The Activity of Cytosolic Phospholipase A₂ Is Required for the Lysis of Adenovirus-Infected Cells by Tumor Necrosis Factor

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Most cell types are resistant to apoptosis induced by tumor necrosis factor (TNF) unless the cells are treated with a sensitizing agent. Inhibitors of transcription or translation act as sensitizing agents, as do adenoviruses lacking one or more resistance genes. We have reported recently that the activity of cytosolic phospholipase A₂ (cPLA₂) is necessary for the TNF-induced lysis of cells that are sensitized by inhibitors of transcription or translation (C. Voelkel-Johnson, T. E. Thorne, and S. M. Laster, *J. Immunol.* 156:201–207, 1996). In this report we have asked whether the lysis of cells infected by the adenovirus *dl758* (which lacks the E3 14.7-kDa resistance gene product) also involves the activity of cPLA₂. We report that a phosphorothioate-modified antisense oligonucleotide specific for cPLA₂, but not the control oligonucleotide, inhibited the TNF-induced release of both [³H]arachidonic acid and ⁵¹Cr from infected cells. Arachidonyltrifluoromethyl ketone (AA COCF₃), an inhibitor of cPLA₂, also inhibited the release of ⁵¹Cr, and we found that the release of [³H]arachidonic acid was highly selective and was preferred over the release of [³H]palmitic acid. Taken together, these results suggest strongly that cPLA₂ is indeed the phospholipase responsible for the release of [³H]arachidonic acid during the lysis of infected cells and that its activity is necessary for cell death. Finally, since arachidonic acid serves as the substrate for the synthesis of inflammatory lipids, our results suggest a possible link between the TNF-induced lysis of infected cells and inflammation. The E3 14.7-kDa resistance protein may, therefore, play two roles: preventing TNF-induced cell death and, as our results show, preventing the TNF-induced release of arachidonic acid.

Tumor necrosis factor alpha (TNF) is a 17-kDa inflammatory cytokine which is known to mediate a variety of different immune and inflammatory reactions (36). With certain cell types, the binding of TNF to its receptors causes cell death. Included in this category are tumor-derived cell lines such as L929 fibroblasts or Meth A sarcoma cells (3). Cells that have been treated with inhibitors of transcription (28) or translation (19) also become susceptible to TNF, as do cells that have been infected with certain viruses, such as adenovirus (5, 8), Newcastle disease virus (25), human immunodeficiency virus (27), and herpesvirus (17). In addition, infections with the intracellular bacteria *Salmonella typhimurium*, *Shigella flexneri*, and *Listeria monocytogenes* have been reported to induce susceptibility to TNF (16). In most cases, TNF-induced lysis includes nuclear disintegration (7, 22), inactivation of the mitochondria (20), and plasma membrane blebbing (22) and is, therefore, categorized as apoptosis, although a necrotic form of lysis has also been reported (22).

The phenomenon of microorganism-induced susceptibility to TNF has been studied most extensively in cells infected by the human adenovirus. Transfection experiments (5) and studies using adenovirus deletion mutants (8) have revealed that the adenovirus E1A protein is responsible for inducing susceptibility to TNF. Deletion mutants have been utilized further to show that susceptibility to TNF is induced by the CD1 region of E1A (9). This region of E1A is also responsible for binding a number of cellular proteins, such as p300 (1) and the retinoblastoma protein (2), and either of these binding sites in CD1 is sufficient to induce susceptibility to TNF (33). Adenovirus also encodes a number of proteins to protect its host cell from TNF, presumably to counter the sensitizing action of

E1A. The E1B 19-kDa protein (39), the E3 14.7-kDa protein (12), and the E3 10.4-kDa–14.5-kDa dimer (13) function in different cell types to keep the infected cell alive in the presence of TNF.

The TNF-induced lysis of susceptible tumor cells is accompanied by the release of arachidonic acid into the culture media (14). The release of arachidonic acid also accompanies the lysis of cells rendered sensitive to TNF by inhibitors of transcription or translation (38). Transfection experiments (14, 38) and antisense technology (38) have been used to show that the high-molecular-weight cytosolic form of phospholipase A₂ (PLA₂), known as cytosolic PLA₂ (cPLA₂), is responsible for this effect. These studies have also revealed that the activity of cPLA₂ is necessary for cell death. In fact, measurements of cPLA₂ can be used to predict TNF susceptibility among human melanoma tumor cells (38). In this study we have examined the death of cells rendered sensitive to TNF by infection with an adenovirus lacking the E3 14.7-kDa gene product. Our results show that [³H]arachidonic acid is released from these cells as they die. We find that cPLA₂ is responsible for this effect, and as with tumor-derived cell lines, its activity is necessary for cell lysis.

