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Apoptosis is a pathway responsible for the resolution of endotoxin-induced alveolar type II cell hyperplasia in the rat

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Summary. Previous studies showed that intratracheal instillation of endotoxin induces transient type II cell hyperplasia in the rat lung and described some of the mechanisms involved in the proliferative response of type II cells. The purpose of the present study was to investigate how long the type II cell hyperplasia persists and how it is resolved. The portion of epithelial cells in hyperplastic lesions of the rat lung expressing cyclin D1, an indicator for cells in the G1 phase of the cell cycle, was greatest at 3 d post instillation and decreased after 4 and 6 d. The fate of the proliferating epithelial cells was traced by injecting the rats with 5-bromo-2′deoxy uridine (BrdU) 2 d post instillation, the peak time point for maximum incorporation of BrdU. Exfoliated BrdU-positive epithelial cells were detected in the alveolar spaces in tissue sections from rats 4, 5, and 6 d post instillation. BrdU-positive epithelial cells showed flattened nuclei at 6 and 10 d post instillation. Expression of the 116 kD poly(ADP-ribose) polymerase (PARP) was low in type II cells from control rats, and was increased at 3, 4, and 6 d post instillation. In cells obtained by lavage, only a 35 kD cleavage product of PARP was detected, which is an indicator of necrotic cell death. In isolated type II cells from rats 3, 4, and 6 d post endotoxin instillation, progressive cleavage of the PARP to its 89 kD residual fragment was detected, which is a direct evidence for the activation of caspases. Furthermore, apoptotic epithelial cells with condensed nuclei were identified by electron microscopy in rats 4 d post instillation. These results indicate that apoptosis is an additional mechanism for the resolution of endotoxin-induced lung epithelial hyperplasias.

Keywords: endotoxin, type II cells, hyperplasia, resolution, cell death

Cell populations in tissues of multicellular organisms are controlled by a coordinated interaction of positive and negative growth regulators. Positive signals stimulate cell proliferation, whereas negative signals either prevent this progression, induce cell differentiation, or induce cell death. Repair of injured tissues is tightly connected with such population control. Understanding processes involved with these controls is of significance, because their disruption is implicated in a wide variety of pathological conditions, including the uncontrolled proliferation of cells resulting in cancer (Green & Martin 1995).

It has been shown in various animals that intratracheal

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instillation of endotoxin, a lipoposysaccharide component of gram-negative bacterial cell wall, induces bronchial epithelial cell hyperplasia, mucous-secretory cell metaplasia (Harkema & Hotchkiss 1992; Stolk *et al.* 1992), hyperplasia of type II pneumocytes (Tesfaigzi *et al.* 1996), and hypertrophy of interstitial fibroblasts (Domenici-Lombardo *et al.* 1995). The number of proliferating alveolar epithelial cells as shown by 5-bromo-2′deoxy uridine (BrdU) nuclear incorporation reaches maximum levels 2 d after endotoxin treatment and decreases to background levels after 4 (Tesfaigzi *et al.* 1996). At 7 d after instillation, the hyperplasia regresses to nearly normal-appearing alveolar epithelium with few focal lesions.

Based on distinctive changes that take place within cells, two forms of cell death have been described. Necrosis is usually accompanied by cellular swelling, organelle swelling and increased membrane permeability (Schwartz & Osborne 1995). The prominent structural changes characteristic of programmed cell death, also called apoptosis, are shrinkage of cell volume, morphological conservation of most cytoplasmic organelles, and peripheral condensation of nuclear chromatin (Majno & Joris 1995). Apoptosis is an energy-dependent and genetically controlled process (White 1996) that can be induced by a number of molecular tools (Wertz & Hanley 1996).

Apoptosis has been implicated as the mechanism responsible for reducing cell numbers during the regression of parenchymal hyperplasia in many organs, including rat liver (Columbano *et al.* 1985), pancreas (Oates *et al.* 1986), and submandibular glands (Chisholm & Adi 1995). Apoptosis is a selective process of physiological cell deletion that eliminates usually normal cells that are unwanted for a number of reasons (Raff 1992). Some cells must be sacrificed in the process of sculpting the body (Saunders 1966); others may have functioned at some time during evolution or development but are no longer needed, as in the tadpole tail at metamorphosis (Raff 1992).

Stimuli trigger a cell to undergo apoptosis by engaging one or more signal transduction pathways. The signals converge in the activation of a set of pro-apoptotic enzymes which play a key biological role in the manifestation of the death phenotype. These enzymes are a family of cysteine proteases that cleave after aspartic acid and are therefore designated caspases (Alnemri *et al.* 1996). A number of proteins, including but not restricted to poly(ADP-ribose) polymerase (PARP) (Kaufmann *et al.* 1993; Lazebnik *et al.* 1994), lamin B1, and topoisomerase I, are degraded in association with apoptosis, thereby providing direct evidence for the activation of one or more caspases (Martin & Green 1995).

