Apoptosis of odontoclasts under physiological root resorption of human deciduous teeth

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Summary

This study was designed to establish apoptosis of odontoclasts under physiological root resorption of human deciduous teeth. Deciduous teeth were fixed, decalcified, and embedded in paraffin for immunohistochemical (IHC) observations and in Epon for transmission electron microscopy (TEM). Apoptotic cells were identified by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-digoxigenin nick end labeling (TUNEL), and then the tartrate-resistant acid phosphatase (TRAP) activity was determined on the same sections. Epon embedded specimens were sectioned serially into 0.5-μm semithin sections, and some of the sections were re-embedded into Epon, sectioned into 0.1-μm ultrathin sections, and then observed by TEM. By IHC, the nuclei of TRAP-positive odontoclasts on the dentine were generally TUNEL-negative. Around these odontoclasts, there were a few TRAP-positive structures with TUNEL-positive structures: A TRAP-positive structure with one TUNEL-positive nucleus, a TRAP-positive structure with one TUNEL-positive nucleus and one or two TUNEL-negative nuclei, and a TRAP-positive structure without any nucleus. By TEM, some odontoclasts showed nuclear fragments including compacted chromatin. The results suggest that, in apoptosis, odontoclasts fragment into variously sized cellular parts including three or fewer nuclei.

Introduction

It has been generally accepted that cell death of mononuclear cells displays two commonly occurring patterns of morphological change, apoptosis and necrosis (Kerr et al., 1972; Walker et al., 1988). Kerr et al. (1972) defined an ultrastructural feature of cells in apoptosis as the presence of nuclear fragments including compacted chromatin by transmission electron microscopy (TEM). Gavrieli et al. (1992) reported the immunohistochemical (IHC) method for detecting DNA fragmentation of nuclei of cells in apoptosis by using terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-digoxigenin nick end labeling (TUNEL).

Odontoclasts resorbing tooth are cells which have the common morphological features of osteoclasts resorbing bone: multinucleation, the presence of a ruffled border observed by TEM (Scott and Pease, 1956; Ten Care and Anderson, 1986; Marks and Popoff, 1988; Pierce et al., 1991). Although the cellular differentiation of osteoclasts and odontoclasts is well documented (Ejiri, 1983; Takahashi et al., 1988; Sahara et al., 1996; Domon et al., 1997, 1998), the fate of these cells, their apoptosis, has not been documented. Fuller et al. (1993) first reported apoptosis of osteoclasts in vitro by TEM, and Hughes et al. (1995) reported that bisphosphonate promoted apoptosis in murine osteoclasts in vivo and in vitro by the TUNEL method. Vaahtokari et al. (1996) observed apoptosis of mouse osteoclasts on the bone surfaces around the tooth germ by the TUNEL method. Rice et al. (1999) examined whole mount and sectioned calvariae from mice aged between 14th embryonic day and 6th day postnatal by the TUNEL

method, and reported that osteoclasts undergoing apoptosis were relatively rarely detected. Ito et al. (1999) observed apoptosis of rat osteoclasts after administration of bisphosphonate by TEM and the TUNEL method, but they reported that, morphologically, dead or dying osteoclasts were rarely observed in normal bone tissue that was not affected by pathological conditions (in the following termed “normal”). Unlike osteoclasts, apoptosis of odontoclasts is not well documented. There is one report by Watanabe et al. (2000) who observed rabbit odontoclastic apoptosis induced by bisphosphonate administration by TEM and the TUNEL method. They reported that a TUNEL reaction was not observed evenly throughout the nuclei of apoptotic odontoclasts. Therefore, apoptosis of odontoclasts, especially in normal conditions, has not been established.

Odontoclasts at a variety of resorptive stages are observed on the resorbing surfaces of human deciduous teeth under physiological root resorption (Sahara et al., 1996). On the resorbing surfaces there were mononuclear odontoclasts with resorptive ability, and they also participate in the tooth resorption (Domon et al., 1994, 1997, 1998). Therefore, detailed observations of the resorbing surfaces of human deciduous teeth under physiological root resorption suggest a potential site for encountering apoptosis of odontoclasts in normal conditions. The present study aims to establish the apoptosis of odontoclasts under physiological root resorption of human deciduous teeth by TEM and the TUNEL method. In this study, tartrate-resistant acid phosphatase (TRAP) activity which is specific to osteoclasts and odontoclasts (Minkin, 1982;

Domon et al., 1994; Sahara et al., 1996) was used for the identification of odontoclasts.

Materials and methods

Deciduous tooth

Twenty four deciduous teeth from 6-10-year-old males and females were used in this study. After obtaining informed consent from all patients and their parents for the use of the teeth in this study, the teeth were extracted under local anesthesia at Hokkaido University Hospital (Sapporo, Japan). After extraction, ten specimens were immediately fixed with 4% formaldehyde in 0.1M phosphate buffer (pH 7.4) overnight at 4 °C for IHC, and fourteen specimens were fixed with 2% formaldehyde and 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4) overnight at 4 °C for light microscopic (LM) and TEM observations. Specimens were then decalcified with 5% EDTA (pH 7.4) for two months at 4 °C. The research protocol for the present study was approved by the ethics committee of Hokkaido University Graduate School of Dental Medicine.

Immunohistochemistry (IHC)

Specimens for IHC observations were dehydrated in a graded series of ethanol, and then embedded in paraffin. The specimens were cut into 7-μm sections in the direction perpendicular to the resorbing surface on a microtome. Apoptotic cells were identified by the modified TUNEL method (Gavrieli et al., 1992) using an Apop Tag Peroxidase In Situ Apoptosis Detection Kit (Chemicon, USA). According to the guidelines for the

TUNEL method, after deparaffinization the sections were treated with 100% ethanol for 10 minutes at room temperature and then placed in 95% ethanol, 70% ethanol, and phosphate-buffered-saline (PBS) for 5 minutes, respectively. Proteinase K (20 μg/ml) treatment was performed at room temperature for 15 minutes before treating with 3.0% H_2O_2 in PBS for 5 minutes. After rinsing with PBS, equilibration buffer in the kit was applied on the sections for 30 seconds at the room temperature. The prepared sections were incubated with TdT and dUTP-digoxigenin for 1 hour at 37 ℃. After rinsing with PBS, anti-digoxigenin-peroxidase sheep polyclonal antibody was applied for 30 minutes at room temperature. After rinsing with PBS, the reaction was visualized by diaminobenzidine tetrahydrochloride (DAB) (DAKO JAPAN, Japan). Positive control was included by treating four number of sections with DNase following proteinase K treatment in each of specimens. Negative control sections were incubated with distilled water in the absence of TdT.

After detection of apoptotic cells by the TUNEL, the TRAP activity was histochemically detected with the azo dye method (Burston, 1958) on the same sections. Paraffin sections were incubated in 0.1M acetate-buffered medium (pH 5.2) containing naphtol AS-MX (Sigma, USA) as a substrate, and fast blue BB salt (Sigma) as a diazonium chloride, and 50mM L(+) sodium tartrate (Sigma). After the detection of TRAP activity, sections were photographed with a light microscope (NIKON FX-A, Japan).

Light microscopy (LM) and transmission electron microscopy (TEM)

After decalcification, following the method mentioned above, TRAP activity was detected with fast red violet as a diazonium chloride for the identification of odontoclasts on specimens for LM and TEM observations. After observing the sites of TRAP-positive cells on the resorbing surfaces, specimens with TRAP-positive cells were photographed by LM. Specimens were postfixed with 1% osmium tetroxide in 0.05M sodium cacodylate buffer (pH 7.4) for 3 hr at room temperature. They were block-stained with 4% uranyl acetate for 30 min, dehydrated in a graded series of ethanol, and then embedded in Epon 812.

Two specimens chosen at random were sectioned with diamond knives on a Reichert-Nissei ultramicrotome into 0.5-μm semithin sections. The sections were stained with toluidine blue, and observed by LM. After observation of the sections, 0.1-μm ultrathin sections were sectioned with diamond knives on an ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a TEM (HITACHI H-7000, Japan) at an operating voltage of 75kV.

