Involvement of the ICE family of proteases in silica-induced apoptosis in human alveolar macrophages

RASHI IYER AND ANDRIJ HOLIAN
Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Toxicology Program, University of Texas Medical School, Houston, Texas 77030

Iyer, Rashi, and Andrij Holian. Involvement of the ICE family of proteases in silica-induced apoptosis in human alveolar macrophages. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L760–L767, 1997.—Exposure to silica dust can result in lung inflammation that may progress to fibrosis for which there is no effective clinical treatment. The mechanisms involved in the development of pulmonary silicosis have not been well defined; however, most current evidence implicates a central role for alveolar macrophages in this process. We have previously demonstrated that fibrotic agents, such as asbestos and silica, induce apoptosis in human alveolar macrophages. The goal of this study was to identify molecular events in the silica-induced apoptotic process to better understand the mechanism by which fibrotic agents may be inducing apoptosis in human alveolar macrophages. To elucidate the possible mechanism by which silica causes apoptosis, we investigated the involvement of the interleukin-converting enzyme (ICE) family of proteases. Human alveolar macrophages were treated with silica in vitro and were examined for the involvement of ICE, Ich1L, and cpp32β in silica-induced apoptosis. Pretreatment of cells with 10 µM of the ICE inhibitor z-Val-Ala-Asp-fluoromethyl ketone and the cpp32β inhibitor Asp-Glu-Val-Asp-fluoromethyl ketone completely blocked silica-induced apoptosis. Additionally, an increased formation of the active p20 fragments of ICE and Ich1L, as well as degradation of the inactivezymogen form of cpp32β protein were observed in silica-treated human alveolar macrophages, indicating activation of these proteases. Furthermore, degradation of the nuclear protein poly(ADP-ribose) polymerase was observed within 2 h of silica treatment. These results suggest that silica-induced apoptosis involves activation of the ICE family of proteases and is the first step in elucidating the intracellular mechanism of particulate-induced apoptosis in human alveolar macrophages.

interleukin-converting enzyme; Ich1L; cpp32β

Apoptosis or programmed cell death is a mechanism of cellular death believed to play an important role in a wide variety of physiological conditions (36). Deregression of apoptosis has been proposed to contribute to the pathogenesis of many diseases ranging from cancer to acquired immunodeficiency syndrome (26). Shrinkage of cells accompanied by unique DNA fragmentation, about 180–200 bp, caused by the activation of endonucleases are characteristic features of cells undergoing apoptosis (38). Although the detailed mechanism of apoptosis has not been established, proteases are thought to play an important role in the regulation of programmed cell death (23).

It appears that a family of cysteine proteases of the interleukin-converting enzyme (ICE) family are involved in programmed cell death or apoptosis (7, 23, 35, 37, 38). ICE is a cytoplasmic cysteine protease synthesized as an inactive 45-kDa precursor that is proteolytically cleaved to the active 20-kDa (p20) and 10-kDa (p10) heterodimer form. The primary known function of ICE is to cleave the inactive 31-kDa pro-interleukin (IL)-1β to generate the active 17.5-kDa form of IL-1β (25). In addition, several lines of evidence indicate that proteases such as ICE may be important modulators of apoptosis. A number of inhibitors of the caspase family have been used to study the involvement of these proteases in apoptosis. Cytokine response modifier A (CrmA) is a cytokine response modifier gene encoded by cowpox virus that preferentially inhibits ICE over cpp32 and p35, a baculovirus that inhibits both ICE and cpp32. Additionally, synthetic tetrapeptides, such as Tyr-Val-Ala-Asp (YVAD) and Asp-Glu-Val-Asp (DEVD), specifically inhibit ICE and cpp32, respectively. Studies with inhibitors of ICE, such as cowpox virus protein CrmA, and the tetrapeptides YVAD (7) and DEVD show that they dramatically block apoptotic cell death (35, 37), implicating the involvement of ICE in apoptosis.