Evans *et al.* (1976) have shown that during the resolution of epithelial hyperplasia from exposure to ozone and nitrate, type II pneumocytes differentiate into type I cells and allow the reconstitution of normal alveolar structure. However, the mechanisms responsible for the disappearance of hyperplastic type II pneumocytes after endotoxin instillation are not clear, because a single dose of lipopolysaccharide to the lung has not been shown to induce cell injury in the broncho-alveolar space (Lopez & Yong 1986; Shami *et al.* 1986; Tesfaigzi *et al.* 1996). To address these issues, we have investigated the persistence of type II cell hyperplasia after endotoxin instillation and whether the apoptotic pathway is involved in the remodeling process.

Materials and methods

Animals and intratracheal instillation

Male F344/N rats, 8–10 weeks of age, were purchased from Harlan Sprague Dawley Laboratories (Germantown, NY). Rats were housed 2–3 per polycarbonate cage and supplied with sterilized hardwood chip bedding and filter tops in animal rooms maintained at 20–22°C, with a relative humidity of 20–50% and a 12 h light/dark cycle starting at 6:00 AM. Food (Lab Blox, Allied Mills, Chicago, IL) and water from a centralized distribution system with sipper tubes were provided ad libitum. Rats were weighed prior to the experiment, then randomly assigned to various treatment groups by body weight.

Rats were lightly anaesthetized with 5% halothane in oxygen before instillation. Thirty rats received intratracheal instillation of 0.5 mg endotoxin (lipopolysaccharide from *E. coli* 0111:B4, Sigma Chemical Co., St. Louis, MO) in 0.5 ml pyrogen-free 0.9% NaCl solution. Three rats were intratracheally instilled with saline as control.

Necropsy and tissue preparation for histopathology

Our previous study has shown that BrdU incorporation is maximum at 2 d post endotoxin instillation (Tesfaigzi *et al.* 1996). To label cells undergoing DNA synthesis and to trace the fate of the newly formed epithelial cells (Harkema & Hotchkiss 1993), all rats were injected intraperitoneally (IP) with BrdU (Sigma Chemical Co.) at 50 mg/g body weight in saline 48 h post instillation. Six rats were killed at each time point starting at 3, 4, 5, 6, and 10 d after instillation by IP injection of 50 mg sodium pentobarbital (Abbott Laboratories, Chicago, IL). The

saline-instilled control rats were sacrificed 3 d post instillation. Their thoracic contents were exposed and lungs perfused through the pulmonary artery with phosphate buffered saline (PBS) without calcium and magnesium ions (GIBCO Laboratories, Grand Island, NY). Lungs were removed, lavaged and the left lung was expanded to inspiratory volume by intratracheal instillation of 10% zinc formalin (Stephens Scientific, Riverdale, NJ) at 25 cm of constant water pressure for 6 h (Herbert *et al.* 1994). Then the lung was immersed overnight in a large volume of the same fixative.

A stratified random sampling scheme was used to cut the fixed left lung into slices, each about 0.5 cm thick. Six or seven slices were prepared, depending on the size of the lung, and numbered from cephalad (slice 1) to caudad (slice 6 or 7). Slices were embedded in paraffin, and sections (5 μm) from the cardial aspect were used for haematoxylin and eosin (H & E) and immunohistochemical staining. Because tissue sections from slices 1 and 2 showed no variability, H & E sections from slices 3, 4, 5, and 6 were randomized and evaluated by a pathologist blinded to section and rat identity so that the severity of inflammation and epithelial cell hyperplasia could be evaluated objectively. Each endpoint was scored as absent (0) or, when present, was graded from minimal (1) to marked (4), indicating the extent and intensity of the reaction.

Electron microscopy

Two lungs from each time point were removed from the thoracic cavity as just described and perfused with PBS. The lungs were expanded to inspiratory volume by intratracheal instillation of a modified Karnovsky's fixative (0.6% glutaraldehyde, 0.45% paraformaldehyde in cacodylate buffer, pH 7.4) at 25 cm constant water pressure for 6 h and immersed in fixative (Harkema & Hotchkiss 1992). Tissue from rats 3 and 4 d post instillation was postfixed in 1% osmium tetroxide, dehydrated in a graded series of alcohol solutions, infiltrated with propylene oxide solutions, embedded in epon araldite, and cut into thin sections with a diamond knife on an Ultracut E ultramicrotome (Reichert-Jung, Cambridge Instruments, Inc., Deerfield, IL). These sections were mounted on Formvar-coated, slotted grids, stained with uranyl acetate and lead citrate, and examined with a Hitachi H7000 scanning/TEM (Hitachi Ltd, Tokyo, Japan).

Immunohistochemistry

Sections from days 3, 4, 5, 6, and 10 were placed on Probe-On Plus slides (Fisher Scientific, Pittsburgh, PA). Tissue sections were double immunostained with BrdU antibody followed by a monoclonal antibody to the cytokeratin Lu-5 (unpublished observations), to identify replicating epithelial cells. Cyclin D1 expression of epithelial cells was also determined by double staining with a polyclonal antibody to cyclin D1 (Santa Cruz Biotechnology, Santa Cruz, CA) followed by the cytokeratin antibody to identify cells in the G1 phase. The percentage of cyclin D1-positive cells was determined from approximately 200 cytokeratin-positive cells (epithelial pneumocytes) in different areas of the lung with extensive hyperplasia.