Four specimens chosen at random were serially sectioned with diamond knives on a Reichert-Nissei ultramicrotome into 0.5-μm semithin sections. The sections were placed on glass slides and then stained with toluidine blue, and observed by LM at a magnification of x125 under oil-immersion. Some sections were used for further TEM investigation.

Re-observation of semithin sections by TEM

Selected 0.5-µm semithin sections were observed by TEM after the LM observations. These sections on the glass slides were dehydrated in a graded series of ethanol, and the sections were placed in the BEEM capsules (NISSIN EM, Japan) filled with Epon 812. After polymerization of the Epon, the BEEM capsules including the sections were removed from the surfaces of the slide glasses. The 0.5-µm semithin sections re-embedded in the Epon of the BEEM capsules were sectioned into 0.1-µm ultrathin sections in the direction parallel to their surfaces with diamond knives on an ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by TEM.

Three-dimensional reconstruction (3DR)

An odontoclast that appeared to be in the process of apoptosis (shown in Figure ~~11~~5) was selected for 3DR investigation. The photographs of the serial sections of the cell were enlarged on printing paper; and the cell surface, dentine surface, and nucleus were traced on tracing paper. Based on both the outline of the section and round cells which were identified as leukocytes and/or lymphocytes, these elements were input into a personal computer (NEC PC-9801 DX) with the Three-Dimensional Graphic Analytic System, COSMOZONE 2SA (NIKON, Japan). The reconstructed image was directly photographed from the computer display.

Results

Immunohistochemistry (IHC)

Odontoclasts were observed on the root dentine surfaces in four deciduous teeth of ten investigated ones. Odontoclasts were multinucleated cells and usually fitted into the resorptive lacunae on the dentine. Odontoclasts were TRAP-positive but their nuclei were TUNEL-negative (Fig. 1a~~1~~). Around odontoclasts, there were a few TRAP- and TUNEL-positive structures: A TRAP-positive structure with one TUNEL-positive nucleus (Fig. 1b), and a TRAP-positive structure with one TUNEL-positive nucleus and one or two TUNEL-negative nuclei ~~(Figs. 2 and 3)~~ (Fig. 1c). These TRAP- and TUNEL-positive structures were apart from the dentine surfaces and some of them were observed near blood vessels ~~(Fig. 3)~~. There were also TRAP-positive structures without any nucleus ~~(Fig. 4)~~ (Fig. 1d). Macrophages phagocytosed small TRAP- and TUNEL-positive structures in the cell bodies, and some of these cells were observed in blood vessels (Figs. 1d and 1e~~4 and 5~~). The TRAP- and TUNEL-positive structures and TRAP-positive structures without any nucleus showed a variety of the staining of TRAP, and there was no apparent difference between the TRAP staining of odontoclasts and that of TRAP- and TUNEL-positive structures (Fig. 1~~s. 1–5~~).

LM, TEM, and 3DR

With a whole-mount light microscopy, numerous TRAP-positive cells with

different sizes and shapes appeared red on the resorbed surfaces in six teeth of fourteen investigated ones (Fig. 2a~~6~~). To understand the ultrastructure of these TRAP-positive cells, six teeth with TRAP-positive cells were used for further investigation.

On 0.5-µm semithin sections, odontoclasts were observed on the resorbed surfaces. The cells were multinucleated and formed resorptive lacunae on the dentine surfaces under light microscopic brush borders (Fig. 2b~~7~~). There were often odontoclasts with single nucleus on a section, and they also formed resorptive lacunae on the dentine surfaces under brush borders (Fig. 3a~~8~~). On 0.1-µm ultrathin sections, odontoclasts had electron microscopic ruffled borders and clear zones facing the resorptive lacunae, and in the cytoplasm there were several nuclei with Golgi apparatus surrounding these, as well as numerous mitochondria, rough-surfaced endoplasmic reticulum (rER), and vacuoles (Fig. 3b~~9~~).

Figure 4~~10~~ shows serial semithin sections of an odontoclast. The odontoclast had irregular contours fitting into the resorptive lacuna on the dentine surfaces but did not have a brush border. The cell had cytoplasm darkly stained with toluidine blue and there were many vacuoles. A pycnotic nucleus was observed in the cytoplasm but other nuclei in the cell did not show a pycnotic shape. Adjacent to the odontoclast, there was an apoptotic body that had a nuclear fragment with a peripheral crescent of compacted chromatin, which was darkly stained with toluidine blue. A leukocyte was in close contact with the cell surfaces of the odontoclast. Observations of serial sections showed that the apoptotic body connected with the odontoclast. The cell had six nuclei

including one pycnotic nucleus and there were two narrowed parts of the cell body (Figs. 4a and 4d~~Fig 10b and 10d~~).

Figure 5~~11~~ shows serial semithin sections of an odontoclast. The odontoclast was situated on the dentine but did not form a resorptive lacuna, and the cell had cytoplasm darkly stained with toluidine blue and there were many vacuoles. Adjacent to this cell, there was a structure that had features similar to that of the odontoclast, and this structure was away from the dentine surfaces. Observations of serial sections showed that the odontoclast connected with a structure similar to the odontoclast. The cell had three nuclei in the cytoplasm and none of the nuclei showed a pycnotic shape. Leukocytes were in close contact with the cell surfaces of the odontoclast. The TEM image showed that the cytoplasm was condensed and there were small protuberances or microvillus-like structures on the cell surfaces (Fig. 6~~s. 12 13~~). The cellular organelles, like mitochondria and rER, were well preserved, and there were many vacuoles in the cytoplasm. The 3DR image of the odontoclast showed that a large pedunculated protuberance extended from the cell body on the dentine surfaces (Fig. 7~~14~~).

Figure 8~~15~~ shows serial semithin sections of an apoptotic body of an odontoclast. Observations of the serial sections showed that the apoptotic body had rounded contours and was away from the dentine surface. The apoptotic body had many vacuoles and a nuclear fragment-like structure in the cytoplasm which was darkly stained with toluidine blue. The TEM image showed that a nuclear fragment-like structure in the apoptotic body was characteristic compacted chromatin (Fig. 9~~16~~). The cell organelles

of the apoptotic body were indistinct and many electron dense structures were observed here. A leukocyte was in close contact with the apoptotic body and phagocytosed small nuclear fragment-like structures in a lysosome.

Figure 10~~17~~ shows serial semithin sections of an apoptotic odontoclast with severe cellular degeneration. The odontoclast was situated away from the dentine surface. Observations of serial sections showed that this cell had one nucleus, structures strongly stained with toluidine blue, and some lightly stained with toluidine blue. The TEM image showed that the odontoclast had an intact nucleus, and that the structure strongly stained with toluidine blue was a characteristic nuclear fragment with compacted chromatin, and that structures lightly stained with toluidine blue were degenerated nuclear fragments (Fig. 11~~18~~). In the cell, the structures of cell organelles were indistinct. On the cell surfaces, several pedunculated protuberances including small degenerated nucleus-like structures can be observed. No special structures between the juxtaposed inner membranes of nuclei were observed in this cell.

214 odontoclasts were investigated in this study, and 3.7% had features of apoptosis.

Discussion

The present study observed odontoclasts under physiological root resorption of human deciduous teeth, and showed the presence of TRAP- and TUNEL-positive structures by IHC and odontoclasts with nuclear fragments with compacted chromatin by TEM. These findings show the characteristic features of DNA fragmentation of a nucleus in apoptosis by TEM (Kerr et al., 1972) and by IHC (Gavrieli et al., 1992). Therefore, the study here is the first to report the apoptosis of odontoclasts in normal conditions. The sequence of changes in apoptosis of odontoclasts in normal conditions will be discussed.

The present results showed that the TRAP-positive structures had a TUNEL-positive nucleus and one or two TUNEL-negative nuclei by IHC. By TEM, an apoptotic odontoclast had all of an intact nucleus, degenerated nuclei, and nuclear fragments with compacted chromatin in the cytoplasm. These findings indicate that not all nuclei of multinucleated odontoclasts in apoptosis show a similar appearance of DNA degeneration. Honma and Hamasaki (1996) have reported that multinucleated giant cells in foreign-body glanuloma show differences in the apoptotic changes occurring in individual nuclei. Watanabe et al. (2000) observed rabbit odontoclastic apoptosis induced by bisphosphonate administration and speculated that the degeneration of nuclei may not be synchronous but occur independently in apoptosis. In agreement with these reports, the present results suggest that the degeneration of nuclei occurs independently in a multinucleated odontoclast in apoptosis. The reasons for this

will be considered next.

