Impact of atopy, asthma, and asthma treatment on nasal epithelial wound healing

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Abstract

Background: Abnormal wound repair is implicated in asthma pathogenesis. The nose is the point of first contact with the environment, yet wound repair ability of the nasal epithelium has received little attention. We sought to determine the impact of atopy and asthma on wound healing of nasal epithelium. Methods: Primary nasal epithelial cells harvested from adult volunteers classified into mutually exclusive groups [healthy (H), atopic non-asthmatic (ANA), non-atopic asthmatic (NAA) and atopic asthmatic (AA)] were grown into well-differentiated epithelium at the air-liquid interface. The ability of the epithelium to heal a mechanical wound was determined under various conditions. Results: Wound healing rate (%/hour) was slowest in ANA (2.9±1.8, vs 4.3±1.9 in H, p=0.02). Healing rates of AA (3.8±1.0) and NAA (4.1±1.1) were not different from H. Exogenous IL-13 slowed healing (2.2±1.1 vs 4.0±1.3, p<0.002) across all subject groups (p<0.001). However, blocking endogenous IL-13 had no effect on wound healing (p=0.68). Blocking endogenous EGF markedly slowed wound healing (0.6±0.4 vs 4.1±1.9, p=0.006), whereas adding exogenous EGF had no effect (p=0.58). Wound healing was significantly faster (4.4±1.0 vs 3.4±0.9, p=0.013) in subjects (6 AA, 9 NAA) who took regular inhaled corticosteroids prior to cell harvesting. Infecting epithelial cultures with RSV 6 days prior to wounding slowed healing in all groups (p<0.001). Prior inhaled steroids also improved wound healing following RSV infection (P<0.001). Conclusion: Nasal epithelium from atopic adults heal wounds more slowly. Inhaled corticosteroids taken in vivo prior to harvest influence their responses in vitro, improving wound healing.

Introduction

The nasal epithelium represents the point of first contact between the respiratory system and the external environment. The nasal epithelium is comprised of goblet, basal and ciliated cells, which are essential in establishing mucociliary clearance, producing anti-microbial peptides, chemokines and cytokines, and forming a protective barrier. The normal respiratory epithelium has the ability to remodel and repair its integrity after epithelial damage [1,2]. Persistent asthma is associated with epithelial dysfunction, associated with a disturbance of the normal ability to repair wounds [3]. Studies using primary bronchial epithelial cells, obtained from children with atopy and/or "asthma" and grown in submerged, monolayer cultures, suggest an inability to heal wounds (reviewed in [4]). While these results are consistent with observations from bronchial biopsies in adults with persistent asthma [3], submerged, monolayer cultures do not adequately represent the conditions seen in a fully differentiated pseudostratified epithelium *in vivo* [5]. To minimize these deficiencies primary airway epithelial cells can be grown at the air-liquid interface (ALI) into a fully differentiated pseudostratified epithelium consisting of basal, goblet and ciliated cells [6].

Respiratory viral infections are involved in the induction and progression of asthma [7,8]. Wheezing and asthma exacerbation, especially in children and the elderly, are often associated with respiratory infections due to respiratory viruses such as respiratory syncytial virus (RSV) and human rhinovirus infection[9,10]. Debate exists about whether the asthmatic respiratory epithelium has an exaggerated response to respiratory viral infections but evidence from *in vivo* and *ex vivo* studies show a different pattern of cytokine secretion from

"asthmatic" epithelial cells under baseline and stimulated conditions[1,11]. Certainly, the inflammatory milieu differs in the "asthmatic" airway when compared to that in healthy individuals[3]. How this milieu influences wound healing is little studied.

We have recently developed an *ex vivo* model for assessing wound healing in a well-differentiated epithelium, grown at the air-liquid interface (ALI), using primary nasal epithelial cells (NECs)[6]. The aim of the present study was to use our model to determine: a) the ability of a nasal epithelium, grown in ALI culture, from cells obtained from subjects with asthma and/or atopy, to heal a wound, and b) to determine the influence of the "asthmatic" airway milieu on wound healing. We chose to use primary NECs obtained from adults to avoid the uncertainties in asthma diagnosis in children.

