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Role of Neutrophil Apoptosis in Vanadium-Induced Pulmonary Inflammation in Mice

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Pulmonary exposure to airborne vanadium and vanadium-containing compounds is associated with acute pulmonary inflammation, characterized by a rapid influx of neutrophilic polymorphonuclear leukocytes with a peak response at 6 hours and resolution by 3 days. We hypothesized that neutrophil apoptosis is involved in the resolution of vanadium-induced lung inflammation. To test this hypothesis, mice were exposed to inspired vanadium or saline control and the bronchoalveolar lavage (BAL) cells were examined at various times for apoptosis using terminal deoxyribonucleotidyl transferase-mediated nick end labeling (TUNEL). Control mice showed only resident alveolar macrophages in the BAL with no evidence of apoptosis. In contrast, vanadium-treated mice showed clear apoptosis of BAL cells, which were predominantly neutrophils. The number of apoptotic cells gradually increased and reached a maximal level by 24 hours with subsequent decline. After 24 hours, when the vanadium-induced lung inflammation was in the resolution phase, we observed an increased number of alveolar macrophages in BAL and the engulfment of apoptotic bodies by these macrophages. At 72 hours, the total number of neutrophils in BAL fell to the baseline level, and the number of apoptotic cells was reduced. Clearance of the apoptotic product was demonstrated by the presence of apoptotic bodies in the cytoplasm of alveolar macrophages. We conclude that apoptosis of neutrophils and clearance by alveolar macrophages are important mechanisms in the resolution of vanadium-induced lung inflammation.

KEY WORDS: vanadium, apoptosis, inflammation, alveolar macrophage, neutrophil leukocyte

Introduction

There is well-documented evidence suggesting that pulmonary exposure to environmental and occupational airborne particulate matter can cause a number of human diseases, including cardiopulmonary diseases and lung cancer.[1] Chemical analysis of this particulate matter reveals that it can contain various trace metal ions such as vanadium, chromium, and nickel.[2,3] While these metal ions have been postulated to contribute to the pathologic effects of in-

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haled particles, the biological mechanisms by which metal ions may be involved in the disease process remain unclear. The present study investigated the role of apoptosis during the development and resolution of vanadium-induced lung inflammation in mice. Vanadium is a transitional metal that exists in various oxidative states ranging from −1 to +5. The pentavalent form of vanadium, V(V), is one of the most abundant forms in nature and is also the most toxic.[4] Previous studies have shown that V(V) can cause apoptosis in a variety of cells including lymphoid cells,[5] fibroblasts,[6] and epidermal cells.[6] However, there have been no reports showing the existence of this process in vivo. Furthermore, the role of metal-induced apoptosis in the disease process has yet to be delineated.

Apoptosis or programmed cell death is an active process in which cell death is initiated and completed in an orderly fashion. It is characterized by cellular shrinkage, nuclear fragmentation, and formation of apoptotic bodies that are ingested by neighboring cells and phagocytes, such as macrophages.[7–9] Because the cell membranes of apoptotic cells remain intact and there is no release of inflammatory and toxic contents,[9] apoptosis is thought to provide a general clearance mechanism that tends to limit tissue injury and promote resolution. Increasing evidence also indicates that apoptosis may play a role in disease processes. For example, failure to clear unwanted cells by apoptosis can cause tissue inflammation because of the release of inflammatory mediators and toxic intracellular contents. Uncontrolled or excessive apoptosis may also cause disease. The administration of the apoptosis-inducing agent, Fas (CD95/APO1) antibody, into the lung of mice causes inflammatory cell influx and apoptosis, which subsequently leads to pulmonary inflammation and fibrosis.[10] Apoptosis has also been observed in a variety of inflammatory lung disorders, including acute lung injury,[7,8,11] diffuse alveolar damage,[12] and idiopathic pulmonary fibrosis.[13,14] While studies of a variety of pulmonary diseases demonstrate an association with apoptosis, the role of apoptosis and/or clearance of apoptotic products is unknown.