It is well known that osteoclasts increase the number of nuclei by cell fusion of mononuclear precursors (Baron et al., 1984, 1986). Under physiological root resorption of human deciduous teeth, we have reported that odontoclasts increase the number of nuclei by cell fusion and this fusion occurs among various odontoclasts with different numbers of nuclei including mononuclear odontoclasts (Domon et al., 1998). Accordingly, it appears that older and newer nuclei are mixed simultaneously in an odontoclast after cell fusion of newly recruited mononuclear precursors. This suggests that the ages of different nuclei in an odontoclast are various. It may be that there is a relationship between the age of a nucleus and nuclear degeneration in apoptosis of multinucleated odontoclasts, however this cannot be substantiated, as there is no data of the age of nuclei.

The 3DR image of the osteoclast in this study shows that a large pedunculated protuberance extended from the cell body on the dentine surfaces. The TEM image of this cell shows that the cytoplasm was condensed and there were small protuberances or microvillus-like structures on the cell surfaces although such structures were not observed on the cell surfaces of odontoclasts resorbing the dentine. It has been reported that at the earliest stages of apoptosis of mononuclear cells, the cytoplasm is beginning to compact. This change is followed by further condensation of the cytoplasm and convolution of the nuclear and cell outlines. Rapid progression of convolution of nuclear outlines and cell ones culminates in nuclear fragmentation and separation of

protuberances respectively, and finally membrane-bounded apoptotic bodies develop on the cell surface (Walker et al., 1988). According to this sequence, it appears that microvillus-like structures on the cell surfaces result from a condensation of the cytoplasm in apoptosis. It also appears that the 3DR image of a large pedunculated protuberance extending from the cell body of the odontoclast shows convolution of the cell outline and that this protuberance will become an apoptotic body after separating from the cell body. Therefore, it is suggested that microvillus-like structures on the cell surfaces are TEM features of the earliest stages of apoptosis in multinucleated odontoclasts in normal conditions.

It has been reported that cell organelles in apoptotic bodies remain well preserved after budding from the cell body (Walker et al., 1988). The present study showed that cell organelles in odontoclasts of the earliest stages of apoptosis remain preserved but that the ones in both apoptotic bodies of odontoclasts and apoptotic odontoclasts with severe cellular degeneration were indistinct. These results may suggest that cell organelles at the early stages of apoptosis in odontoclasts remain well preserved but that at later stages of apoptosis they gradually become indistinct, although we observed only few cases of this in this study.

Ito et al. (1999) examined apoptotic osteoclasts of rats after administration of bisphosphonate by TEM and observed that there were ladder-like structures with striations between the juxtaposed inner membranes of nuclei. Watanabe et al. (2000) also observed such structures in apoptotic odontoclasts of the rabbit after administration

of bisphosphonate by TEM. However, the present study did not observe such structures in odontoclasts in apoptosis. The ladder-like structures between the juxtaposed inner membranes of nuclei may be a specific feature of osteoclasts and/or odontoclasts in apoptosis after administration of bisphosphonate.

The present results showed that around resorbing odontoclasts on the dentine surfaces there were a few TRAP- and TUNEL-positive structures. The significance of these structures will be considered here. It has been reported that TRAP activity is a specific marker for human odontoclasts (Domon et al., 1994; Sahara et al., 1996). This indicates that the TRAP-positive structures with a TUNEL-positive nucleus as shown in this study are apoptotic odontoclasts. This leads a hypothesis that only odontoclasts with three or fewer nuclei may be subject to apoptosis under physiological root resorption. However, it has been reported that the majority of human odontoclasts are cells with ten or fewer nuclei (Domon et al., 1997). Therefore, it is necessary to consider why only odontoclasts with three or fewer nuclei appear to be involved in apoptosis.

The present results showed that TRAP-positive structures without a nucleus were situated away from the dentine surfaces on the sections. As TRAP activity is a specific marker for human odontoclasts, these TRAP-positive structures may be cellular parts sectioned in the sites of the cell bodies of odontoclasts without nucleus. However, such TRAP-positive structures were rarely observed on the dentine surfaces. In this study, the 3DR image of the odontoclast at the earliest stages of apoptosis showed that a large pedunculated protuberance extended from the cell body on the dentine surfaces. This

protuberance would be an apoptotic body that is separated from the cell body. Considering the presence of this protuberance, it is suggested that in apoptosis odontoclasts fragment into variously sized cellular parts including three or fewer nuclei. This is a possible explanation why there were a TRAP-positive structure with one TUNEL-positive nucleus, a TRAP-positive structure with one TUNEL-positive nucleus and one or two TUNEL-negative nuclei, and a TRAP-positive structure without any nucleus as observed by IHC in this study.

Boyce et al. (1995) reported that the intensity of TRAP staining in apoptotic osteoclasts was consistently stronger than that in viable osteoclasts. They considered that this intensity of TRAP in apoptotic osteoclasts might be a reflection of cytoplasmic condensation during cell death or could be due to increased production or decreased secretion of TRAP enzyme by the cells. Rice et al. (1999) observed that some osteoclasts were TRAP- and TUNEL-positive but many TUNEL-positive osteoclasts were TRAP-negative. They considered this reason as follows: dying osteoclasts would cease to function and thereby stop producing TRAP enzyme. In the present study, apoptotic bodies of odontoclasts showed a variety of the staining of TRAP and there was no apparent difference between the TRAP staining of resorbing odontoclasts and that of apoptotic cells. These results may suggest that the intensity of TRAP staining shows a reflection of cytoplasmic condensation at the early stage of apoptosis, and that the fading of TRAP staining is due to ceasing or decreasing TRAP enzyme during cell death.

It is generally accepted that apoptotic bodies are rapidly phagocytosed by macrophages and they are digested within lysosomes (Kerr et al., 1972; Walker et al., 1988). The present results showed that macrophages phagocytosed small TRAP- and TUNEL-positive structures, and that leukocytes phagocytosed small nuclear fragment-like structures in lysosomes. These leukocytes were in contact with apoptotic bodies of odontoclasts. These findings indicate that apoptotic bodies of odontoclasts are phagocytosed by macrophages as well as leukocytes. Therefore, under physiological root resorption of human deciduous teeth, it is suggested that phagocytosis of mononuclear phagocytic cells is the main route for elimination of apoptotic bodies of odontoclasts. The present results showed some macrophages phagocytosing apoptotic bodies of odontoclasts in blood vessels. This means that some macrophages migrate into blood vessels after phagocytosing apoptotic bodies of odontoclasts, and it suggests a minor route for removal of apoptotic bodies of odontoclasts to eliminate them quickly from the dentine surfaces. It has been reported that apoptotic bodies without phagocytosis degenerate spontaneously (Searle et al., 1975). The results here also suggest that apoptotic bodies of odontoclasts without phagocytosis may spontaneously degenerate. There is no information of the life span of odontoclasts in normal conditions. To clarify the details of apoptosis of odontoclasts under physiological root resorption of human deciduous teeth, elucidation of the life span of odontoclasts will be necessary.

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Legends

Fig. 1. Light micrographs showing odontoclasts ~~(Ocl)~~ and various TRAP-positive structures on the dentine surface (~~D~~d). Odontoclasts (ocl) show ~~as~~ TRAP-positive (blue), but the nuclei are TUNEL-negative. Around the odontoclasts, there are a few TRAP- and TUNEL-positive (brown) structures (small arrows) (a: ~~.~~ x 400, ~~20.~~ bar = 20 µm.). There are a TRAP-positive structure with one TUNEL-positive nucleus (arrows~~b~~) (b), and a TRAP-positive structure with one TUNEL-positive nucleus and one TUNEL-negative nucleus (nu) near blood vessel (~~v~~v) (~~e~~c). There is also a TRAP-positive structure without any nucleus (arrow head, ~~Fig. 4~~d). Macrophage (mp~~M~~p) phagocytoses TRAP- and TUNEL- positive structures in the cell body (~~Fig. 4~~d), and one such cell is observed in a blood vessel (~~Fig. 5~~e) (b-d: x 680, bar = 10 µm).

