



Review

Processes Involved in the Repair of Injured Airway Epithelia

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Abstract. Recent studies have uncovered many aspects of the repair processes that follow airway epithelial injury. Although the repair process has common elements among various epithelia, such as the ones lining the airways, skin, and gut, there are differences based on their diverse functions. Whenever possible, similarities are pointed out that could help researchers further investigate their application to airway epithelia, although it would be beyond the scope of this review to cover the processes that may occur during the repair of all types of epithelia. In general, five major steps are involved in the recovery of airway epithelia from injury: 1) epithelial cells migrate to cover denuded areas within minutes, and certain proteins, such as the trefoil factor family proteins, are crucial to this process; 2) epithelial cells start to proliferate in order to replace injured cells and to differentiate to establish squamous or mucous cell metaplasia; 3) because more epithelial cells are present after proliferation, some of the cells must be discarded to restore the epithelium to the original condition; 4) once the cell numbers have been reduced to those found in unexposed individuals, the normal proportions of cell types are restored; 5) finally, studies from exposures of rats to ozone show that epithelial cells can adapt and develop a memory of the chronic exposure to which they were exposed. This adaptation allows the epithelium to respond quickly, thus minimizing further injury. Although the molecular mechanisms involved in these major steps of the recovery process are largely unknown, disruption of these steps clearly causes the permanent changes observed in diseases such as asthma, chronic bronchitis, and cancer; therefore, extensive research of these mechanisms may provide ideas for novel therapies.

Key words: epithelium; proliferation; cell cycle; apoptosis; differentiation; homeostasis.

Introduction

Epithelia of the skin, cornea, and the digestive, respiratory, and reproductive tracts are constantly exposed to insults from the outer world. These insults range from mechanical injury of epithelial cells to irritation by environmental pollutants and infectious agents. Such exposures initiate inflammatory responses and result in protective responses, including clearance of inflamma-

tory cells and repair of the damaged area. Understanding the events that lead to a repaired epithelium is critical, because disruption of these regulated events may cause unregulated cell growth and deadly cancers or chronic disease, including bowel disease, Crohn's disease, asthma, and chronic bronchitis. This review focuses on the events involved in the repair of respiratory epithelia and uses examples from other organs to describe the mechanisms involved.

Abbreviations used: MCM – mucous cell metaplasia, BrdU – bromodeoxy uridine, LPS – lipopolysaccharides, AB/PAS – alcian blue/periodic acid schiff.

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Migration of Epithelial Cells to Cover Denuded Areas

Because injury often leads to desquamation of cells from the epithelium, the migration of neighboring cells is an important component of the repair process. Within minutes after injury, epithelial cells from neighboring areas migrate to the denuded area to form the initial coverage⁴. Microcirculation-derived factors along the intact basement membrane promote rapid re-epithelialization¹⁸. Epithelial cells start to migrate within minutes after damage, long before cells initiate proliferation, and this process is termed restitution³. The family of trefoil factor family (TFF) peptides, TFF1, TFF2, and TFF3, act as motogens during *in vitro* wound-healing assays of respiratory epithelia¹⁶ by activating the protein kinase C and extracellular signal-regulated kinase (ERK) pathway⁵. The TFF proteins show protective or healing effects *in vivo* for the gastrointestinal tract¹³, and on the basis of their mitogenic activity *in vitro*¹⁶, one assumes that similar roles also would be observed in respiratory epithelia.

The transmembrane receptor subunit gp130 activates two signaling modules in cells of the gastrointestinal tract. The signal transducers and activators of transcription 3 (STAT3) and the Src-homology tyrosine phosphatase 2 (SHP2)-Ras-ERK pathways emanate from this receptor and maintain cellular homeostasis by balancing positive and negative signals²⁰. Targeted ablation of each pathway by generating knock-in mutations in mice shows that the mucosal wound healing in the gut depends on activation of STAT3, whereas gastric hyperplasia ensues when activation of both pathways is not coordinated²⁰. Simultaneous and balanced activation of the two signaling pathways is essential not only to mediate epithelial migration, but also to stop cytokine-mediated proliferation of these cells. These studies show a close interaction of the mechanisms that control migration and proliferation of epithelial cells following injury.