Methods (full details can be found in the online supplement)

Study population

Primary human NECs were collected from 70 adults aged 18-55 years old; 23 healthy adults (14 female), 14 non-atopic asthmatics (3 female), 22 atopic non-asthmatics (20 female) and 11 atopic asthmatics (7 female). Atopy, asthma and medication use were confirmed by self-report. The project was approved by the human research ethics committees of Children's Health Queensland (2011000058) and The University of Queensland (2017000520). All volunteers gave written consent.

Cell culture

Primary NECs were harvested from the inferior surface of the anterior turbinate using a purpose designed curette (ASI Rhino-Pro, Arlington Scientific, USA). Scrapings were suspended in 2ml of RPMI (Roswell Park Memorial Institute**)** media and immediately transported to the laboratory [6]. Cells were pelleted by centrifugation, grown in submerged culture until they reached passage 2 (approximately 3 weeks) and then cryopreserved [6]. When required, cells were thawed and grown in ALI culture into a well differentiated epithelium, validated by the presence of beating cilia under light microscopy (see online supplement). Epithelial integrity was validated by measuring the resistance to an electric current travelling across the cell culture (Trans-epithelial electrical resistance, TEER) using an EVOM2 Epithelial Voltohmmeter (World Precision Instruments Inc., Sarasota, FL, USA) connected to STX2 Chopstick Electrode (World Precision Instruments Inc.).

Wound healing

When the epithelium was fully differentiated, hydrocortisone was withdrawn from the basolateral media for three days prior to wounding. Mechanical injury was performed by scraping a P10 sterile pipette tip across the cell layer creating a wound with a diameter ranging from 600 to 1500μm. The initial wound area was expressed as 100% to remove variability from wounds of different size [6]. Wounds were considered to be closed when the calculated area fell below 3%, the effective limit of detection for image processing. Wound closure was calculated as follows: Wound closure (%) = 100 - ((Area/Initial Area)*100). Wound closure (%), plotted as a function of time (hour), was used to calculate the rate of wound closure (%/hour).

Cytokine assays

The apical surface of ALI cultures was washed with 100μl PBS and analysed for secretion of Interleukin (IL)-13, Interferon beta (INFβ), IL-1β, IL-33 and Transforming growth factor beta (TGFβ), using the Luminex Assay (Millipore) or AlphaLISA kits (Perkin Elmer) following manufacturer's recommendations. Limits of detection for the different assays are shown in Table E1.

Influence of IL-13 on wound healing

To determine the role of IL-13 on wound healing, exogenous IL-13 (10ng/ml) [12] was added to the basolateral media 7 days prior to wounding and replenished every two days with media changes. In separate cultures IL-13 was neutralized by adding human IL-13 Mab (Clone 31606, R & D Systems) to the basolateral media 7 days prior to wounding and replenished every two days when media was changed.

Influence of Epidermal Growth Factor (EGF) on wound healing

To determine the role of the EGF pathway on wound healing, EGF (25ng/ml, StemCell) [13] was added to the basolateral media 7 days prior to wounding and replenished every two days with media changes. In separate experiments EGF was neutralized by adding the specific EGF receptor blocker, Erlotinib HCl (2μg/ml) (OSI-744, Shelleckchem)[14], to the basolateral media 7 days prior to wounding. Media with inhibitor was replenished every two days.

Respiratory syncytial virus infection

Primary NECs grown at ALI were infected with green fluorescent tagged RSV-A2 at a multiplicity of infection (MOI) of 1 plaque-forming unit (pfu) per epithelial cell. Cells were incubated with virus for 2 hours in PBS at 37°C in the apical chamber, after which they were washed with PBS and TEER recorded. RSV load was quantified (pfu/ml) using a plaque assay in confluent monolayers of Hep-2 cells. Cultures were maintained in hydrocortisone free media for 6 days before wounding. Apical and basal wash was collected at 6 days to determine viral titre and toxicity of the virus.

Statistical analysis

Data were generally not normally distributed. Comparisons between groups were undertaken using Kruskal-Wallis, Mann-Whitney, Fishers-Exact Test, Analysis of Variance on Ranks, and Repeated Measures Analysis of Variance on Ranks as appropriate using GraphPad Prism 7.0 (GraphPad Inc., La Jolla, CA, USA) or SigmaPlot V13 (Systat Software Inc. San Jose, CA, USA). Statistical significance was accepted at the 5% level.

