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Necrosis versus Apoptosis as the Mechanism of Target Cell Death Induced by *Entamoeba histolytica*

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The human pathogen *Entamoeba histolytica* is known to kill a variety of host cells, including leukocytes. Using human myeloid cells as targets, we studied whether cytotoxicity of amoebic trophozoites in vitro is equivalent to the induction of apoptosis or whether these target cells die via necrosis. Based upon morphological criteria, incubation of target cells with amoebae resulted in necrosis, with cell swelling, rupture of plasma membrane, and release of cell contents including nucleic acids being detected by light and transmission electron microscopy. On the other hand, the characteristic features of apoptosis such as cell shrinking, surface blebbing, and chromatin condensation were not observed. Moreover, internucleosomal fragmentation of genomic DNA within target cells as a characteristic feature of apoptotic cell death did not occur as judged by the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling technique in combination with flow cytometry. Consistently, cleavage of DNA was detectable upon agarose gel electrophoresis only after a substantial part of the target cell population had already been lysed. We also analyzed the mechanism of cell death induced by amoebapores, pore-forming peptides and primary candidate molecules for mediating the cytolytic activity of *E. histolytica*. At a time point at which the majority of target cells showed membrane injury upon incubation with purified amoebapores, no DNA degradation was detectable in the victim cells. The data suggest that the target cells used in our study undergo necrosis rather than apoptosis when they are killed by viable trophozoites as well as by isolated amoebapores.

The enteric protozoon *Entamoeba histolytica* is the cause of worldwide human amoebiasis. Infection with the pathogenic amoebae may result in massive destruction of host tissues and life-threatening disease (20). The name-giving histolytic activity of the amoebae is considered to result from degradation of extracellular matrix proteins by secreted cysteine proteases and from lysis of surrounding host cells. Upon invasion, amoebae may lyse a variety of host cells, i.e., colonic epithelial cells, endothelial cells, and also cellular effector cells of the defense system. Accordingly, the extraordinary cytolytic capacity of *E. histolytica*, which in vitro is reflected in the killing of almost every cell type, appears to be a major pathogenic function of the parasite.

It was shown previously that contact of amoeba with the target cell is obligatory and is mediated primarily via a Gal- or GalNAc-specific adherence lectin on the amoebic surface (16). Several notions of how amoebae actually kill their target cells have been reported, but the cytocidal mechanism has not yet been determined (1). Nonetheless, the rapid cytolytic reaction of amoebae observed in vitro after target cell contact has been established (particularly against polymorphonuclear granulocytes, lymphocytes, and macrophages) suggests that a cytolytic machinery is activated in the amoebae which is equally as efficient as that found within cytolytic lymphocytes.

In analogy to the lytic (perforin-mediated) mechanism of lymphocytes, one of the primary candidate principles for mediating the amoebic killing of target cells is the exocytosis of pore-forming molecules to the target cell membrane. These molecules are commonly named amoebapores and represent a family of three 77-residue peptides which are characterized by their disulfide-cross-linked all-alpha helical structure (12, 14). They exist as mature and potentially active peptides inside the cytoplasmic granules of the amoebae (13). Interestingly, amoebapores were found to be structurally and functionally related to NK-lysin (11), an effector peptide of mammalian lymphocytes discovered in pigs (3). Moreover, a recently detected human T-cell- and NK-cell-specific granule protein has homology with them (18).

It has been reported recently that killing of the murine myeloid cell line induced by *E. histolytica* resulted in a ladder-like DNA fragmentation, indicating that an apoptotic pathway of cell death was triggered (19). Apoptosis (programmed cell death) is a common form of eukaryotic cell death which also may be induced by cytotoxic effector cells. The morphological characteristics of apoptosis are cell shrinkage, surface blebbing, and chromatin condensation (23, 24). Moreover, apoptosis is characterized by prelytic fragmentation of genomic DNA into internucleosomal fragments. In contrast, upon necrosis, cleavage of DNA may be observed only after membrane damage has occurred (23, 24). It may be assumed that killing through the formation of a stable pore within the target cell membrane mostly results in cell death with the characteristics of necrosis. As the plasma membrane loses its function as a permeability barrier, the attacked cell will swell and eventually lyse, releasing its intracellular contents into the surrounding medium. However, internucleosomal DNA fragmentation is also triggered by small amounts of pore-forming proteins, e.g., *Staphylococcus* alpha-toxin (9), leukotoxin of *Actinobacillus actinomycetemcomitans* (15), and listeriolysin (8). Target cells affected by sublytic concentrations of such bacterial toxins may be capable of repairing their plasma membranes, but their apoptotic death program apparently has been switched on by the exogenous stimulus.

The evidence that some percentage of the murine target cells killed by amoebae undergoes apoptosis intrigued our interest to analyze the mechanism of target cell death in our in vitro systems employing human myeloid cell lines in combina-

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tion with viable trophozoites or purified amoebapores. The leukemia cell lines HL-60 and Jurkat used in this study resemble undifferentiated granulocytes and T lymphocytes, respectively, and function here as substitutes for the more fragile effectors of the cellular immune system of the host. To enhance the significance of our findings, we also introduced freshly isolated human neutrophil granulocytes (PMN) in the study. Our classification of cell death was based upon four different methods and the established morphological and biochemical criteria for necrosis and apoptosis mentioned above.

