Biochemical and Biophysical Research Communications 421 (2012) 808–812

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Nanosecond pulsed electric fields (nsPEFs) activate intrinsic caspase-dependent and caspase-independent cell death in Jurkat cells

Wei Ren [1], Nova M. Sain, Stephen J. Beebe *

Frank Reidy Research Center for Bioelectric, Old Dominion University, Norfolk, VA 23508, United States

ARTICLE INFO

Article history:
Received 17 April 2012
Available online 25 April 2012

Keywords:
APAF-1
Apoptosis
Caspases
Cytochrome c death induced signaling complex
Electric fields
Electroporation
Jurkat clones

ABSTRACT

NsPEF ablation induces apoptosis markers, but specific cell death pathways have not been fully defined. To identify nsPEF-activated cell death pathways, wildtype human Jurkat cells and clones with deficiencies in extrinsic and intrinsic apoptosis pathways were investigated. NsPEFs activated caspase isozymes and induced identical electric field-dependent cell death in clones deficient in FADD or caspase-8, indicating that extrinsic apoptosis pathways were not activated. This was confirmed when cytochrome *c* release was shown to be unaffected by the pan caspase inhibitor, z-VAD-fmk. NsPEF-treated APAF-1-silenced cells did not exhibit caspase-3/7 and -9 activities and corresponding electric field-dependent cell death in this clone was attenuated compared to its vector control at low, but not at high electric fields. These data demonstrate that nsPEFs induce intrinsic apoptosis activate by cytochrome c release from mitochondria through an APAF-1- and caspase-dependent pathway as well as through caspase-independent mechanisms that remain to be defined. Furthermore, the results establish that nsPEFs can overcome natural and oncogenic mechanisms that promote cell survival through inhibition of apoptosis and other cell death mechanisms.

1. Introduction

Nanosecond pulsed electric field (nsPEF) technology has recently been investigated as an extension of conventional electroporation with a hypothesis that sub-microsecond pulses with fast risetimes have advantages for manipulating intracellular structures that determine cell viability [1]. Modeling studies provided an analysis of nsPEF outcomes causing supra-electroporation with very high densities of very small nanopores in all cell membranes, which were hypothesized to account for apoptosis [2,3]. Given that nsPEFs do not exist in nature and the potential for nsPEF cancer ablation exists [4–10], it is of interest to determine more specifically which mechanisms and pathways are recruited in response to nsPEF stimuli and what initiates and promotes loss of cell viability. Since apoptotic cell death has been of great interest in eliminating cancer, several studies found evidence for nsPEF-induced apoptosis in cell lines *in vitro* [11–15] and in tumor tissues *in vivo* [9,10]. While other mechanisms of cell death are likely operative [14,16], most attention has focused on apoptosis programs in response to nsPEF treatment as a means of cell demise.

Apoptosis induction occurs through two major pathways [16]. Extrinsic mechanisms occur through death receptors that form death induced signaling complexes (DISCs) composed of cytoplasmic domains of death receptors, FADD and caspase-8. In type I cells, large amounts of caspase-8 are activated in DISCs and caspase-8 directly activates caspase-3 without involvement of mitochondria. In type II cells, caspase activation requires amplification steps. Very few DISCs are formed and small amounts of caspase-8 cleave Bid to form a pro-apoptotic, truncated Bid (t-Bid) that collaborates with Bax or Bak to promote cytochrome *c* release from mitochondria. Intrinsic pathways induce cytochrome *c* release from intracellular signals that disrupt homeostasis and share a mitochondria pathway with extrinsic type II cells. Cytochrome *c*, APAF-1, ATP and caspase-9 form an apoptosome that activates caspase-9, which then activates caspase-3.

It was previously reported in Jurkat calls that cytochrome c was released and executioner caspases were activated [11]. However,

Abbreviations: nsPEFs, nanosecond pulsed electric fields; ΔC-8, caspase-8 deficient Jurkat clone; ΔAPAF-1, APAF-1 deficient Jurkat clone; APAF-1, apoptotic protease activating factor 1; FADD, fas-associated protein with death domain; DISC, death-induced signaling complex; Bcl-2, B-cell lymphoma 2; Bid, BH3 interacting-domain death agonist; Bax, Bcl-2–associated X protein; Bcl-xl, B-cell lymphoma-extra-large; ΔΨm, mitochondria membrane potential.