The neutrophil polymorphonuclear leukocyte is a key cellular effector of the immediate host response to injury and infection. However, uncontrolled release of its formidable array of toxic substances may inflict damage on surrounding tissue and propagate the inflammatory response, leading to scarring and tissue destruction.[15] Indeed, neutrophils and neutrophil products have been implicated in the pathogenesis of a variety of inflammatory diseases, including the adult respiratory distress syndrome, idiopathic pulmonary fibrosis, ulcerative colitis, and rheumatoid arthritis. Apoptosis of neutrophils is associated with down-regulation of many cellular functions, such as chemotaxis, enzyme secretion, generation of a respiratory burst, and responsiveness to external stimuli.[9,16] The present study was undertaken to test the hypothesis that pulmonary exposure to V(V) is associated with inflammatory neutrophilic and apoptotic responses, and that clearance of apoptotic neutrophils by macrophages plays an important role in the resolution of V(V)-induced lung inflammation.

Materials and Methods

Animals

Male BALB/cJ mice (4–6 weeks old) were obtained from Jackson Laboratories (Bar Harbor, ME). They were acclimated in an AAALAC-accredited facility for at least a week before use. The mice were provided water and food ad libitum. Intratracheal instillations of test agents were performed by anesthetizing the animals with a mixture of ketamine and xylazine (45 and 8 mg/kg, i.p., respectively) and challenging the mice by inspiration. The animals were placed on a board in a supine position. The animal's tongue was extended with lined forceps and 50 µL of the test solution containing V(V) (25–150 µg) was placed on the back of the tongue, from where it was inspired rapidly. Control animals were given an equal volume of saline in a similar manner.

Bronchoalveolar Lavage

At selected time intervals, treated mice were killed by exsanguinations under anesthesia. An intratracheal cannula was inserted and the lungs were lavaged with ice-cold Ca^{2+} and Mg^{2+}-free phosphate-buffered saline (PBS). The lungs were lavaged four times (0.9 mL/lavage) to collect cellular and soluble constituents. Each lavage was centrifuged, and the cell pellets were combined. The cell pellet was resuspended in 1 mL of PBS buffer, centrifuged, and the

supernatant was decanted and discarded. The BAL cells were then resuspended in 1 mL PBS buffer, and cell counts and differentials were determined using a Coulter Multisizer II (Coulter Electronics, Hialeah, FL).

Lung Histology
To obtain tissues for histologic examination, a separate group of animals was treated with V(V) but not subjected to BAL. After sacrifice, the lungs were inflated in situ with 10% formalin solution instilled through the trachea for 5 minutes; they were then removed, and fixed in buffered 10% formalin solution for 24 hours. After embedding in paraffin, the samples were sectioned, mounted on glass slides, and stained with hematoxylin and eosin for light microscopic examination.

TUNEL Assay
Analysis of lung cell apoptosis was performed using a terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) kit (Promega, Madison, WI), according to the manufacturer's protocols. Briefly, cytospin preparations of the BAL cells were fixed in 4% paraformaldehyde at 4°C for 30 min, washed with PBS, and incubated with 1% Triton X for 10 minutes. Lung-tissue sections were deparaffinized, rehydrated, and incubated with 100 μg/mL proteinase K at 37°C for 30 minutes. Thereafter, the following steps were the same for both cytospins and lung-tissue sections: after 10 minutes incubation at room temperature with equilibration buffer provided, the slides were immersed in TdT and fluorescein-dUTP, which were diluted in equilibration buffer and allowed to incubate for 60 minutes at 37°C. After being washed with 2% SSC washing buffer provided in the kit and PBS, the slides were counterstained with propidium iodide (Molecular Probes, Eugene OR).