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MATERIALS AND METHODS

Cultivation and harvesting of *E. histolytica*. Trophozoites of the *E. histolytica* isolate HM-1:IMSS were cultured in plastic tissue culture flasks axenically with TYI-S-33 medium (5). The cultured amoebae do not represent cloned cells. Trophozoites from cultures in the late logarithmic phase were harvested after being chilled on ice for 10 min, sedimented at 430 × *g* at 4°C for 3 min, and washed three times in ice-cold phosphate-buffered saline adjusted to amoebae (PBS-A; 4.5 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 147 mM NaCl, 2.5 mM KCl [pH 7.4], 320 mosmol kg^{-1}).

Proteins. Amoebapores were isolated from trophozoites of *E. histolytica* HM-1:IMSS by using reversed-phase high-performance liquid chromatography as the final purification step as described previously (13). They were used in the assays in a mixture of isoforms A, B, and C at a ratio (35:10:1) that was found in the amoebae (13). Cecropin A was purchased from Sigma. Streptolysin O (SLO) in a highly purified form (4) was a generous gift of S. Bhakdi, Institute for Medical Microbiology and Hygiene of the University of Mainz.

Target cells. The human leukemic cell lines HL-60 and Jurkat (American Type Culture Collection, Rockville, Md.) were grown in RPMI 1640 medium (GIBCO BRL, Grand Island, N.Y.) supplemented with 10% inactivated fetal bovine serum, 2 mM L-glutamine, penicillin (100 U/ml), streptomycin sulfate (100 μg/ml), and amphotericin (0.25 μg/ml). Cells were maintained at 37°C in a humidified 5% CO_2 atmosphere and passaged three times a week. PMN were isolated from the blood of apparently healthy volunteers on two-layer Ficoll-Hypaque gradients as described previously (6).

Microscopic analysis. Trophozoites were incubated with target cells in PBS-A at a ratio of 1:10 at 37°C for 20 min. For induction of apoptosis, target cells were incubated in the absence of trophozoites with 5 μg of actinomycin D (ActD) per ml of PBS-A for 4 h. As a criterion for viability of target cells, the exclusion of trypan blue (2.5 mg/ml) and of the fluorescent probe propidium iodide (PI; 5 μg/ml) was used. Light and fluorescence microscopy analyses were done with a Leica (Bensheim, Germany) DMR microscope. For transmission electron microscopy, cells were fixed with 2% glutaraldehyde in PBS-A. Cells were washed twice in PBS-A, postfixed with 1% OsO_4, treated with 1% tannic acid, and dehydrated with graded ethanol solutions and propylene oxide. After the cells were embedded in an epoxy resin (Epon), 70-nm ultrathin sections were cut (Ultra Cut E; Reichert/Leica, Bensheim, Germany) and counterstained with uranyl acetate and lead citrate. Sections were examined with a Philips CM 10 transmission electron microscope at an acceleration voltage of 80 kV.

Measurement of cytotoxicity by BCECF release. Jurkat cells or HL-60 cells (5×10^6/ml) were labeled with 10 μM 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF AM; Molecular Probes Europe BV, Leiden, The Netherlands) in PBS-A (pH 7.4) for 20 min (10). Amoebae (3×10^4) plus target cells (3×10^5) in 200 μl of PBS-A (pH 7.4) were sedimented at 150 × *g* at 4°C for 3 min and incubated at 37°C for different time intervals. In other assays, target cells (2×10^5) were incubated with 10 μM amoebapores in MES buffer (20 mM morpholineethanesulfonic acid, 150 mM NaCl [pH 5.5]) or with 0.4 μM SLO (~12 hemolytic units [HU]) in PBS-A at 37°C for 2 h. Subsequently, the cells were centrifuged at 3,000 × *g* for 10 min and the supernatant was removed. The fluorescence of supernatants was measured in a fluorescence microtiter plate reader (Fluorotec; Merlin, Bornheim-Hersel, Germany) with excitation and emission wavelengths of 485 and 538 nm, respectively. Cytolysis is expressed as percentage of released fluorescence. One hundred percent dye release was achieved with 0.1% Triton X-100 for target cell lysis. Spontaneous release of target cells incubated in buffer only was subtracted from the maximal and experimental values. Experiments were done at least in triplicate. For parallel analysis of membrane injury and DNA degradation, the sediments of the same samples were pooled and used for DNA extraction.

Electrophoretic analysis of DNA fragmentation. After incubation of target cells with trophozoites under the conditions described above, cell sediments were washed with cold PBS-A and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.4]) supplemented with 0.3% sodium dodecyl sulfate and 300 μg of proteinase K (Boehringer, Mannheim, Germany) per ml. After incubation at 56°C for 12 h, DNA was extracted with phenol, precipitated with 2.5 volumes of ethanol at −20°C, pelleted, dried, and incubated with heat-treated RNase A (Boehringer; 10 μg/ml in TE buffer) at 37°C for 30 min. DNA samples were loaded onto a 1% agarose gel containing ethidium bromide (0.5 μg/ml) and separated by electrophoresis. The length of fragmented DNA was compared with a standard 100-bp ladder marker (Bethesda Research Laboratories, Gaithersburg, Md.).