Isolation of normal and hyperplastic type II cells

Type II cells were isolated from at least three rats each before and 3, 4, and 6 d post endotoxin instillation as described (Tesfaigzi *et al.* 1996) with minor modifications. Briefly, lungs were lavaged three times via the trachea with 5 ml Dulbecco's PBS. Lavaged cells were centrifuged at 1000 g for 5 min and stored at −80°C for further analysis. Lungs were digested by instilling with elastase (Worthington Biochem. Corp., Freehold, NJ) via the trachea at a specific activity of 4.3 units/ml in JMEM supplemented with 1 mg DNase (Sigma Chemical Co.) and incubated at 37°C for 35 min. Five ml of the same elastase solution was reinstilled into the lung every 10 min to ensure maximum cell dissociation. Lungs were minced with scissors; the suspension was agitated on ice and filtered through nylon mesh with pore sizes of 150 μm and 70 μm, sequentially. Cells were separated from red blood cells by layering over a percoll gradient with a density of 1.08 g/ml, and centrifuging for 20 min at 2500 g. After two washes, type II cells were separated from macrophages by panning on rat IgG-coated Petri dishes. The cell viability was >90%, and the purity of type II cells ranged from 65 to 70% as determined by a modified Papanicolaou staining (Tesfaigzi *et al.* 1996); contaminating cells were largely inflammatory cells (lymphocytes, neutrophils, and macrophages).

Western blot analysis

Protein was extracted from isolated type II pneumocytes and lavaged cells by homogenizing in lysis solution containing 10 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X100, 1% deoxycholate, 0.1% sodium dodecyl sulphate, and 5 mM EDTA (Krajewski *et al.* 1994), supplemented with the protease inhibitors PMSF (1 mM), pepstatin (10 μg/ml), aprotinin (2 μg/ml), and benzamidine (2 μg/ml). The protein concentration was determined using the BCA assay kit (PIERCE, Rockford, IL);

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100 μg of protein from control and endotoxin-exposed lungs was loaded on each lane. Western analysis was carried out as described by Tesfaigzi *et al.* (1994) and filters stained with the reversible stain, Ponceau S, confirmed that equivalent amounts of protein had been loaded on each lane. The antibody to PARP (PharMingen, San Diego, CA) was used at a 1:2000 dilution.

Results

In a previous study, we reported that intratracheal instillation of endotoxin induces type II cell hyperplasia in the rat lung (Tesfaigzi *et al.* 1996). In the present study, we examined the events that may be responsible for the resolution of these hyperplasias. At 3 d post instillation, three control lungs that were instilled with saline showed no significant inflammation or epithelial cell hyperplasia. Therefore, investigations from endotoxin-instilled rat lungs are described in detail. At 3 d post endotoxin instillation, most tissue sections exhibited multifocal, moderate-to-marked alveolar epithelial hyperplasia with a concomitant inflammation primarily consisting of neutrophils, macrophages, and proteinacious exudate. Both the inflammation (data not shown) and the pneumocyte hyperplasia (Figure 1) decreased significantly within the endotoxin-exposed lungs from days 4–10 post instillation. Cyclin D1, a gene expressed in the G1 phase of the cell cycle (Sherr 1995), was used to identify actively cycling cells and to determine the number of proliferation cycles after endotoxin instillation (Uhal 1997). Cytokeratin, which is expressed only in epithelial cells, was used as a marker to distinguish epithelial from inflammatory and interstitial cells, because the proteinaceous exudate can obscure the normal architecture of the large number of inflammatory cells. The fraction of cyclin D1-positive epithelial cells in foci of hyperplasia was greatest at 3 d post-instillation and decreased at 4 and 6 d post-instillation (Figure 2). The fact that one cell division takes at least 24 h suggests that the pneumocytes underwent one or two proliferative cycles in response to the endotoxin instillation.

Mechanisms underlying the reduction of hyperplastic alveolar type II cells were examined by a series of experiments. The fate of the proliferating epithelial cells was followed by nuclear staining with BrdU, a marker for DNA synthesis. The tissue sections were double-immunostained with BrdU antibodies followed by an antibody to cytokeratins to distinguish epithelial cells from inflammatory and interstitial cells. A high number of cytokeratin-positive and interstitial cells showed BrdU positivity (Figure 3a). A low percentage (< 5%) of the BrdU-labelled epithelial cells in tissue sections from rats