Fluorescence Microscopy
After staining, the slides were examined under a fluorescence microscope using standard fluorescein excitation and emission wavelengths of 495 nm and 525 nm, respectively. A bright green fluorescence signal in the nucleus indicated TUNEL-positive apoptotic cells. Propidium iodide was viewed with excitation at 595 nm, and a bright red emission was seen in all nuclei present. The counterstain propidium iodide was used to aid the identification of cell type, i.e., neutrophil or macrophage, based on their size and morphology. The percentage of apoptotic cells on cytospin slides was counted by examining at least 1,000 cells per sample. For lung tissue–section slides, at least 20 views were determined using standard morphometric methods.

Results

Pulmonary Inflammatory Response to Vanadium
The effect of vanadium on the pulmonary inflammatory response was studied by monitoring inflammatory cell influx following intratracheal V(V) ad-

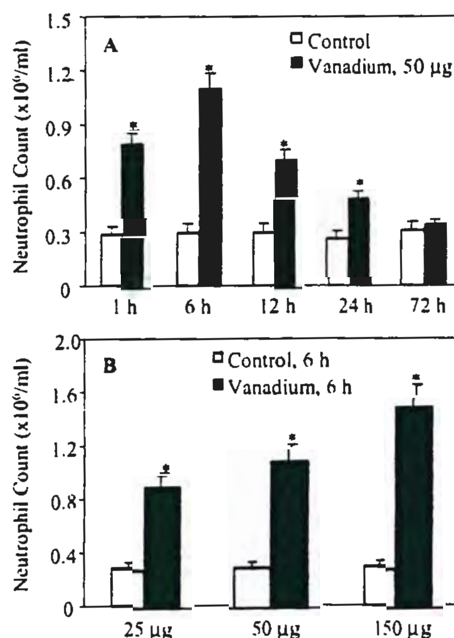

FIGURE 1. Neutrophil cell number analysis after vanadium treatment. Mice were treated with V(V) or saline via intratracheal instillations. BAL was performed at indicated times and analyzed for number of neutrophils. (A) Time-course of neutrophils after V(V) treatment (50 μg/mouse). (B) Dose effect of V(V) (25–150 μg/mouse) determined at 6 h after the treatment. Data are the mean ± SEM, n = 4 mice/group. *$p < 0.05$ vs. saline-treated control groups.

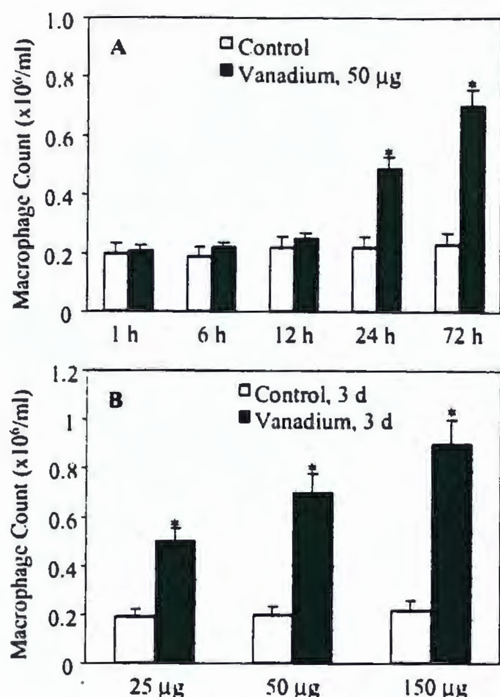

FIGURE 2. Macrophage cell number analysis after vanadium treatment. Mice were treated with V(V) or saline via intratracheal instillations. BAL was performed at indicated times and analyzed for number of alveolar macrophages. (A) Time-course of alveolar macrophages after V(V) treatment (50 μg/mouse). (B) Dose effect of V(V) (25–150 μg/mouse) determined at 6 h after the treatment. Data are the mean ± SEM, n = 4 mice/group. *$p < 0.05$ vs. saline-treated control groups.