Results

Ninety-seven ALI cultures were attempted using primary NECs obtained from 70 adults, with an overall success rate of 72.16% (70/97). When classified into four mutually exclusive groups, there were no significant differences (p=0.067) in culture success with cells obtained from healthy (23/37), non-atopic asthmatics (14/16), atopic non-asthmatic (22/26) or atopic asthmatic (11/18) adult subjects (Table E2).

Group median (25%-75%) TEER was 293.0 (198.0 – 544.0) $\Omega.cm^2$ for cultures from healthy subjects, 316.0 (263.5 – 427.8) $\Omega.cm^2$ for non-atopic asthmatics, 265.0 (82.0 – 348.0) $\Omega.cm^2$ for atopic non-asthmatics, and 160.0 (132.0 – 207.0) $\Omega.cm^2$ for atopic asthmatics. TEER values were significantly different between groups (p=0.01), with the difference explained by the lower resistance in atopic asthmatics (p=0.02). Basal cytokine secretion was low and below the limit of detection for most cytokines in most cultures (data not shown).

Wound healing

Wound healing rates in the mutually exclusive groups are shown in Figure 1. The healthy subjects healed fastest, with a mean rate of 4.3± (SD) 1.9 %/hour. The slowest rate was seen in atopic adults (2.9±1.8 %/hour) (p=0.02 compared to healthy subjects), with rates of the atopic asthmatics (3.8±1.0 %/hour) and non-atopic asthmatics (4.1±1.1 %/hour) falling in-between, but not statistically different from the healthy subjects.

Impact of IL-13 on wound healing

Exogenous IL-13 caused a reduction in wound healing from 4.0±1.3 %/hour to 2.2±1.1 %/hour (p<0.002) (Figure 2A), consistent across groups (p<0.001) (Figure 2). However, inhibiting the action of IL-13 had no influence on wound closure (control 3.4±1.8 %/hour vs IL-13 inhibited 3.2±1.8 %/hour, p=0.68, Figure 2B).

Impact of EGF on wound healing

Exogenous EGF had no effect on wound closure, with a group mean closure rate of 3.2±1.7 %/hour in the presence of EGF compared to 3.4±1.8 %/hour without EGF (p=0.58) (Figure 3A). However, inhibiting EGF had a profound effect, slowing wound closure (control 4.1±1.9 %/hour vs 0.6±0.4 %/hour in the presence of Erlotinib, p=0.006, Figure 3B).

Impact of inhaled corticosteroid use in vivo on wound healing

Fifteen asthmatics, 6 atopic and 9 non-atopic, were taking regular inhaled corticosteroids. In addition, one atopic non-asthmatic took regular nasal corticosteroids. Wound closure occurred at a faster rate in those asthmatics taking inhaled corticosteroids (p=0.013). The difference was greater amongst non-atopic asthmatics (Table 1).

Impact of RSV infection on wound healing

Infecting ALI cultures with RSV 6 days prior to wounding resulted in delayed healing in all groups ($p<0.001$ for RSV vs control, $p<0.001$ for all groups, Table 2). Wound healing was significantly faster for those subjects taking inhaled corticosteroids (difference of means 2.25, $t=5.31$, $p<0.001$, Figure E1). There were no differences in RSV titre between groups nor in those with or without prior use of inhaled corticosteroids ($p=0.22$).

Discussion

The results from the present study demonstrate that wounds inflicted on well-differentiated respiratory epithelium, grown from primary NECs in ALI culture, heal at different rates depending on the atopic and asthmatic status of the individual donors. Healing is slowest in atopic asthmatics. However, atopic status appears to have more influence than asthmatic status. In addition, our data strongly suggest that the regular use of inhaled corticosteroids prior to nasal cells being harvested improves wound healing in our *ex vivo* model. Finally, wounded epithelium takes longer to heal when the wound occurs at the peak of an RSV infection, regardless of health status, and that prior regular use of inhaled corticosteroids improves wound healing under these circumstances as well.