MATERIALS AND METHODS

Cell culture. C3HA and NIH 3T3 cell lines are murine 3T3-like cells that were cultured in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum and maintained at 37°C and 8% CO₂. NR-5 cells are NIH 3T3 cells transfected with the T24 ras vector. NER-7 cells were transfected with the same vector containing the gene for E1A. NR-5 and NER-7 cells were also cotransfected with pSV2-neo plasmid. These cells were grown as described above with the addition of G418 (200 µg/ml). All cells were supplied to us by L. Gooding, Emory University, Atlanta, Ga.

Reagents. Media and chemicals were purchased from Sigma Chemical Co., St. Louis, Mo. TNF and other cytokines were purchased from Quality Control Biochemicals, Inc., Hopkinton, Mass. Radiolabeled compounds were purchased from Dupont NEN Corporation, Boston, Mass.

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Viruses and infections. The adenovirus *rec700* is a full-length virus whose E3 region is from adenovirus type 2 and the rest of which is from adenovirus type 5. The mutant *dl758* was derived from *rec700* and lacks the E3 14.7-kDa-protein gene. The construction of these viruses has been described in detail previously (40). The viruses were supplied to us by W. S. M. Wold, St. Louis University, St. Louis, Mo. For infections, C3HA cells were plated overnight, washed, and incubated with 200 PFU of virus per cell for 1.5 h in serum-free media. Cells were then returned to media with serum and incubated 24 h before treatment with TNF or other cytokines.

⁵¹Cr-release assays. Cells were labeled overnight and harvested, and 10⁴ were added to 96-well flat-bottom assay plates with media and TNF. Then, following 16 h of incubation, ⁵¹Cr release in 100-μl aliquots was determined by gamma counting (Autogamma; Packard Instrument Co., Downers Grove, Ill.). The percent specific ⁵¹Cr release was calculated by the following formula: [(experimental release - spontaneous release)/(maximum release - spontaneous release)] × 100. Maximum release was determined with 1 N HCl, and all treatments were done in triplicate.

Radiolabeled fatty acid release assays. A total of 10⁵ cells per well were plated in 12-well tissue culture plates and labeled overnight with 0.1 μCi of [³H]arachidonic acid or [³H]palmitic acid per ml. The following morning, the cells were washed twice with Hanks balanced salt solution, incubated in media for 2 h, and washed two more times with HBSS; cytokines were then added. Radiolabel release in supernatants was determined by scintillation counting (Beckman Instruments, Inc., Schaumburg, Ill.). All treatments were done in triplicate, and 1% sodium dodecyl sulfate was used to determine total incorporation.

sn-2 fatty acid analysis. Cells were lysed by sonication, and membranes were recovered by centrifugation. Membranes were then resuspended and incubated with 15 enzyme units of PLA₂ from *Naja naja* for 4 h at 37°C. The reaction was terminated with EDTA, and the mixture was extracted with Dole's reagent. Silicic acid was used to remove any undigested phospholipids, and the resulting fatty acids were dried under nitrogen. Free fatty acids were converted to methyl esters in acidic methanol and quantitated by using an HP 5890 gas chromatograph with a 30-m Carbowax column. Helium was used as the carrier gas, and the oven temperature was programmed from 100 to 240°C (8°C/min).

Antisense oligonucleotide experiments. Oligonucleotides were purchased from Midland Certified Reagent Co., Midland, Tex. The murine cPLA₂ antisense oligonucleotide (5'-ATAAGGATCTATGAAAGACAT-3') was directed against the initiation codon and the 18 nucleotides downstream. The nonsense oligonucleotide (5'-TAGGTAGGAATCACTAAATA-3') was derived randomly from the antisense sequence and was used as the control. Both oligonucleotides were phosphorothioate modified and added to the cells at a concentration of 150 μM for 36 h prior to the start of treatments with TNF. The expression of cPLA₂ was monitored by Western blotting (immunoblotting) with an anti-cPLA₂ antiserum supplied by J. Clark, Genetics Institute.