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. The method originally used for detection of apoptosis in tissue sections (7) was based on deoxynucleoside triphosphate binding to 3′-hydroxyl ends of DNA by a terminal deoxynucleotidyl transferase in a template-independent manner. Here, amoebae (3×10^4) plus target cells (3×10^5) or amoebae and target cells separately were incubated in 200 μl of PBS-A (pH 7.4) at 37°C for 5, 10, or 30 min after contact had been established by sedimentation (150 × *g* at 4°C for 3 min). Subsequently, the cells were centrifuged at 1,000 × *g* for 5 min and the supernatant was removed. Cells were resuspended in 0.5 M *N*-acetylgalactosamine (GalNAc) for 10 min and fixed in 500 μl of 4% phosphate-buffered paraformaldehyde (pH 7.4) containing 0.2 M GalNAc for 30 min. After fixation, the cells were washed with PBS-A, permeabilized by incubation with 100 μl of 0.1% Triton X-100–0.1% sodium citrate at 4°C for 2 min, and then washed twice with PBS-A. DNA breaks of cells were stained with the in situ cell death detection kit fluorescein (Boehringer) as described in the manufacturer's instructions. As a positive control, fixed and permeabilized cells were incubated with 50 μg of DNase I (Boehringer) per ml at 37°C for 20 min and then processed in a similar manner. Samples were washed twice with PBS-A and analyzed with a FACScan (Becton Dickinson, Heidelberg, Germany) flow cytometer with computer-assisted evaluation of data (Lysis II software). Aliquots of samples were stained with propidium iodide (5 μg/ml), air dried on slides, and analyzed by confocal microscopy with the Leica TCS NT confocal laser scanning system in combination with a Leica DMR microscope.

RESULTS

Necrotic morphology of amoeba-killed target cells. Human myeloid cells killed by *E. histolytica* trophozoites showed the characteristic morphological features of necrosis. When target cells, e.g., Jurkat T cells, were observed under the light microscope after they had come into intimate contact with amoebae, they appeared markedly swollen and had compromised membranes as evidenced by staining with trypan blue (Fig. 1). In contrast, Jurkat cells, in which apoptosis was induced by incubation with ActD, were shrunken, partially fragmented, and excluded the dye. Notably, target cells destroyed by amoebae were found in a mass of debris, which consisted predominantly of nuclei and nucleic acid as evidenced by staining with PI (Fig. 1). Examination by electron microscopy revealed that target cells, e.g., HL-60 cells, and their nuclei were often already markedly swollen after a 20-min period of contact with amoebae (Fig. 2). Many cells showed lesions of the plasma membrane and lost their cytoplasmic contents. In contrast to the control cells, in which apoptosis was triggered by ActD, victim cells with apoptotic surface convolutions and chromatin condensation were not observed (Fig. 2).

Parallel analysis of membrane injury and DNA fragmentation. Apoptosis is most often demonstrated through the presence of a characteristic ladder formation of genomic DNA upon electrophoresis due to internucleosomal fragmentation of target cell DNA. We wanted to determine whether and, if so, when such a DNA cleavage occurred in human target cells incubated with trophozoites. The DNA analysis of agarose gels revealed that amoeba-induced killing is accompanied by DNA degradation (Fig. 3). However, the kinetics of this process suggested that a substantial part of the target cell population had already been lysed when degraded DNA was detected. Consequently, disruption of plasma membrane integrity and DNA damage of target cells induced upon contact with trophozoites were monitored in time course experiments in parallel. It became apparent that prior to the degradation of their DNA, a substantial part of target cells possessed compromised plasma membranes as judged by BCECF release: Jurkat cells, for example, released about 30 and 50% of the fluorescent dye already after 10 and 20 min of incubation with amoebae, respectively, whereas DNA degradation was hardly detectable at