Before discussing the implications of our findings, some technical issues need to be acknowledged. Wound healing involves three processes: spreading, migration and proliferation [4,6]. Our model only addresses spreading and migration as our end-point is when the wound is physically closed. Thus we are not able to comment on proliferative events. Our *ex vivo* model of wound closure uses a fully-differentiated epithelium grown from primary NECs in ALI culture. Many previous studies have wounded monolayer cultures of tracheal epithelial cells [15-18] . A major advantage of ALI cultures is they contain the full variety of epithelial cells[6,19]; this is not the case with monolayer cultures, which consist of basal cells only. Thus the results from the present study may differ from monolayer wounding studies. However, the general impairment of wound repair we report following RSV infection in ALI culture is similar to previous reports of delayed wound repair following rhinovirus infection of monolayer cultures [16]. Finally, ALI cultures are not well suited to measuring cytokine secretion into the surface lining fluid. The surface lining fluid layer*in vitro* is thin and the maximum volume that can be removed is around 1-2µL without the addition of a wash solution and even this stimulates the cells [20] . Thus lack of cytokine responses in the present study should be treated with caution and is unlikely to reflect *in vivo* conditions.

Previous monolayer culture studies, reported incomplete wound healing in asthmatic children [15,17,18] . In the present study, delayed wound repair was related more close to atopy than asthma; cultures from non-atopic asthmatics healed at similar rates to those from healthy subjects and delayed healing was seen in atopic non-asthmatics. Atopy is associated with increased levels of the T-helper(Th)-2 cytokine, IL-13 [12]. IL-13 induces epithelial proliferation by inducing TGF ∝, which in turn binds to the EGF receptor (EGFR) on epithelial cells [12]. In the present study, we confirm that signalling through EGFR is necessary for epithelial wound repair; repair rate was markedly reduced by blocking EGFR. However, adding additional EGF did not hasten wound repair. The role of IL-13 is more difficult to understand. Exogenous IL-13 did reduce wound healing, however, the amount added was at least six orders of magnitude (ng/ml) greater than endogenous production by epithelial cells (pg/ml). Blocking the effects of endogenous IL-13 had no effect on the rate of wound healing. Taken together, these data suggest that excessive production of IL-13 could influence wound healing but not in concentrations made by epithelial cells. One caveat to that premise is that epithelial cells grown in monolayer culture have been shown to secrete high enough levels of IL-13 to facilitate wound healing[21]. However, that study used 1HAEo cells, a SV-40 transformed cell line. How these results relate

to normal epithelial cells is uncertain. Monocyte derived macrophages secrete IL-13 in ng/ml quantities, especially when polarized to the alternatively activated phenotype [22] . IL-13 is also secreted by Th-2 T cells. As such, IL-13 secreted into the airway *in vivo* could be involved in an exaggerated delay in wound healing in atopic non-asthmatics as well as in atopic asthmatics and warrants further investigation.

The data from the present study confirm previous reports, using different respiratory viruses [16,23], that infection of the respiratory epithelium impairs wound repair in all subjects, but especially in asthmatics. In the context of the present study, these results serve to demonstrate the utility of the model rather than advancing knowledge about epithelial response to external stressors.

Perhaps the most surprising result from the present study was the improved wound healing in subjects taking regular inhaled corticosteroids prior to cell harvesting. There is an implied, if not written, "belief" that harvested cells are unlikely to be influenced by *in vivo* exposures after several culture passages *in vitro* . Our data definitely challenge this premise. In our case, these cells can only have been exposed via the systemic circulation, either by the extremely low levels of corticosteroid absorbed into the circulation or by some downstream effect of corticosteroids in the lung. Despite this, the effects of prior use of inhaled corticosteroids in improving the rate of wound healing, including after RSV infection, are clear and indisputable (Table 2). The real challenge is in understanding what these findings mean! By definition, innate immune responses, such as those from epithelial cells, do not have a response memory. However, this concept has recently been challenged by elegant studies showing that skin wounds in mice heal more quickly when the mouse had previously been wounded in the same area [24-26]. Whether similar mechanisms operate in human respiratory epithelium remains to be demonstrated.

In summary, data from the present study have demonstrated that atopic subjects *per se* are more likely to have delayed healing of a wounded respiratory epithelium, however the mechanisms involved remain unclear. Exposure to inhaled corticosteroids prior to harvest improve wound healing *in vitro* , including following RSV infection.