ministration. Figures 1 and 2 show that V(V) was able to increase the yield of both neutrophils and macrophages harvested by BAL in a dose-dependent manner; however, the kinetics of the two events were quite different. Whereas neutrophil influx occurred rapidly within the first hour and peaked at 6 hours after the treatment (Fig. 1A), alveolar macrophage yield was not significantly elevated until 24 hours; it further escalated on day 3 (Fig. 2A). Increasing or decreasing the dose of V(V) (25–150 μg/mouse) correspondingly affected the cell number of neutrophils (Fig. 1B) and alveolar macrophages harvested by BAL (Fig. 2B). Treatment of mice with saline had no significant effect on either neutrophil or alveolar macrophage cell counts at any time point.

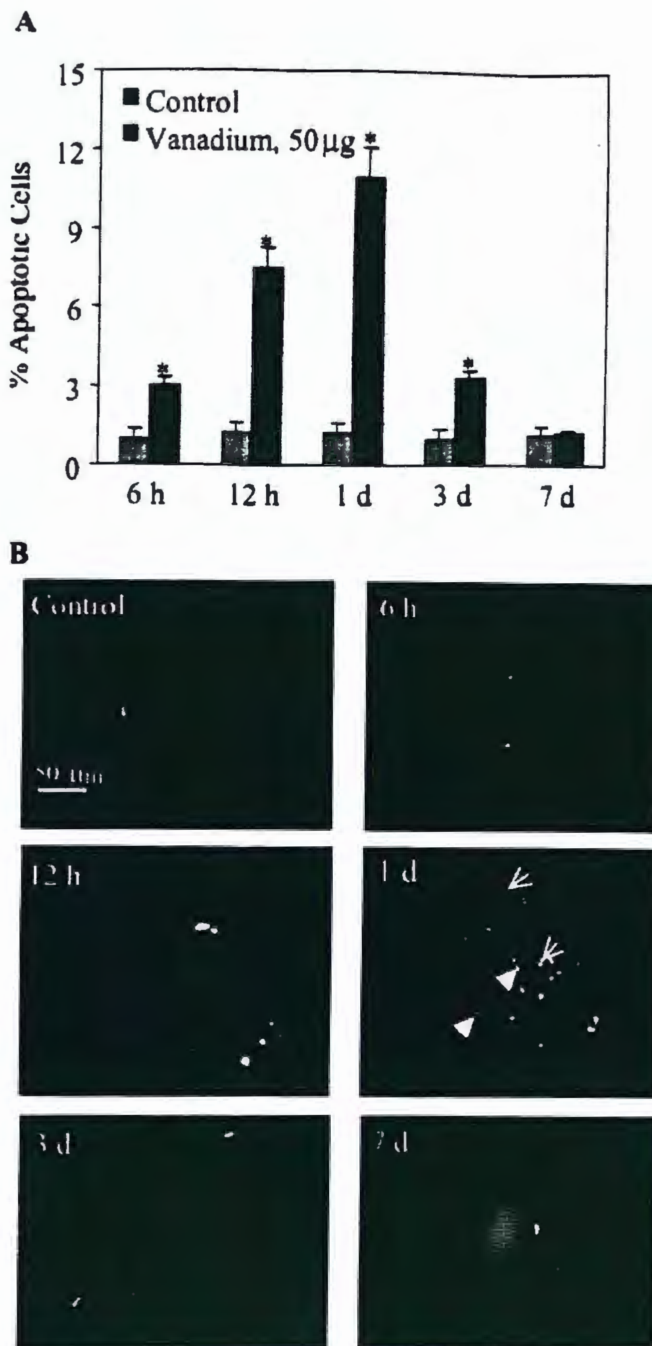

FIGURE 3. Time course of vanadium-induced BAL cell apoptosis. Mice were treated with V(V) (50 μg/mouse) or saline, and BAL (A) was performed at 6 h, 12 h, 1 d, 3 d, and 7 d after the treatment. Apoptosis of BAL cells was determined by TUNEL assay. Data are the mean ± SEM, n = 4 mice/group. *$p < 0.05$ vs. saline-treated control groups. Similar dose response and time course were also showed in lung tissue sections stained by TUNEL (B). Both airspace cell (open arrows) and alveolar wall epithelium cell (close arrows) apoptosis were identified. Under high magnification examination with oil emission, a small percentage of the apoptotic cells were identified as epithelial and/or interstitial cells (not shown).

