

Dispatches

Allergy: Airway Epithelial Rac1 Suppresses Allergic Inflammation

Loss of Rac1 in airway epithelial cells has demonstrated its participation in the ingestion of apoptotic cells and, more importantly, has revealed an unexpected role for Rac1 in maintaining the anti-inflammatory and non-immunogenic environment of the lung and reducing the intensity of allergic sensitization.

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In metazoa, effete, unwanted or damaged cells undergo various forms of programmed cell death, including apoptosis, and are removed from the body following uptake into phagocytic cells and subsequent intraphagosomal digestion. This process is highly efficient; so much so, that the presence of apoptotic cells in tissues can often only be detected if the clearance process is blocked, such as by disruption of genes involved in the uptake process, for example in *Caenorhabditis elegans* [1] or mice [2]. There are numerous mechanisms by which the dying cells are recognized for clearance, but key amongst them is the exposure in the outer plasma membrane of the phospholipid phosphatidylserine (PS) [3], which is normally actively maintained on the inner membrane leaflet. Redundancy is also evidenced by the many molecules and receptors that have been shown to recognize the PS, as well as the subsequent signaling pathways that contribute to the eventual uptake of the apoptotic cell [4]. Importantly, these receptors and pathways merge to initiate a highly conserved uptake mechanism, a form of stimulated macropinocytosis, that shows an absolute requirement for Rac1, a key member of the Rho family of low molecular weight GTPases. For simplicity, we term the process efferocytosis, in part to distinguish the recognition and uptake mechanisms from those of other forms of phagocytosis, such as those involved in clearance of pathogens.

A further element of redundancy in the process is evident from the many different cell types that can perform apoptotic cell clearance, not merely the

so-called 'professional' phagocytes. For example, in many tissues in *C. elegans* it is the neighboring cells that carry out the ingestion of apoptotic cells. In mammals, epithelial cells, fibroblasts and endothelial cells can all perform this function, though, for the most part, probably less efficiently than macrophages and dendritic cells. A striking example is involution of the mammary gland after lactation has ceased, wherein the secretory glandular epithelial cells undergo apoptosis and are then rapidly cleared by the surviving epithelial cells themselves — a process that in mice can result in complete resolution of the glandular epithelium in as little as four days [5]. An additional critical element of the removal of cells undergoing programmed cell death is that the process is usually quiet, i.e. without initiation of either inflammatory or immunological consequences. In part, this has been ascribed to the release of immunosuppressive and inflammasuppressive mediators, such as transforming growth factor (TGF) β , interleukin-10 (IL-10) and inhibitory eicosanoids, from the ingesting cell and, once again, PS recognition by the ingesting cell is a major stimulus for this effect [6].

These observations set the stage for the new study reported by Juncadella *et al.* [7], which initially set out to assess the ability of epithelial cells lining the airways of the murine lung to recognize and remove apoptotic cells, as well as the consequences of this efferocytosis in the possible context of an important human airways disease — asthma. The technique used to explore these questions — specific and inducible knockdown of Rac1 in airway epithelial cells in the mouse — not only addressed the issue of efferocytosis in these cells, but also opened up a number of additional and more

interesting activities for Rac1 in the normal programming and function of the airway epithelial cells. First, given the critical role of Rac1 in cytoskeletal organization, it was of substantial importance for the investigation to demonstrate that the epithelium *in vivo* was not obviously altered by Rac1 deletion in the epithelial cells. Thus, the animals were allowed to mature for 8 weeks before the deletion and a substantial series of studies supported the apparent normality of epithelial cell numbers, differentiation, and function, including assays for junctional integrity, which is critical for an epithelium that is exposed to the outside environment of air-borne particulates and potential allergens. However, it could be noted that the permeability assay likely primarily assessed the alveolar epithelium, which represents the major surface area of the lung and whose cells do not normally express the promoter used for Rac1 deletion. As the study went on to show, Rac1 deletion certainly changed the properties of the airway epithelium, even if not obviously altering its overall composition, structure and barrier function.