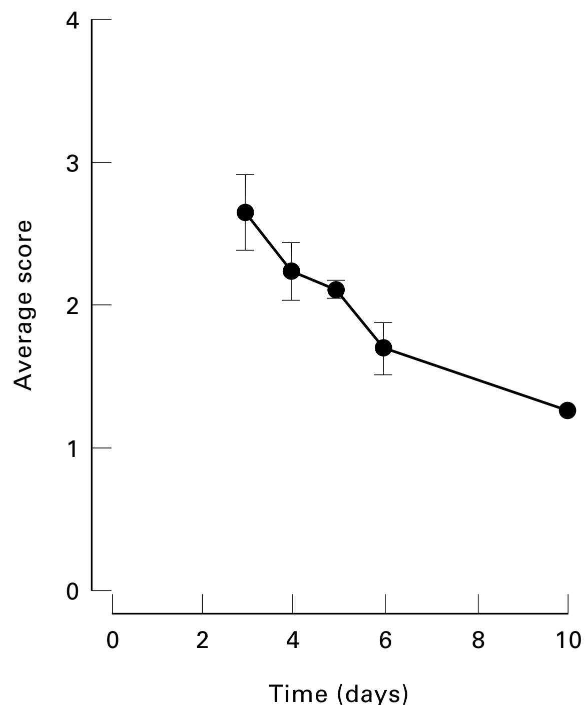

Figure 1. Severity scoring of alveolar epithelial hyperplasia in tissue sections of rat lungs 3, 4, 5, 6, and 10 d after intra-tracheal endotoxin instillation. The mean of the scores for each group ($n = 6$) was calculated from the mean severity score for each rat. Bars represent standard error of the mean.

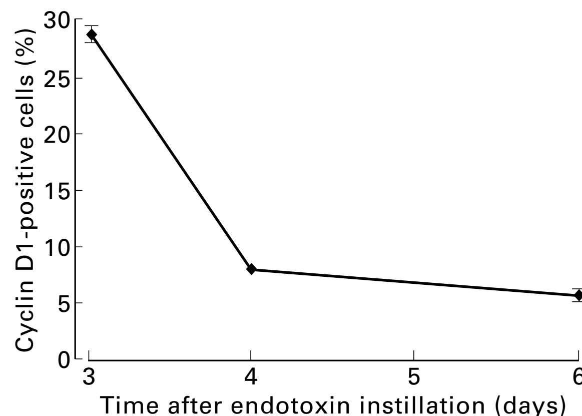

Figure 2. Cyclin D1 positive cells as indicators for endotoxin-induced type II cell proliferation at 3, 4, and 6 d post instillation. The mean percentage values of D1-positive epithelial cells from a total of 200 cells were determined in three areas of hyperplasias from at least two different rats per time point. Error bars represent the standard error of the mean.

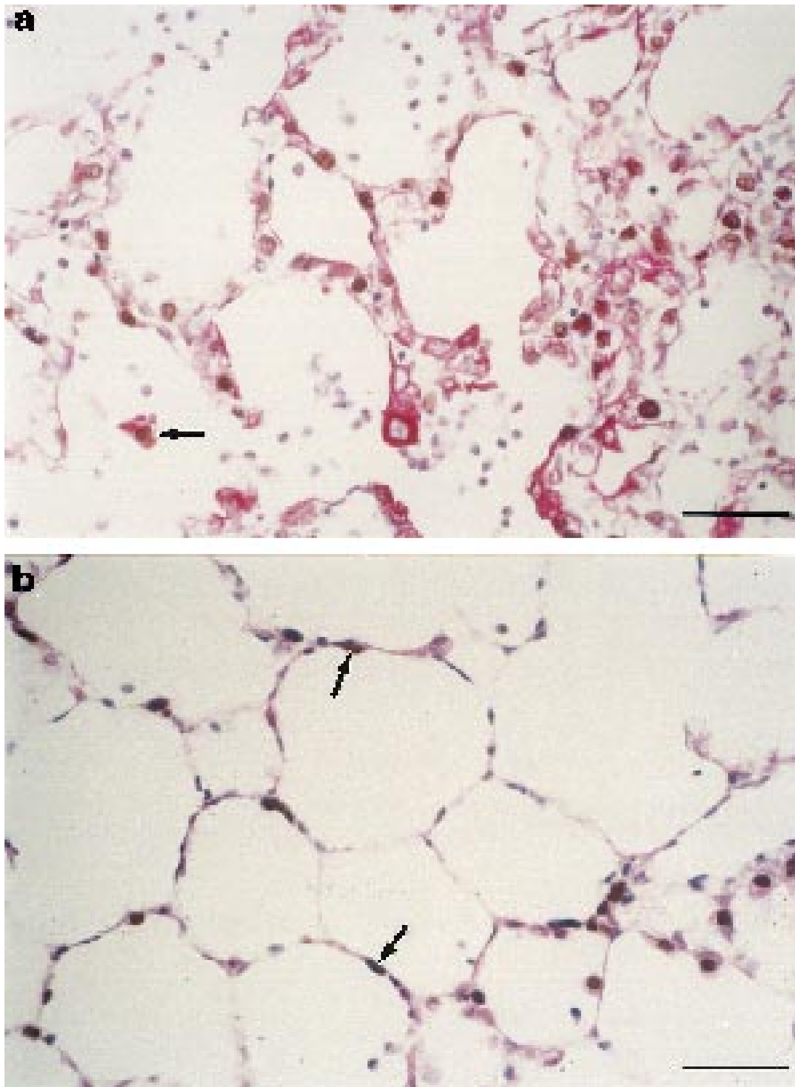

Figure 3. Examples of processes that may be involved in reducing the number of hyperplastic epithelial cells during the remodeling process of rat lung hyperplasias. To label cells undergoing DNA synthesis and to trace the fate of the newly formed epithelial cells, all rats were injected IP with BrdU 48 h post instillation. Tissue sections were immunostained with BrdU antibody (brown colour) to label replicating cells and further stained with cytokeratin antibody (red colour) to distinguish between epithelial, inflammatory, and interstitial cells. a. Tissue sections from rat lungs 3 d postintillation show large, round nuclei of BrdU-positive epithelial cells. Arrow shows an exfoliated BrdU-positive epithelial cell. b. At 6 and 10 d post-instillation, some BrdU-positive epithelial cells show flattened nuclei (arrows).