Apoptosis of BAL Cells
Apoptosis of BAL cells was determined using a TUNEL assay at 6 hours, 12 hours, 1 day, 3 days, and 7 days after V(V) administration. Figure 3A shows that treatment of mice with V(V) caused a time-dependent increase in the level of cell apoptosis in BAL, whereas saline control treatment had no effect at any time point. V(V)-induced apoptosis increased significantly within 6 hours, peaking around 1 day, and decreasing to the baseline level by 7 days. Cell apoptosis determined by TUNEL assay of lung tissue sections exhibited a similar pattern of response (Fig. 3B).

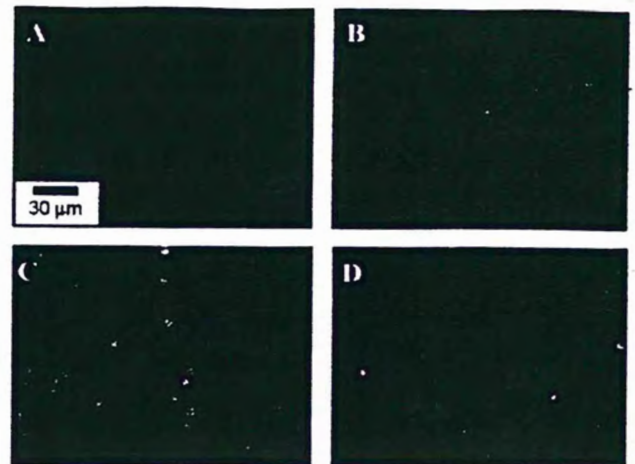

FIGURE 4. Vanadium-induced cell apoptosis in BAL and phagocytosis by alveolar macrophages. Mice were treated with V(V) (50 µg/mouse) or saline, and BAL cells were processed for TUNEL using fluorescein-labeled dUTP and counterstained with propidium iodide. (A) Control mice 1 d after saline treatment. BAL cells were predominantly alveolar macrophages with no or minimum apoptosis. (B) Mice treated with V(V) for 6 h. Note the presence of neutrophils, which are characterized by their small nucleus and distinct nuclear lobes. Some TUNEL-positive cells can also be seen. (C) Mice treated with V(V) for 1 d. Apoptotic cells, which are primarily neutrophils. (D) Mice treated with V(V) for 3 d. Note the disappearance of neutrophils and the presence of some apoptotic bodies in alveolar macrophages.

Identification of Apoptotic BAL Cells
The cells in BAL fluids were identified as neutrophils and alveolar macrophages on the basis of their characteristic nuclear morphology and size with propidium iodide fluorescence. Neutrophils are smaller in size compared to alveolar macrophages and have characteristic nuclear lobes. In control mice at all times, the BAL cells were predominantly resident alveolar macrophages with no or minimum apoptosis. Figure 4A shows representative result of the control mice at day 1. In contrast, V(V)-treated mice showed increasing numbers of apoptotic BAL cells with time. At 6 hours, extensive neutrophil accumulation in the BAL fluids was observed with a small number of TUNEL-positive cells (Fig. 4B). At 1 day, the peak time for BAL cell apoptosis, the presence of a considerable number of TUNEL-positive cells was observed (Fig. 4C). These cells were identified on the basis of their nuclear morphology as neutrophils. By day 3, the number of apoptotic cells was greatly reduced, but the presence of apoptotic bodies could still be seen in the cytoplasm of alveolar macrophages (Fig. 4D). At this time point, the neutrophils were virtually absent from the BAL samples. These results indicate that neutrophils are the primary cells undergoing apoptosis during V(V)-induced lung inflammation and that alveolar macrophages play an important role in the clearance of apoptotic neutrophils.

