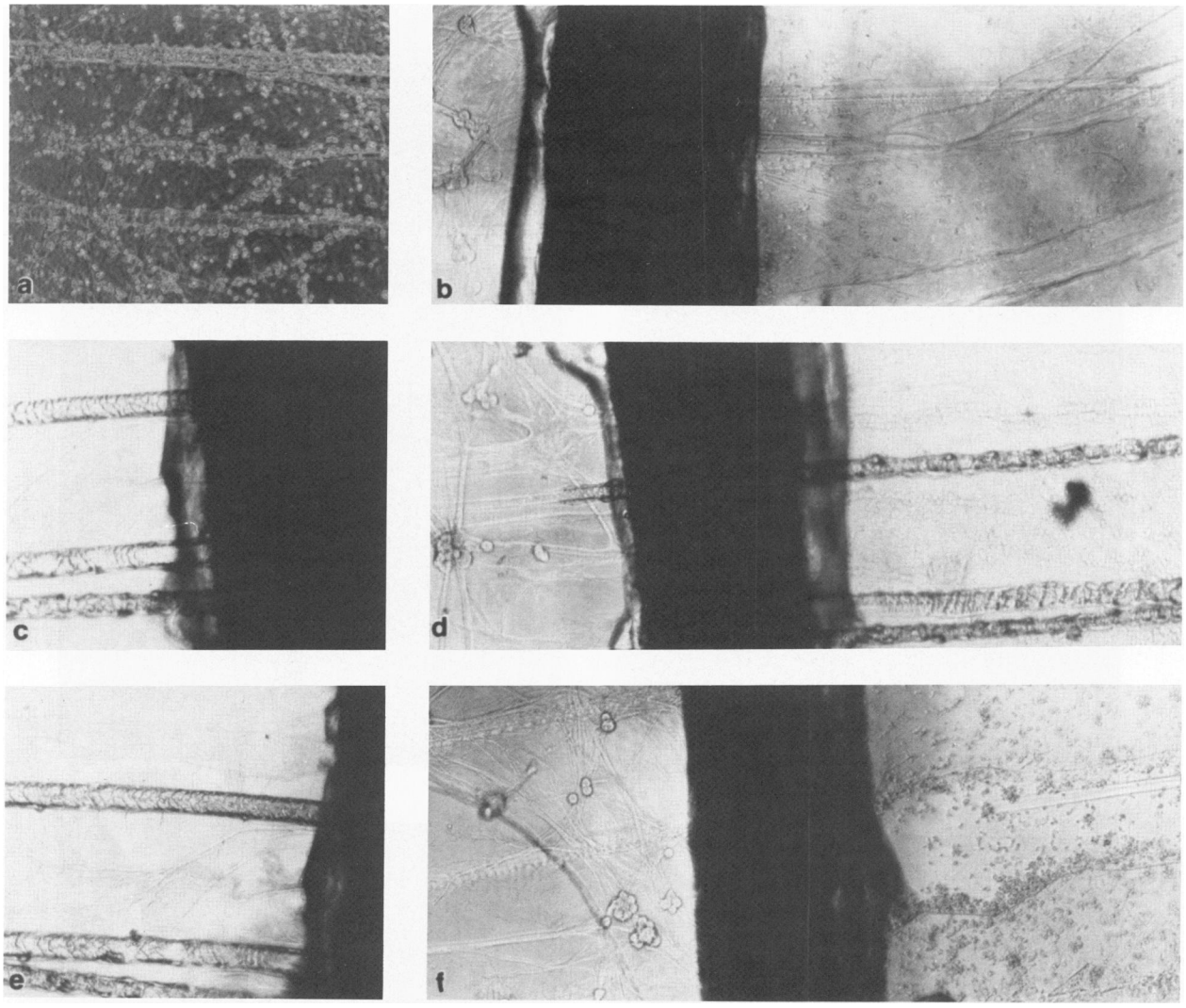
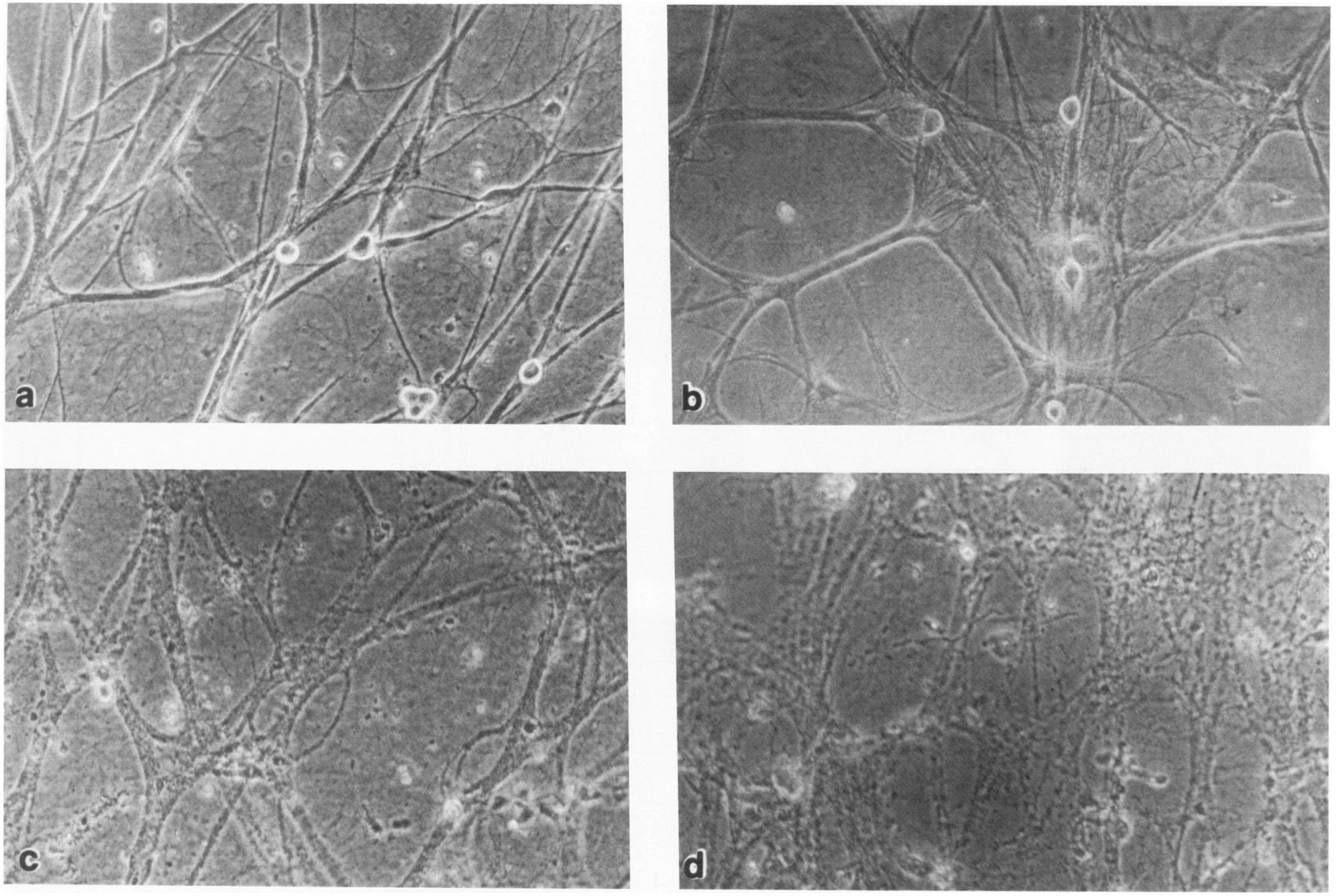