Fig. ~~6~~2. Light micrographs showing TRAP-positive cells and odontoclasts on the resorbed surfaces of a lower first deciduous molar. Whole mount preparation show~~sing~~ many TRAP-positive cells (red spots) ~~activity. Red spots~~ with various sizes and shapes ~~are TRAP-positive cells~~on the root dentine surfaces (d) (~~.~~a: x45, bar = ~~–~~200 µm). ~~–~~A semithin section of TRAP-positive cells on the root dentine surfaces in Fig. 2a shows that ~~most~~ odontoclasts (ocl~~ocl~~) with several nuclei (nu~~nu~~) have light microscopic brush borders (bb~~bb~~) facing resorptive lacunae (asterisk~~s~~) on the dentine (d~~d~~). The cells contain many darkly stained granules scattered in the cytoplasm, and vacuoles are seen

near brush borders (b: x 830, bar = 12 µm).

Fig. 3s. 7-8. MLight micrographs showing an odontoclast onin the resorbed surfaces in Fig.ure 2a6. A semithin section shows that an odontoclast (ocl) with single nucleus has brush border (bb) facing resorptive lacuna (asterisk) on the dentine (d) (a: x 830, bar = 12 µm).- An ultrathin section shows that the cell has nucleus, and the ruffled border (rb) and clear zone (cz) are seen at the dentine surface. There is a small resorptive lacuna (asterisk) under the ruffled border. Endoplasmic reticulum (erER), Golgi apparatus (goGo), many mitochondria (mt(Mt) and vacuoles (vV) are observed in the cytoplasm (b: x 4600, bar = 4 µm).

Fig. 410. Serial semithin sections showing an odontoclast with an apoptotic body: **a-f** respectively, 5.0, 7.0, 10.0, 15.5, 20.0, and 26.5 µm from the cell surface. The apoptotic body (**c**, arrow head) connects with the odontoclast (**a-c**, arrows). The odontoclast has irregular cellular contours and there are two narrow parts of the cell body (between arrows, **b, d**). The odontoclast fits with the resorptive lacuna (asterisks) on the dentine surface (Dd) but there is no brush border. A pycnotic nucleus (**b**, nuNu) is observed in the cytoplasm but other nuclei show no pycnotic shape. Leukocyte (lL) is in contact with the cell surface. x 11001500,- bar = 10 µm.

Fig. 5.11. Serial semithin sections showing an odontoclast: **a-f** respectively, 2.5, 12.0, 16.0, 23.5, 27.5, and 35.0 µm from the cell surface. Two white arrows (**a**, **f**)

indicate the edges of the odontoclast. This odontoclast does not form a resorptive lacuna on the dentine (~~D~~d), and the cell has the cytoplasm darkly stained with toluidine blue and there are many vacuoles. Adjacent to this odontoclast, there is a structure that had features similar to that of the odontoclast (arrows, **d**, **e**). Leukocytes (~~L~~l) are in contact with the cell surfaces of the odontoclast. Two boxed areas (**d**) indicate the sites of the TEM micrographs shown in Figs. 6a~~12~~ and 6b~~13~~. ~~—~~x 860,~~1200.~~ bar = 10 µm.

Fig. 6~~s. 12 and 13~~. Electron micrographs showing~~of~~ the boxed areas in Fig. 5d.~~Fig. 11d.~~ Both an odontoclast on the dentine (d) and a structure similar to the odontoclast have condensed cytoplasm with many vacuoles and there are small protuberances ~~(Fig. 12)~~ or microvillus-like structures ~~(Fig. 13)~~ on the cell surfaces. The cell organelles are well preserved. Insert: boxed area in Fig. 6a ~~in Fig. 12~~ enlarged. ~~—~~x 5000,~~4900.~~ bar = 2 µm, Insert: x 13400~~13000~~.

Fig. 7.~~14.~~ Model showing the three-dimensional reconstruction of the odontoclast and a structure similar to the odontoclast in Fig. 5~~11~~. This image consists of the dentine (~~D~~d, yellow), cellular surfaces (blue), and the nuclei (red). The odontoclast extends a large pedunculated protuberance (arrow) without a nucleus. The three nuclei (~~Nu~~nu) are scattered in the cell body on the dentine. ~~–~~x 1200,~~1600.~~ bar = 5 µm.

Fig. 8.~~15.~~ Serial semithin sections showing an apoptotic body of an odontoclast:

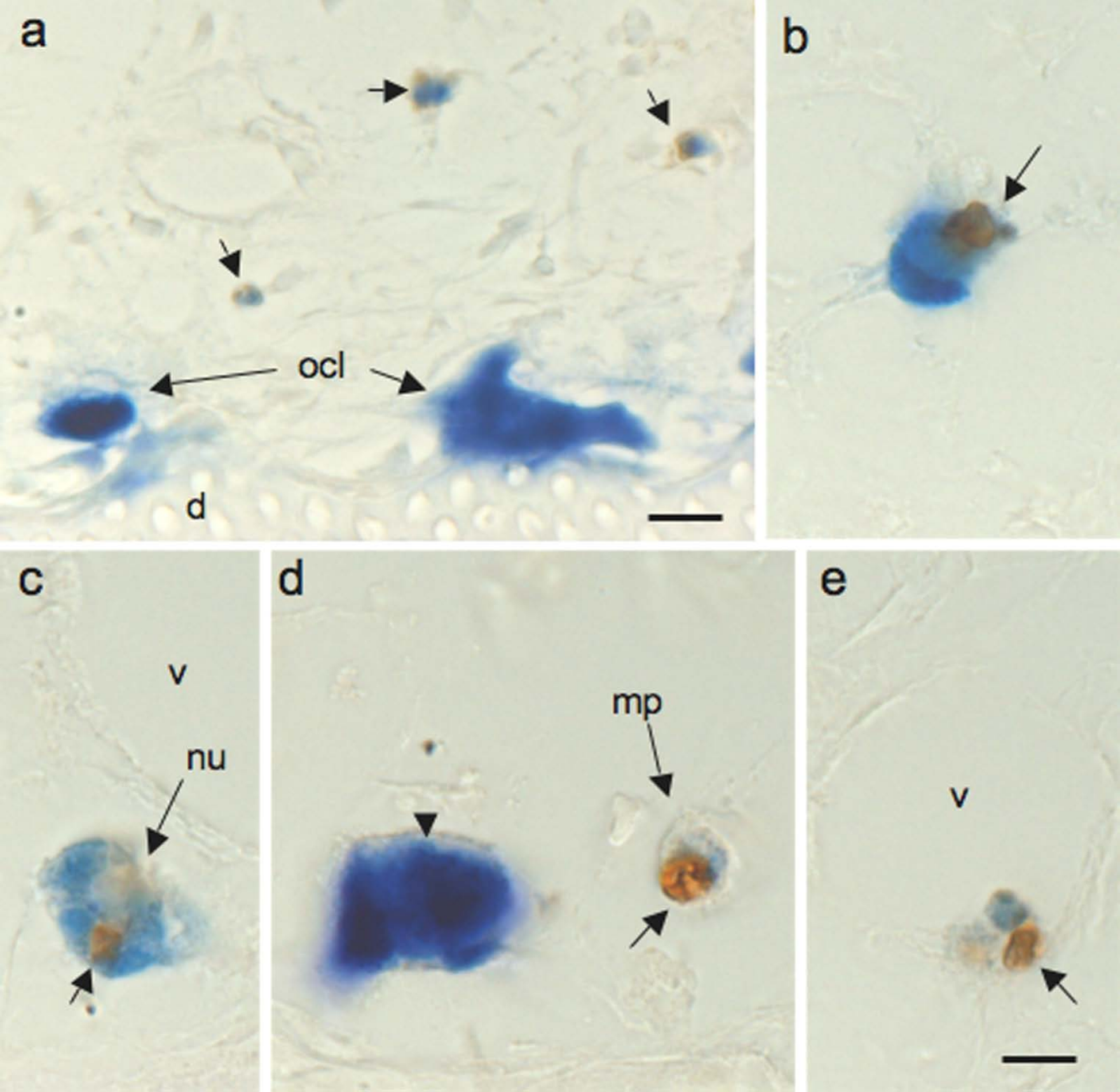
a-f respectively, 3.5, 4.5, 5.5, 6.5, 9.0, and 13.0 µm from the cell surface. The apoptotic body is away from the dentine surfaces (~~D~~d) and has a nuclear fragment-like structure (arrows) and many vacuoles in the cytoplasm. A leukocyte (~~L~~l) is in contact with the apoptotic body. The boxed area (**e**) indicates the site of the TEM micrograph shown in Fig. 9~~16~~. –x 1300~~1500~~,~~.~~ bar = 10 µm.