* Corresponding author. Address: Frank Reidy Research Center for Bioelectric, 4211 Monarch Way, Suite 300, Old Dominion University, Norfolk, VA 23508, United States. Fax: +1 757 451 1010.

E-mail address: sbeebe@odu.edu (S. J. Beebe).

[1] Present Address: Division of Molecular Carcinogenesis and Targeted Therapy for Cancer, State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing 100101, PR China

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.04.094

initiator caspases were not identified and it was not determined if this occurred through a type II cell extrinsic pathway by affecting plasma membranes or through intrinsic pathways by affecting intracellular structures. Furthermore, caspase activities were coincident with cell death, but cell death was not shown to be caspase-dependent. In this study, we more clearly determine which apoptosis pathways are activated by nsPEFs and determine more specifically whether caspase-independent pathways are present and whether they function in parallel with caspase-dependent pathways in Jurkat cells. In another study that complements with this one [17], we identify nsPEF cellular targets and primary and secondary mechanisms that likely initiate and propagate cell death pathways that are identified here. It is possible that these nsPEF-induced mechanisms can be insensitive to cancer causing mechanisms irrespective of their mutation.

2. Materials and methods

2.1. Cell culture

Wild type Jurkat T-lymphocytes (clone A3) and mutant cell lines deficient for FADD (clone I2.1) or caspase-8 (clone I9.2) were purchased from ATCC (Manassas, VA). These clones were grown and cultured in RPMI 1640 medium (ATCC) containing 10% fetal bovine serum (FBS) (Atlanta Biologicals), 1% L-glutamine and 1% penicillin and streptomycin. The clone with APAF-1 silenced (ΔA-PAF-1) and its vector control (in Jurkat clone E6.1) were generous gifts from Dr. John Robertson, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas. They were grown in the same media indicated above except 1 mg/ml Geneticin (Invitrogen) was substituted for penicillin and streptomycin [18–20]. All cell lines were maintained in a humidified incubator with 5% CO_2 at 37 °C. All experiments were carried out with cells at 60–70% confluency.

2.2. Treatment of cells with nsPEFs

Cells was treated in cuvettes with nsPEFs as previously described [14,15]. Jurkat cells were exposed with or without ten pulses with durations of 60 ns (risetime ~5 ns) and electric field strengths ranging from 0 to 60 kV/cm using pulse generators as previously described [1,5].

2.3. Determination of cell viability

Cell viability was determined 24 h after treatment with nsPEFs according to the Manufacturer's protocol by CellTiter-Glo Luminescent Cell Viability Assay Kit (Promega, Madison, WI). The luminescent signals for ATP levels were directly proportional to cell numbers. Luminescence was analyzed in luminometer (Gemini XPS, Molecular Devices, CA).

2.4. Determination of Caspase-3/7, -8, and -9 (-like) activities

Caspase-3/7, -8, and -9 (-like) catalytic activities were assayed by Caspase-Glo 3/7, -8, -9 kits using tetrapeptide substrate sequences DEVD, LETD and LEHD, respectively (Promega, Madison, WI). According to manufacturer's instruction, cells (10^5 cells/well) were placed in triplicate into 96-well plates after treatment with nsPEFs. Plates were kept at 37° C for various times as indicated and then 100 μl of specific Caspase-Glo reagent was added into each well. Luminescence was read in a luminometer (Gemini XPS, Molecular Devices, CA) after 1 h incubation at room temperature.

2.5. Flow cytometry analysis of cytochrome c release

Cytochrome *c* was assessed using the Innocyte Flow Cytometric Cytochrome *c* Release assay (Calbiochem) as previously described [15]. When indicated, cells were pre-incubated for 30 min and treated in the presence of 50 μM z-VAD-fmk (300 μM z-VAD-fmk gave identical results). At 0, 1.5, 3, 6 h post-pulse cells were prepared for analysis by flow cytometry as described by the manufacturer. Cells (10,000) were analyzed by Becton Dickinson FACS Aria flow cytometer.

2.6. Statistical analysis

All experiments were performed at least three times independently in this study. Data were expressed as mean ± standard error (S.E.). Statistical differences between control and treated groups were analyzed by a paired Student's *t*-test (two-tailed).