Several ICE homologs have been identified, such as cpp32β, Mch2, Mch3, Mch4, Tx (ICE rel III), Ich-2 (ICE rel II), Ich-1s, and Ich-1L. ICE and cpp32β have been demonstrated to play an important role in Fas-induced apoptosis, the latter also being inhibitable by CrmA. An overproduction of Ich-1L has been shown to induce apoptosis, whereas Ich-1L serves to protect against cell death (37). Each member of this family of proteases is synthesized in a pro (zymogen) form, which is required to be cleaved for activation (37). Therefore, multiple members of the ICE family appear to be cleaved and activated in the apoptotic process (4, 8). It has been proposed that members of the ICE family are involved in a chain of events leading to apoptosis (35, 37). For example, ICE has been shown to process cpp32β to its
active form in vitro (35). Additionally, Ich-1, appears to be also processed by ICE (22). Therefore, all of these proteases are capable of autocleavage and/or can serve as substrates.

As a consequence of the activation of the ICE family of proteases, a number of nuclear proteins such as nuclear lamins, U1 ribonucleoprotein, and poly(ADP) ribose polymerase (PARP) have been reported to be degraded in apoptosis. PARP, a 116-kDa nuclear protein, is cleaved to an 85-kDa fragment in apoptosis induced by a number of agents (19). Studies have demonstrated that members of the ICE family, such as cpp32β (35) and Ich-1 (22), may be responsible for the formation of the 85-kDa PARP fragment.

Previous studies have demonstrated that silica treatment of human AM results in a slightly increased release of IL-1β (15). This increased IL-1β release may have been due to the activation of ICE by silica. The purpose of this study was to test the hypothesis that silica-induced apoptosis of human AM may involve the activation of ICE and/or other members of its family.

MATERIALS AND METHODS

Cell cultures. Human AM were obtained by bronchoalveolar lavage of normal, nonsmoking adult volunteers of either gender as previously described (5). This procedure has been approved by the University of Texas Committee for the Protection of Human Subjects. Installations of sterile saline resulted in recoveries of 240–260 ml of lavage fluid that was kept at 4°C until cells were isolated from the lavage fluid by centrifugation. The saline supernatant was aspirated and the cell pellet was resuspended in a small volume (1–5 ml) of N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid-buffered medium 199 (GIBCO-BRL, Gaithersburg, MD) with 10% fetal bovine serum (Sigma) and antibiotics (50 U/ml penicillin, 50 µg/ml gentamicin, and 50 µg/ml streptomycin). The cell count was determined with a ZBI Coulter Counter (Coulter Electronic, Hialeah, FL). Lavages yielded an average of 20 × 10⁶ cells that were >92% AM, as verified by leukostat staining (Fisher Scientific, Houston, TX). Viability was >90% as determined by trypan blue exclusion.

Particulate. Crystalline silica (average size 5 µm, acid-washed Min-U-Sil-5 from Pennsylvania Glass Sand, Pittsburgh, PA), at a concentration of 133 µg/ml, was used in all experiments. We have established that 133 µg/ml is a bioactive but nonnecrotic dose of crystalline silica (18).

Cytokentrifugation and morphological differentials. Immediately after cell culture, 30 × 10⁶ cells were incubated with phosphate-buffered saline (PBS) for 5 min in sterile disposable cytofunnels (Shandon, Pittsburgh, PA) and were centrifuged at 1,500 revolutions/min (rpm) for 5 min on positively charged glass slides (Probe On Plus; Fisher Scientific, Pittsburgh, PA) using a Shandon cytopsin 2 (Shandon). Slides were stored at 25°C until leukostat fixation, and staining was performed.

Leukostat staining. After cytokentrifugation, cells were fixed in cold methyl alcohol for 5 min, stained in leukostat (Fisher Scientific) eosin stain for 2 min, and then stained in leukostat methylene blue stain for 4 s. The slides were air-dried and were examined by light microscopy at ×630 (dry objective).