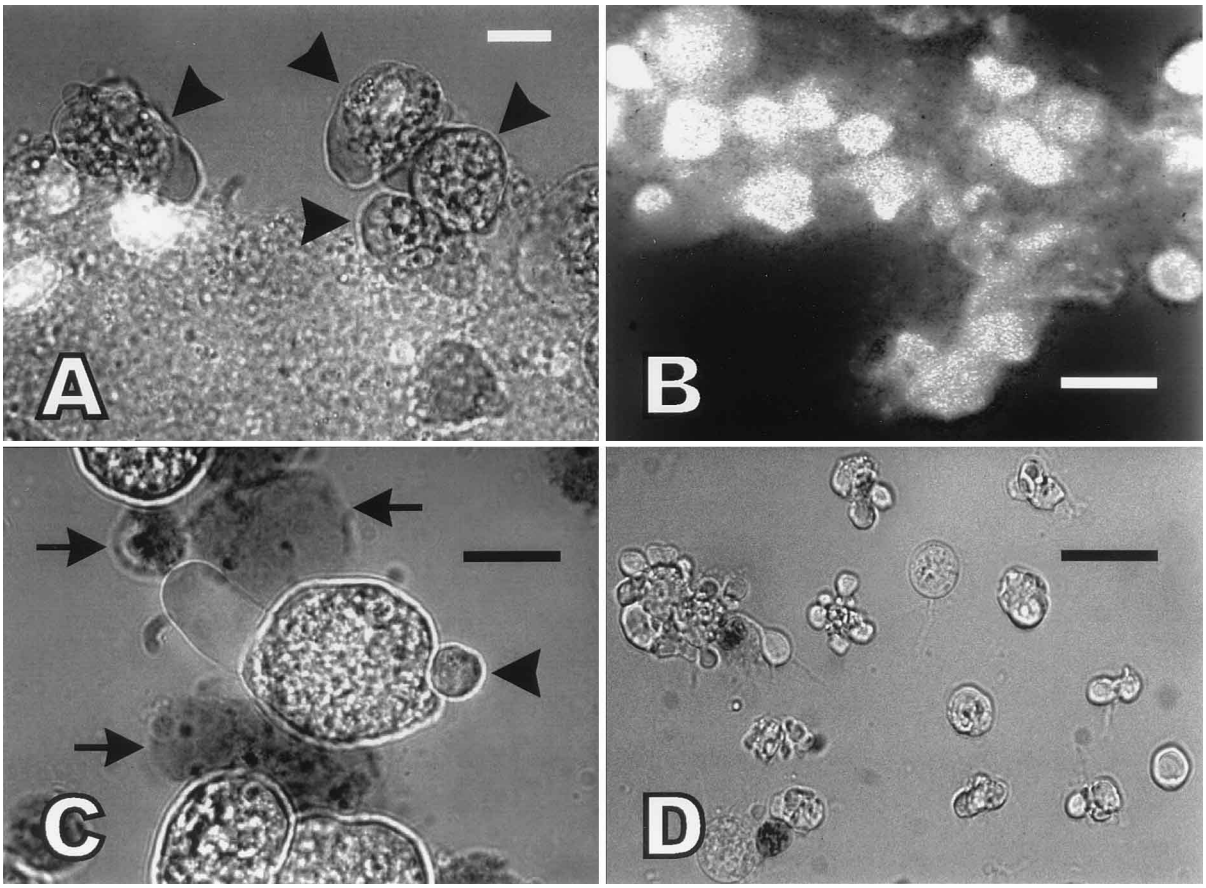

FIG. 1. Analysis of target cell death by light microscopy. (A) Amoebae (arrowheads) in a mass of target cell debris after incubation with BCECF-prelabeled Jurkat cells for 20 min. Viable Jurkat cells appear bright under fluorescent light. (B) Another aliquot of the cell debris shown in panel A, stained with PI and indicating a high content of nuclei and of released DNA. (C) Unlabeled Jurkat cells in close contact with a pseudopod-extruding trophozoite showing heavy staining by trypan blue. An individual target cell situated at the uroid of the amoeba appears viable since it excluded the dye (arrowhead). (D) ActD-treated Jurkat cells with an apoptotic appearance. They excluded the dye but show blebbing and fragmentation. Bars, 20 μm.

such early time points (Fig. 4). Similar results were obtained with other myelocytic targets such as HL-60 cells and freshly isolated PMN.

Failure to detect amoebae-induced apoptosis in target cells by the TUNEL technique. To answer the question whether DNA degradation occurred within target cells, we used the highly sensitive TUNEL technique in combination with fluorescence-activated cell sorter (FACS) analysis. After 30 min of incubation of Jurkat cells with amoebae, the cells were separated from the trophozoites with *N*-acetylgalactosamine, the monosaccharide that inhibits adherence of amoebae to target cells by interfering with the surface lectin of *E. histolytica*. After dissolution of amoebae-target cell conjugations was verified by light microscopy, amoebae and target cells were subjected to the TUNEL assay and analyzed by FACS. Both amoebae and the smaller target cells were gated by forward and side scatter to receive regions of distinct cell populations. The cell populations representing amoebae and target cells were analyzed separately, and the fluorescent signals were quantified. After the incubation period (30 min), the initial amoeba/target cell ratio of 1:10 decreased to a ratio of approximately 1:2, indicating complete lysis of many target cells.

Whereas the fluorescent signals of the remaining target cells were found to be unchanged compared to that of the control, amoebae showed a slight increase in fluorescence upon incubation with target cells (Fig. 5). Fluorescence microscopy revealed that several amoebae had engulfed target cells (as shown in Fig. 6), which may explain this phenomenon.

To exclude the possibility that apoptotic features of dying cells were not observed after the 30-min incubation with amoebae, because the majority of the victim cells were already completely disrupted, shorter incubation times were also used. At earlier time points (5 and 10 min), the majority of target cells still have intact membranes as revealed by parallel measurement of BCECF release. Nonetheless, a TUNEL-positive subpopulation of cells could not be detected (Fig. 6A). Analysis of samples by confocal microscopy revealed that single TUNEL-positive cells can be found but are also present in the control incubated without amoebae (Fig. 6B). The appearance of massive DNA breaks was observed only in segments of a viscous mass (Fig. 6B) resulting from complete cell disruption and indicates extracellular DNA degradation. This phenomenon had already been observed under the light microscope (Fig. 1).