3. Results and discussion

3.1. NsPEFs selectively induce caspase catalytic activities in a time-dependent manner

It was previously shown that caspase-3-like activity was increased in Jurkat cells, but active initiator caspases were not identified and it was not determined whether apoptotic cell death was due to intrinsic and/or type II cell extrinsic apoptosis [11]. This question became more intriguing when nsPEF-stimulated E4 cells released cytochrome c [15] and B16f10 cells did not [14], yet both exhibited decreases in mitochondria membrane potential ($\Delta\Psi m$). To determine whether nsPEFs activated executioner and initiator caspases, Jurkat clones were treated with ten 60 ns pulses at 60 kV/cm. All caspases were activated in an electric field-dependent (data not shown) and time-dependent manner (Fig. 1). The assays for each clone are shown with caspase isozyme activities for wild type (WT, panel A), FADD-deficient clone (ΔFADD, panel B) and caspase-8-deficient clone (ΔC-8, panel C). Caspase-9 was activated slightly before and to a greater extent than caspase-3 with these assays; peaks were at 4–5 and 6–9 h, respectively. This suggests activation through a mitochondria-dependent pathway. Caspase-8 was only weakly activated in WT and ΔFADD, which is typical of type II cells, like Jurkat cells, where activation of caspase-8 is relatively low and initiation of apoptosis requires amplification steps through the mitochondria pathway [16]. As expected, caspase-8 was essentially absent in ΔC-8. Somewhat surprisingly, caspase-9 and caspase-3 activities were greatest in ΔFADD, suggesting that the presence of FADD attenuates caspase activity in Jurkat cells in response to nsPEFs. Interestingly, caspases-3/7 and -9 were more weakly activated in ΔC-8, suggesting that caspase-8 may be important for robust activation of the other caspases. This is reminiscent of activation through the extrinsic pathway. Regardless of the activated pathways, which are investigated in more detail below, this is the first report showing that caspase isozyme activities can be differentiated in response to nsPEFs.

3.2. NsPEFs induce electric field-dependent cell death in the presence and absence of death induced signaling complex (DISC) in Jurkat cells

Given previous results suggesting nsPEF-induced extrinsic apoptosis [14,15] and showing that cytochrome c was released [11,15], using the clones analyzed in Fig. 1, the possibility was considered that nsPEF-mediated membrane charging events initiated changes in plasma membrane structures, perhaps aggregation of cell death receptors, activated a type II extrinsic apoptosis, DISC-mediated pathway. To confirm that ΔFADD and ΔC-8 were in

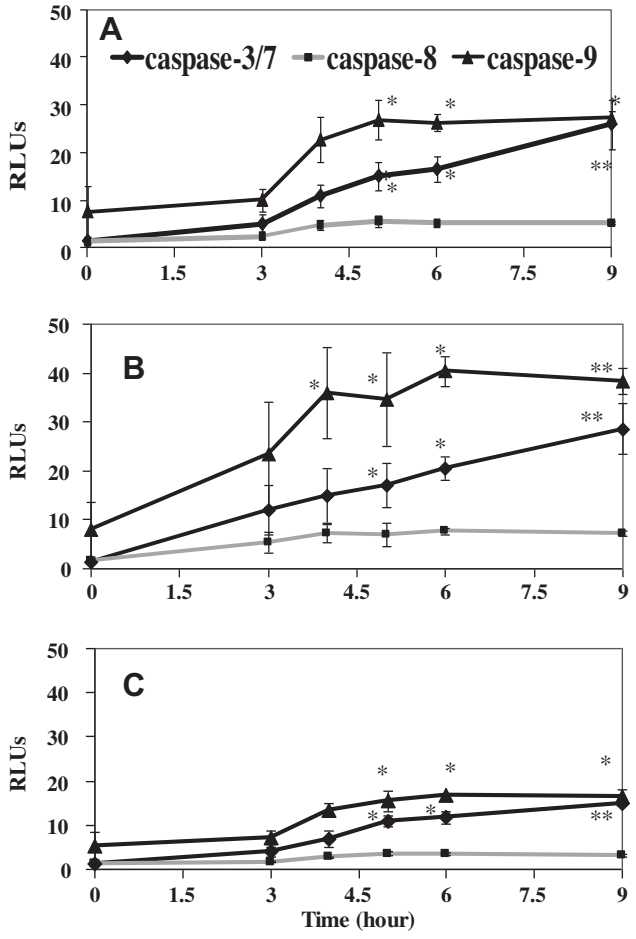

Fig. 1. NsPEFs increased catalytic activities of caspase proteases in Jurkat cells. Jurkat clones (all clones in A3) were exposed to 10 pulses at 60 ns and 60 kV/cm, a lethal treatment. The activities of caspase-3/7 (♦), -8 (■), -9 (▲) were measured at various times as indicated using Caspase-Glo assay with DEVD-, LETD-, LEHD-aminoluciferin as substrates, respectively. (A) Wild type (WT), (B) FADD deficient clone (ΔFADD), (C) Caspase-8 deficient clone (ΔC-8). Data represent the mean ± SE ($n = 3$). $^{*}p < 0.05$; $^{**}p < 0.01$.