Figure 6—Phase-contrast micrographs of C3H/He neurons grown in Campenot chambers after addition of CTL-3 to either the center (**A**) or side chambers (**B, D,** and **F**). **A**—Center chamber 10 hours after addition of CTLs directly to the center. Note complete neuronal destruction. Center and right side of chamber (black area is the barrier between the chambers under which the neurites grow) 2 (**B**), 10 (**D**), and 26 hours (**F**) after addition of 6×10^5 CTLs to the right side chamber. Note progressive degeneration of neurites in the right side chamber as a function of time. No changes are visible in either the cell bodies within the central chambers or the neurites extending into the left side chambers at 10 (**C**) or 26 hours (**E**) after CTL addition to the right side chamber. (×80)

Figure 5—Nuclear changes occurring in neuronal targets during the CTL lytic process. **A**—CTL-3 with normal-appearing nucleus and nuclear pores (*arrow*). **B**—C3H/He neuron 2 hours after CTL addition; note the normal-appearing nuclear pore (*arrow*) and mitochondria, whereas chromatin has begun to condense. **C**—C3H/He neuron after addition of antibody and complement. Note the absence of cytoplasmic organelles but normal-appearing nuclear pore. **D**—Late changes in CTL lysis of C3H/He neuron. Note the condensed chromatin and irregular nuclear shape. No apparent nuclear pores are visible. (Bars = 1 μ)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striking differences from those following CTL addition. The first observable changes that took effect were swelling of mitochondria (Figure 8B and C, arrows) and the appearance of numerous cytoplasmic vacuoles. Swollen mitochondria were present in the absence of any nuclear changes and did not occur in neurons that were incubated with complement alone (Figure 8A) or with antibody alone (not shown). Nuclear changes, including condensation of chromatin, happened only when marked cytoplasmic changes had occurred such that mitochondria were no longer recognizable and the cytosol was filled with large, numerous vacuoles (Figure 8D). Normal-appearing nuclear pores were still evident at the time of marked cytosolic change (Figure 5C). Thus, the nuclear changes were apparently secondary to the primary cytoplasmic lesion.

Discussion