Proliferation and Regulated Differentiation of Epithelial Cells Following Injury

The rapid migration of epithelial cells is followed by proliferation of epithelial cells to replace the injured cells. VERMEER et al.²⁸ recently reported that in differentiated airway epithelia the ligand for erB2, heregulin α , is present exclusively in the apical membrane, and the overlying airway surface liquid is physically separated from the receptor, which is segregated to the ba-

solateral membrane. Whenever epithelial integrity is disrupted, heregulin α can access its receptor at the edge of the wound and promote cell proliferation. This mechanism allows epithelial cells to stand poised and promote their own healing. The significance of this finding lies in the concept that, in general, compartmentalizing the signaling components may control responses of the epithelial cells¹⁵.

Interestingly, little is known about the cell types that proliferate at this stage. They are believed to be derived from stem-like cells, also called "early and late transient amplifying cells" that are directly derived from stem cells². Studies using denuded tracheal grafts showed that both basal and secretory cells "dedifferentiate" into a similar highly proliferative phenotype and "redifferentiate" to and from a mucociliary epithelium¹². Therefore, in the tracheal epithelium, both basal and secretory cells appear to have the potential of proliferating to reconstitute the epithelium¹. Interestingly, these stem cells seem to have the ability to produce the appropriate mixture of cell types in the course of such regeneration.

To restore the function of the epithelium, proliferating cells undergo differentiation into the cell types of interest. This differentiation is affected by the type of injury and the cell types required for protection from further insult. For example, lipopolysaccharide (LPS) instillation causes extensive inflammation and proliferation followed by a massive mucous cells metaplasia (MCM)²³. Alternatively, exposure to high concentrations of cigarette smoke for an extended time changes the mucociliary epithelium lining the proximal septum of the nose into a squamous phenotype, which is called squamous metaplasia²⁵. It will be crucial to identify the local chemical signals released in tissues that can activate such differentiation pathways. In general, all findings agree that proliferation must stop before cells can differentiate to carry out the appropriate function.

Our recent study in LPS-instilled rats shows that bromodeoxy uridine (BrdU)-positive mucous cells are essentially absent in non-instilled control rats, but are increased 5-fold from 5 to 26 mucous cells/mm basal lamina 48 h after LPS instillation²³. Adjacent cells show BrdU positivity, suggesting that the two cells were a result of cell division. The number of BrdU-positive cells at 48 h is similar to the increase of total epithelial cells²³. In addition, the sum of the decrease in serous cells and the increase in the total numbers of epithelial cells approximate the numbers of MCM at 48 h post instillation. These observations show that the increase in epithelial cell number consists of MCM.

Many questions remain to be answered to clarify the molecular mechanisms regulating differentiation in airway epithelia: 1) the proliferating transient amplifying cells are multipotent and can differentiate into several types of cells, such as mucous cells, squamous cells, or ciliated cells; 2) in addition, these cells must sense the proportion of the cell types in such epithelium to form the original composition of cell types in the right proportion¹⁹. How the population of proliferating epithelial cells decides which cells should become mucus-producing, ciliated, or another type of cell is still a puzzle to airway biologists. Errors during such a regeneration process may be associated with tissue damage and metaplastic changes in chronic diseases, including asthma and chronic bronchitis²⁶.

The proliferation of airway epithelial cells is stopped regardless of whether the injury was transient or is chronic. One can appreciate that these regulatory mechanisms are very robust and minimally prone to error. Only <15% of smokers eventually develop cancers of the airways⁶, although epithelia are repeatedly exposed to carcinogens for years. Mechanisms involved in the cell cycle arrest are possibly related to cell-cell interactions and are of great interest, particularly for understanding the dysregulation during cancer progression.

Cell Death during Resolution of Airway Epithelial Hyperplasia

Our findings suggest that 2–3 days following injury to the epithelium the number of epithelial cells per unit area of epithelium exceeds the numbers found in normal epithelium by approximately 20–30%²³. Interestingly, this increase in numbers indicates that the epithelium initially produces more cells and then discards some to reconstitute the healed epithelium. This process implies that the organism must have established mechanisms to selectively remove certain cells.