Necrosis assay (trypan blue exclusion). Cells were exposed to trypan blue dye (0.04% in PBS), placed on a hemocytometer, and examined under light microscopy. Only necrotic cells internalize the dye. Two-hundred random cells were counted after each treatment, and the percentage of “blue” cells was expressed as the percentage of necrotic cells for any given condition. There was no evidence of significant necrosis in silica-treated human AM at 6 h as seen by trypan blue staining.

DNA fragmentation assay. Particulate-treated cells were washed one time with PBS before DNA isolation. Genomic DNA was isolated by using the DNA isolator (Genosys, Woodlands, TX). The isolated genomic DNA was dissolved in 10 mM tris(hydroxymethyl)aminomethane (Tris) and 1 mM EDTA buffer and was 3’-end labeled with [α-32P]dCTP (ICN Pharmaceuticals, Costa Mesa, CA) by incubation of 1 µg of DNA in 50 µl of reaction buffer (50 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 200 µM dATP, 200 µM dGTP, 200 µM dTTP, 2 µl [α-32P]dCTP, and 2 units klenow) at 37°C for 30 min. The [α-32P]dCTP-labeled DNA was mixed with 10 µl loading buffer (0.25% bromphenol blue, 100 mM EDTA, and 30% glycerol). The same amount of [α-32P]dCTP-labeled DNA (50 ng) for each sample was loaded onto a 2% agarose gel and was run at 5 V/cm for 5 h in 40 mM Tris-acetate buffer, pH 8.0, with 1 mM EDTA. The gel was dried at 60°C for 4 h under vacuum in a gel drier and was exposed to X-ray film for documentation of labeled DNA fragments.

Cell death enzyme-linked immunosorbent assay. Cytosolic histone-bound DNA fragments were detected by cell death enzyme-linked immunosorbent assay (ELISA). Cells from control and particulate treatments were processed according to the manufacturer’s protocol and were analyzed for cytosolic histone-bound DNA fragments using the cell death ELISA kit (Boehringer Mannheim, Indianapolis, IN). Briefly, 1 × 10⁶ cells from each condition were washed one time with PBS; cells were then lysed in 1 ml lysis buffer, incubated on ice for 30 min, and then centrifuged at 14,000 rpm for 15 min at 4°C. Lysate were retained, and 100 µl of lysate were used for each reaction. A 96-well ELISA plate was coated with blocking buffer for 2 h, wells were washed, and 100 µl of anti-histone antibody were added per well and incubated for 90 min at room temperature; wells were washed, and 100 µl of cell lysate (triplicate assays were performed for each condition) were added and incubated for 90 min at room temperature. Wells were washed, and 100 µl of mouse IgG were added and incubated. The reaction was allowed to occur for 15 min, and optical density was read at 405 nm using an Emax precision microplate reader (Molecular Devices, Sunnyvale, CA).

ICE and cpp32β in silica-induced apoptosis. Cells were pretreated with and without ICE and cpp32β inhibitors z-Val-Ala-Asp-fluoromethyl ketone (zVAD-FMK; 10 µM; Enzyme Systems, Livermore, CA) and Asp-Glu-Val-Asp-fluoromethyl ketone (DEVD-FMK; 10 µM; Enzyme Systems) at 25°C for 30 min. Cells were cultured at 1 × 10⁶ cells/ml in the presence or absence of silica for 4 h at 37°C. Cells were maintained in suspension by slow end-over-end tumbling in sterile polypropylene tubes. Cultures were assayed for apoptosis by cell death ELISA and DNA fragmentation assay.

Immunoprecipitation. Approximately 3 × 10⁶ cells were cultured at 1 × 10⁶ cells/ml with or without zVAD-FMK in the presence or absence of silica for 4 h at 37°C. Cells were lysed with 250 µl sodium dodecyl sulfate (SDS) lysis buffer, incubated for 30 min on ice, and centrifuged at 14,000 g for 15 min, and supernatant was retained. Rabbit immunoglobulin G was added (for nonspecific binding) and was incubated for 1 h after which 20 µl of 50% Protein A/G beads were added and allowed to incubate at 4°C for 1 h with slow end-over-end tumbling. Samples were centrifuged, and beads were discarded. Antibody to the protein of interest (anti-ICE p20 or anti-Ich-1, p20, Santa Cruz Biotechnologies, Santa Cruz, CA;
anti-cpp32 p20, Transduction Laboratories, Lexington, KY) at a concentration of 20 µg/ml was added to the supernatant and was incubated overnight at 4°C. The p20 protein-antibody complex was isolated by incubation with 50% Protein A/G beads for 1 h at 4°C. Samples were washed and centrifuged three times, and the Protein A/G beads with the bound p20 protein were retained. Protein was denatured with denaturing buffer and was boiled for 5 min to dissociate the p20 from the beads.