Cell death induced by amoebapores is not accompanied by internucleosomal DNA fragmentation. To determine whether the pore-forming peptides of *E. histolytica* can induce apoptotic cell death, target cells were subjected to purified amoebapores. Incubation of Jurkat cells and HL-60 cells with 10 μM amoebapores resulted in lysis of the majority of target cells (~75% BCECF release in 2 h), but DNA fragmentation was not detectable upon agarose gel electrophoresis (Fig. 7).

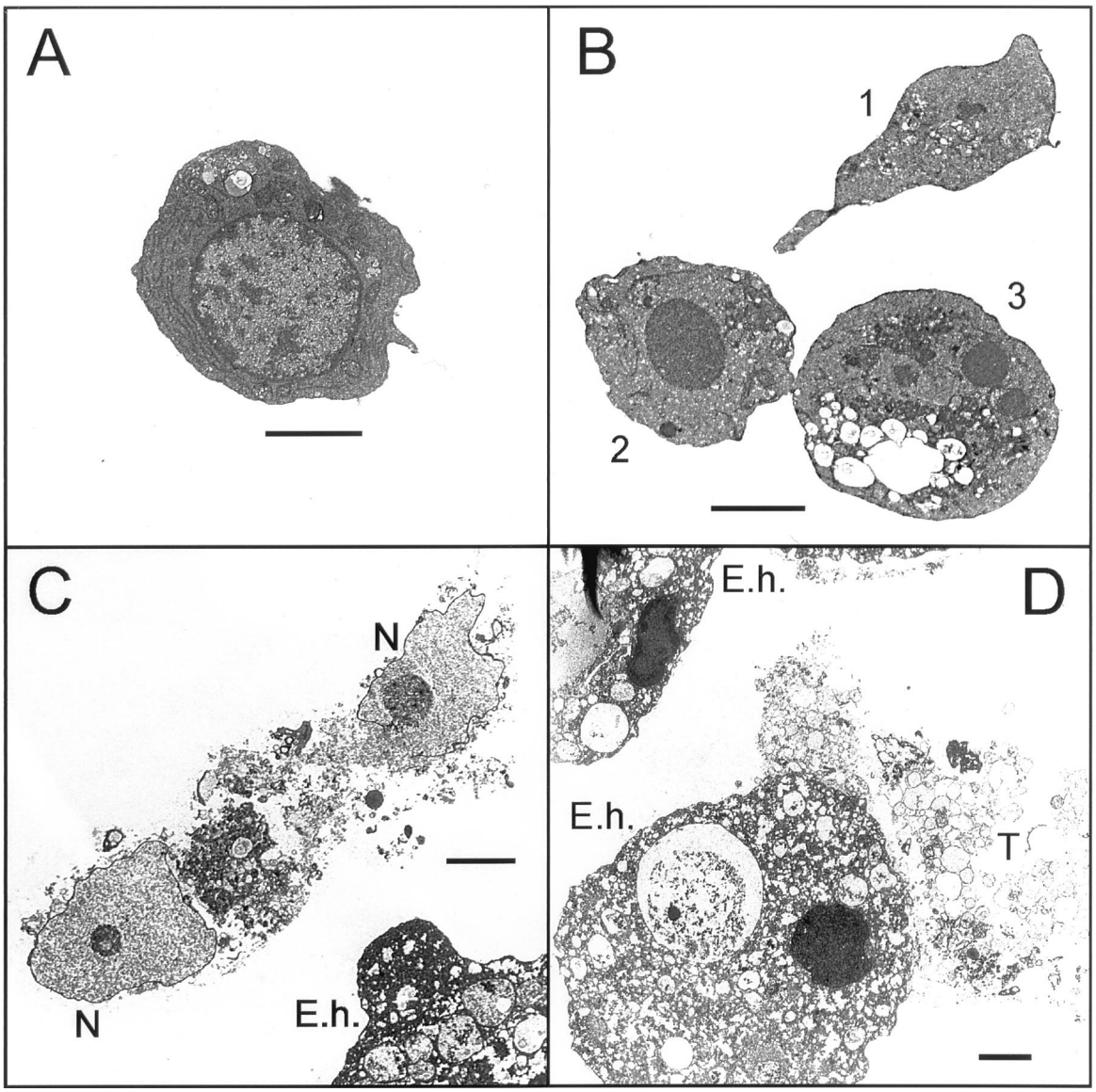

FIG. 2. Analysis of target cell death by transmission electron microscopy. (A) Untreated HL-60 cells with heterogeneous chromatin and intact mitochondria. (B) HL-60 cells treated with ActD for 4 h demonstrating the characteristics of apoptotic death. Cell fragments are visible (no. 1), cells are shrunken and their chromatin is condensed (no. 2), and many cells are heavily vacuolated and possess fragmented nuclei (no. 3). (C) Disruption of plasma membranes of HL-60 cells after incubation with *E. histolytica* (E.h.) for 30 min, resulting in the release of cell contents. Swollen nuclei (N) and cytoplasmic material are visible. (D) A disintegrated target cell (T) in close contact with a trophozoite. The amoeba appears to have engulfed a target cell, at least in part, which is contained in a large vacuole. E.h., *E. histolytica*. Bars, 4 μm.