RESULTS

Sensitization by adenovirus. In this report we focus on the ability of the adenovirus *dl758*, which lacks the gene for the E3 14.7K protein, to cause TNF susceptibility in C3HA fibroblasts (5, 8, 12, 22). The virus *rec700*, which is the parent of *dl758* and which expresses a 14.7K protein and does not cause susceptibility, was included as a control. Previous studies have shown that the death of C3HA cells that are infected with *dl758* is apoptotic and includes membrane blebbing (22), protease activation, and breakdown of the nuclear membrane (37), although fragmentation of the DNA does not occur (22). The sensitizing effect of E1A itself was studied with the cell line NER-7, which expresses E1A in the T24 ras vector and is killed by TNF (8). Cells of the NIH 3T3 cell line, the parent of NER-7, and NR-5 cells, which express only the vector, were also included as controls. Neither of these cell lines is killed by TNF (8).

Release of [³H]arachidonic acid. PLA₂-dependent (14, 38) and PLA₂-independent (30) pathways of TNF-induced lysis have both been reported. Which pathway is active in C3HA cells that are infected with *dl758* and killed by TNF? We addressed this question by studying the release of [³H]arachidonic acid, which accompanies PLA₂-dependent (14, 38) but not PLA₂-independent (30) cell death. These experiments were performed in two phases: we examined first the effects of adenovirus itself and then the effects of TNF on the release of [³H]arachidonic acid. In phase 1, C3HA cells were labeled with [³H]arachidonic acid for 24 h, washed, exposed to adenovirus for 1.5 h in serum-free media, washed again, and returned to

FIG. 1. Release of [³H]arachidonic acid (³H-AA) from adenovirus-infected cells. C3HA cells were labeled overnight with [³H]arachidonic acid and then infected; radiolabel release into the media was assayed during the course of infection. This experiment was repeated three times with identical results.

media containing serum. The release of [³H]arachidonic acid into the media was then monitored for 24 h. Control cells were treated in the same manner except that adenovirus was not included. As shown in Fig. 1, returning washed cells to media with serum, without virus infection, did stimulate the release of [³H]arachidonic acid. Release began rapidly after reexposure to media with serum and reached a maximum after approximately 6 h. However, this pattern did not change when C3HA cells were infected with either *rec700* or *dl758*, indicating that infection per se does not activate a PLA₂-type enzyme.

After this the cells were washed once again and then exposed to serum-containing media, with or without TNF. As shown in Fig. 2A, at this time, serum-containing media did not cause the release of [³H]arachidonic acid from uninfected cells, nor did treatment with TNF. In contrast, TNF did stimulate the release of [³H]arachidonic acid from cells that had been infected with *dl758* (Fig. 2B), suggesting that the death of these cells is accompanied by activation of a PLA₂-type enzyme. The release of [³H]arachidonic acid began approximately 6 h after TNF was added (2 to 4 h before ⁵¹Cr release began) and continued throughout the incubation period. This effect was not seen with C3HA cells that were infected with *rec700* (Fig. 2C). Note also that both viruses caused a 30 to 40% decrease in the level of background release.

The effect of TNF on the release of [³H]arachidonic acid from NIH 3T3, NER-7, and NR-5 cell lines is shown in Fig. 2D through F. A 10 to 20% increase in radiolabel release was seen with NIH 3T3 cells, but this effect occurred rapidly and did not increase with time (Fig. 2D). In contrast, TNF treatment of NER-7 cells did cause sustained release of [³H]arachidonic acid (Fig. 2E). Radiolabel release began 2 to 4 h after treatment was initiated, and by 24 h, levels were approximately 200% above background. TNF did not cause the release of [³H]arachidonic acid from the NR-5 vector control cell line (Fig. 2F). Finally, note that the background level of radiolabel release is decreased by approximately 50% in NER-7 cells, compared with levels in either the NIH 3T3 or the NR-5 cell line.

Identification of cPLA₂. Which form of PLA₂ is responsible for the TNF-induced release of [³H]arachidonic acid from cells infected with *dl758*? We have shown previously that C3HA fibroblasts express cPLA₂ but do not express detectable levels

FIG. 2. TNF-induced release of [^3H]arachidonic acid (^3H -AA) from adenovirus-infected or E1A-expressing cells. C3HA cells, either uninfected (A), infected with *dl758* (B), or infected with *rec700* (C), were treated with 20 ng of TNF per ml. NIH 3T3 cells, either untreated (D) or transfected with the T24 ras vector plus the gene for E1A (E) or vector alone (F), were treated similarly. The complete time course was repeated twice, along with numerous endpoint determinations.