SDS-polyacrylamide gel electrophoresis and immunoblotting. Aliquots of the above denatured protein were run on 12% SDS Ready Gels (Bio-Rad, Hercules, CA) in a mini-gel apparatus (Bio-Rad). Resolved proteins were transferred using a wet minitranfer unit (Bio-Rad) to a nitrocellulose membrane (Amersham, Arlington Heights, IL). Membranes were blocked by overnight incubation at 4°C in blocking buffer (5% dry nonfat milk in 10 mM Tris and 150 mM NaCl, pH 7.2). Membranes were then incubated with the relevant antibodies (anti-ICE p20, anti-Ich-1L p20 from Santa Cruz Biotechnologies; and anti-cpp32β from Transduction Laboratories) at 1 µg/ml dilution in blocking buffer for 16 h at 4°C and were washed extensively with Tris-buffered saline (0.05% Tween 20, pH 8.0) (TBST). Blots were incubated with peroxidase-linked anti-rabbit/mouse immunoglobulin (Amersham) in TBST for 1 h at 25°C and then was washed extensively with TBST. Blots were placed in enhanced chemiluminescence (ECL) reagents (Amersham) for 1 min, followed by exposure to autoradiographic film (ECL film; Amersham) for 3 min, and were developed using an automated film processor (Kodak).

Cleavage of PARP and cpp32β degradation. Approximately 2 x 10^6 cells were cultured at 1 x 10^6 cells/ml in the presence or absence of silica (133 µg/ml) for 2, 4, and 6 h at 37°C. Samples were denatured, and aliquots of the denatured protein were run on 12% SDS Ready Gels (Bio-Rad) in a mini-gel apparatus (Bio-Rad). Immunoblotting was performed as described above using the anti-cpp32β monoclonal antibody (Transduction Laboratories) at 1 µg/ml and anti-PARP monoclonal antibody (Enzyme Systems, Livermore, CA) at 1:1,000 dilution.

Statistical analysis. Values are presented as means ± SE. The number of individuals whose cells were used for a given experiment is denoted by n in the legends for Figs. 1–9. For each experiment, statistical treatment included a one-way analysis of variance followed by a Student-Newman-Keuls test for post hoc pairwise comparisons.

RESULTS

ICE in silica-induced apoptosis. In a previous study, preliminary data were presented demonstrating the partial inhibition of silica-induced apoptosis by the ICE inhibitor zVAD-FMK (2 µM) in human AM (18). This result suggested the involvement of ICE. To establish more firmly the role of ICE in silica-induced apoptosis, a higher concentration of zVAD-FMK was used. Human AM were treated with 133 µg/ml silica with and without 10 µM zVAD-FMK for 6 h at 37°C and were evaluated for apoptosis by cell death ELISA (Fig. 1).

The cell death ELISA assay detects cytosolic histone-bound DNA fragments formed in cells undergoing apoptosis. The results revealed that treatment of human AM with silica resulted in a significant increase in cytosolic histone-bound DNA fragments compared with control. However, significant inhibition of silica-induced apoptosis was observed in cells incubated with 10 µM zVAD-FMK.