Lung Histopathology
Figure 5 shows changes in lung histopathology after V(V) treatment using H&E-stained lung sections. Increased cellularity was seen at day 1 (Fig. 5A), which corresponded to the peak neutrophil immigration. Cellularity decreased in subsequent days and returned to a control level by day 7 after V(V) treatment (Fig. 5B).

Discussion

During the process of acute inflammation, inflammatory cells are recruited from the circulation to the inflamed site through a series of coordinated signals. In vanadium-induced lung inflammation, we have shown that neutrophils are the primary inflammatory cells that first immigrate into the lung tissue. The number of neutrophils obtained by BAL increased substantially as early as 1 hour post exposure and reached a maximum level by 6 hours. The neutrophilic inflammatory response completely

and spontaneously resolved by day 3, a process that involved a series of cellular events including (1) induction of neutrophil apoptosis, which lagged behind neutrophil influx, (2) alveolar macrophage infiltration, which increased substantially at the peak of neutrophil apoptosis, and (3) phagocytosis of apoptotic neutrophils by alveolar macrophages, which cleared most of apoptotic bodies by day 3. While apoptosis has been shown to be associated with a variety of pulmonary inflammatory disorders, there is no evidence to date for the in vivo induction of apoptosis by vanadium. In the present study, we have demonstrated that V(V) can induce a time- and dose-dependent increase in neutrophil apoptosis, a critical process that may play a key role in the resolution of acute lung inflammation.

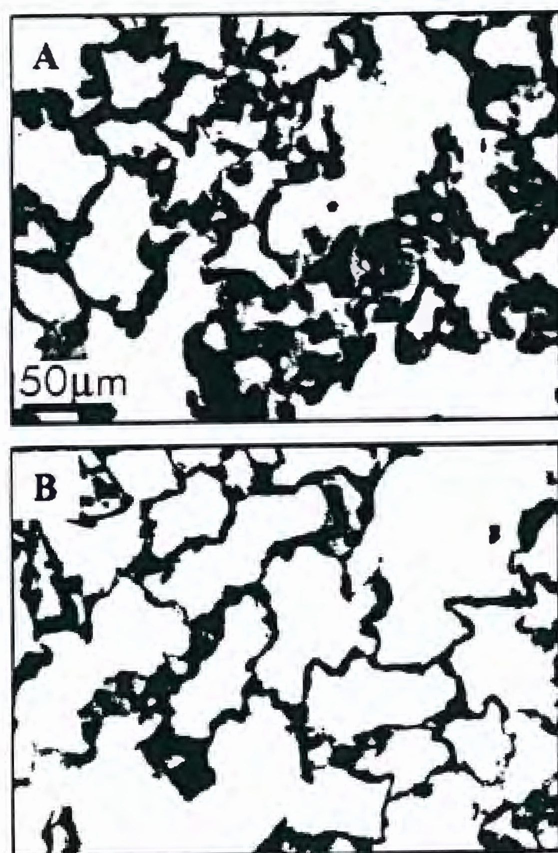

FIGURE 5. H&E-stained lung sections showing V(V)-induced inflammatory response and its resolution. Mice were treated with V(V) (50 µg/mouse) and lung sections were prepared for H&E staining. (A) Inflammatory response at 1 d after V(V) treatment. (B) Resolving inflammatory response at 7 d after V(V) treatment.