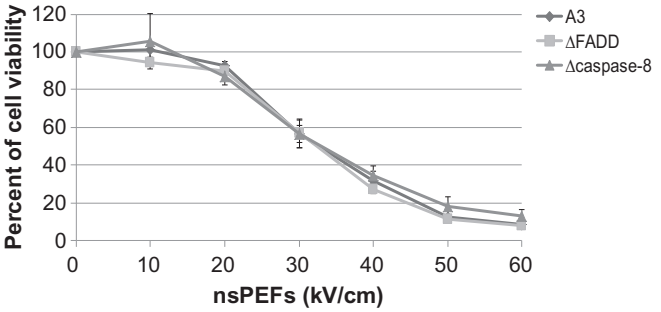

Fig. 2. Determination of nsPEFs- induced cell death in Jurkat clones. NsPEFs induced cell death in all Jurkat clones in an electric field amplitude-dependent manner. Three Jurkat clones were pulsed ten times at 60 ns and electric field strengths between 0 and 60 kV/cm. Cell viability was determined by CellTiter-Glo luminescent cell viability assay 24 h post pulse. Values from each point were normalized to control values. Values represent the mean ± SE ($n = 3$), $^{*}p < 0.05$; $^{**}p < 0.01$.

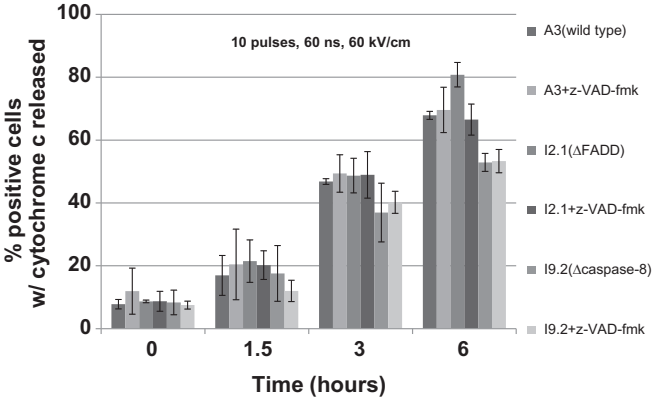

Fig. 3. NsPEFs induce caspase-independent cytochrome *c* release in Jurkat cells. Jurkat clones were preincubated with 50 μM pan caspase inhibitor z-VAD-fmk for 30 min followed by exposure to ten 60 ns pulses at 60 kV/cm and then analyzed by flow cytometry at indicated times. Cytochrome *c* release was evaluated by measuring percentage of cells that exhibited decreased levels of cytoplasmic cytochrome *c* at the indicated time point post pulse using the InnoCyte Flow Cytometric cytochrome *c* release assay. The graph represents the mean ± SE ($n = 3$). There were no statistical difference between presence and absence of z-VAD-fmk and statistical differences were observed for cytochrome c release 3 and 6 h after pulse treatment ($p < 0.5$).

indeed defective, we first showed that unlike WT, both of these clones were resistant to Fas-induced cell death (data not shown) as had been previously demonstrated [21]. To determine if nsPEFs required DISCs to induce cell death, cell viability was determined after nsPEF treatment in WT, ΔFADD and ΔC-8 clones (Fig. 2).

The cells were exposed to ten 60 ns pulses with increasing electric fields from 0 to 60 kV/cm and cell viability was determined 24 h post pulse. Results indicated that nsPEFs induced cell death in all clones with identical electric field dependence. The electric field LD_{50} was between 30 and 40 kV/cm and pulsing at 60 kV/cm resulted in ⩽10% survival in all three clones. Thus, cell death in Jurkat clones treated with nsPEFs was independent of DISC or other mechanisms using FADD or caspase-8. This suggests that nsPEF-induced cell death does not occur through the type II cell extrinsic apoptosis pathway in Jurkat cells. However, in ΔC-8 cells, which expressed very low levels of caspase-3/7 and -9, cell death was not different than WT, suggesting that apoptosis is activated, but is not required for cell death. When Jurkat cells were exposed to the pan caspase inhibitor z-VAD-fmk and stimulated with nsPEFs, cell death was still evident (data not shown). It was previously shown that when Fas-stimulated Jurkat cell caspases were inhibited by z-VAD-fmk and when these same FADD or capsase-8 deficient clones were stimulated with Fas, a caspase independent cell death was evident [21]. It was further shown that TNFα sensitized these cells to caspase-independent cell death pathway(s) [22] that were characterized as programmed necrosis [23,24]. Taken together these results indicate that nsPEFs can induce caspase-independent cell death pathways that may be common to cell death receptor pathways when caspase activity is absent.