Should the injury or insult to the epithelium be transient, then several mechanisms start to decrease the cell numbers. Whether some of the metaplastic cells slough off during this process or in part dedifferentiate to reconstitute the proportion of cell types similar to that of the original condition is unknown.

MCM is induced in nasal epithelia following exposure of rats to ozone^{9, 24} or in airway epithelia following intratracheal instillation of rats with LPS^{7, 22}. Pre-existing and proliferating airway epithelial cells can differentiate into mucous cells following exposure to these agents, cigarette smoke, or allergens²³. When investi-

gating the involvement of cell death programs in the recovery process from these hyperplastic stages, we found that Bcl-2 is expressed in approximately 20–30% of LPS-induced metaplastic mucous cells in airway epithelia²² or in approximately 50% of ozone-induced MCM in epithelia lining the maxilloturbinates²⁴. Interestingly, adjacent MCM in these epithelia are heterogeneous in their expression of Bcl-2; some cells express high levels, whereas others express low levels or no Bcl-2 (Fig. 1). These observations demonstrate that some epithelial cells are singled out to express Bcl-2 although they appear similar in histological preparations. The fact that they do not express the same genes despite their identical morphology and their role as mucus-storing cells indicates that mechanisms must be in place to discriminate between epithelial cells in metaplastic epithelia. Furthermore, the percentages of cells to be eliminated in these respiratory epithelia and the percentage of mucous cells that express Bcl-2 are strikingly similar, which may indicate that Bcl-2 expression is associated with the recovery process and the marking of cells for elimination.

Interestingly, only half of the Bcl-2-positive mucous cells are BrdU-positive, suggesting that the BrdU-negative but Bcl-2-positive mucous cells must have been nonproliferating, pre-existing cells that were present before LPS injury²³. Bcl-2 can inhibit cell-cycle progression in various systems^{11, 14, 17}. However, the presence of Bcl-2-positive mucous cells that are BrdU-negative shows that Bcl-2 does not have a cell-cycle regulatory function in this system. In another study, we have shown that mucous cell numbers are decreased to background levels at least 2 days after the percentage of Bcl-2-positive cells has decreased to levels found in control animals²². These data support the hypothesis that Bcl-2, an inhibitor of apoptosis, must be down-regulated before the numbers of mucous cells are reduced.

Prolonged exposure of mice to allergen causes the cytokine interleukin 13 that is secreted by T helper cell type 2 (Th2) to decrease and interferon γ , which is secreted by Th1 cells, to increase in the bronchoalveolar lavage fluid²⁹. During this time, the percentage of Bax-immunopositive mucous cells increases from approximately 3–25%, while the number of MCM is decreased²¹. However, Bcl-2 is not detected in these mucous cells. The fact that Bcl-2 expression is associated with the appearance of LPS-induced MCM and Bcl-2 is not detected in allergen-induced MCM in mice suggests different resolution mechanisms in these two experimental systems. Overall, approximately 25–35% of mucus cells expressed Bax after repeated exposure