of low-molecular-weight PLA_2 s (38). Is cPLA_2 the enzyme activated in this response? To test this hypothesis, we monitored the release of [^3H]arachidonic acid from C3HA cells treated with an antisense oligonucleotide specific for cPLA_2 . We have used this method previously to inhibit the expression of cPLA_2 in uninfected C3HA fibroblasts and found (38), as others have reported (24, 31), that the expression of cPLA_2 could be inhibited by 50 to 70%. As shown in Fig. 3, the antisense oligonucleotide works similarly in C3HA cells that are infected with *dl758*. In this experiment immunoreactive material was suppressed by 70% (lane C), while the control oligonucleotide had no effect (lane B). Expression of cPLA_2

FIG. 3. Suppression of cPLA_2 by antisense oligonucleotides. C3HA cells were treated with oligonucleotides and infected by adenovirus. Subsequently, lysates were prepared and analyzed for the expression of cPLA_2 by Western blotting. The protein in lane A comes from cells that were infected but did not receive oligonucleotide, while lanes B and C show results for cells treated with nonsense and antisense oligonucleotides, respectively. The 75-kDa band represents a proteolytic fragment of cPLA_2 (37).

was inhibited by 57% in a second experiment with infected cells (data not shown).

As shown in Fig. 4A, the antisense oligonucleotide completely prevented the TNF-induced release of [^3H]arachidonic acid from C3HA cells infected with *dl758*, providing strong evidence that cPLA_2 is indeed the form of PLA_2 that is activated during this response. The antisense oligonucleotide also completely prevented the release of ^{51}Cr (Fig. 4B), indicating that the activity of cPLA_2 is necessary to this response. To confirm this observation, we also tested the effects of arachidonyltrifluoromethyl ketone (AACOCF_3), a fluorinated derivative of arachidonic acid that selectively inhibits the activity of cPLA_2 (34). As shown in Fig. 4C, this compound also prevented the TNF-induced lysis of cells infected with *dl758*, supporting the conclusion that the activity of cPLA_2 is necessary for cell death.

Ligand specificity. As shown in Fig. 5, the ability of *dl758* to prime C3HA cells for the release of [^3H]arachidonic acid is restricted to TNF. Infection with *dl758* did not augment responsiveness to interleukin 1, interleukin 6, or gamma interferon. Responsiveness to thrombin, A23187, or ATP, which have all been used previously to induce the release of [^3H]arachidonic acid (23), also was not enhanced in C3HA cells infected with *dl758* (data not shown).

Fatty acid selectivity in dying cells. The enzyme cPLA_2 is important for inflammatory responses because it is selective for arachidonic acid (6). To determine whether that selectivity is expressed, in situ, in dying cells, we used the following proce-

FIG. 4. Suppression of the release of [³H]arachidonic acid (³H-AA) and ⁵¹Cr. C3HA cells were treated with either antisense or nonsense oligonucleotides (as described in Materials and Methods), radiolabeled, infected with *dl758* or left uninfected (Uninf.), and treated with TNF (20 ng/ml). The releases of [³H]arachidonic acid (A) and ⁵¹Cr (B) were then measured after 10 and 16 h, respectively. (C) Effect of AACOCF₃ on the release of ⁵¹Cr. Infected cells were pretreated for 1.5 h with AACOCF₃, and treatment was continued throughout the 16-h ⁵¹Cr release assay. The data shown in panels A, B, and C are means $\pm$ standard errors of the means from two, three, and three experiments, respectively.

First, we examined the fatty acids that are found at the sn-2 position in C3HA membranes. A nonspecific form of PLA₂ was used to obtain sn-2 fatty acids, which were analyzed by gas chromatography. As summarized in Table 1, the major fatty acids we found were myristic acid (26%), oleic acid (24%), palmitic acid (17%), and palmitoleic acid (15%), while arachidonic acid accounted for only 7%. Activation of a nonspecific form of PLA₂ during lysis would, therefore, be predicted to release free fatty acids in those proportions, i.e., 2.4-fold more palmitic acid than arachidonic acid ($17/7 = 2.4$). In contrast, as shown in Table 1, we found that [³H]arachidonic acid was released preferentially over [³H]palmitic acid. At 24 h, for example, levels of [³H]arachidonic acid in the media had increased by 207%, while levels of [³H]palmitic acid had increased by 37% (a 5.6-fold preference for arachidonic acid over palmitic acid). We conclude, therefore, that cPLA₂ indeed selectively cleaves arachidonic acid. In this case, for example, the enzyme selects arachidonic acid for cleavage 13.4 times more frequently than palmitic acid. We used [³H]palmitic acid in these experiments because it was available at the same molar concentration and specific activity as [³H]arachidonic acid.