4, 5, and 6 d post-instillation was found exfoliated in the alveolar air spaces (Figure 3a), indicating that some hyperplastic cells may be exfoliated and cleared through the airways. BrdU-positive pneumocytes at 3, 4, and 5 d showed round nuclei and a more abundant granular cytoplasm, and some of the BrdU-immunostained cells at 6 and 10 d post-instillation showed flattened nuclei (Figure 3b).

Apoptosis as another mechanism for reducing the number of unneeded cells was investigated, because the number of exfoliated hyperplastic epithelial cells was low, and only a fraction of the hyperplastic type II pneumocytes may have been recruited to replace damaged type I cells. Evidence for the apoptotic process was assessed through examining the activation of caspases in the remodeling lung. Type II cells were isolated from control rats before endotoxin-instillation and at 3, 4, and 6 d post instillation. To rule out the involvement of inflammatory cells in modulating caspase activity, we used a pure population of inflammatory cells obtained by lavage of the respective lungs. The cleavage of PARP was analysed by western blotting to assess the activation of caspases (Figure 4). Interestingly, a minor fragment at 43 kD and a major band at 35 kD reacted with the PARP antibody in lavaged cells. Compared to control rats, PARP levels were increased in type II cells at 3, 4, and 6 d post endotoxin instillation, the highest level being detected at day 4. In these type II cell preparations, PARP was progressively cleaved to the 89, 50, 43, and 35 kD fragments (Figure 4).

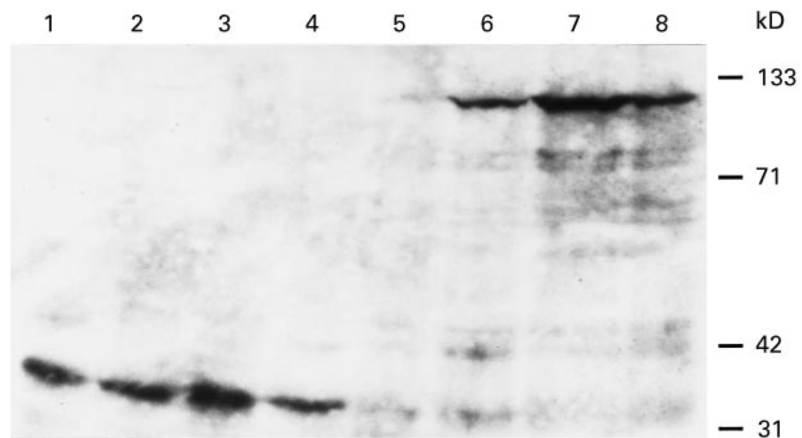

Figure 4. Extent of PARP degradation as an indicator of ced3-type caspases activation in type II cells isolated from the remodeling lung. Protein extracts from lavaged (lanes 1–4) and isolated type II cells (lanes 5–8) from rat lungs before and 3, 4, and 6 d post endotoxin instillation were analysed by western blotting. Staining with the reversible stain, Ponceau S, confirmed that equivalent amounts were loaded on each lane (not shown). The PARP antibody was used at 1:2000 dilution.

The presence of apoptotic epithelial cells was confirmed by electron microscopy. Cells with condensed nuclei, a sign of apoptosis, were identified in areas of extensive hyperplasia on tissue sections (1 μm thick) prepared from glutaraldehyde-fixed lung tissues from rats 4 d post-instillation. Identification of tight junctions established the epithelial nature of the apoptotic cells (Figure 5). These results confirm that programmed cell death must occur during the resolution of endotoxin-induced hyperplasia.