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Impact Statement

Results from the present study demonstrate the impact of atopy on delaying wound healing in nasal epithelium. Further, regular use of inhaled corticosteroids improve wound healing, even during respiratory viral infections, despite having no direct exposure to the nasal epithelium. Atopic asthmatics are likely to benefit from regular use of inhaled steroids.

References

1. Proud D, Leigh R. Epithelial cells and airway diseases. Immunol Rev 2011;242:186-204.

2. Yan Y, Gordon WM, Wang DY. Nasal epithelial repair and remodeling in physical injury, infection, and inflammatory diseases. Curr Opin Otolaryngol Head Neck Surgery 2013;21:263-70.

3. Holgate S, Sly P. Asthma Pathogenesis. In: Adkinson N, Bochner B, Burks A, et al., eds. Allergy Principles and Practice 8th Edition Philadelphia. : Elselvier Inc.; 2014:812-35.

4. Iosifidis T, Garratt LW, Coombe DR, Knight DA, Stick SM, Kicic A. Airway epithelial repair in health and disease: Orchestrator or simply a player? Respirol 2016;21:438-48.

5. Karp PH, Moninger TO, Weber SP, et al. An in vitro model of differentiated human airway epithelia. Methods for establishing primary cultures. Methods Mol Biol 2002;188:115-37.

6. Schagen J, Sly PD, Fantino E. Characterizing well-differentiated culture of primary human nasal epithelial cells for use in wound healing assays. Lab Invest 2018;98:1478-86.

7. Kusel MMH, de Klerk NH, Kebadze T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J Allergy Clin Immunol 2007;119:1105-10.

8. Teo SM, Mok D, Pham K, et al. The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. Cell Host Microbe 2015;17:704-15.

9. Abe N, Yasudo H, Fukano R, et al. Multi-season analyses of causative pathogens in children hospitalized with asthma exacerbation. Pediatr Allergy Immunol 2019;30:724-31.

10. Bjerregaard A, Laing IA, Poulsen N, et al. Characteristics associated with clinical severity and inflammatory phenotype of naturally occurring virus-induced exacerbations of asthma in adults. Respir Med 2017;123:34-41.

11. Kicic A, Sutanto EN, Stevens PT, Knight DA, Stick SM. Intrinsic biochemical and functional differences in bronchial epithelial cells of children with asthma. Am J Respir Crit Care Med 2006;174:1110-8.

12. Booth BW, Adler KB, Bonner JC, Tournier F, Martin LD. Interleukin-13 induces proliferation of human airway epithelial cells in vitro via a mechanism mediated by transforming growth factor-alpha. Am J Respir Cell Mol Biol 2001;25:739-43.

13. Wijayagunawardane MP, Hambruch N, Haeger JD, Pfarrer C. Effect of epidermal growth factor (EGF) on the phosphorylation of mitogen-activated protein kinase (MAPK) in the bovine oviduct in vitro: Alteration by heat stress. J Reprod Dev 2015;61:383-9.

14. Parker JC, Douglas I, Bell J, et al. Epidermal Growth Factor Removal or Tyrphostin AG1478 Treatment Reduces Goblet Cells & Mucus Secretion of Epithelial Cells from Asthmatic Children Using the Air-Liquid Interface Model. PloS one 2015;10:e0129546-e.

15. Kicic A, Hallstrand TS, Sutanto EN, et al. Decreased fibronectin production significantly contributes to dysregulated repair of asthmatic epithelium. Am J Respir Crit Care Med 2010;181:889-98.

16. Kicic A, Stevens PT, Sutanto EN, et al. Impaired airway epithelial cell responses from children with asthma to rhinoviral infection. Clin Exp Allergy 2016;46:1441-55.

17. Ling KM, Sutanto EN, Iosifidis T, et al. Reduced transforming growth factor beta1 (TGF-beta1) in the repair of airway epithelial cells of children with asthma. Respirol 2016;21:1219-26.

18. Stevens PT, Kicic A, Sutanto EN, Knight DA, Stick SM. Dysregulated repair in asthmatic paediatric airway epithelial cells: the role of plasminogen activator inhibitor-1. Clin Exp Allergy 2008;38:1901-10.