To confirm the cell death ELISA results, silica-treated cells were also examined morphologically. Figure 2 is a representative photomicrograph of cells incubated for 4 h and represent control (A), 133 µg/ml silica treated (B), 10 µM zVAD-FMK treated (C), and 10 µM zVAD-FMK treated and 133 µg/ml silica treated (D). Control AM are rounded with uniformly large, light purple nuclei and normal cytoplasms. In contrast, Fig. 2B shows that human AM treated with silica have dark, shrunken nuclei indicative of nuclear condensation commonly seen in apoptosis. Nuclear disintegration is also apparent in some cells, which is characterized by a very dark, condensed, segmented nucleus. In contrast, cells treated with silica in the presence of zVAD-FMK have a morphology similar to control cells, indicating the absence of apoptosis (Fig. 2D). Therefore, zVAD-FMK was effective in blocking silica-induced apoptosis, further implicating ICE involvement in the apoptotic process.

Because internucleosomal DNA fragmentation is a characteristic feature of apoptotic cells, a DNA agarose gel was used to detect the presence of DNA fragments in 133 µg/ml silica-treated human AM with and without 10 µM zVAD-FMK. As can be seen in Fig. 3, treatment of human AM with silica resulted in significant DNA ladder formation at 6 h. However, pretreatment with 10 µM zVAD-FMK blocked silica-induced DNA fragmentation. Consequently, the results from cell death ELISA assay (measuring cytosolic histone-bound DNA), morphological characterization, and the DNA ladder formation demonstrated that zVAD-FMK...
was an effective inhibitor of silica-induced apoptosis. Taken together, these results demonstrate the involvement of ICE in regulating silica-induced apoptosis of human AM.

Formation of the p20 fragment of ICE. As described earlier, the zymogen form of ICE is cleaved to a heterodimeric form (p20 and p10) to be activated (10). Therefore, to determine whether ICE is activated in silica-induced apoptosis, the formation of the active component of ICE, i.e., p20, was assessed. This was accomplished by immunoprecipitation with ICE p20 antibody followed by Western analysis as described in MATERIALS AND METHODS. The level of the p20 "active" form of ICE was determined in human AM treated with silica with or without 10 µM zVAD-FMK pretreatment. As shown in Fig. 4, there was an increase in the level of p20 after silica treatment within 1 h. In contrast, when cells were stimulated with silica in the presence of zVAD-FMK, the level of p20 was only marginally higher than control. These results provide evidence for silica-induced activation of ICE and for ICE activation being one of the crucial events in the induction of apoptosis by silica. Collectively, the above results (Figs. 1–4) strongly support a central role for ICE in silica-induced apoptosis.

Involvement of Ich-1 L. In a similar manner to other members of the ICE family, Ich-1 L is present as an inactive p48 zymogen form that is cleaved to the active p20 and p10 heterodimer form. Therefore, the involvement of Ich-1 L activation in silica-treated human AM cells was examined, using the same procedure as used for detecting ICE activation (immunoprecipitation and Western analysis). As shown in Fig. 5, an increase in Ich-1 L p20 levels was detected as early as 1 h in the 133 µg/ml silica-treated cells. Furthermore, pretreatment (30 min) of human AM with 10 µM zVAD-FMK blocked the silica-induced increase of Ich-1 L. These results suggest that silica increased Ich-1 L activity in human AM, implicating the involvement of this protease in the sequence of events that result in silica-induced apoptosis.

Fig. 2. Photomicrographs of leukostat-stained human AM. A: control; B: 133 µg/ml silica-treated cells; C: 10 µM zVAD-treated cells; D: 10 µM zVAD-pretreated silica-treated cells. Cells were cultured for 4 h. Silica induced morphological signs of apoptosis in human AM, whereas pretreatment with the ICE inhibitor zVAD-FMK inhibited silica-induced apoptosis. Apoptotic cells (indicated by arrows) have a dark, condensed or segmented nuclei indicative of nuclear condensation common to apoptosis. Magnification is ×630 (dry) in bright field.
Activation of cpp32\textsuperscript{b}. As described earlier, cpp32\textsuperscript{b} is considered a terminal member of the ICE family of proteases and has been shown to cleave the "death substrate" PARP to an 85-kDa inactive form during apoptosis (35). To examine the involvement of cpp32\textsuperscript{b} in silica-induced apoptosis, human AM were treated with and without the cpp32\textsuperscript{b} peptide inhibitor DEVD-FMK (10 µM) for 30 min at 37°C and were cultured at $1 \times 10^6$ cells/ml in the presence or absence of silica (133 µg/ml) for 6 h. Apoptosis was detected by cell death ELISA assay. The results shown in Fig. 6 illustrate that pretreatment with DEVD-FMK resulted in significant inhibition of apoptosis in silica-treated human AM. These results are consistent with the potential involvement of cpp32\textsuperscript{b} in the induction of apoptosis by silica.