These experiments make several important points. There have been conflicting reports in the literature about the expression of Class I MHC proteins on neural tissue.[9,11,25] Although we have not made an exhaustive study of different nervous tissues as targets for CTLs which are directed at different parts of the MHC region, it is clear that primary cultures of SCG neurons (nontransformed) do serve as targets for clones whose specificity has been mapped to Class I MHC antigens. Thus, it is probable that neurons, like every other somatic cell examined to date, do express Class I antigens and can serve as targets for traditional CTLs after viral infection or transplantation or possibly as targets of autoimmune destruction.

In addition, we have demonstrated that it is possible to separate the plasma membrane and nuclear lesions within the same cell. Before the nuclear lesion was discovered, it was known that CTLs could inflict membrane damage on enucleated cytoplasts.[26–28] Therefore, whether the membrane damage was a consequence of the nuclear lesion was not an issue. However, an important question remained as to whether the nuclear and membrane lesions were end points of a single or of multiple primary events. The demonstration that the two lesions can be separated in a CTL attack on a single neuron offers the suggestion that multiple pathways of damage are initiated at the site of attack.

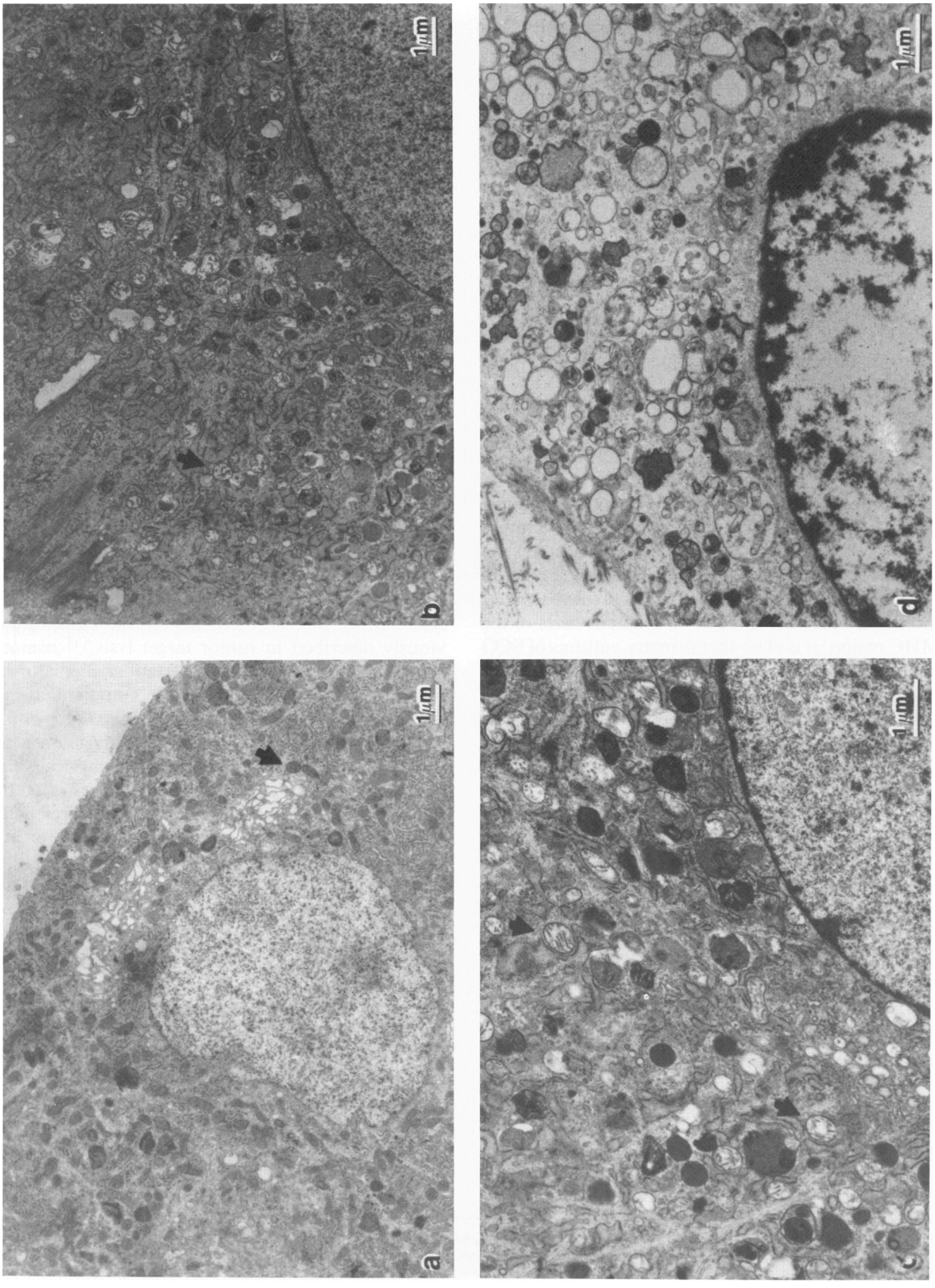
A remaining issue is whether the nuclear lesion is the result of damage by material injected from the CTL into the target or represents an autolytic response of the target after a "signal" from the CTL.[16] Gromkowski et al[23] recently demonstrated that the degree of nuclear damage is a function of the target cell used. The magnitude of DNA damage of a single target cell population was independent of the attacking CTL population. The suggestion of Christiaansen and Sears that the nuclear lesion was a species-specific phenomenon[29] may reflect the relatively small number of target cells tested, rather than a species-restricted effect. In any event, the association of the nuclear damage with target cell type, independent of effector source, suggests that target cell mechanisms, rather than CTL-injected materials, are responsible for the nuclear destruction.