To further investigate whether nsPEF-induced cell death occurred through an extrinsic apoptosis pathway, we considered that if a cytochrome-c mediated cell death pathway was activated through the type II extrinsic pathway, cytochrome c release would be sensitive to caspase inhibition and should not occur in ΔFADD or ΔC-8. Therefore, we treated WT, ΔFADD and ΔC-8 to ten 60 ns pulses at 60 kV/cm and determined cytochrome c release in the presence and absence of the pan caspase inhibitor z-VAD-fmk (Fig. 3). In all clones, cytochrome *c* release was unaffected by caspase inhibition. This demonstrates that nsPEF-induced cell death does not occur through a type II extrinsic apoptosis pathway that leads to cytochrome c release.

To further investigate possible activity through this pathway, we reasoned that Bid cleavage should be absent if it were downstream of DISC. However, if Bid cleavage were present, it is

expected to occur as part of an amplification pathway downstream of cytochrome c release and activation of caspase-9 and -3. In data not shown, the presence of t-Bid was evident on immunoblots in all three nsPEF-stimulated clones. Bid cleavage was partially caspase-dependent using Z-VAD-fmk and partially calpain-dependent using a calpain-specific inhibitor, Z-LLY-fmk. Given that Bid cleavage could not occur in ΔFADD and ΔC-8, it must result from caspase activity downstream of cytochrome c release, which is the amplification pathway in Fas-mediated apoptosis in type II cells. Thus, even though nsPEFs do not activate the extrinsic type II pathway through DISC, this amplification step is activated. However, because cytochrome c release was not altered in response caspase inhibition, nsPEF-induced Bid cleavage appears to be inconsequential for cytochrome c release. This suggests that in response to nsPEFs, it is sufficient to activate caspase-dependent pathways and amplification through Bid cleavage is superfluous. This is in contrast to etoposide-induced apoptosis in Jurkat cells where caspase-mediated amplification of initial mitochondria apoptotic events are an essential part of cell death. Thus, nsPEFs can activate this step that may determine cell death in response to stress [10].

3.3. The absence of APAF-1 abolishes caspase-3/7 activity and promotes survival at low electric fields, but not at high electric fields

Cytochrome c release and caspase activation were associated with nsPEF-induced cell death in Jurkat cells; however, cell death was not shown to depend on these events [11], and Figs. 1–3. Given that nsPEFs do not activate type II extrinsic apoptosis (Fig. 3), the possibility was investigated that nsPEF-induced cell death was through an intrinsic, cytochrome c-, APAF-1-dependent activation of caspase-9 and caspase-3. To determine whether nsPEF-induced cell death was caspase-dependent and whether caspase activation and cell death required formation of apoptosomes, ΔAPAF and its control vector clone were exposed to electric fields between 0 and 60 kV/cm and catalytic activities of caspase 3/7 were determined 6 h after treatment (Fig. 4A), and cell viability was determine 24 h after treatment (Fig. 4B). Time courses indicated that nsPEF induced a robust caspase-3/7 activation in WT clones (data not shown) with a peak 6 h after treatment. Fig. 4A shows that caspase-3 catalytic activity in the ΔAPAF-1 was absent at all electric fields tested, confirming that APAF-1 was silenced, as previously demonstrated [18]. In contrast, nsPEFs activated caspase-3/7 in an electric field-dependent, biphasic manner. There was no caspase-3 activation at 10 kV/cm. As electric fields increased to 40 kV/cm, there was an electric field dependent increase in caspase-3/7 activity. However, at higher electric fields, caspase-3 activity decreased such that at 60 kV/cm it was similar to control.

