Acute lung injury in children: from viral infection and mechanical ventilation to inflammation and apoptosis
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Full color illustration section
Figure 1-1. Pathways of caspase-(in)dependent apoptosis in lung epithelial cells mediated by mitochondrial disruption or death receptor stimulation initiated by recruited effector lymphocytes, neutrophils (PMN) or macrophages. Members of the Bcl-2 family including Bcl-2, Bax and Bcl-XL and p53 regulate cytochrome c release from the mitochondria in response to stimuli such as DNA damage, reactive oxygen species (ROS) or calcium overload. Cytochrome c in the cytosol assembles with apoptotic peptidase activating factor 1 (Apaf-1) to activate initiator caspase-9. Adaptor proteins interact through their death domain upon activation of the death receptor pathway by FasL or TRAIL, leading to activation of initiator caspase-8. The mitochondrial and death receptor pathway can interact through Bid. The activation of caspase-8 or caspase-9 leads to the downstream activation of the caspase cascade including effector caspase-3, -6 and -7, resulting in apoptosis. Granzymes delivered to the cytosol by effector lymphocytes can activate caspases and Bid to induce apoptosis. The inhibitor-of-apoptosis proteins (IAPs) can block several caspases thereby inhibiting cell death.

Figure 2-2. A, representative composite images of cytokeratin (blue) and active caspase-3 (red) double staining of lung tissue after unmixing of the individual color spectra, showing co-localization (yellow) (zoomed from 200x magnification images); B, original RedGreenBlue-bright field images (upper left) and composite images: without highlighted co-localization (upper right), active caspase-3 images (lower left) and cytokeratin images (lower right); C, percentage of cytokeratin (ck) positive staining co-localized with positive cleaved caspase-3 staining in alveolar tissue sections of ARDS patients with indirect lung injury (n=4) or direct lung injury (n=12); D, correlation between the percentage of cytokeratin (ck) positive staining co-localized with positive cleaved caspase-3 staining and age (r=0.5, p<0.05).
Figure 2-1. A-C, lung tissue sections of ARDS patients stained with haematoxylin and eosin (magnification 100x) showing changes characteristic of exudative ARDS (protein-rich alveolar exudates) (A), hyaline membranes (arrow) (B), and marked fibroproliferative changes (C). D-F, Immunohistochemistry for cleaved (active) caspase-3 (magnification 500x) showing the wide range of positive staining in lung tissue sections of patients with ARDS: from widespread positive cells in the alveolar walls (arrows) (D), to almost no positive cells in the alveolar walls (E). Note that the latter picture is comparable to the finding in lung tissue of a patient who died with sepsis, but with normal lung architecture and without clinical ARDS (F).
Figure 3-1. Total alveolar macrophages (A) and PMN (B) counts in the BALF of mice 24, 48 or 72 hr after intratracheal instillation of liposomes containing PBS (white) or clodronate (gray) (n=3/group for each time). Panel C and D show representative lung tissue sections from mice studied 24 hr after intratracheal instillation of liposomes containing PBS or clodronate respectively (H&E staining, magnification 400x). Note the absence of macrophages and neutrophils in the lungs from the mouse treated with clodronate liposomes (D).
Figure 3-4. Lung tissue sections from mice treated with intratracheal liposomes containing PBS or clodronate, followed 24 hr later by intratracheal instillations of Jo2 mAb or control IgG mAb, 2.5 μg/g, and studied 18 hr later. The right column shows representative lung tissue sections stained with H&E (400x, insets 200x). The left column shows immunohistochemistry for AM (arrows), using anti mac-2 antibody.
Figure 4-3. A, immunocytochemistry for TRAIL-R1 and -R2 of cytospins of primary bronchial epithelial cells of children (magnification 500x). B, representative examples of FACS histogram plots of primary bronchial epithelial cells of children stained for membrane bound TRAIL-R1 and -R2. Negative control (grey surface plot) is without primary antibody. C, representative examples of FACS histogram plots for membrane bound TRAIL-R1 and -R2 staining on primary bronchial epithelial cells of children, 3 and 6 days post-infection (dpi) with RSV-A in vitro. Uninfected cells are shown in grey surface plots.
