Differentiation between apoptotic and necrotic cell death in airway epithelial cells in response to viral infection and anaerobic conditions using flow cytometry

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Introduction/Aim:
Necrosis of airway epithelial cells (AEC) resulting in airway inflammation is a characteristic finding in cystic fibrosis (CF), driven by mucus obstruction of the airway and previously suggested as a potential response to respiratory viral infection. Current methodologies to measure apoptotic and necrotic cell death using flow cytometry in AEC are not adequate to completely differentiate between the two. The aim here was to determine whether a novel flow cytometry methodology described in other cell types could be optimized and adapted to AEC to sufficiently differentiate apoptotic and necrotic AEC.

Methods:
Non-CF and CF AECs were permeabilised, infected with human rhinovirus for 24 hours (MOI 1 & 3), or incubated in a limited O_2 environment (0% O_2 for 15 hours). Cells were then collected and stained with Annexin-V (A5) and TO-PRO-3 (TP3) before analysis via flow cytometry. Data was analysed using a seven-step gating process to differentiate six different populations from AEC.

Results:
Flow cytometry using A5 and TP3 was able to differentiate viable, apoptotic, and necrotic cells, plus apoptotic bodies and cellular debris in stimulated and unstimulated epithelial samples.

		Viable	A5+ Apoptotic	A5- Apoptotic	Necrotic	Apoptotic Bodies	Debris
non-CF	Control	82.43%	1.88%	0.50%	9.82%	3.92%	1.44%
	Permeabilised	3.01%	75.21%	0.23%	18.64%	3.16%	0.26%
	Virus	76.91%	1.97%	0.49%	11.01%	5.23%	3.51%
CF	Control	59.46%	8.83%	1.28%	17.92%	6.81%	2.56%
	Permeabilised	0.98%	60.43%	2.09%	26.94%	7.51%	0.89%
	Virus	58.10%	9.63%	1.88%	15.51%	8.31%	2.75%

Conclusion:
Flow cytometry utilising A5 and TP3 in conjunction with a seven-step gating process is sufficient to differentiate six populations from both stimulated and unstimulated AEC of healthy and diseased individuals. Further studies utilising this technique will allow quantification of AEC necrosis in patients with and without CF, with the aim to investigate differences in mechanisms driving cell death and airway inflammation.

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