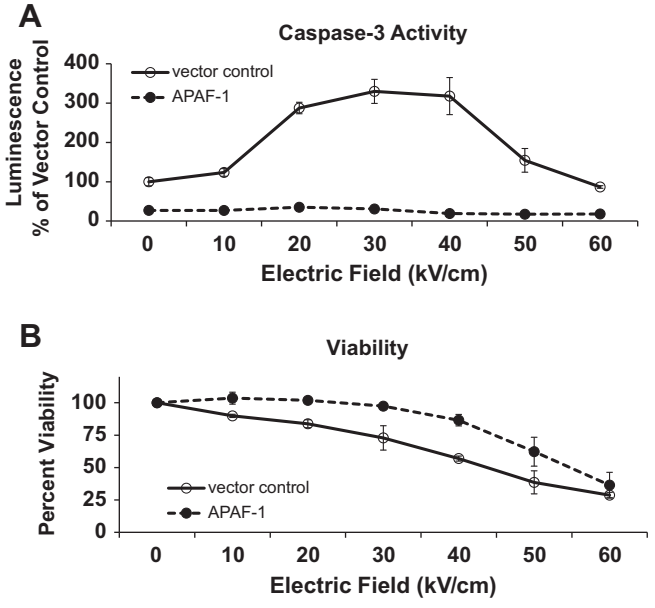

Fig. 4. The absence of APAF-1 abolishes caspase-3/7 and -9 activities and promotes survival at low electric fields. The Jurkat clone deficient in APAF-1 (●) and its corresponding vector control (○) were sham treated or treated with ten 60 ns pulses at increasing electric fields analyzed for caspase-3/7 catalytic activity (A) as described in Experimental Procedures 6 h after treatment. (B) With the same treatments, both clones were treated with nsPEFs and analyzed for cell viability determined by CellTiter-Glo luminescent assay 24 h post pulse.

To determine cell death under these same conditions, cells were analyzed for survival 24 h after treatment with nsPEFs (Fig. 4B). Cell death in the vector control was electric field-dependent with a threshold for cell death at 20 kV/cm, a LD_{50} between 40 and 50 kV/cm and 75% cell death at 60 kV/cm. In contrast, cell death in ΔAPAF was shifted significantly to higher electric fields; the threshold for cell death was between 40 and 50 kV/cm with an electric field LD_{50} between 50–60 kV/cm. This indicates that at these electric fields, cell death is APAF-1- and caspase-3-dependent and suggests that cell death is induced at least in part by apoptosis at electric fields between 10 and 40 kV/cm. However, at 60 kV/cm when caspase-3 activity was similar to control, cell death was not significantly different between vector control and ΔAPAF. This indicates that at electric fields $\geqslant$50 kV/cm, cell death is caspase-independent.

Data indicate that nsPEFs activate intrinsic cell death; however, these programs are activated by diverse intracellular signals that can originate from endoplasmic reticulum (ER), mitochondria, and nucleus or indirectly through events downstream of plasma membrane permeabilization, among others. ER stress can induce cell death by releasing intracellular calcium, which we did not detect. A possible reason for these results may be due to release of calcium in micro-domains with local concentrations high enough to activate calcium uniporters in outer inner mitochondria membranes [25,26], but below the sensitivity of Fluo-4. To determine nsPEF-induced ER stress it will be helpful to analyze ER stress markers. DNA damage is another possible activator of intrinsic apoptosis. In data not shown, increases in Noxa and Puma, which would induce cytochrome c release in response to DNA damage, were not observed, suggesting that these DNA damage pathways were not activated by nsPEFs under these conditions. Also, increases in Bcl-2, Bcl-xl or Bak we not observed.

In a study related to this one [17], analysis of nsPEF effects on plasma membranes and mitochondria membranes indicated that mitochondria are primary nsPEF targets resulting in dissipation of $\Delta\Psi$m and cell death. Cell death was independent of calcium and insensitive to Bcl-2 and Bcl-xl. Plasma membranes were also shown to be targets in that permeabilization of them allowed calcium influx, which enhanced dissipation of $\Delta\Psi$m.

