**Introduction**

Influenza A is typically thought to be a seasonal virus which demonstrates symptoms of coughing and sneezing through irritation of the respiratory tract. Mutant strains such as H1N1 for example, while much less common than the common cold, wreaks havoc upon societies plagued by it, and these strains are typically followed by high death rates due to the inability of the human immune system to interact with these unknown antigens.

Despite waves of influenza A spreading every few years (some notable cases include the spanish flu and 2009), very little is still understood about their mechanism of destructive action, except for lung damage. Case studies and cadaver reports suggest a few common observations. Most notably, like all Influenza A, alveolar edema is observed [citation] which involves a dysregulation of water transporters in the alveoli. Similarly, diffuse alveolar damage may result from edema but may also have other possible causes.

Acute respiratory distress syndrome has been observed in rat models of Influenza A and is also of particular interest as it may provide evidence for the legitimacy of the rat influenza model, etc.

Finally, whilst quite restricted in occurrence, it is interesting to note that encephalitis has been simultaneously been observed with influenza A.

Together, these pathologies may ultimately be due to cellular or epithelial damage to the cells, etc. As such, it is of particular interest to invest their mechanisms of action in order to understand not only the predictors of negative survival outcome but also to enhance our understanding of the cellular nature of the human lung. Here, we also discuss the possible risk factors associated with influenza A pathology and negative outcomes, and propose possible treatments based on the cellular mechanism of pathophysiology.

**Discussion**

* The fatal negative outcomes of pulmonary pathologies lies in the downregulation, dysregulation, inhibition, or damage to fluid transport mechanisms within the lung
* In particular, we should understand the cellular mechanism epithelial fluid resorption across the alveoli to understand alveolar edema and how it can mediate diffuse alveolar damage (and acute respiratory distress?)
* [encephalitis no info yet – not looked into, possibly no space either]

<http://ajplung.physiology.org.ezproxy1.library.usyd.edu.au/content/294/2/L149.short> issue for animal model

1. **Alveolar edema**

<http://erj.ersjournals.com/content/early/2016/01/07/13993003.01282-2015>

* Flooding of alveolar lumen with proteinaceous oedema fluid, erythrocytes and inflammatory cells
* H1N1 in seeded in vitro human alveolar resulted in barrier damage, damage to epithelial cells occurred independently of endothelial cells (which were pro-inflammatory and pro-coagulant)
* Barrier damage associated with loss of **claudin-4**
* Human lung (Adult) studies