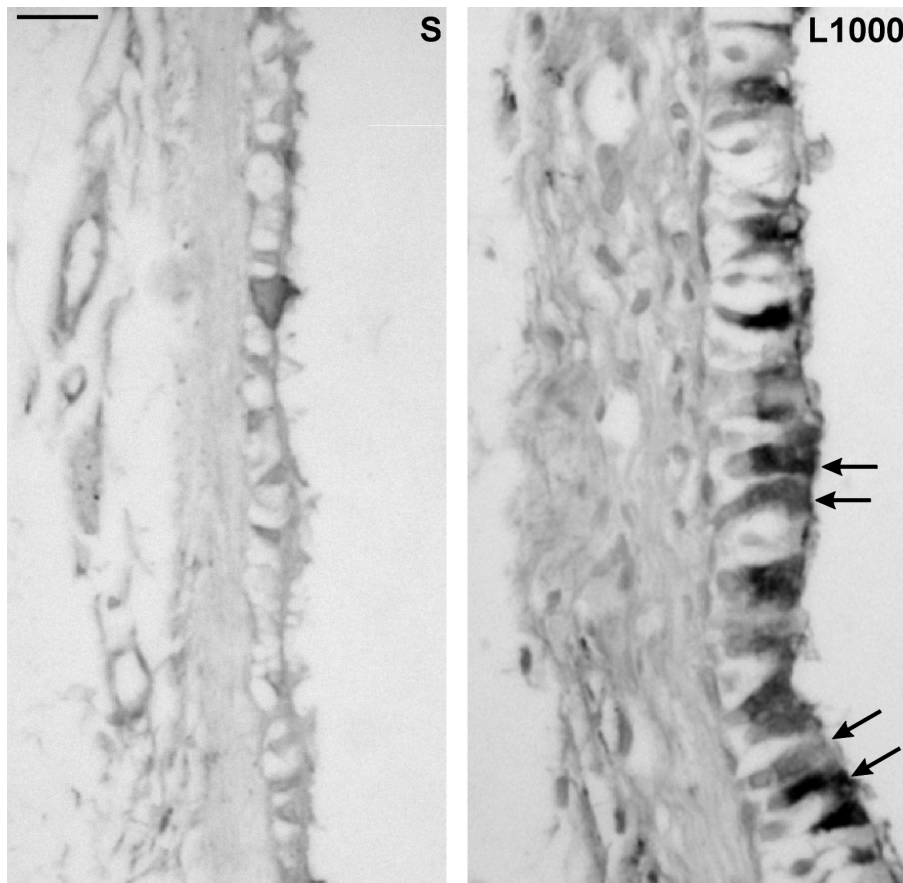


Fig. 1. Representative photomicrographs of axial airways at generation 5 in the left lung lobes of rats 2 days after intratracheal instillation with saline (S) or 1000 µg LPS (L1000). Bcl-2 detected by immunohistochemistry and diaminobenzidine shows dark brown; mucous cells were identified by staining with alcian blue. Bar = 20 µm. Arrows show Bcl-2-positive and -negative mucous cells

to allergen for 15 days. TRIFILIEFF et al.²⁷ found that by 3-day post allergen exposure, approximately 30% of epithelial cell nuclei were BrdU-positive, a marker for cells that undergo DNA synthesis during the cell cycle. Taken together, the observed Bax positivity in approximately 25–35% of mucus cells during the resolution of allergen-induced MCM suggests that the Bax-positive mucous cells may represent cells that must be eliminated to reconstitute the original cell number of the repaired epithelium. Further studies are needed to determine whether only newly formed cells express Bax and undergo apoptosis during the resolution of metaplasia or whether pre-existing cells also undergo apoptosis during this resolution process. Furthermore, detailed studies are underway to determine what distinguishes the mucous cells that express these regulators of the cell death program from neighboring mucous cells that do not express these proteins. These studies may help us elucidate the signals that determine which epithelial cells are discarded during the recovery process.

Increased proliferation and decreased apoptosis induce epithelial hyperplasia in the epidermis and the upper digestive tract of Smad7 overexpressing mice¹⁰. Together with studies in the airways, these observations suggest that epithelial diseases in various organs may

result from disruption of regulated cell death and prolonged persistence.

Many questions in this area remain unanswered. Some of the questions are listed as follows: how does the epithelium determine the number of cells that must be eliminated to restore the original condition? Do increased numbers of cells that populate the epithelium signal that there is not enough room by gauging the intercellular pressure? What are the signals that determine which of the epithelia cells should be discarded? Whether the discarded cells represent abnormal or damaged epithelial cells is unknown? How does the epithelium restore the normal proportions of epithelial cell types? For example, what signals determine that a certain number of the remaining cells differentiate into serous, Clara, ciliated, and mucous cells to reconstitute the proportions found in normal epithelia?

Adaptive Changes in Airway Epithelia

Dr. Harkema's studies show that although MCM may resolve after a recovery period post injury, the epithelium may not revert to exactly the original condition at that time⁸. Rats exposed to 0.5 ppm ozone show

epithelial cell hyperplasia associated with concomitant increase in the number of AB/PAS-positive mucous secretory cells in the epithelia lining the maxilloturbinate of the proximal region of the nose. After a 13-week recovery period, MCM is reduced by 96%, but the epithelial cell numbers are still 25% greater than air-exposed controls. Interestingly, an acute exposure to 0.5 ppm ozone after the recovery period increases stored mucosubstances in rats previously exposed to 0.5 ppm ozone, but not in rats exposed to filtered air. In addition, BrdU incorporation is absent in rats recovered from the ozone exposure, but is present in control rats. These studies show that a transient state of the epithelium maintains increased numbers compared with untreated controls. It appears that some of the cells retain a partially differentiated state, which could be defined as adaptation or a memory response to the last exposure. This transient state may be important for a quick response to subsequent exposures and for reducing injury by quickly producing mucus. It is, however, not known how the epithelium responds to repeated inflammation and denudation.