The data in this report indicate that like other apoptotic stimuli, nsPEFs can activate more than one cell death program. As defined here, nsPEFs induce both caspase-dependent (apoptosis) and caspase-independent cell death, possibly by programmed necrosis or necroptosis, which can be activated by other apoptotic stimuli in Jurkat cells when caspases are inhibited [21–23,27]. A focus on apoptosis pathways delineate an intrinsic, APAF-1- and caspase-dependent apoptosis pathway, which is sufficient for inducing cell death at lower electric fields. This is highly likely to occur downstream of cytochrome c release induced by direct and/or indirect nsPEF effects on mitochondria that are sufficiently intense to forgo the need for amplification through Bid cleavage. Although cell death is diminished at lower electric fields in the ΔAPAF-1, indicating caspase-dependent cell death, cell death was not diminished in ΔFADD, which exhibited high caspase activity, or in ΔC-8, which

exhibited low caspase activity. Taken together, caspase-dependent and –independent cell death pathways are likely functioning simultaneously. Superficially, cell death in cancer may be considered to be sufficient regardless of the mechanism. However, increasing understanding of cell death and immune mechanisms indicate that caspase-dependent death appears to be required for immunogenic cell death and dendritic cell antigen presentation to T-cells [28]. This has critical implications for cancer treatment.

Overall these studies demonstrate that nsPEFs are a potent activator of cell death, supporting the successes observed in using them to eliminate cancer [4–10]. This study shows that cancer causing mechanisms that prevent death receptor-induced apoptosis as well as those that could inhibit apoptosis by blocking cytochrome c release and APAF-1 can be displaced by effects of nsPEFs through multiple cell death pathways. This should provide important advantages for nsPEF ablation of cancer.

Acknowledgments

We thank Drs. John Robertson and Shary Shelton for providing the Jurkat clones that are deficient in APAF-1. We thank the Center for Bioelectrics and Mr. Frank Reidy for support of this work.