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| Results suggested from h1n1 studies | Results suggested from all studies |
| <http://www.nature.com.ezproxy1.library.usyd.edu.au/nm/journal/v17/n2/abs/nm.2262.html>  C4d deposition—a marker of complement activation mediated by immune complexes—was present in lung sections of fatal cases  IMMUNE SYSTEM MEDIATED PROGRESSION OF INFLUENZA DAMAGE? | Amiloride (apical Na+ uptake inhibitor) and ouabain (NaKATPase inhibitor) reduced alveolar clearance by 40 and 49%  Terbutaline doubled alveolar clearance (12+/2 – 28+/9%  Propranolol and amiloride inhibited terbutaline-induced increase  🡺 alveolar liquid dependent on apical Na uptake, NaKATPase activity and beta-adr stimulation increases rate of alveolar liquid clearance  (resected human lung w/ lung cancer)  <http://www.atsjournals.org.ezproxy1.library.usyd.edu.au/doi/abs/10.1164/ajrccm.150.2.8049807> |
|  | Fluid transport dependent on active na+ transport (primary mechanism) to drive cluid from distal air spaces  Rate increased by cAMP stimulation  Hormones, GFs, cytokines upregulate epithelial fluid clearance in lung  <http://jap.physiology.org.ezproxy1.library.usyd.edu.au/content/93/4/1533.full.pdf+html> |
|  | Mouse study **(???)**  AQP provide pw for osmotic water movement across epithelial and microvascular barriers in lung  Wet-to-dry weight ratio decreased after 1st hr and AQP1/4/5 deletion did not affect W/D  Fluid clearance inhibited by amiloride, but AQP deletion did not affect clearance  In established models of ALI, W/D increased but not affected by AQP deletion  Thiourea increased W/D and pleural effusions appeared – no difference with AQPKO  AQP no effect on hyperoxic subacute lung injury induced by 95% oxy  AQP no importance in lung water clearance in neonatal or adult lung  <http://onlinelibrary.wiley.com.ezproxy1.library.usyd.edu.au/doi/10.1111/j.1469-7793.2000.00771.x/full>  AQP4 basolateral membrane, AQP5 apical membrane T1 epithelial, AQP1/4 upregulated near birth (separate paper, AQP1/5 found after acute viral infection in lung Towne 2000)  Paracellular water permeability probably substantially greater than transceullular water permability in lung microvessels – non-osmotic fluid movement in lung (hydrostatic filtration, lymphatic drainage)  <https://www-ncbi-nlm-nih-gov.ezproxy1.library.usyd.edu.au/pubmed/10781416> AQPs in edema after lung infection? |
|  | <http://www.sciencedirect.com.ezproxy1.library.usyd.edu.au/science/article/pii/S1569904807000626>  AQP additional roles |
|  | <http://jap.physiology.org.ezproxy1.library.usyd.edu.au/content/87/4/1301.short>  Catecholamine-independent factors are important in the regulation of alveolar fluid clearance in patients with severe hydrostatic pulmonary edema |
|  | [**http://www.sciencedirect.com.ezproxy1.library.usyd.edu.au/science/article/pii/S1569904807001486**](http://www.sciencedirect.com.ezproxy1.library.usyd.edu.au/science/article/pii/S1569904807001486) **lit review nice pics** |
|  | <http://ajplung.physiology.org.ezproxy1.library.usyd.edu.au/content/290/2/L242.short> cultured human lung CTFR contribution to fluid clearance |