Fig. 9.~~16.~~ Electron micrograph showing~~of~~ the boxed area in Fig. 8e~~15e~~. The apoptotic body has a nuclear fragment (nu~~Nu~~) with compacted chromatin (arrows). The cell organelles are indistinct and there are many electron dense structures. A leukocyte (l~~L~~) is closely in contact with the apoptotic body and phagocytoses a small nuclear fragment-like structure (arrowhead) in a lysosome. x 8900,~~8500.~~ bar = 1 µm.

Fig. 10.~~17.~~ Serial semithin sections showing an apoptotic odontoclast with severe cellular degeneration: **a-f** respectively, 0.5, 5.5, 10.5, 12.0, 17.5, and 20.0 µm from the cell surface. The odontoclast was situated away from the dentine surface (d~~D~~), and the cell has one intact nucleus (~~Nu~~nu), structures strongly stained with toluidine blue (small arrows) and lightly stained ones (white arrows). Large arrows (**a**, **f**) indicate the edges of the cell. The boxed area (**c**) indicates the site of the TEM micrograph shown in Fig. 11~~18~~. –x 970, ~~1200.~~ bar = 10 µm.

Fig. 11.~~18.~~ Electron micrograph showing~~of~~ the boxed area in Fig. 10c~~17c~~. The

