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# Two cell populations participate in clearance of damaged hair cells from the sensory epithelia of the inner ear

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ABSTRACT

The cochlea and the vestibular organs are populated by resident macrophages, but their role in inner ear maintenance and pathology is not entirely clear. Resident macrophages in other organs are responsible for phagocytosis of injured or infected cells, and it is likely that macrophages in the inner ear serve a similar role. Hair cell injury causes macrophages to accumulate within proximity of damaged regions of the inner ear, either by exiting the vasculature and entering the labyrinth or by the resident macrophages reorganizing themselves through local movement to the areas of injury. Direct evidence for macrophage engulfment of apoptotic hair cells has been observed in several conditions. Here, we review evidence for phagocytosis of damaged hair cells in the sensory epithelium by tissue macrophages in the published literature and in some new experiments that are presented here as original work. Several studies also suggest that macrophages are not the only phaogocytic cells in the inner ear, but that supporting cells of the sensory epithelium also play an important role in debris clearance. We describe the various ways in which the sensory epithelia of the inner ear are adapted to eliminate damaged and dying cells. A collaborative effort between resident and migratory macrophages as well as neighboring supporting cells results in the rapid and efficient clearance of cellular debris, even in cases where hair cell loss is rapid and complete.



**Contents**



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## 1. Introduction

The events leading to hair cell loss have been rigorously studied, and the role of supporting cells in the sensory epithelium has been shown to be interesting and important during the loss of the sensory cells. In order to maintain the separation between perilymph and endolymph, the gaps that form in the sensory epithelium from loss of hair cells must be filled by the adjacent supporting cells. Recent studies indicate that hair cell death is indeed accompanied by a coordinated series of events that involve both hair cells and supporting cells. After injury, supporting cells expand to fill the space that remains after hair cell shrinkage and apoptosis, and restores the integrity of the reticular lamina. Furthermore, a number of studies have shown that supporting cells participate in clearing cellular debris during the process of hair cell death (Li et al., 1995; Abrashkin et al., 2006).

While the supporting cells are the closest neighbors to the hair cells, macrophages also live within the membranous labyrinth and are capable of responding to hair cell injury. Tissue macrophages are often the first-responders to local injury in many organ systems, where they contribute to the removal of cellular debris (Wynn and Vannella, 2016). The inner ear is populated by resident macrophages and additional mononuclear phagocytes are readily recruited into the ear after injury. Activation of such cells could be an excellent method to augment and hasten the process of debris clearance (Savill and Fadok, 2000). Our laboratories have reported the recruitment of phagocytes into the mouse cochlea after acoustic injury and aminoglycoside ototoxicity (Hirose et al., 2005; Sato et al., 2010) and into the mouse cochlea and vestibular organs after selective hair cell ablation (Kaur et al., 2015a, 2015b). Using immunohistochemistry of fixed tissue, we have noted macrophage engulfment of vestibular hair cells, but have rarely observed macrophage-mediated phagocytosis in the injured cochlea. One possible explanation is that cochlear phagocytosis occurs rapidly and is difficult to capture in fixed tissue where only one time point is sampled.

All of the mechanisms that lead to the removal of hair cell debris appear to be initiated during the early phases of cell death, but the molecular signals that trigger such responses have not been identified. It is notable that there are different strategies for removal of cellular debris in different inner ear sensory organs. In some cases, dying hair cells remain in place and become condensed and fragmented, and are then surrounded and partially engulfed by nearby supporting cells. Other sensory epithelia appear to eject injured, but apparently intact hair cells from the sensory epithelium into the endolymph. Macrophages have also been observed to engulf hair cell debris. As described below, the precise method of debris clearance varies by species and by sensory organ (mammals versus birds and amphibians, cochlear versus vestibular organs).

In this review, we examine the role of both supporting cells and macrophages in the clearance of hair cell debris. We also report data from several new studies that further characterize the interaction between macrophages and dying hair cells. In one study, we have used time-lapse imaging of organotypic cultures of the mouse organ of Corti, in order to observe the actions of macrophages after aminoglycoside ototoxicity. Under these conditions, we show that macrophages are able to both identify and phagocytose dying hair cells. Previous studies have also indicated that CD36, which is expressed on the surface of macrophages, is critical for identifying cellular targets for phagocytosis and that CD36 knockout mice suffer from impaired phagocytosis (Silverstein and Febbraio, 2009). With time-lapse recording of organotypic cultures, we assessed whether CD36 is necessary for phagocytosis of damaged sensory cells, and whether deletion of CD36 would influence cochlear repair. Finally, we have characterized macrophage activity in lateral line neuromasts of larval zebrafish following ototoxic injury. We show that macrophages enter lesioned neuromasts and engulf the debris of dying hair cells. These new findings, combined with previous data, support the notion that cellular debris is removed from the sensory epithelia of the inner ear by both supporting cells and macrophages.

## 2. Phagocytosis by professionals: macrophages

### 2.1. Macrophages are present in the inner ear during development

Macrophages populate the central nervous system during early development, and we have limited data suggesting that macrophages are also present in the inner ear during early development. We have observed macrophages associated with the otic vesicle at

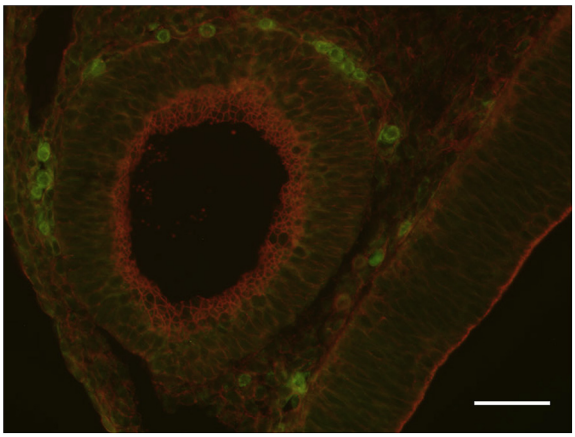

**Fig. 1.** E10 mouse embryo. Macrophages are observed adjacent to the otic vesicle at embryonic day 10 suggesting that macrophages may play a significant role during this period of inner ear development. Selection and elimination of unwanted cells may be undertaken by these local tissue macrophages. Green: CX3CR1$^{GFP}$(macrophages), Red: phalloidin (f-actin). Scale bar: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)