* Rat lung (Adult) studies

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| Results suggested from h1n1 studies | Results suggested from all studies |
| <http://www.sciencedirect.com.ezproxy1.library.usyd.edu.au/science/article/pii/S0092867411011731>  We show here that p63-expressing stem cells in the bronchiolar epithelium undergo rapid proliferation after infection and radiate to interbronchiolar regions of alveolar ablation  (TRY TO UNDERSTAND?? REGENERATION OF LUNG TISSUE) | Amiloride inhibited alveolar fluid clearance 46+/15, diltiazem (cyclic nucleotide gated cation channel inhibitor) did nothing  Terbutaline and DBcGMP increased alveolar fluid clearance by 85+-3 and 36+-5  Amiloride and diltiazem inhibited terbutaline-stimulated alveolar clearance  Terbutaline stimulated amiloride sensitive and diltiazem sensitive pathways  Na+ driven alveolar fluid clearance mediated thru cyclic nucleotide gated cation channels (activated by increased cGMP??)  Rat study  <http://jap.physiology.org.ezproxy1.library.usyd.edu.au/content/90/4/1489.short> |
|  | <https://link-springer-com.ezproxy1.library.usyd.edu.au/article/10.1007/s00134-007-0662-7> review paper on fluid clearance and methods ofresolving alveolar oedema (Good flow chart) |
|  | In ATII, beta adrenergic agonists activate ENaC and CFTR  ENaC activated by dopamine in ATI and ATII but not CFTR  [ion channels, hypoxia and ENaC regulation]    <http://ajplung.physiology.org.ezproxy1.library.usyd.edu.au/content/291/3/L301.full.pdf+html> |
|  | NAKATPAse important in clearance of lung edema  <http://jap.physiology.org.ezproxy1.library.usyd.edu.au/content/93/5/1860.short> |
|  | AQPs not as important for clearance of oedema but for submucosal gland function  <http://jap.physiology.org.ezproxy1.library.usyd.edu.au/content/93/6/2199.short> |
|  | Beta 2 AR overexpression improves Na+ transport by improving responsiveness to endogenous catecholarmines (exogenous procaterol did not improve fluid clearance (is beta receptor rate limiting? )  <http://circres.ahajournals.org/content/89/10/907> |
|  | AFC with CFTR 92% compared to control  No albumin flux measured  NPPB, glibenclamide, or bumetanide added, blocked Cl- effect, suggested AFC increase due to Cl- function  CFTR effect on active Na+ transport requires beta AR  <http://circres.ahajournals.org/content/96/9/999.full> |
|  | <http://jap.physiology.org.ezproxy1.library.usyd.edu.au/content/93/5/1852.short>  ENaC review importance |
|  | Importance of alpha2 NaKATPase in AT1 cells contribute to active Na+ transport and lung liq clearance  <http://circres.ahajournals.org/content/92/4/453.short> |
|  | Role of adenosine in alveolar clearance  These findings suggest that physiologic concentrations of adenosine allow the alveolar epithelium to counterbalance active Na+ absorption with Cl− efflux through engagement of the A1R  <http://www.pnas.org.ezproxy1.library.usyd.edu.au/content/104/10/4083.short> |
|  | Virus reduced the open probability of single ENaC channels in apical cell-attached patches  U-73122, a phospholipase C (PLC) inhibitor, and PP2, a Src inhibitor, blocked the effect of virus on ENaC  influenza virus rapidly inhibits ENaC in ATII cells via a PLC- and Src-mediated activation of PKC but does not increase epithelial permeability in this same rapid time course  <http://ajplung.physiology.org.ezproxy1.library.usyd.edu.au/content/287/2/L366.short> |
|  | NAKATPase effect on alveolar clearance and endocytosis dus to thrombin  <http://www.atsjournals.org.ezproxy1.library.usyd.edu.au/doi/abs/10.1165/rcmb.2004-0407OC>  more nakatpase <http://ajplung.physiology.org.ezproxy1.library.usyd.edu.au/content/289/1/L104.short> |
|  | <http://jap.physiology.org.ezproxy1.library.usyd.edu.au/content/81/4/1723.short> more terbutaline |
|  | <http://journals.lww.com/anesthesia-analgesia/Abstract/2009/01000/The_Effect_of_Endothelin_1_on_Alveolar_Fluid.34.aspx>   ET-1 reduced alveolar fluid clearance by about 65%, an effect that was related to a decrease in amiloride-sensitive transepithelial Na+ transport |

* Foetal lung

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|  | <http://www.sciencedirect.com.ezproxy1.library.usyd.edu.au/science/article/pii/S0146000506000073?np=y&npKey=66b07858043271e638b691ced3830f8fbe9326fdfea07700d01d3915032c27f2>  possibly not as useful review paper  ENaC and amiloride sensitive Na+ channels |
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1. **Diffuse alveolar damage**

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0166184>

* Increased expression of AQP3, AQP5, NaKATPase in DAD group
* Decreased expression of ENaC in DAD group
* No difference in protein expression and AQP1 expression between DAD and normal group

<http://www.atsjournals.org/doi/abs/10.1164/rccm.200909-1420OC>

* DAD present in 20 individuals
* 6 associated with necrotizing bronchiolitis, 5 showed extensive hemorrhage
* Human lung (Adult) studies

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* Rat lung (Adult) studies

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|  | <http://ajplung.physiology.org.ezproxy1.library.usyd.edu.au/content/286/4/L679.short>  proteins effect on alveolar clearance? |
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* Foetal lung

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1. **Acute respiratory distress syndrome**

Acute lung injury / respiratory destress syndrome:

Early – alveolar epithelial and lung endothelial injury 🡪 increased permeability pulmonary edema, alveolar filling, respiratory failure

Little known about capacity of injured alveolar epithelium to resolve pulmonary edema in patients with ALI/ARDS

<http://www.atsjournals.org.ezproxy1.library.usyd.edu.au/doi/full/10.1164/ajrccm.163.6.2004035>

alveolar capillary barrier permeability measured using initial edema fluid-to-plasma protein ratio