odontoclast has nucleus (nu~~Nu~~), a nuclear fragment with compacted chromatin (arrow), and degenerated nuclear fragments (arrowheads). In this cell, cell organelles are indistinct. On the cell surfaces, several pedunculated protuberances including small degenerated nucleus-like structures (asterisks) can be observed. x 6300,~~7600.~~ bar = 2 µm.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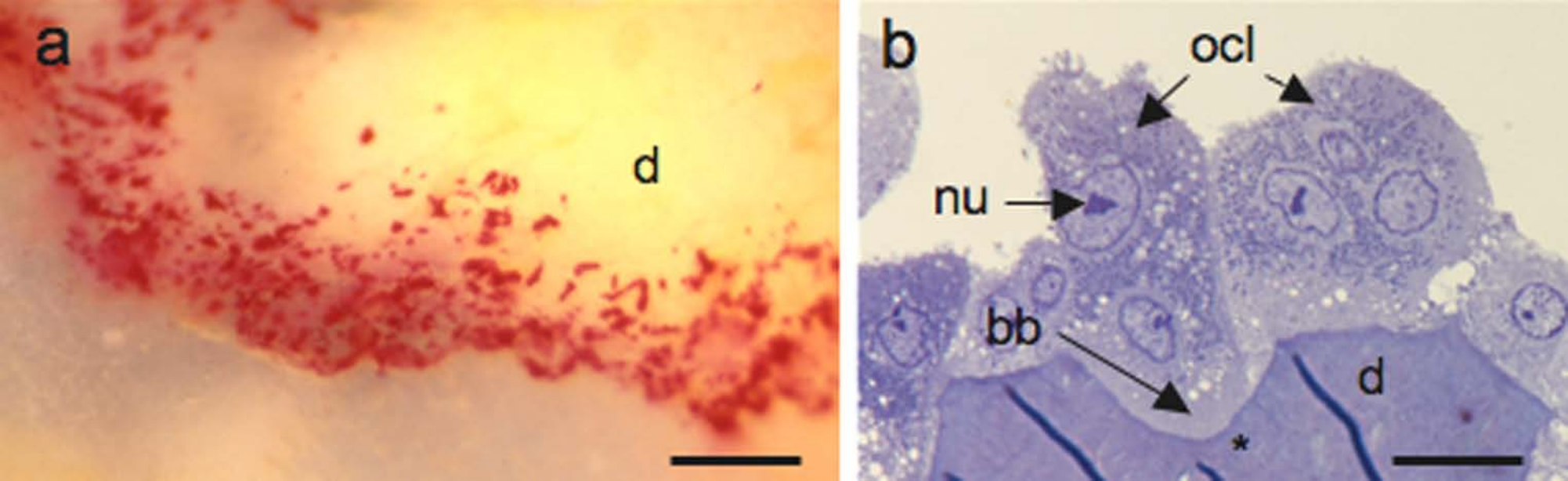