To further confirm the role of cpp32\textsuperscript{b} in silica-induced apoptosis, the disappearance of the zymogen form of the protease (p32) was assayed by Western analysis. Cells were treated with silica for 1 and 3 h. As shown in Fig. 7, a decrease in the p32 form of cpp32\textsuperscript{b} was detected at both time points in the silica-treated cells. Additionally, as seen in Fig. 8, a corresponding increase in the p20 form of cpp32\textsuperscript{b} was observed at 1 h (immunoprecipitation and Western analysis) in silica-treated human AM, indicative of the activation of cpp32\textsuperscript{b} by silica. These results are in agreement with the cpp32\textsuperscript{b} inhibitor-mediated abolition of silica-induced apoptosis and further support the role of this protease.

PARP degradation. As described previously, the nuclear protein PARP is cleaved by cysteine proteases, such as cpp32\textsuperscript{b}, to an inactive 85-kDa fragment by several apoptotic agents (35). Therefore, to determine whether PARP is hydrolyzed during silica-induced activation by one or more members of the ICE family of proteases, degradation of PARP was assessed by Western analysis. Cells were treated with silica for 2, 4, and...
6 h. As shown in Fig. 9, an increase in the 85-kDa degradation product of PARP is observed within 2 h of silica treatment of cells. The degradation of PARP is consistent with the results describing the activation of this family of cysteine proteases, specifically cpp32β and Ich-1L.

**DISCUSSION**

In previous studies, we demonstrated that in vitro treatment of human AM with fibrogenic particulates such as silica (18) and asbestos (14) resulted in cell death by apoptosis, whereas nonfibrogenic particulates did not induce apoptosis. The results suggested that the fibrogenicity of a particulate correlated with its ability to cause apoptosis of AM. This was further supported by studies demonstrating that other fibrotic agents also induce apoptosis in human AM (13). In addition, BéruBé et al. (1) reported that asbestos-induced apoptosis in rat mesothelial cells. However, the possible mechanism by which fibrotic agents such as silica induce apoptosis is unclear. This is the first study that provides insight to the possible mechanism by which fibrotic particulates, such as asbestos and silica, induce apoptosis.

Results from a number of laboratories have demonstrated that cysteine proteases play an important role in the regulation of programmed cell death by some apoptotic agents (7, 23, 35, 37, 38). In this study, we provide results supporting the direct involvement of the ICE family of proteases in silica-induced apoptosis. Figures 1–3 show a significant inhibition of silica-induced apoptosis in cells pretreated with the ICE inhibitor zVAD-FMK. The ICE inhibitor blocked silica-induced DNA cleavage, DNA ladder formation, and morphological changes characteristic of apoptosis. The involvement of ICE was further confirmed by detecting increased levels of ICE p20 (Fig. 4) in silica-treated cells, implying the activation of ICE by silica. These results directly implicate ICE in silica-induced apoptosis. In addition to ICE, other members of the ICE family of proteases also appeared to contribute to silica-induced apoptosis. Both cpp32β and Ich-1L have been proposed to be activated in other model systems, and the current studies implicate their activation in silica-induced apoptosis. An increase in the level of p20 Ich-1L and degradation of cpp32β were detected in silica-treated human AM within 1 h (Figs. 5 and 8), indicating that both of these proteases are rapidly activated by silica treatment. Furthermore, inhibition of cpp32β by DEVD-FMK resulted in a corresponding inhibition of apoptosis in silica-treated human AM (Fig. 6). Therefore, it can be concluded that silica induces apoptosis in human AM by stimulating members of the ICE family of cysteine proteases, namely ICE, Ich-1L, and cpp32β. Consequently, this pathway may be an important apoptotic mechanism used by other fibrogenic agents, such as asbestos.