<http://journals.lww.com/co-criticalcare/Abstract/2011/02000/H1N1__viral_pneumonia_as_a_cause_of_acute.12.aspx>

Human lung (Adult) studies

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| Results suggested from h1n1 studies | Results suggested from all studies |
|  | Net alveolar fluid clearance reduced from patients with acute respiratory distress / acute lung injury  Endogenous and exogenous catecholamines did not improve alveolar fluid clearance  Lower alveolar fluid clearance = lower mortality  Reduced alveolar fluid clearance not due to protein accumulation  Females had better alveolar clearance than males  No effect using diuretics (furosemide), exogenous glucocorticoids (methylprednisolone, hydrocortisone) [is 4hrs enough to test with glucocorticoid?] or beta agonists (alupent, albuterol) or vasoactive agents (Dobutamine, dopamine, epinephrine, norep, neosynephrine)  <http://www.atsjournals.org.ezproxy1.library.usyd.edu.au/doi/full/10.1164/ajrccm.163.6.2004035> |
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Rat lung (Adult) studies

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Foetal lung

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mice studies:

<http://onlinelibrary.wiley.com.ezproxy1.library.usyd.edu.au/doi/10.1111/j.1750-2659.2012.00414.x/full>

* Shows low PaO2: FiO2 ratios in affected mice, suggesting it can be used to track ARDS development
* Also suggests that the mouse model of H1N1 (at a lethal dose of mouse-adapted virus) validly also shows ARDS
* Progressive increase in pulmonary edema also found
* Conducted in 8-12w/o BALB/cAnNCr mice

<http://ajplung.physiology.org/content/311/6/L1160>

* Reduced surfactant phospholipids in ATII cells, abnormal ultrastructure of ATII cell lamellar bodies
* Altered ATII cell surface lipid metabolism, resulting in surfactant dysfunction and development of ARDS

**Encephalitis**

<http://pediatrics.aappublications.org.ezproxy1.library.usyd.edu.au/content/129/4/e1068.short>

* Autoantibodies against NDMA elevated or positive in cerebrospinal fluid
* Normalized in the 3 follow-up studies

**Epidemiology and possible risk factors**

<http://journals.lww.com/pccmjournal/Citation/2012/09000/H1N1_in_Japanese_children_More_data_but_even_more.35.aspx>

* Insignificant results due to small sample (8 patient sample), but on better mortality in jap children

<http://www.scielo.br/scielo.php?pid=S0103-51502016000400805&script=sci_arttext>

Comorbidities associated with H1N1 (11 patient sample)

**Possible treatments?**

**Conclusion**

**Histological findings**

<http://www.sciencedirect.com.ezproxy1.library.usyd.edu.au/science/article/pii/S0344033810002633>

http://www.sciencedirect.com.ezproxy1.library.usyd.edu.au/science/article/pii/S0344033810002633

<http://www.atsjournals.org/doi/abs/10.1164/rccm.200909-1420OC>

* Cytopathic effect in alveolar cells
* As well as necrosis
* Epithelial hyperplasia, squamous metaplasia of large airways
* Expression of TLR-3 and IFN-yamma
* Large amounts of CD8+ Tcells, granzyme B+ within lung tissue

<http://www.sciencedirect.com/science/article/pii/S0002944010600739?np=y&npKey=44a8ba7100e0df7f947e34db67ad1091a6cf17cf033f44191ca26234ca5c8ea9>

http://www.sciencedirect.com/science/article/pii/S0210569111002944

<http://www.nejm.org/doi/full/10.1056/NEJMc0907171#t=article>

* Epithelium necrosis
* hemmorrhage
* Inflammation and edema of lung
* Diffuse alveolar damage, hyaline membranes, inflammation, fibrosis
* No signs of myocarditis or encephalitis however not all samples had heart/brain samples available.
* Viral distribution
  + Epithelial cells in airways, sub
* Alveolar damage associated with viral antigen localization in T2 pneumocytes and alveolar lining cells
* Increased deaths associated with obesity and asthma – perhaps immunological factors and these medial conditions affect the outcome