In this report, we have demonstrated that terminally differentiated neurons as well as tumor cells and activated lymphoblasts have the capacity to carry out this nuclear destruction. As has been described earlier with tumor and lymphoblast targets,[6,14] nuclear destruction is minimal in neurons undergoing attack by serum complement even when the attack is directed to the cell body (Figure 8). Similarly, mitochondrial swelling is an early event in complement-mediated lysis but not in CTL-mediated attack. Thus, as previously described in tumor target lysis,[6,14] osmotic changes appear to be less important as mediators of early damage in CTL lysis than in complement-mediated lysis in neuronal targets.

The demonstration that nuclear damage does not occur when the point of CTL attack is physically distanced from the cell body (Figure 6) suggests that the putative signal for nuclear destruction has a restricted range within the target cell cytoplasm. Sanderson and Glauert[4] previously observed cytoplasmic projections from the CTL into the vicinity of the target cell nucleus. We observed similar projections into the neuronal targets (Figure 3). In no case have we or others actually observed direct contact between the CTL membrane and the target cell nucleus, nor is there evidence of secretory activity in this region. Thus, it is difficult to assess the significance of these cytoplasmic projections, but they serve to illustrate the minimal intracytoplasmic distances required for any putative signal or injected material to act on the nucleus.

In conclusion, these experiments demonstrate that neurons in culture can serve as targets for MHC-spe-

Figure 7—Phase-contrast micrographs of C3H/He neurons treated with antibody and complement. **A**—Three hours after the addition of complement alone to C3H/He neurons. No apparent changes are visible. One (**B**), 2 (**C**) and 3 (**D**) hours after addition of antibody and complement. Swelling and lysis (**B**) of neuronal cell bodies precede neuritic degeneration (**C**). No viable neurons are present after 3 hours (**D**). (×160)


1 μm
b
1 μm
d
1 μm
a
1 μm
c

cific CTLs and thus express functional levels of Class I antigens. The signal for complete cell destruction is not retrogradely transported, but is local; and morphologic changes following CTL-neuron interaction are similar to those that occur in dividing tumor target cell populations.

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Figure 8—Electron micrographs of C3H/He neurons 1 hour after addition of antibody and complement. **A**—No changes are visible after addition of, complement alone; mitochondria (*arrow*) appear normal. **B–D**—Neurons at various stages of lysis after addition of antibody and complement. Note swelling of mitochondria (*arrows*, **B** and **C**) and the absence of chromatin condensation until very late in the lytic process (**D**). (Bars = 1 μ)