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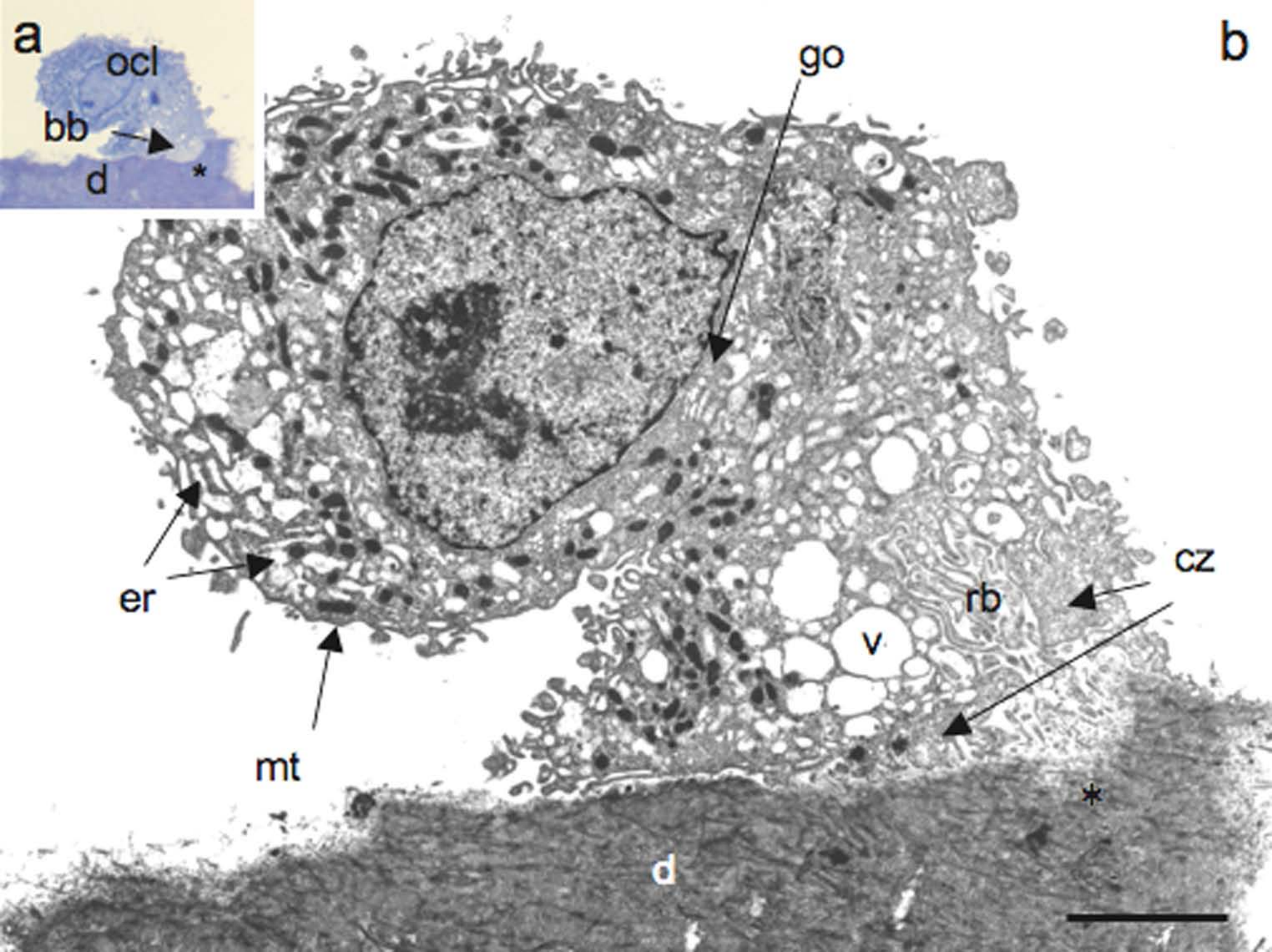
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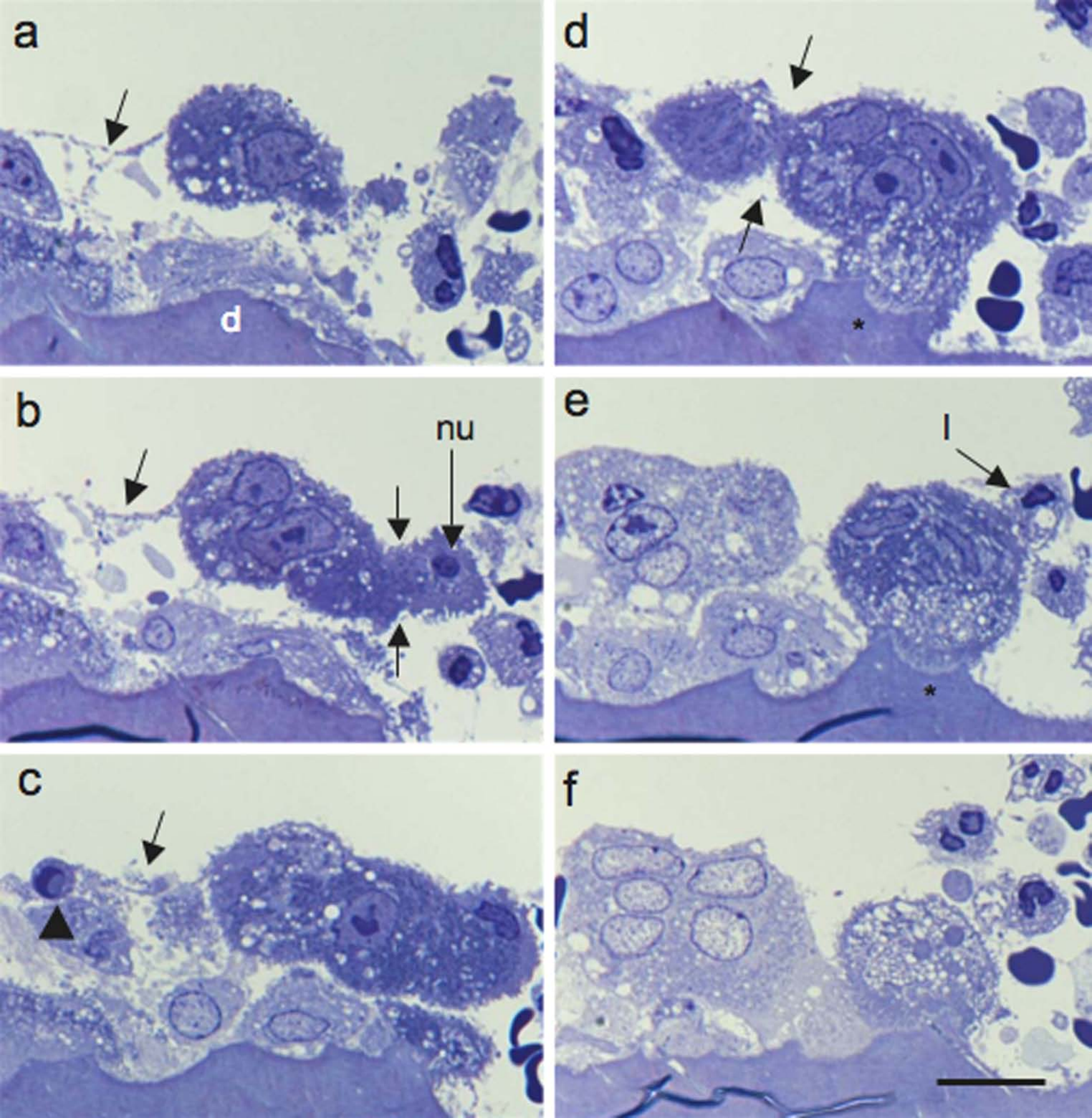
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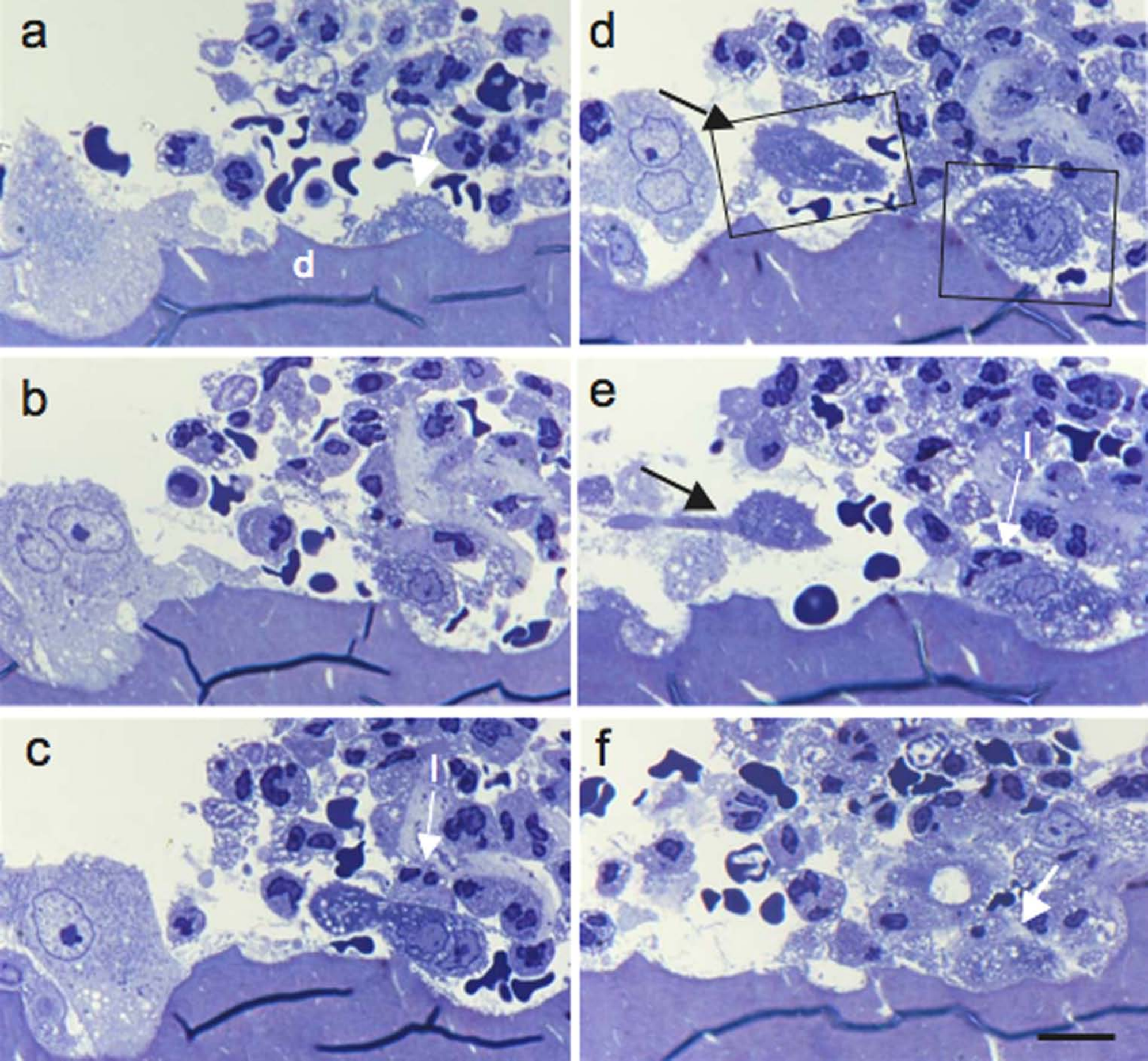
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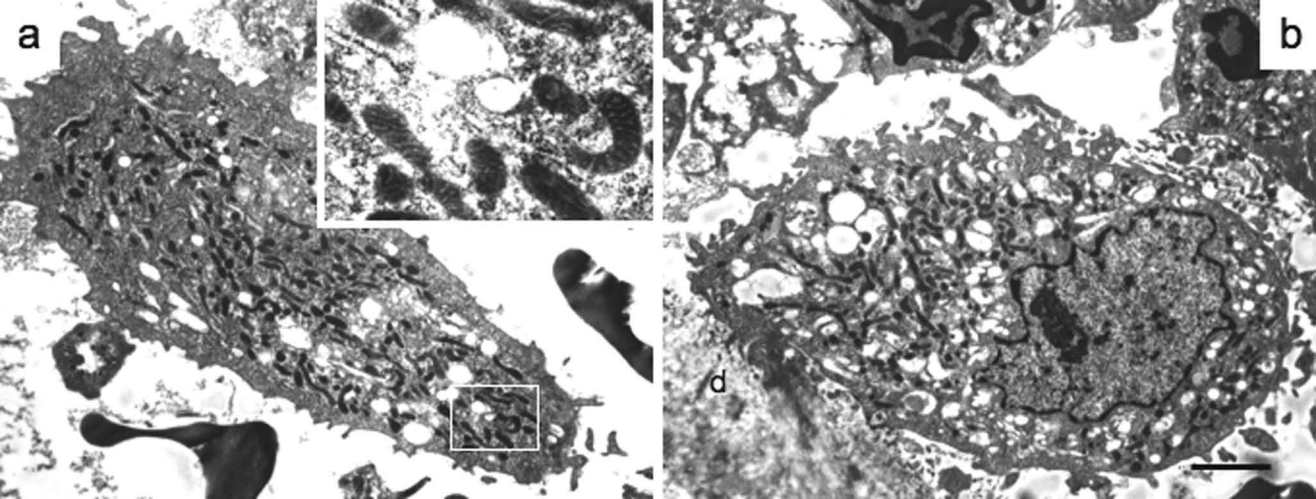