The neutrophil is an archetypical cellular effector of acute lung inflammation. For the lung to return to normal following the course of inflammation, these cells must be disposed of locally, since there is no evidence that the immigrated neutrophils can reenter the circulation. The possibility of neutrophil clearance by mucociliary transport mechanisms is also unlikely, since such transport systems only exist in larger airways and not in the pulmonary alveolar region. Since one of the primary roles of alveolar macrophages is phagocytosis and these cells have been shown to actively phagocytose apoptotic neutrophils both in vitro[16] and in vivo,[7,8] we suggested that macrophages may play an important role in the phagocytic clearance of apoptotic neutrophils and in the resolution of lung inflammation induced by V(V). The following experimental observations support these notions: (1) alveolar macrophages are recruited during and after the peak time of neutrophil apoptosis, (2) apoptotic neutrophils are cleared by alveolar macrophages during this period, and (3) both neutrophil cell number and apoptotic cells are greatly and concomitantly reduced during the resolution phase of inflammation, i.e., on day 3.

Previous studies have shown that vanadium-containing particles in polluted air can induce the synthesis and expression of proinflammatory cytokines such as TNF-α, IL-6, and IL-8.[17–19] These cytokines may directly or indirectly trigger the recruitment of neutrophils into the airspaces. The delayed response in alveolar macrophage recruitment, which did not occur until after neutrophil apoptosis had fully developed, suggests that such recruitment may be dependent on apoptosis-related events. Phagocytosis of apoptotic neutrophils by macrophages has been shown to result in a suppression of proinflammatory cytokines, including TNF-α and IL-8.[20] Such events have also been shown to cause an increase in macrophage release of anti-inflammatory mediators, such as TGF-β and PGE2, which have suppressive effects on the inflammatory response.[20]

Vanadium is widespread in the environment including in water, soil, and air. An in vitro study showed that vanadium can induce cell apoptosis,[21] but little is known about the relationship of V(V) and apoptosis of the lung epithelial cells in vivo. In this paper, apoptosis induction by V(V) in mouse lung was examined both by dose and by time. In addition to the induction of neutrophil apoptosis by

V(V) in vivo and the clearance function of alveolar macrophages, the analysis of apoptosis analysis in lung tissue suggested that V(V) may also induce apoptosis of other lung cells such as epithelial cells (Fig. 3B). This induction would contribute to the phagocytic burden of alveolar macrophages. Apoptosis and injury to lung epithelial cells have been reported in a variety of pulmonary disorders,[12–15] and macrophage clearance of these cells has also been shown to be crucial to the resolution of inflammation and to the remodeling of the lung.[22]

In conclusion, the present study has demonstrated: (1) that pulmonary exposure to V(V) resulted in pulmonary inflammatory cell influx and induction of neutrophil apoptosis; (2) that apoptosis occurred after neutrophil infiltration and was followed by alveolar macrophage influx and neutrophil clearance; and (3) that the observed temporal relationship among neutrophilia, neutrophil apoptosis, and macrophage ingestion of apoptotic neutrophils suggests a critical role of macrophage clearance of apoptotic cells in the resolution of V(V)-induced lung inflammation.