References

[1] K.H. Schoenbach, S.J. Beebe, E.S. Buescher, Intracellular effect of ultrashort electrical pulses, Bioelectromagnetics 22 (2001) 440–448.
[2] D.A. Stewart, T.R. Gowrishankar, J.C. Weaver, Transport lattice approach to describing electroporation: use of local asymptotic model, IEEE Trans. Plasma Sci. 32 (2004) 1696–1708.
[3] T.R. Gowrishankar, A.T. Esser, Z. Vasilkoski, K.C. Smith, J.C. Weaver, Microdosimetry for conventional and supra-electroporation in cells with organelles, Biochem. Biophys. Res. Commun. 341 (2006) 1266–1276.
[4] S.J. Beebe, W.E. Ford, W. Ren, X. Chen, Pulse Power Ablation of Melanoma with Nanosecond Pulsed Electric Fields, in: R. Morton (Ed.), Treatment of Metastatic Melanoma, InTech, Croatia, 2011, pp. 231–268.
[5] S.J. Beebe, P.M. Fox, L.J. Rec, E.S. Buescher, K. Somers, K.H. Schoenbach, Nanosecond pulsed electric field (nsPEF) effects on cells and tissues: Apoptosis induction and tumor growth inhibition, IEEE Trans. Plasma Sci. 30 (2002) 286–292.
[6] R. Nuccitelli, U. Pliquett, X. Chen, W. Ford, R.J. Swanson, S.J. Beebe, J.F. Kolb, K.H. Schoenbach, Nanosecond pulsed electric fields cause melanomas to self-destruct, Biochem. Biophys. Res. Commun. 343 (2006) 351–360.
[7] R. Nuccitelli, X. Chen, A.G. Pakhomov, W.H. Baldwin, S. Sheikh, J.L. Pomicter, W. Ren, C. Osgood, R.J. Swanson, J.F. Kolb, S.J. Beebe, K.H. Schoenbach, A new pulsed electric field therapy for melanoma disrupts the tumor's blood supply and causes complete remission without recurrence, Int. J. Cancer 125 (2009) 438–445.
[8] E.B. Garon, D. Sawcer, P.T. Vernier, T. Tang, Y. Sun, L. Marcu, M.A. Gundersen, H.P. Koeffler, In vitro and in vivo evaluation and a case report of intense nanosecond pulsed electric field as a local therapy for human malignancies, Int. J. Cancer 121 (2007) 675–682.
[9] X. Chen, J.F. Kolb, R.J. Swanson, K.H. Schoenbach, S.J. Beebe, Long term survival of mice with hepatocellular carcinoma after pulse power ablation with nanosecond pulsed electric fields, Pigment Cell Melanoma Res. 23 (2010) 554–563.
[10] X. Chen, J. Zhuang, J.F. Kolb, K.H. Schoenbach, S.J. Beebe, Apoptosis initiation and angiogenesis inhibition: melanoma targets for nanosecond pulsed electric fields, Technol. Cancer Res. Treat. 11 (2012) 83–93.
[11] S.J. Beebe, P.M. Fox, L.J. Rec, E.L. Willis, K.H. Schoenbach, Nanosecond, high-intensity pulsed electric fields induce apoptosis in human cells, FASEB J. 17 (2003) 1493–1495.
[12] P.T. Vernier, Y. Sun, L. Marcu, S. Salemi, C.M. Craft, M.A. Gundersen, Calcium bursts induced by nanosecond electric pulses, Biochem. Biophys. Res. Commun. 310 (2003) 286–295.
[13] E.H. Hall, K.H. Schoenbach, S.J. Beebe, Nanosecond pulsed electric fields induce apoptosis in p53-wildtype and p53-null HCT116 colon carcinoma cells, Apoptosis 12 (2007) 1721–1731.
[14] W.E. Ford, W. Ren, P.F. Blackmore, K.H. Schoenbach, S.J. Beebe, Nanosecond pulsed electric fields stimulate apoptosis without release of pro-apoptotic factors from mitochondria in B16f10 melanoma, Arch. Biochem. Biophys. 497 (2010) 82–89.
[15] W. Ren, S.J. Beebe, An apoptosis targeted stimulus with nanosecond pulsed electric fields (nsPEFs) in E4 squamous cell carcinoma, Apoptosis 16 (2011) 382–393.
[16] C. Scaffidi, I. Schmitz, J. Zha, S.J. Korsmeyer, P.H. Krammer, M.E. Peter, Differential modulation of apoptosis sensitivity in CD95 type I and type II cells, J. Biol. Chem. 274 (1999) 22532–22538.
[17] W. Ren, S.M. Sain, S.J. Beebe, Mitochondria are primary targets for nanosecond pulsed electric fields nsPEFs in Jurkat cells, submitted for publication.
[18] M.E. Shawgo, S.N. Shelton, J.D. Robertson, Caspase-mediated Bak activation and cytochrome c release during intrinsic apoptotic cell death in Jurkat cells, J. Biol. Chem. 283 (2008) 35532–35538.
[19] M.E. Shawgo, S.N. Shelton, J.D. Robertson, Caspase-9 activation by the apoptosome is not required for Fas-mediated apoptosis in type II Jurkat cells, J. Biol. Chem. 284 (2009) 33447–33455.
[20] S.N. Shelton, C.D. Dillard, J.D. Robertson, Activation of caspase-9, but not caspase-2 or caspase-8, is essential for heat-induced apoptosis in Jurkat cells, J. Biol. Chem. 285 (2010) 40525–40533.
[21] C. Vonarbourg, M.C. Stolzenberg, E. Hölzelova, A. Fischer, F.L. Deist, F. Rieux-Laucat, Differential sensitivity of Jurkat and primary T cells to caspase-independent cell death triggered upon Fas stimulation, Eur. J. Immunol. 32 (2002) 2376–2384.
[22] C.P. Lawrence, S.C. Chow, FADD deficiency sensitises Jurkat T cells to TNF-alpha-dependent necrosis during activation-induced cell death, FEBS Lett. 579 (2005) 6465–6472.
[23] F.K. Chan, J. Shisler, J.G. Bixby, M. Felices, L. Zheng, M. Appel, J. Orenstein, B. Moss, M.J. Lenardo, A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses, J. Biol. Chem. 278 (2003) 51613–51621.
[24] G. Kroemer, S.J. Martin, Caspase-independent cell death, Nat. Med. 11 (2005) 725–730.
[25] J.M. Baughman, F. Perocchi, H.S. Girgis, M. Plovanich, C.A. Belcher-Timme, Y. Sancak, X.R. Bao, L. Strittmatter, O. Goldberger, R.L. Bogorad, V. Koteliansky, V.K. Mootha, Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter, Nature 476 (2011) 341–345.
[26] D. De Stefani, A. Raffaello, E. Teardo, I. Szabò, R. Rizzuto, A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter, Nature 476 (2011) 336–340.
[27] G. Kroemer, S.J. Martin, Caspase-independent cell death, Nat. Med. 11 (2005) 725–730.
[28] O. Kepp, L. Galluzzi, I. Martins, F. Schlemmer, S. Adjemian, M. Michaud, A.Q.Sukkurwala, L. Menger, L. Zitvogel, G. Kroemer, Molecular determinants of immunogenic cell death elicited by anticancer chemotherapy, Cancer Metastasis Rev. 30 (2011) 61–69.