Although it is well established that the ICE proteases play a role in apoptosis, the individual roles are not clear. It is still not known whether one or all of these proteins may be required in the apoptotic process. In silica-induced apoptosis, at least three members of this family were activated. If these events occur in parallel, it might indicate a redundancy of function in these proteases. However, it has been demonstrated that cpp32β and Ich-1L can be cleaved by ICE, thus suggesting that this pathway may be sequential. Consistent
with this proposal, pretreatment of cells with the ICE inhibitor blocked the formation of the p20 form of Ich-1L in silica-treated cells (Fig. 5), implying that Ich-1L may be downstream of ICE. Conversely, the inhibitor used may not be specific for ICE and may inhibit Ich-1L itself. In that regard, zVAD-FMK has been suggested to inhibit both ICE and cpp32 (28). It could also be speculated that this rapid and wide response of the ICE proteases to silica treatment may overwhelm the survival mechanism of the cell thus totally committing the AM to die by apoptosis.

Many apoptotic agents induce cell death by interacting with surface receptors stimulating one or more intracellular signaling pathways. Previous studies in our laboratory have reported the involvement of the scavenger receptor (SR) in silica-induced apoptosis (18). The SR is a membrane receptor involved in the uptake of a broad array of negatively charged ligands and is speculated to play a role in atherosclerosis (2, 11, 12). Additionally, it has been proposed to be involved in the uptake of silica by the AM (18, 21). However, the intracellular signal transduction pathways stimulated by ligand-SR interaction have not been elucidated. One of the better characterized mechanisms by which ligand-receptor interaction leads to apoptosis is the tumor necrosis factor (TNF) and Fas pathways. It has been reported that the Fas and TNF receptors associate with intracellular proteins Fas-associated death domain and TNF receptor-associated death domain, respectively, which trigger a death stimulating pathway, ultimately resulting in activation of ICE proteases leading to apoptosis (3, 17). It is possible, therefore, that binding of silica to the SR results in the activation of ICE proteases by a similar mechanism; however, a direct relationship between the SR and the cysteine proteases needs to be demonstrated.

Generally, apoptosis is a normal physiological process; however, extensive injury to selective cell populations has been observed in certain disease conditions. Preferential cell death of a specific cell population could provide a mechanistic linkage between apoptosis and fibrosis. Studies have demonstrated that the AM is a heterogenous population of several functionally distinct subpopulations (32–34), mostly comprising of a large immunosuppressive (RFD1+RFD7+) and a relatively smaller immune inducer (RFD1+RFD7−) population (16, 20). It is generally believed that there is dynamic balance between the suppressive and inductive AM populations and that a perturbation in this ratio due to targeted cell death may result in altered lung homeostasis; conversely, the ratio of these subpopulations could be altered by disease and by therapeutic regimes (33). Taking into consideration these two AM subpopulations and applying our in vitro results to the in vivo situation, it is possible that chronic exposure to crystalline silica may lead to the depletion of the predominant suppressor AM population by apoptosis, resulting in a predominance of the inducer AM population. The combined events of a change in the relative ratio of the suppressor to inducer AM populations and the consequent decrease or absence of the immune suppressive factors released by the suppressor population may result in an inflammatory response, ultimately causing fibrosis.

In summary, these studies demonstrate the role of multiple members of the ICE family of proteases in silica-induced apoptosis. If apoptosis is a critical step in the fibrogenic process then a better understanding of the regulation of these proteases may lead to important therapeutic intervention.

This work was supported by National Institutes of Health Grants ES-04804 Clinical Research Center M01-RR-02558.

Address for reprint requests: A. Holian, Dept. of Internal Medicine, University of Texas Medical School, 6431 Fannin, Rm. 1.276, Houston, TX 77030.

Received 10 February 1997; accepted in final form 25 June 1997.

REFERENCES