Whether these changes that occur in response to an injury completely reverse over a life time is not known. It is possible that certain changes are permanent and induce a memory, similar to changes that occur in adaptive immunity. The nature of such a memory and how it is stored in epithelial cells has not been examined. However, one can easily envision that disruption of such mechanisms may make certain individuals susceptible to developing chronic responses to environmental insults.

In summary, the research to date shows that various steps occur during the repair process of injured epithelia. However, the regulatory mechanisms of these processes still remain poorly understood and will require intensive research. This research will require expertise from many different backgrounds and will result in understanding the basis for debilitating chronic diseases such as asthma and chronic bronchitis and for deadly lung cancers.

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References

- BORTHWICK D. W., SHAHBAZIAN M., KRANTZ Q. T., DORIN J. R. and RANDELL S. H. (2001): Evidence for stem-cell niches in the tracheal epithelium. *Am. J. Respir. Cell Mol. Biol.*, **24**, 662–670.
- ENGELHARDT J. F. (2001): Stem cell niches in the mouse airway. *Am. J. Respir. Cell Mol. Biol.*, **24**, 649–652.
- ERJEFALT J. S., ERJEFALT I., SUNDLER F. and PERSSON C. G. (1995): *In vivo* restitution of airway epithelium. *Cell Tissue Res.*, **281**, 305–316.
- ERJEFALT J. S. and PERSSON C. G. (1997): Airway epithelial repair: breathtakingly quick and multipotentially pathogenic. *Thorax*, **52**, 1010–1012.
- GRANESS A., CHWIERALSKI C. E., REINHOLD D., THIM L. and HOFFMANN W. (2002): Protein kinase C and ERK activation are required for TFF-peptide-stimulated bronchial epithelial cell migration and tumor necrosis factor- α -induced interleukin-6 (IL-6) and IL-8 secretion. *J. Biol. Chem.*, **277**, 18440–18446.
- GREENLEE R. T., HILL-HARMON M. B., MURRAY T. and THUN M. (2001): Cancer statistics, 2001. *CA Cancer J. Clin.*, **51**, 15–36.
- HARKEMA J. R. and 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., BARR E. B., BENNETT C. B., GALLUP M., LEE J. K. and BASBAUM C. (1999): Long-lasting effects of chronic ozone exposure on rat nasal epithelium. *Am. J. Respir. Cell Mol. Biol.*, **20**, 517–529.
- HARKEMA J., HOTCHKISS J. and GRIFFITH W. (1997): Mucous cell metaplasia in rat nasal epithelium after a 20-month exposure to ozone: a morphometric study of epithelial differentiation. *Am. J. Respir. Cell Mol. Biol.*, **16**, 521–530.
- HE W., LI A. G., WANG D., HAN S., ZHENG B., GOUmans M. J., TEN DIJKE P. and WANG X. J. (2002): Overexpression of Smad7 results in severe pathological alterations in multiple epithelial tissues. *EMBO. J.*, **21**, 2580–2590.
- LINETTE G. P., LI Y., ROTH K. and KORSMEYER S. J. (1996): Cross talk between cell death and cell cycle progression: BCL-2 regulates NFAT-mediated activation. *Proc. Natl. Acad. Sci. USA*, **93**, 9545–9552.
- LIU J. Y., NETTESHEIM P. and RANDELL S. H. (1994): Growth and differentiation of tracheal epithelial progenitor cells. *Am. J. Physiol.*, **266**, L296–307.
- MASHIMO H., WU D. C., PODOLSKY D. K. and FISHMAN M. C. (1996): Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. *Science*, **274**, 262–265.
- MAZEL S., BURTRUM D. and PETRIE H. T. (1996): Regulation of cell division cycle progression by bcl-2 expression: a potential mechanism for inhibition of programmed cell death. *J. Exp. Med.*, **183**, 2219–2226.
- MOSTOV K. and ZEGERS M. (2003): Cell biology: Just mix and patch. *Nature*, **422**, 267–268.
- OERTEL M., GRANESS A., THIM L., BUHLING F., KALBACHER H. and HOFFMANN W. (2001): Trefoil factor family-peptides promote migration of human bronchial epithelial cells: synergistic effect with epidermal growth factor. *Am. J. Respir. Cell Mol. Biol.*, **25**, 418–424.
- O'REILLY L. A., HARRIS A. W., TARLINTON D. M., CORCORAN L. M. and STRASSER A. (1997): Expression of a bcl-2 transgene reduces proliferation and slows turnover of developing B lymphocytes *in vivo*. *J. Immunol.*, **159**, 2301–2311.
- PERSSON C. G., ERJEFALT J. S., GREIFF L., ANDERSSON M., ERJEFALT I., GODFREY R. W., KORSGREN M., LINDEN M., SUNDLER F. and SVENSSON C. (1998): Plasma-derived proteins in airway defense, disease and repair of epithelial injury. *Eur. Respir. J.*, **11**, 958–970.
- SLACK J. M. (2000): Stem cells in epithelial tissues. *Science*, **287**, 1431–1433.
- TEBBUTT N. C., GIRAUD A. S., INGLESE M., JENKINS B., WAR-