Figure 4-4. A, fold increase in the percentages of annexin V positive cells upon 6 hr exposure to superkillerTRAIL, as compared to cells treated with dilution buffer only. Experiments were performed in duplicates with primary bronchial epithelial cells obtained from three different children. B, example of FACS histogram plots from primary bronchial epithelial cells stained for annexin V upon 6 hr exposure to superkillerTRAIL, as compared to cells treated with dilution buffer only. C, percentages of PI positive primary bronchial epithelial cells from one child upon 48 hr exposure to non cross-linked FLAG-TRAIL with or without M2-anti-FLAG antibody to establish crosslinking. D, percentages of PI positive cells upon 48 hr exposure to non-cross linked FLAG-TRAIL (33 ng/ml) or concentrated BALF from a RSV patient with or without DR5-Fc to neutralize TRAIL signalling. Experiments were performed in duplicates with primary bronchial epithelial cells obtained from two different children. * p <0.05 (paired t-test). Data are shown as bars depicting the mean and standard error. E, representative light microscopy photograph showing the apoptotic morphological appearance of primary bronchial epithelial cells of children in TRAIL cytotoxicity assays (zoomed in 250x magnification). Inset shows normal unexposed control cells (250x magnification).
Figure 6-1. A, gene expression profiles of transcripts encoding GzmA and GzmB in total lung RNA of C57Bl/6 mice (RNA from 5 mice pooled per time point) at time zero and days 1-7, 10, 14, 21 and 28 after inoculation with PVM J3666 (30 pfu). Peak of GzmA and GzmB expression at day 7 (green highlight). B, histogram plots of intracellular GzmA and GzmB expression in BALF CD8^+CD3^+ cells (CTLs, blue plot), NK1.1^+CD3^- (NK cells, green plot) and CD8^-CD3^+ (CD8^- T lymphocytes, red plot) of PVM-infected mice at day 7 (BALF leukocytes from 3 mice pooled).

Figure 6-6. Representative images of haematoxylin and eosin stained lung tissue from the GzmA^+/ GzmB^+/- mice and GzmA^-GzmB^-/- mice on day 8 after PVM inoculation (6 x 10^3 copies).
Figure 6-3. A, virus titer in the lung, expressed as number of PVM-sh copies per 10^9 gapdh copies; B, lung homogenate caspase-3 activity (arbitrary fluorescence units), in the GzmA+/-GzmB+/- mice and GzmA−/−GzmB−/− mice on day 7 (n=6 per group) and 8 (n=6 per group) after PVM inoculation (6 x 10^3 copies). * p<0.05. Data are shown as box plots depicting the median, interquartile range and full range. C, representative images of cleaved caspase-3 immunohistochemistry in lung tissues of the GzmA+/-GzmB+/- mice and GzmA−/−GzmB−/− mice on day 8 after PVM inoculation. Note the relative positive staining in alveolar wall cells (arrows) as compared to bronchial epithelial cells in the GzmA+/-GzmB+/- mice.
Figure 7-6. Caspase-3 activity in lung homogenates (A); number of TUNEL-positive cells per 12 high-power fields in lung tissue sections (B); BALF soluble FasL concentrations (C) and BALF granzyme B (GrB) concentrations (D) in uninfected mice allowed to breathe spontaneously (SB, n = 6) or subjected to mechanical ventilation (MV, n = 5); and in PVM infected mice allowed to breathe spontaneously (PVM + SB, n = 6) or subjected to mechanical ventilation (PVM + MV, n = 6), * p < 0.05. The cellular distribution of caspase-3 activation is shown in merged differential interference contrast (DIC) and fluorescence images (cleaved caspase-3 in pink, nuclei in blue) from a mouse in the SB group (E); MV group (F), PVM group (G), and PVM + MV group (H). The figures show the presence of signal in cells of the alveolar walls (open arrows) or macrophages (closed arrow).
Figure 7-7. H&E stained lung tissue sections of lung tissue from uninfected mice allowed to breathe spontaneously (SB, A) or subjected to mechanical ventilation (MV, B); and in PVM infected mice allowed to breathe spontaneously (PVM + SB, C) or subjected to mechanical ventilation (PVM + MV, D); 400X magnification. Note the peribronchiolar cellular infiltration in both the PVM + SB and PVM + MV group (arrows). E, mean peak inspiratory pressures in uninfected mice subjected to mechanical ventilation (n = 5) and PVM infected mice subjected to mechanical ventilation (n = 6). Means ± standard error.