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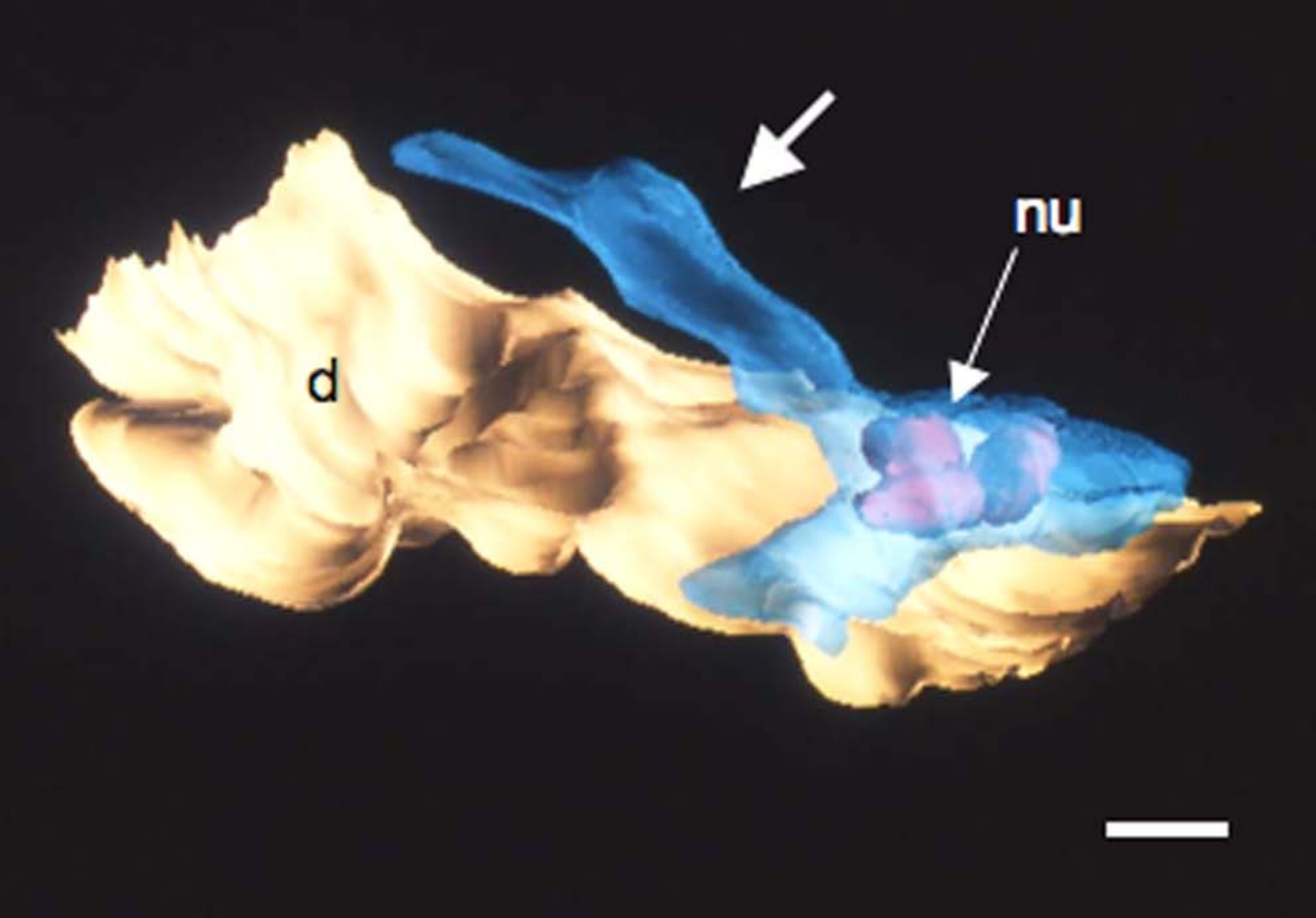
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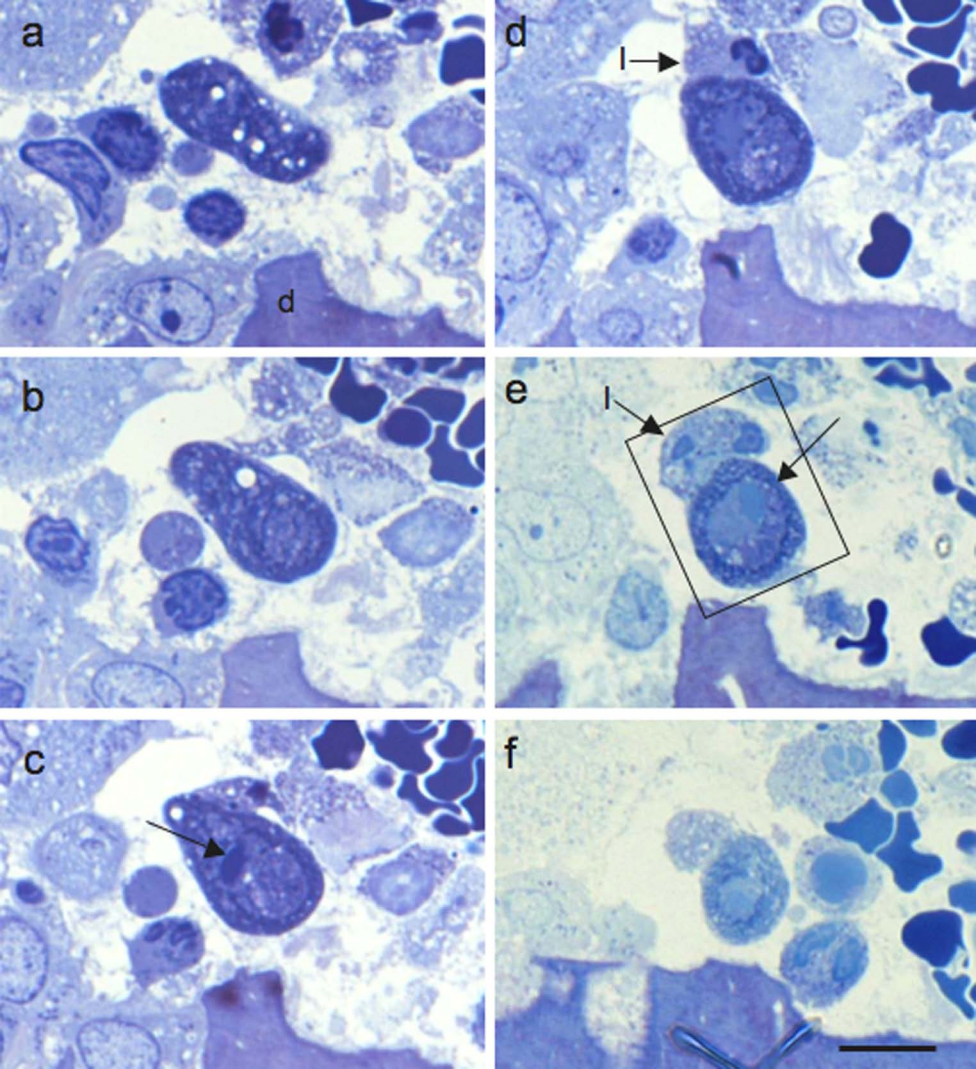
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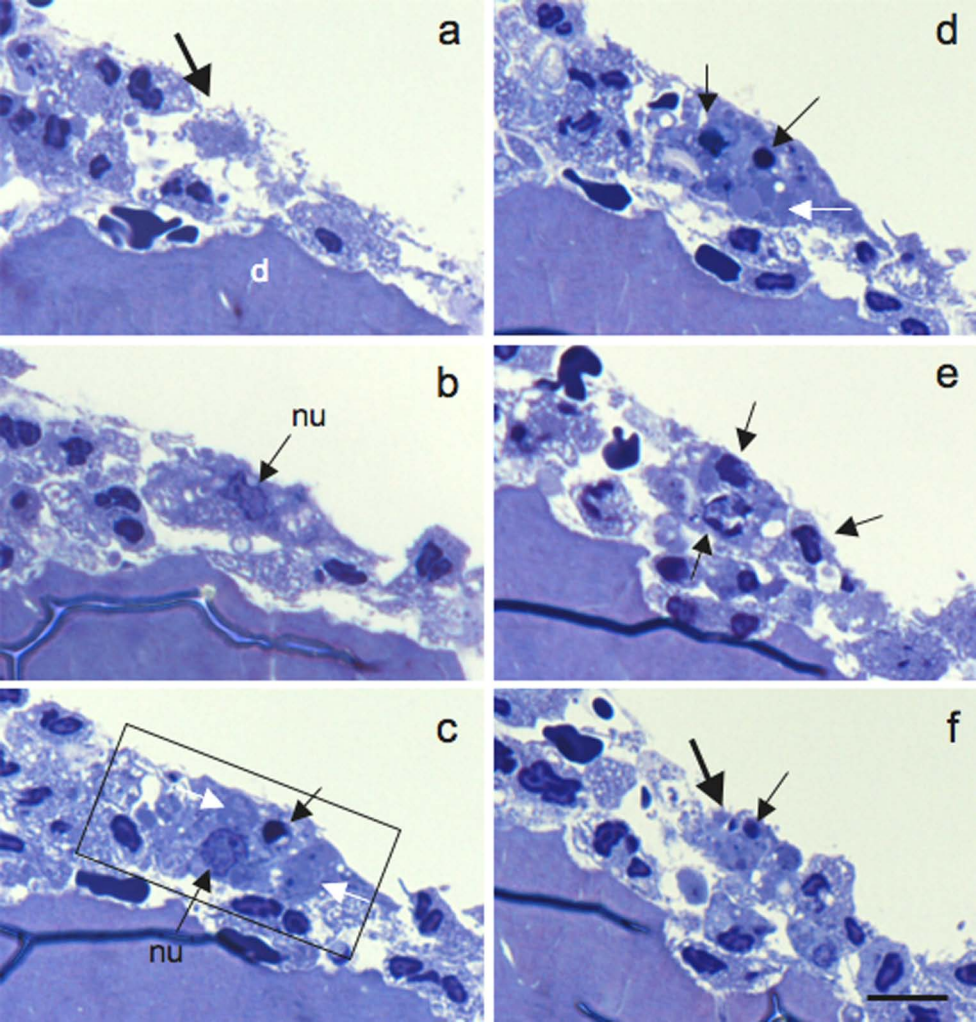
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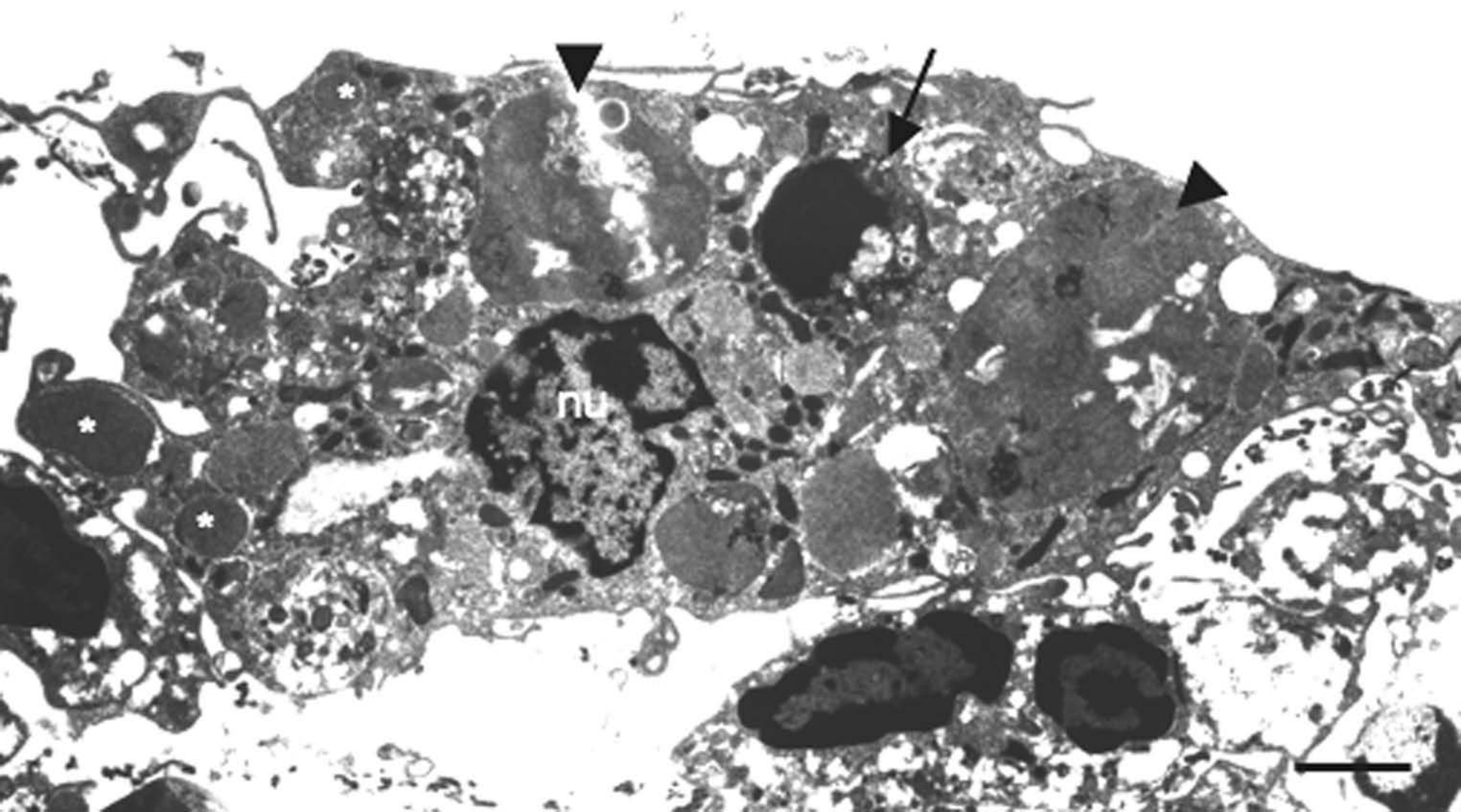
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