19. Muller L, Brighton LE, Carson JL, Fischer WA, 2nd, Jaspers I. Culturing of human nasal epithelial cells at the air liquid interface. J Vis Exp 2013; Oct 8;(80):50646.

20. Schagen J. Establishing a wound healing model in well differentiated cultures of primary human nasal epithelial cells. Brisbane: The University of Queensland; 2019.

21. Allahverdian S, Harada N, Singhera GK, Knight DA, Dorscheid DR. Secretion of IL-13 by airway epithelial cells enhances epithelial repair via HB-EGF. Am J Respir Cell Mol Biol 2008;38:153-60.

22. Tarique AA, Sly PD, Holt PG, et al. CFTR-dependent defect in alternatively-activated macrophages in cystic fibrosis. J Cystic Fibrosis 2017;16:475-82.

23. Moheimani F, Koops J, Williams T, et al. Influenza A virus infection dysregulates the expression of microRNA-22 and its targets; CD147 and HDAC4, in epithelium of asthmatics. Respir Res 2018;19:145.

24. Gourbal B, Pinaud S, Beckers GJM, Van Der Meer JWM, Conrath U, Netea MG. Innate immune memory: An evolutionary perspective. Immunol Reviews 2018;283:21-40.

25. Natoli G, Ostuni R. Adaptation and memory in immune responses. Nat Immunol 2019;20:783-92.

26. Wlodarczyk M, Druszczynska M, Fol M. Trained Innate Immunity Not Always Amicable. Int J Mol Sci 2019;20.

Table 1: Rate of wound closure [%/hour (mean±SD)] in asthmatics stratified by atopic status and prior use of inhaled corticosteroids.

	Regular inhaled steroids	**No inhaled steroids**	**p**
All asthmatics	4.4±1.0 (n=15)	3.4±0.9 (n=10)	0.013
Non-atopic asthmatics	4.7±1.0 (n=9)	3.2±0.7 (n=5)	0.013
Atopic-asthmatics	4.0±1.0 (n=6)	3.5±1.1 (n=5)	0.43

Table 2 . Rate of wound closure [%/hour (mean±SD)] in healthy adults (n=8), non-atopic asthmatics (n=7), atopic non-asthmatics (n=9) and atopics asthmatics (n=9) before and 6 days after infection with respiratory syncytial virus (RSV, multiple of infection 1.0).

	Prior to RSV	Post-RSV^{+}
Healthy adults	5.8±1.7	3.8±1.7
Non-atopic asthmatics	3.4±0.8	2.5±1.1
Atopic non-asthmatics	3.2±1.6	1.6±2.3*
Atopic asthmatics	3.8±0.7	1.7±1.1*

$^{+}$ delayed wound healing in all groups ($p<0.001$ for RSV vs control overall, $p<0.001$ for each group)

* two atopic non-asthmatics and two atopic asthmatics failed to heal post-RSV and have a rate of closure of 0%/hour.

Legends for Figures

Figure 1 : Rate of wound closure (% per hour) of primary nasal epithelial cells grown into a fully-differentiated epithelium at the air-liquid interface from mutually exclusive groups of healthy subjects (n=17), non-atopic asthmatics (n=12), atopic non-asthmatics (n=16) and atopic asthmatics (n=10). The rate of wound healing was significantly lower (p=0.02) in atopic subjects when compared to healthy or non-atopic asthmatics.

Figure 2: Impact of IL-13 on wound closure rate (%/hour). Panel A: exogenous IL-13 (10ng/ml) added to the basolateral media for 7 days prior to wounding. Panel B: IL-13 inhibiting antibody (Clone 31606) added to the basolateral media for 7 days prior to wounding. Wound closure was slower in the presence of exogenous IL-13 ($p<0.001$) but not influenced by anti-IL-13 (p=0.68).

Figure 3: Impact of EGF on wound closure. Panel A: exogenous EGF (25ng/ml) added to the basolateral media for 7 days prior to wounding. Panel B: EGF receptor blocker, Erlotinib (2μg/ml) added to the basolateral media for 7 days prior to wounding. Wound closure was unaffected by exogenous EGF (p=0.58) but markedly slower in the presence of Erlotinib (p=0.006).