DISCUSSION

TNF can induce apoptosis in certain tumor-derived cell lines; however, this phenotype is not typical (10, 35). With most cell types, both normal and transformed, the binding of TNF to its receptors does not cause cell death. This situation changes, however, when cells are infected by certain viruses. In this report we have determined that the activity of cPLA₂ is required for the death of C3HA fibroblasts that are infected with the adenovirus *dl758*. This conclusion is based largely on the activity of an antisense oligonucleotide corresponding to cPLA₂, which completely inhibited the release of both [³H]arachidonic acid and ⁵¹Cr, indicating that cPLA₂ is responsible for the release of arachidonic acid during cell death and that this activity is necessary for the lytic response.

The antisense strategy has been used successfully to investigate cPLA₂'s role in certain monocyte responses, in which a 10 to 20 μ M concentration of oligonucleotide typically inhibits expression by 50 to 75%, as shown by a Western blot assay (24, 31). In C3HA fibroblasts we found that a higher concentration of oligonucleotide was required (150 μ M), apparently because

FIG. 5. Only the cytokine TNF stimulated the release of [³H]arachidonic acid (³H-AA) from infected cells. Cells were labeled with [³H]arachidonic acid, and treated with either TNF (20 ng/ml), interleukin 1 (IL-1; 5 ng/ml), interleukin 6 (IL-6; 5 ng/ml), or gamma interferon (IFN- γ ; 100 U/ml). Radiolabel release into the media was measured after 16 h. The data shown are means $\pm$ standard errors of the means from two experiments.

TABLE 1. Analysis of C3HA fatty acids

Fatty acid	sn-2 membrane fatty acids (% of total) ^a	Free fatty acids (% release) ^b	
		–TNF	+TNF
Myristic (14:0)	25.8 ± 1.4	ND ^c	ND
Palmitic (16:0)	16.8 ± 3.9	22.6 ± 2.9	31.0 ± 2.5
Palmitoleic (16:1)	14.1 ± 1.4	ND	ND
Stearic (18:0)	7.1 ± 0.9	ND	ND
Oleic (18:1)	24.2 ± 2.0	ND	ND
Linoleic (18:2)	5.1 ± 0.5	ND	ND
Arachidonic (20:4)	6.8 ± 1.0	6.8 ± 0.6	20.9 ± 3.3

^a Values are means ± standard errors of the means from four independent isolations.

^b Fatty acids found in media after 24 h of incubation with TNF. Values are means ± standard errors of the means from three experiments.

^c ND, not done.

fibroblasts take up oligonucleotides more poorly than monocytes (26). Interestingly, in monocytes, under conditions in which the expression of cPLA₂ is partially inhibited, chemotaxis (24) and prostaglandin E₂ production (31) were inhibited completely. We have encountered a similar result. The oligonucleotide inhibited cPLA₂ expression by only 60 to 70%, while the release of [³H]arachidonic acid and cytotoxicity were inhibited completely. Taken together, these data may indicate that a threshold level of cPLA₂ needs to be expressed or, alternatively, that only the most recently synthesized cPLA₂ is useful to the cell.

We also tested AACOCF₃, a fluorinated form of arachidonic acid that acts as a selective inhibitor of cPLA₂ (34). This compound inhibited the lysis of C3HA cells that were infected with *dl758*, supporting our conclusion that the activity of cPLA₂ is necessary for this response. We found it impossible to use AACOCF₃ to study the release of [³H]arachidonic acid. It acted competitively with the radiolabel, simultaneously reducing uptake and enhancing the level of nonspecific release. Finally, the highly selective release of arachidonic acid in preference to palmitic acid also supports our conclusion that cPLA₂ is active in this response, since the low-molecular-weight membrane and secreted forms of PLA₂ are not selective for arachidonic acid over other fatty acids (32).

The activity of cPLA₂ has now been linked to TNF-induced death for a number of different target cell types, including spontaneously sensitive tumor-derived cell lines (14, 38), cells rendered sensitive by inhibitors of transcription and translation (38), and cells rendered susceptible by virus infection (reference 40 and this report). Why the activity of cPLA₂ is necessary for TNF-induced cell death is not known. On the basis of inhibitor studies (4), it has been suggested that one of the metabolites of the lipoxygenase pathway may be acting as a second messenger. Alternatively, arachidonic acid has been suggested as the activator for the neutral sphingomyelinase, whose activity has also been linked to the TNF-induced death of several tumor-derived cell lines (15). However, in this study sphingomyelinase activity appeared rapidly, within minutes after treatment with TNF. It is not clear whether cPLA₂ could serve in this role during the death of virus-infected cells, since we did not measure the release of [³H]arachidonic acid until several hours after TNF was added. Finally, it has been suggested that the activity of cPLA₂ and downstream enzymes could damage the cell (4). The loss of fatty acids could damage membranes, as could lipid peroxides, and many intracellular structures could be damaged by reactive oxygen species. Our results may support that hypothesis. We found constitutive activation of cPLA₂ in apoptotic cells, as opposed to the rapid

on-off pattern of activation in cells that are treated with proinflammatory but nontoxic ligands (6).