Cecropin A, an α-helical bacteriolytic 37-residue peptide from insect hemolymph (21), did not induce BCECF release from Jurkat cells at concentrations up to 30 μM. The DNA pattern of amoebapore-killed cells resembled that of target cells completely lysed by SLO, a prototype of a pore-forming protein. Lower concentrations of amoebapores (1, 3, and 5 μM) in combination with a prolonged incubation time resulted in less-pronounced cytolysis (0, 21, and 60% BCECF release, respectively, in 3 h), but internucleosomal DNA fragmentation was not observed in any of the samples (data not shown).

DISCUSSION

In the present study, we raised the question whether *E. histolytica* trophozoites as well as their pore-forming peptides are capable of inducing apoptosis in human myeloid cells as implied by a recent study of cell death of murine myeloid target cells induced by that pathogen. It was reported in that study (19) that most of the cells die with both necrotic and apoptotic characteristics, but at least a small percentage revealed characteristics of apoptotic death exclusively. The cell death of murine neuroblastoma cells induced by another amoeba, the free-living pathogen *Acanthamoeba castellani*, and soluble products thereof has also been attributed, at least in part, to the triggering of programmed cell death (2). However, one may propose that thorough kinetic studies in combination with highly sensitive methods, e.g., the TUNEL assay, are needed to corroborate the hypothesis that a primitive, nonprofessional killer cell such as an amoeba may be capable of inducing apoptosis in a given target cell.

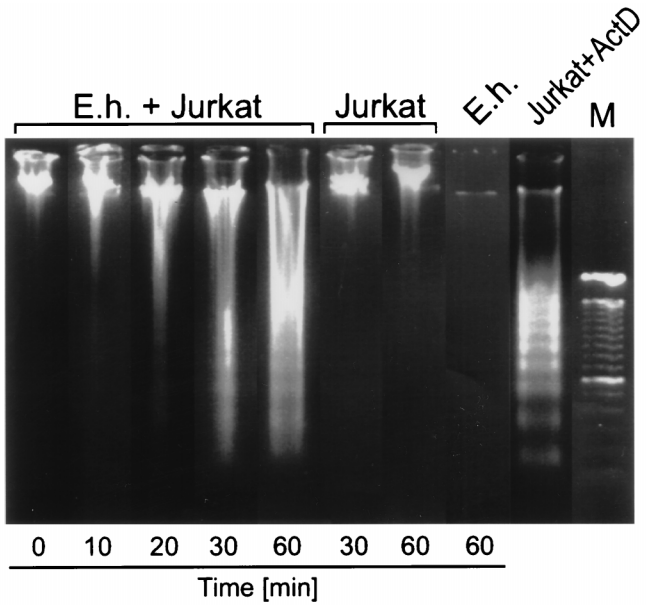

FIG. 3. Time course of DNA fragmentation of target cells killed by *E. histolytica*. Trophozoites (*E. histolytica* [E.h.]) and Jurkat cells at a ratio of 1:10 were incubated for various time periods. For each time point, the DNA of the cell mixture was extracted and analyzed by agarose gel electrophoresis. As a control, Jurkat cells and trophozoites in a number equivalent to that employed in the cytolysis assay were also incubated separately. Jurkat cells incubated with ActD for 4 h served as a positive control. Lane M, 100-bp ladder marker.

One of the most reliable criteria for distinguishing between apoptosis and necrosis is cell morphology. In our study, the death of target cells can be assigned morphologically to necrosis as evidenced by light and electron microscopies. Another criterion for apoptotic cell death is the formation of a ladder pattern of DNA upon gel electrophoresis. When cells undergo

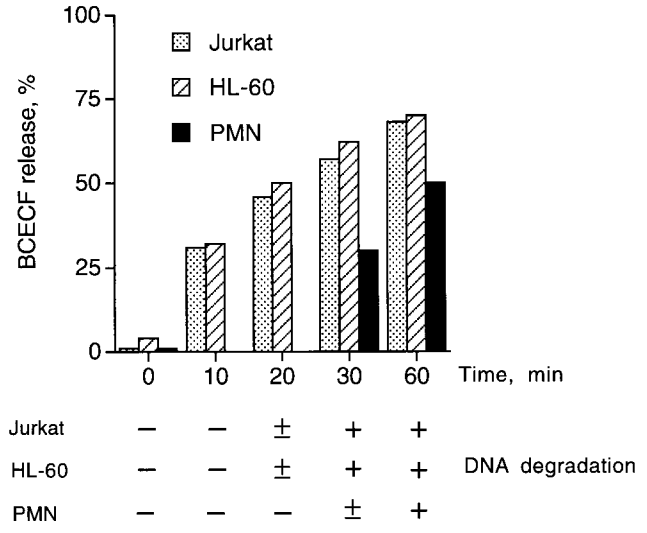

FIG. 4. Kinetics of membrane injury of target cells induced by *E. histolytica* compared to those of DNA degradation. Trophozoites were incubated with BCECF-prelabeled Jurkat cells, HL-60 cells, or PMN at a ratio of 1:10. The release of the fluorescent dye from target cells was measured over time to detect the occurrence of membrane lesions. In parallel, the DNA from cell mixtures representing each time point was extracted and analyzed by agarose gel electrophoresis.