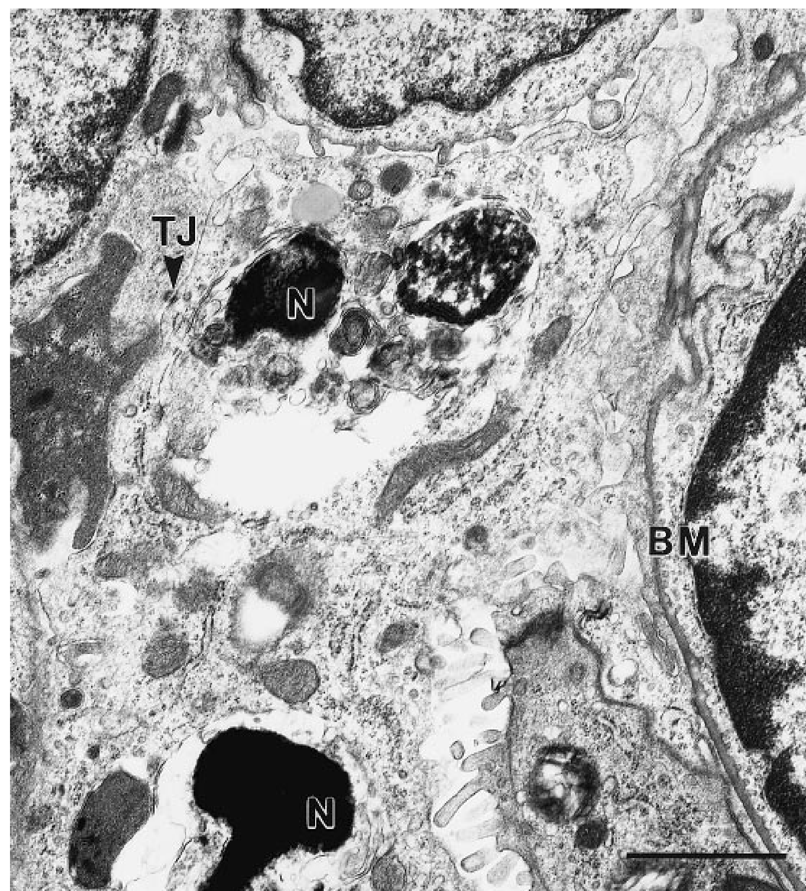

Figure 5. Electron micrograph of a cell with condensed nuclear (N) chromatin in the remodeling lung (4 d post instillation); a sign of cells in apoptosis in the remodeling lung. BM, basement membrane; TJ, tight junction.

Discussion

The present investigation provides evidence that apoptosis may be a factor involved in the resolution of endotoxin-induced lung epithelial cell proliferation. Exfoliation of hyperplastic pneumocytes and differentiation into type I cells appear to also play a role in reducing cell numbers during resolution of these hyperplasias.

A single intratracheal instillation of endotoxin induces alveolar epithelial cells to proliferate in the rat lung (Tesfaigzi *et al.* 1996). More cells immunostained with cyclin D1 antibody at 3 d than at 4 and 6 d. Assuming that the completion of a cell cycle takes 21–24 h (Uhal 1997), this observation suggests that these cells may undergo at most two cell divisions. A previous study showed that the number of BrdU-labelled cells is maximum at 48 h, when rats are injected with BrdU 2 h before sacrifice (Tesfaigzi *et al.* 1996), which supports the theory that the majority of these cells undergoes one or two cell divisions.

It is possible that endotoxin directly stimulates type II cell proliferation. However, it is also likely that neutrophils and macrophages release the cytokines responsible for pneumocyte hyperplasia (Shami *et al.* 1986), because the proliferative response of type II cells is correlated with the influx of inflammatory cells into the alveolar spaces. Cytokines, such as keratinocyte growth factor (Panos *et al.* 1993), epidermal growth factor (Raaberg *et al.* 1992), transforming growth factor-α, and basic fibroblast growth factors (Buch *et al.* 1995), have been shown to induce proliferation of type II cells in short-term cultures. These cytokines may be produced by the inflammatory cells or by activated mesenchymal cells.

It is conceivable that endotoxin would be cleared by inflammatory cells, thereby removing signals for further proliferation. More investigation is necessary to clarify whether the end of proliferation is separate from signals that enable the dissolution of hyperplastic cells. It is also possible that the end of proliferation is secondary to decreased inflammation and levels of stimulatory growth factors and/or increased levels of inhibitory cytokines. Up-regulation of wild-type p53 and p21 in type II pneumocytes after severe lung injury (Guinee *et al.* 1995) indicates that the cell growth arrest may be mediated by the p53 pathway. Whether or not these processes or other mechanisms may be responsible for the end of proliferation is unclear.

Because the hyperplastic cells undergo a maximum of two rounds of cell division, the incorporated BrdU would be reduced to half the amount in the dividing cells. BrdU labelling of newly formed cells was therefore still detectable in the remodeled lung and was useful in following the fate of these cells. This procedure allowed the detection of BrdU-positive cells with morphologic features of type I cells, indicating a differentiation of hyperplastic cells into type I cells. Exposure of adult lungs to oxidant gases results in the damage of a substantial number of alveolar type I cells. In those lungs, hyperplastic type II cells differentiate and replace the damaged type I cells (Adamson & Bowden 1974; Evans *et al.* 1976). In contrast, several studies report that cell injury could not be detected in the bronchoalveolar space after a single dose of lipopolysaccharide to the lung (Lopez & Yong 1986; Shami *et al.* 1986; Tesfaigzi *et al.* 1996). However, the presence of BrdU-positive pneumocytes showing type I cell morphology in the present study indicates that a low number of type I cells must have been damaged by endotoxin instillation. Taken together, only a small number of hyperplastic cells are needed to differentiate into type I cells and reconstitute the normal alveolar architecture. Based on the detection of exfoliated epithelial cells in the alveolar spaces of endotoxin-instilled rat lungs, the second mechanism must be that some hyperplastic cells are cleared through the airways. This finding is consistent with previous results showing that during the resolution of acute lung injury and in the acute exudative early phase exfoliated type II pneumocytes have been found in bronchoalveolar lavage specimens (Stanley *et al.* 1992).