- ING P., CLAY F. J., MALKI S., ALDERMAN B. M., GRAIL D., HOLLANDE F., HEATH J. K. and ERNST M. (2002): Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 mutant mice. *Nat. Med.*, **8**, 1089–1097.
21. TESFAIGZI Y., FISCHER M. J., GREEN F. H. Y., DE SANCTIS G. T. and WILDER J. A. (2002): Bax is crucial for IFN γ -induced resolution of allergen-induced mucous cell metaplasia. *J. Immunol.*, **169**, 5919–5925.
 22. TESFAIGZI Y., FISCHER M. J., MARTIN A. J. and SEAGRAVE J. (2000): Bcl-2 in LPS- and allergen-induced hyperplastic mucous cells in airway epithelia of Brown Norway rats. *Am. J. Physiol.*, **279**, L1210–L1217.
 23. TESFAIGZI Y., HARRIS J. F., HOTCHKISS J. A. and HARKEMA J. R. (2003): DNA synthesis and Bcl-2 expression during the development of mucous cell metaplasia in airway epithelium of rats exposed to LPS. *Am. J. Physiol.* (in press).
 24. TESFAIGZI J., HOTCHKISS J. A. and HARKEMA J. R. (1998): Expression of Bcl-2 during mucous cell metaplasia and remodeling in F344/N rats. *Am. J. Resp. Cell Mol. Biol.*, **18**, 794–799.
 25. TESFAIGZI J., TH'NG J., WRIGHT P. S., HOTCHKISS J. A. and HARKEMA J. R. (1996): A small proline-rich protein, sPRP1, is up-regulated early during tobacco smoke-induced squamous metaplasia in rat nasal epithelia. *Am. J. Respir. Cell Mol. Biol.*, **14**, 478–486.
 26. TOSH D. and SLACK J. M. (2002): How cells change their phenotype. *Nat. Rev. Mol. Cell Biol.*, **3**, 187–194.
 27. TRIFILIEFF A., EL-HASHIM A. and BERTRAND C. (2000): Time course of inflammatory and remodeling events in a murine model of asthma: effect of steroid treatment. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **279**, L1120–1128.
 28. VERMEER P. D., EINWALTER L. A., MONINGER T. O., ROKHLINA T., KERN J. A., ZABNER J. and WELSH M. J. (2003): Segregation of receptor and ligand regulates activation of epithelial growth factor receptor. *Nature*, **422**, 322–326.
- Added in proff:*
29. SHI Z. O., FISCHER M. J., DE SANCTIS G. T., SCHUYLER M. R. and TESFAIGZI Y. (2002): IFN- γ , but not Fas, mediates reduction of allergen-induced mucous cell metaplasia by inducing apoptosis. *J. Immunol.*, **168**, 4764–4771.

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