In pursuing the first objective, the authors confirmed previous studies (for example [8,9]) and demonstrated that airway epithelial cells did show evidence of apoptotic cell uptake *in vitro* and, following instillation of apoptotic cells into the murine airways, *in vivo*, that such uptake was dependent on PS exposure and, importantly herein, was prevented by selective knockdown of Rac1 in the airway epithelium. Nonetheless, although not altering the overall implications of the 'apoptotic cell' uptake observations made in this investigation, it is not clear, especially *in vivo*, what was actually ingested. The FACS-based assay used in this study cannot distinguish between uptake of intact apoptotic cells versus membrane-bound cell fragments or apoptotic bodies. Additionally, whether the airway epithelial cells constitute

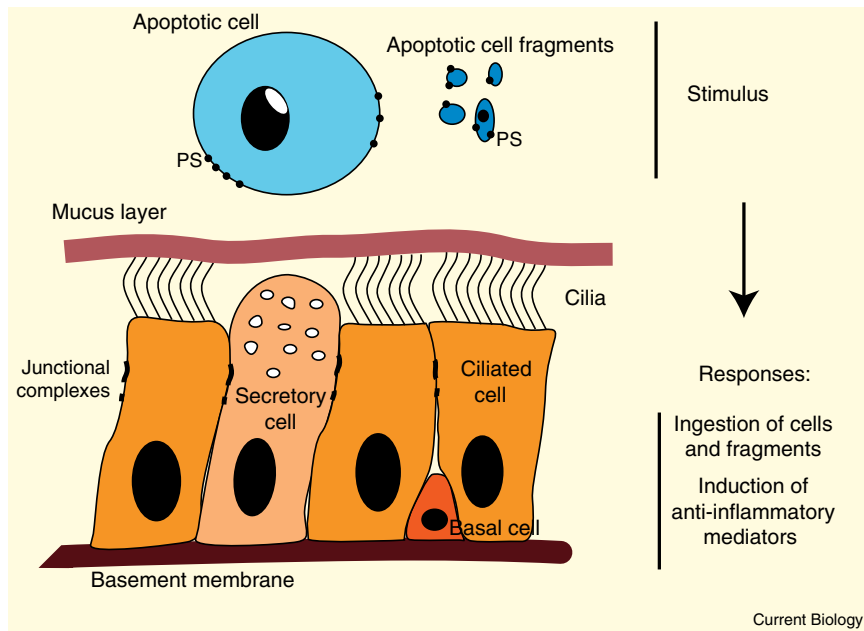


Figure 1. Interaction of PS-exposing apoptotic cells with airway epithelial cells.

an important clearance mechanism for dying cells within the lumen of the lung might be questioned: alveolar epithelium (greater surface area), alveolar macrophages (greater efferocytic activity) and the airway mucociliary escalator driven by the ciliated epithelial cells all likely play important, even dominant, roles in this effect.

The importance of the Juncadella *et al.* [7] study goes substantially beyond the actual uptake of apoptotic cells or fragments, however. As clearly demonstrated, both *in vitro* and *in vivo*, Rac1 expression in the airway epithelial cells was required for their production of IL-10 and TGF β in response to the apoptotic cells. Thus, after Rac1 deletion, the levels of suppressive cytokines were reduced. While at first glance these effects of Rac1 deletion might be attributed to reduced uptake of apoptotic cells, studies in other systems show that actual ingestion of the apoptotic cell or fragment/microparticles is not necessary for the production of anti-inflammatory mediators. As expected, however, the production of these anti-inflammatory mediators by airway epithelial cells as shown in this study was dependent on PS. Additionally, it is not yet clear which airway epithelial cell was responsible; the murine airway barrier epithelium comprises both a ciliated and secretory cell form, with the secretory cell more

likely to be deleted of Rac1 in this experimental system, and both appeared to be present normally after the deletion (Figure 1). To add to the complexity, the Rho family GTPases do not act independently and there is substantial evidence that Rac and Rho function competitively [10,11] (Figure 2). Accordingly, an intriguing line of further investigation would be to assess the precise role of diminished Rac activity versus an expected enhanced effect of Rho within the epithelial cells.

Thus, it is in this context that Juncadella *et al.* [7] tell an even more compelling second story where epithelial Rac1 appears to play a significant role in maintaining the normal non-inflammatory, anti-immunogenic (anti-allergic) environment in the lung. Investigating a commonly used murine hypersensitivity model of human allergic asthma, the absence of airway epithelial Rac1 resulted in heightened immunological and inflammatory responses in a two-step model of airway sensitization with allergen (priming) followed by later inhalational challenge with the allergen to induce allergic inflammation and heightened bronchoconstriction. Following the challenge with dust mite allergen, animals deficient in airway epithelial Rac1 showed increases in lung-infiltrating T lymphocytes and

eosinophils, allergic cytokine production by draining lymph node T cells, serum immunoglobulin E levels, mucus production, airway resistance and hyper-responsiveness. Production of IL-33, a pro-allergic cytokine of substantial current interest, was also enhanced in the absence of Rac1. Importantly, IL-10 was diminished in lung lavage of these animals and this finding proved to be pivotal, in that IL-10 replenishment reversed the heightened allergic responses. By phased timing of the induction of Rac1 deletion, the effect of epithelial Rac1 was shown to act at the sensitization rather than the challenge step. It is also noteworthy that, in the absence of Rac1, allergen priming via the airway was much more effective, and even inhalation of an antigen not normally able to sensitize the mice was now able to prime for subsequent allergic challenge.