Shah *et al.* (1996) reported that apoptotic Hl-60 cells in culture exhibit only the signature 89 kD fragment of PARP, while necrosis of the same cells was accompanied by formation of major fragments at 89 and 50 kD and minor fragments at 40 and 35 kD. This pattern of PARP degradation is suggested to be an indicator for apoptotic or necrotic cell death. Cells obtained by lavage 3, 4, and 6 d post endotoxin instillation showed a major 35 kD fragment and a minor 43 kD fragment. The type II cell preparations, however, showed a major 89 kD fragment accompanied by minor 50, 43, and 35 kD fragments (Figure 4). Because the type II cell preparations were contaminated with nontype II cells to about 30–35%, the necrotic cell death of these cells may be responsible for the appearance of the low molecular weight bands. Although it is possible that a part of the type II cells undergo necrotic cell death, the progressive appearance of the 89 kD band indicates that type II cells undergo programmed cell death. Identification of epithelial cells undergoing apoptosis was confirmed by electron microscopy (Figure 5). In future studies additional methods must be used to determine the number of type II cells that die by apoptosis.

These results suggest that apoptosis is an additional mechanism for the rapid decrease of type II pneumocyte

hyperplasia in the resolution phase after intratracheal endotoxin instillation. Possible mechanisms for induction of apoptosis could include withdrawal of cytokines or growth factors, or activation of a death-inducing cell surface receptor, such as members of the tumour necrosis factor receptor family (Chinnaiyan *et al.* 1996). These apoptotic cells may be cleared by phagocytes or through the airways. The present model may be useful to further clarify the mechanism(s) responsible for inducing cell death to resolve pulmonary hyperplasias *in vivo*.

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References

Adamson, I. & Bowden, C. (1974) The type 2 cell as progenitor of alveolar epithelial regeneration: a cytodynamic study in mice after exposure to oxygen. *Lab. Invest.* **30,** 35–42.

Alnemri, E.S., Livingston, D.J., Nicholson, D.W., Salvesen G., Thornberry, N.A., Wong, W.W. & Yuan, J. (1996) Human ICE/CED-3 protease nomenclature [letter]. *Cell* **87,** 171.

Buch S., Han, R.N., Liu J., Moore, A., Edelson, J.D., Freeman, B.A., Post, M. & Tanswell, A.K. (1995) Basic fibroblast growth factor and growth factor receptor gene expression in 85% O_2-exposed rat lung. *Am. J. Physiol.* **268,** L455–L464.

Chinnaiyan A.M., O'rourke K., Yu, G.L., Lyons, R.H., Garg, M., Duan, D.R., Xing, L., Gentz, R., Ni, J. & Dixit, V.M. (1996) Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. *Science* **274,** 990–992.

Chisholm, D.M. & Adi, M.M. (1995) Cell proliferation and apoptosis in isoprenaline-induced sialosis in the rat submandibular glands. *Int. J. Exp. Pathol.* **76,** 263–269.

Columbano, A., Ledda-Columbano, G.M., Coni, P.P., Faa, G., Liguori, C., Santa Cruz, G. & Pani, P. (1985) Occurence of cell death (apoptosis) during the involution of liver hyperplasia. *Lab Invest* **52,** 670–675.

Domenici-Lombardo, L., Adembri, C., Consalvo, M., Forzini, R., Meucci, M., Romagnoli, P. & Novelli, G.P. (1995) Evolution of endotoxin induced acute lung injury in the rat. *Int. J. Exp. Pathol.* **76,** 381–390.

Evans, M.J., Johnson, L.V., Stephens, R.J. & Freeman, G. (1976) Renewal of the terminal bronchiolar epithelium in the rat following exposure to NO_2 or O_3. *Lab. Invest.* **35,** 246–257.

Green, D.R. & Martin, S.J. (1995) The killer and the executioner: how apoptosis controls malignancy. *Curr. Opin. Immunol.* **7,** 694–703.

Guinee, D.G., Woodwad, M.L. & Walls, J. (1995) p53 nd WAF1 expression in diffuse alveolar damage: An example of p53 upregulation in non-neoplastic pulmonary diease. *Am. J. Clin. Pathol.* **104,** 331.

Harkema, J.R. & Hotchkiss, J.A. (1992) *In vivo* effects of endotoxin on intraepithelial mucosubstances in rat pulmonary airways. Quantitative histochemistry. *Am. J. Pathol.* **141,** 307–317.

Harkema, J.R. & Hotchkiss, J.A. (1993) *In vivo* effects of endotoxin on DNA synthesis in rat nasal epithelium. *Microsc. Res. Tech.* **26,** 457–465.