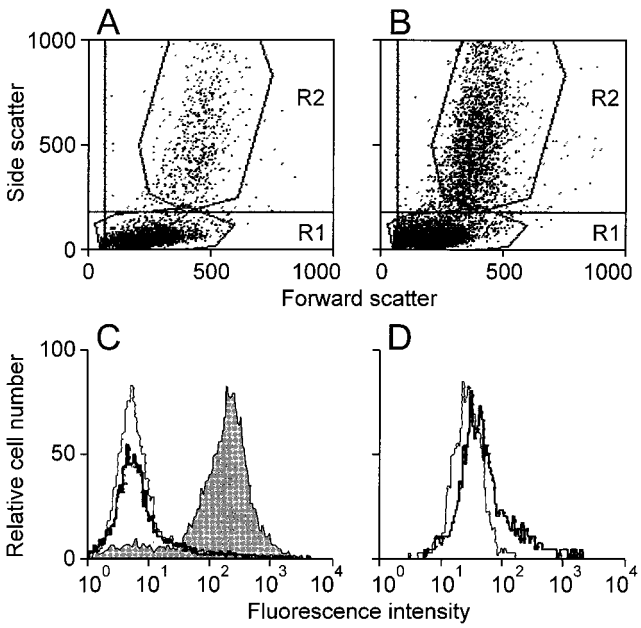

FIG. 5. Use of the TUNEL technique and FACS analysis to detect amoeba-induced apoptosis of target cells. (A and B) Light scatter pattern after 0 (A) and 30 (B) min of amoeba-target cell contact. Events in gate R1 are Jurkat cells; those in gate R2 are amoebae. (C) FACS profile of the Jurkat cell population (encompassed by gate R1) after incubation with amoebae for 30 min (thick line), in buffer alone (thin line) or treated with DNase I (positive control; filled grey area). (D) FACS profile of the amoebae population (encompassed by gate R2) after incubation for 30 min with Jurkat cells (thick line) or in buffer alone (thin line). Increase in fluorescence upon time represents DNA breaks within cells. Due to autofluorescence, amoebae have higher background fluorescence than Jurkat cells.

apoptosis, the DNA will be digested into oligonucleosomal fragments about 180 bp long due to the enzymatic digestion of DNA between the histon complexes by a Ca^{2+}-dependent endonuclease similar to DNase I (17). We also observed cleavage of target cell DNA during the incubation period of 30 min; a smear of degraded DNA with a faint ladder formation became visible upon electrophoresis. However, our kinetic studies revealed that another criterion for apoptosis is not fulfilled (24)—the DNA cleavage did not occur prior to membrane disintegration. Since it is known that the typical DNA ladder can be reproduced in vitro by incubation of nuclei with DNase I which was preincubated with nuclear extracts (17), we assume that cellular contents, including DNA and enzymes which cleave DNA, were released from target cells disrupted by amoebae and thereby DNA became accessible to enzymatic degradation. PI staining of amoeba-target cell conjugates revealed the presence of substantial amounts of nuclei and extracellular nucleic acid, which strengthened the notion that the observed DNA degradation may represent a secondary event. Data from the highly sensitive TUNEL technique further excluded the possibility that an intracellular suicide program is triggered in target cells during incubation with trophozoites. Notably, this observation is valid for the entire cell population encompassed by the gating as target cells. Since cell lysate and released DNA have been excluded from flow cytometric analysis, only cleavage of intracellular DNA is detectable in that assay. Here, the cleavage of DNA within cells having intact plasma membranes, a hallmark of apoptosis, could not be observed.