What is not clear, however, is whether the observed effects of responses to apoptotic cells (or their uptake) play any role in the allergic effects. The observations do clearly support a normally dampening effect of the epithelial Rac1 GTPase, point to its action in this model as anti-immunogenic at the sensitization step (which is critically required for the induction of significant allergic inflammation), and raise intriguing questions about its role in normally preventing sensitization for human asthma. An intriguing implication here, is that altering the functional Rho:Rac effects *in vivo*, for efferocytosis as well as anti-inflammatory mediator

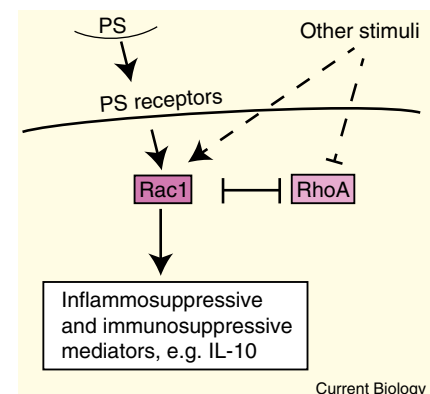


Figure 2. Possible role for Rac1 in airway epithelial cells for maintaining or enhancing the anti-allergic environment in the lung. The Rac:Rho balance is also illustrated and provides a possible point for intervention.

production, might be considered. For instance, this balance can be tipped towards Rac effects by statin drugs [12] acting through their somewhat more selective inhibition of Rho activation. If so, perhaps statins could decrease the sensitization phase in the murine hypersensitivity model employed here, and perhaps even in some circumstances in human asthma. While therapeutics for allergic asthma, particularly inhaled corticosteroids, are largely effective in treating established disease, none currently targets the initial sensitization steps. Thus, enhancing airway epithelial Rac may be a novel target in early intervention, or even prevention, of this disease.

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<http://dx.doi.org/10.1016/j.cub.2012.12.008>

Evolutionary Ecology: Knowing How to Hide Your Eggs

A new study of camouflage in quail shows that individual birds know the appearance of their own eggs and select backgrounds that maximise concealment.

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The study of camouflage has been a central feature of evolutionary theory since Darwin and Wallace [1,2], exemplified by the classic textbook example of evolution: the peppered moth [3,4]. As a phenomenon, camouflage seems intuitively simple — one only needs to find a hidden stick insect or cuttlefish to appreciate its function. Only in the last decade, however, have scientists really got to grips with the different forms of camouflage that exist, tested the survival value that they confer, and how they work in terms of visual processing. In this time, the first experimental support has been found for almost all types of camouflage [5–7], despite most theories existing for more than 100 years [8]. The emphasis in most research has been on the optimization

and tuning of camouflage coloration over successive generations, yet it is well known that individuals of various species, for example many moths, show species-level behavioural preferences for particular backgrounds to rest on [9,10]. But little work has tested whether individuals within a species actively chose backgrounds or microhabitats that best confer camouflage with regards to their own specific appearance. In this issue of *Current Biology*, Lovell *et al.* [11] report that individual Japanese quail (*Coturnix japonica*), when given a choice of substrates of different colours, select the background to lay their eggs on that confers the best camouflage.

Lovell *et al.*'s [11] experiment showed that the individual quail choices were dependent on the appearance of their own eggs, specifically how maculated (patterned)

they are. Individuals with eggs having lots of dark maculation (up to around 50% of the egg surface) selected backgrounds that were dark, and this provided improved concealment through disruptive coloration (breaking up the appearance of body outlines [1,12]) as the dark markings blended into the background and broke up the egg shape. Conversely, individuals with few markings and generally light coloured eggs chose light coloured backgrounds that provided camouflage by background matching (simply matching the colour and pattern of the substrate). This new study has a number of important implications. First, effective camouflage can be a product not just of tuning of appearance over multiple generations, but also the behavioural choices of individual animals. A study of camouflage that simply compares all individuals to randomly chosen background samples could be inappropriate if different individuals show preferences for specific microhabitats that optimize their own concealment. This could be especially important in highly mobile species. A next step in this area of work is to test what specific cues individuals