Herbert, R.A., Stegelmeier, B.S., Gillett, N.A., Rebar, A.H., Carlton, W.W., Singh, G. & Hahn, F.F. (1994) Plutonium-induced proliferative lesions and pulmonary epithelial neoplasms in the rat: immunohistochemical and ultrastructural evidence for their origin from type II pneumocytes. *Vet. Pathol.* **31,** 366–374.

Kaufmann, S., Desnoyers, S., Ottaviano, Y., Davidson, N. & Poirier, G. (1993) Specific proteolytic cleavage of poly (ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. *Cancer Res.* **53,** 3976–3985.

Krajewski, S., Krajewska, M., Shabaik, A., Miyashita, T., Wang, H.G. & Reed, J.C. (1994) Immunohistochemical determination of in vivo distribution of Bax, a dominant inhibitor of Bcl.-2. *Am. J. Pathol.* **145,** 1323–1336.

Lazebnik, Y., Kaufmann, S., Desnoyers, S., Poirier, G. & Earnshaw, W. (1994) Cleavage of poly (ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature* **371,** 346–347.

Lopez, A. & Yong, S. (1986) Injury versus inflammatory response in the lungs of rats intratracheally inoculated with bacterial lipopolysaccharide. *Am. J. Vet. Res.* **47,** 1287–1292.

Majno, G. & Joris, I. (1995) Apoptosis, oncosis, and necrosis. An overview of cell death [see comments]. *Am. J. Pathol* **146,** 3–15.

Martin, S.J. & Green, D.R. (1995) Protease activation during apoptosis: death by a thousand cuts? *Cell* **82,** 349–352.

Oates, P.S., Morgan, R.G.H. & Light, A.M. (1986) Cell death (apoptosis) during pancreatic involution after raw soya flour feeding in the rat. *Am. J. Physiol.* **250,** G9–G14.

Panos, R.J., Rubin, J.S., Csaky, K.G., Aaronson, S.A. & Mason, R.J. (1993) Keratinocyte growth factor and hepatocyte growth factor/scatter factor are heparin-binding growth factors for alveolar type II cells in fibroblast-conditioned medium [published erratum appears in *J Clin. Invest.* Mar 1994; **93** (3),1347]. *J. Clin. Invest.* **92,** 969–977.

Raaberg, L., Nexo, E., Buckley, S., Luo, W., Snead, M.L. & Warburton, D. (1992) Epidermal growth factor transcription, translation, and signal transduction by rat type II pneumocytes in culture. *Am. J. Respir. Cell Mol. Biol.* **6,** 44–49.

Raff, M.C. (1992) Social controls on cell survival and cell death. *Nature* **356,** 397–400.

Saunders, J.W. (1966) Death in embryonic systems. Death of cells is usual accompaniment of embryonic growth and differentiation. *Science* **154,** 604–612.

Schwartz, L.M. & Osborne, B.A. (1995) Cell Death, In *Methods in Cell Biology*, Vol. 46. Eds. L. Wilson & P. Matsudaira. San Diego: Academic Press Inc. pp. 1–27.

Shah, G., Shah, R. & Poirier, G. (1996) Different cleavage pattern for poly (ADP-ribose) polymerase during necrosis and apoptosis in HL-60 cells. *Biochem. Biophys. Res. Commun* **229,** 838–844.

Shami, S.G., Evans, M.J. & Martinez, L.A. (1986) Type II cell

proliferation related to migration of inflammatory cells into the lung. *Exp Mol Pathol* **44,** 344–352.

SHERR, C.J. (1995) D-type cyclins. *Trends Biochem. Sci.* **20,** 187–190.

STANLEY, M.W., HENRY-STANLEY, M.J., GAJL-PECZALSKA, K.J. & BITTERMAN, P.B. (1992) Hyperplasia of type II pheumocytes in acute lung injury. *Am. J. Clin. Pathol.* **97,** 669–677.

STOLK, J., RUDOLPHUS, A., DAVIES, P., OSINGA, D., DIJKMAN, J.H., AGARWAL, L., KEENAN, K.P., FLETCHER, D. & KRAMPS, J.A. (1992) Induction of emphysema and bronchial mucus cell hyperplasia by intratracheal instillation of lipopolysaccharide in the hamster. *J. Pathol.* **167,** 349–356.

TESFAIGZI, J., JOHNSON, N.F. & LECHNER, J.F. (1996) Induction of EGF receptor and erbB-2 during endotoxin-induced alveolar type II cell proliferation in the rat lung. *Int J. Exp. Path.* **77,** 143–154.

TESFAIGZI, J., SMITH-HARRISON, W. & CARLSON, D.M. (1994) A simple method for reusing Western blots on PVDF membranes. *Biotechniques* **17,** 268–269.

UHAL, B.D. (1997) Cell cycle kinetics in the alveolar epithelium. *Am. J. Physiol.* **272,** L1031–L1045.

WERTZ, I.E. & HANLEY, M.R. (1996) Diverse molecular provocation of programmed cell death. *Trends Biochem. Sci.* **21,** 359–364.

WHITE, E. (1996) Life, death and the pursuit of apoptosis. *Genes Dev.* **10,** 1–15.