Taken together, our findings seem to contradict those re-

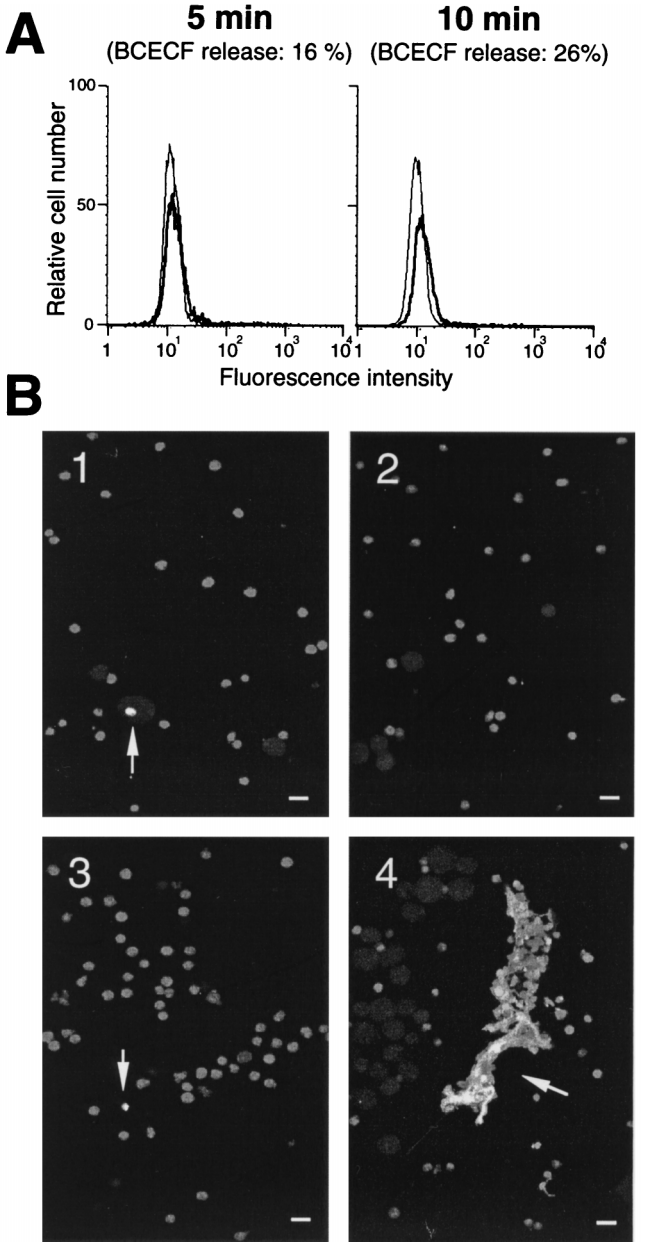

FIG. 6. Analysis for TUNEL positivity at early time points using flow cytometry and confocal microscopy. (A) FACS profiles of Jurkat cell population (gated as shown in Fig. 5) after incubation for 5 and 10 min with amoebae (thick line) or in buffer alone (thin line). It is important to note that the fluorescence intensity of the target cell population in the control and not in the experiment with amoebae differs slightly between time points. The percentages of target cells with membrane lesions, determined by a BCECF release assay made in parallel, are shown above the FACS profiles. (B) Confocal microscopy images of Jurkat cells incubated with amoebae for 5 (panel 1) and 10 (panels 2 and 4) min or with buffer alone for 10 min (panel 3). Nuclei were stained red with PI (moderate brightness). Amoebae are characterized by their larger size and weak (auto)fluorescence. Green-yellow TUNEL-positive events are marked by arrows (intense brightness). The TUNEL-positive cell in panel 1 appears to be a target cell phagocytozed by an amoeba. A segment of a viscous mass containing destroyed target cells is also highly TUNEL positive (panel 4), indicating extracellular DNA fragmentation. Bars, 20 μm.

ported previously. However, it should be noted that conditions which induce apoptosis are cell type specific, and thus it is not excluded that murine cells, at least in part, undergo cell death through a mechanism different from that of the human target

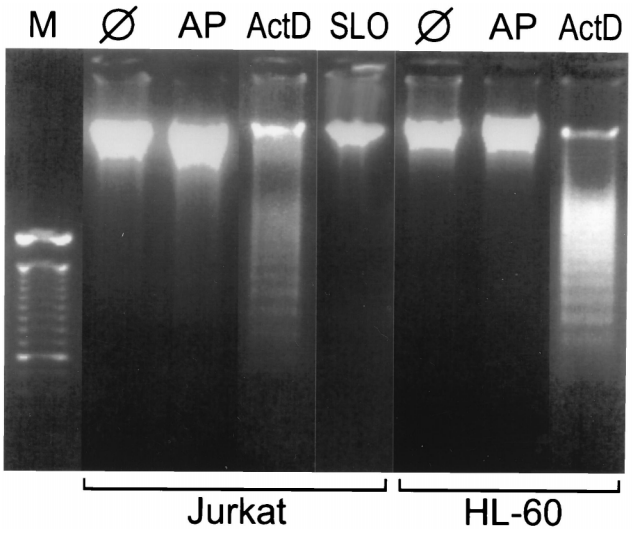

FIG. 7. Analysis of DNA of target cells after exposure to amoebapores. Jurkat cells and HL-60 cells were incubated with 10 μM amoebapores for 2 h, and their DNA was analyzed by agarose gel electrophoresis. As a positive control, target cells were incubated with 5 μg of ActD per ml to induce apoptosis indicated by the characteristic 180-bp ladder. For comparison, Jurkat cells were incubated with 0.4 μM SLO. Target cells were also incubated for 2 h without additives (Ø). The DNA was extracted from each sample and analyzed on an ethidium bromide-stained agarose gel. Lane M, 100-bp ladder marker.

cells used here. We primarily employed the human leukemia cell lines Jurkat and HL-60 in our experiments because they are easy to handle and propagate. However, with freshly isolated human PMN, virtually the same results were obtained in the kinetic experiments. The variations within the cytolytic efficacy of the amoebic effector cells during culture may also have some influence on the results; a highly aggressive trophozoite population may induce rapid lysis of target cells only, whereas a less aggressive population may induce death of some of the target cells with apoptosis-like characteristics.

Nonetheless, the results of our study led us to the assumption that cell death of host cells induced by amoebae occurs via a necrotic mechanism. Since necrotic cell death results in a release of factors that are chemotactic for leukocytes, it typically provokes inflammation (22). Accordingly, the inflammatory symptoms observed with extraintestinal amoebiasis may be due in part to the fatal contact of host cells with amoebae. In contrast, apoptosis is considered to be a mechanism of cell death which avoids inflammation (22). The finding that live trophozoites as well as purified amoebapores evoke a necrotic cell death are in good agreement with our notion that the pore-forming peptides are the primary candidates for conferring the extraordinary cytolytic potential to *E. histolytica.*

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