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References

1. Dockery DW, Pope CA III. Acute respiratory effects of particulate air pollution. Annu Rev Public Health 1994; 15:107–132.
2. Kennedy T, Ghio AJ, Reed W, Samel J, Zagorski J, Quay J, Carter J, Dailey L, Hoidal JR, Devlin RB. Copper-dependent inflammation and nuclear factor-kappaB activation by particulate air pollution. Am J Respir Cell Mol Biol 1998; 19:366–378.
3. Kodavanti UP, Hauser R, Christiani DC, Meng ZH, McGee J, Ledbetter A, Richards J, Costa DL. Pulmonary responses to oil fly ash particles in the rat differ by virtue of their specific soluble metals. Toxicol Sci 1998; 43:204–212.
4. Barceloux DG. Vanadium. J Toxicol Clin Toxicol 1999; 37:265–278.
5. Hehner SP, Hofmann TG, Droge W, Schmitz ML. Inhibition of tyrosine phosphatases induces apoptosis independent from the CD95 system. Cell Death Differ 1999; 6:833–841.
6. Huang C, Ding M, Li J, Leonard SS, Rojanasakul Y, Castranova V, Vallyathan V, Ju G, Shi X. Vanadium-induced nuclear factor of activated T cells activation through hydrogen peroxide. J Biol Chem 2001; 276:22397–22403.
7. Cox G, Crossley J, Xing Z. Macrophage engulfment of apoptotic neutrophils contributes to the resolution of acute pulmonary inflammation in vivo. Am J Respir Cell Mol Biol 1995; 12: 232–237.
8. Hussain N, Wu F, Zhu L, Thrall RS, Kresch MJ. Neutrophil apoptosis during the development and resolution of oleic acid-induced acute lung injury in the rat. Am J Respir Cell Mol Biol 1998; 19: 867–874.
9. Savill J, Fadok V, Henson P, Haslett C. Phagocyte recognition of cell undergoing apoptosis. Immunol Today 1993; 14:131–136.
10. Hagimoto N, Kuwano K, Miyazaki H, Kunitake R, Fugita M, Kawasaki M, Kaneko Y, Hara N. Induction of apoptosis and pulmonary fibrosis in mice in response to ligation of Fas antigen. Am J Respir Cell Mol Biol 1997; 17:271–278.
11. Bardales RH, Xie S, Schaefer RF, Hsu S. Apoptosis is a major pathway responsible for the resolution of type II pneumocytes in acute lung injury. Am J Pathol 1996; 149:845–852.
12. Guinee D, Fleming M, Hayashi T, Woodward M, Zhang J, Walls J, Koss M, Ferrans V, Travis W. Association of p53 and WAF1 expression with apoptosis in diffuse alveolar damage. Am J Pathol 1996; 149:531–538.
13. Kuwano K, Kunitake R, Kawasaki M, Nomoto Y, Hagimoto N, Nakanishi Y, Hara N. p21(Waf1/Cip1) and p53 expression in association with DNA strand breaks in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1996; 154: 477–483.
14. Kuwano K, Miyazaki H, Hagimoto N, Kawasaki M, Fugita M, Kunitake R, Kaneko Y, Hara N. The involvement of Fas-Fas ligand pathway in fibrosing lung diseases. Am J Respir Cell Mol Biol 1999; 20:53–60.
15. Anderson BO, Brown JM, Harken AH. Mechanisms of neutrophil mediated tissue injury. J Surg Res 1991; 51:170–179.
16. Haslett C. Granulocyte apoptosis and its role in the resolution and control of lung inflammation. Am J Respir Crit Care Med 1999; 160:S5–S11.
17. Carter JD, Ghio AJ, Samet JM, Devlin RB. Cytokine production by human airway epithelial

cells after exposure to an air pollution particle is metal-dependent. Toxicol Appl Pharmacol 1997; 146:180–188.

18. Pierce LM, Alessandrini F, Godleski JJ, Paulauskis JD. Vanadium-induced chemokine mRNA expression and pulmonary inflammation. Toxicol Appl Pharmacol 1996; 138:1–11.
19. Dye JA, Adler KB, Richards JH, Dreher KL. Role of soluble metals in oil fly ash-induced airway epithelial injury and cytokine gene expression. Am J Physiol 1999; 277:L498–L510.
20. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE2 and PAF. J Clin Invest 1998; 90: 1513–1522.
21. Huang C, Zhang Z, Ding M, Li J, Ye J, Leonard SS, Shen HM, Butterworth L, Lu Y, Costa M, Rojanasakul Y, Castranova V, Vallyathan V, Shi X. Vanadate induces p53 transactivation through hydrogen peroxide and causes apoptosis. J Biol Chem 2000; 275:32516–32522.
22. Kuwano K, Hagimoto N, Tanaka T, Kawasaki M, Kunitake R, Miyazaki H, Kaneko Y, Matsuba T, Maeyama T, Hara N. Expression of apoptosis-regulatory genes in epithelial cells in pulmonary fibrosis in mice. J Pathol 2000; 190:221–229.

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