Infecting C3HA cells with *dl758* causes susceptibility to TNF. Previous studies have shown that the E1A gene product is responsible for this effect (5, 8). Our experiments with NER-7 suggest that E1A is also responsible for TNF's ability to activate cPLA₂. How the presence of E1A enables TNF to activate cPLA₂ is not clear. Using an in vitro assay, we have not found any change in total cPLA₂ activity either in infected C3HA fibroblasts or in NER-7 cells, compared with activity in the appropriate controls. It is unlikely, therefore, that the ability of TNF to activate cPLA₂ arises from enhanced expression of cPLA₂. It is more likely that E1A, via the changes in gene expression it produces, is altering the signaling pathway from the type I TNF receptor to cPLA₂. Experiments are now under way to examine the effects of E1A on MAP kinase activity and on levels of intracellular Ca²⁺, the two signals reported previously to activate cPLA₂ (6, 23).

We also show in this report that after 24 h of infection by adenovirus, the background level of [³H]arachidonic acid release was inhibited by 30 to 40%. This effect was equivalent with *dl758* and *rec700*, indicating that the 14.7K protein was not responsible. We found the same effect in the NER-7 transfectant (but not NR-5), suggesting that E1A is sufficient to cause this effect. At this time it is not clear if decreased levels of background release are due to an effect on cPLA₂. Background release was unaffected by the antisense oligonucleotide treatment, suggesting perhaps that another enzyme system(s) may be responsible for the release of [³H]arachidonic acid from unstimulated cells. E1A could, for example, be altering the rate at which arachidonic acid is reincorporated into phospholipid by affecting the activity of Δ6-desaturase (29).

We have suggested previously that the release of arachidonic acid from infected cells that are killed by TNF might serve as a proinflammatory signal (21). Linking cPLA₂ to this response strengthens that hypothesis because cPLA₂ is selective for arachidonic acid over other fatty acids. In studies in vitro with vesicles made from U937 membranes, cPLA₂ displayed roughly a 20:1 preference for the cleavage of arachidonic acid over other fatty acids (6). In our studies, we examined cPLA₂ activity in apoptotic cells, where the normal architecture of the cell is disrupted and regulatory controls may be aberrant. We felt it important, therefore, to demonstrate that the key attribute of cPLA₂ vis-à-vis inflammation, its selectivity, was expressed in apoptotic cells. We found that during apoptosis cPLA₂ retained a strong preference, albeit somewhat less than in vitro, for arachidonic acid. This finding suggests that during the TNF-induced death of cells infected with *dl758*, cPLA₂ could provide sufficient quantities of arachidonic acid for eicosanoid biosynthesis. On the other hand, cPLA₂ was not activated in cells infected with *rec700*, suggesting that the 14.7-kDa gene product may have evolved to prevent inflammation, in addition to protecting its host cell from lysis. In this context it is interesting that polymorphonuclear leukocytes failed to appear in lungs of cotton rats infected with wild-type adenoviruses but did appear in lungs of cotton rats infected with *sub304*, an adenovirus mutant lacking the right-hand end of the E3 region, including the gene expressing the 14.7-kDa protein (11).

TNF was described originally for its antitumor activity and its ability to kill selectively transformed cells in vitro (3). Subsequent studies showed, however, that susceptibility to TNF was rare among tumor-derived or transformed cells (10, 35), leaving the apoptosis-inducing activity of TNF without biological significance. The killing of virus-infected cells by TNF (5, 8, 17, 25) and the possession of resistance genes by adenovirus

(12, 13, 39) and cytomegalovirus (41) now indicates that the apoptosis-inducing activity of TNF exists to aid in host defense against viral infection. Connecting cPLA₂ to this system raises the possibility that the apoptotic death of virus-infected cells generates signals that can be perceived by immune and inflammatory cells, signals that might indicate the location of a virus infection or, alternatively, target infected tissue for removal by phagocytes.

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