

# Extracellular vesicles: important players in immune homeostasis

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Exosomes are nano-sized extracellular vesicles (EVs) released from cells that act as mechanisms of cellular communication both between the surrounding cells that make up the cellular milieu and cells at distant locations in the body (1). Not only do they play many different roles in normal physiology, exosomes have been shown to exacerbate the progression of numerous different pathologies (1), including playing dual roles in immune regulation (2). Exosomes activate immunity by way of transferring antigens and promoting activation of antigen presenting cells. They also have been shown to participate in immunosuppression under different circumstances (2). For example, exosomes released from intestinal epithelial cells have the ability to suppress global immune function and are critical players in immune evasion in tumor models. Additionally, exosomes released by mesenchymal stem cells suppress immune function in various animal models of disease and tissue damage when used for therapeutic cellular regeneration (2).

An activated immune state contributes to the progression of many different types of inflammatory diseases, namely those irreversible degenerative eye diseases such as age-related macular degeneration (AMD). In the case of this disease, retinal pigment epithelium (RPE) cells, which are critical for maintaining integrity of the blood-retinal barrier, become damaged in an inflammatory environment. Unfortunately, regeneration does not occur in RPEs. Under normal conditions, RPE cells suppress the inflammatory response, protecting themselves and the retina from immune mediated damage. However, RPEs release both

immunosuppressive and inflammatory cytokines that are important for immune responses, as they have the ability to initiate a rapid defense system to protect the retina from pathogens (3). Although RPEs play a critical role in the protection of the retina by upregulating the inflammatory response, immunoregulation creates a balance in inflammatory states, and is a vital mechanism that protects both RPE cells and the retina from excessive cellular damage potentially caused by an activated immune response.

Korthagen *et al.*, demonstrate modified gene expression in RPE cells when stimulated by TNF- $\alpha$ , one of the cytokines involved in ocular inflammation. Other altered gene expressions include those involved in apoptosis, cell motility, immune response, protein transport, and cell signaling, suggesting TNF- $\alpha$  mediates numerous RPE cellular functions (4). Furthermore, it was proposed that vesicular trafficking within the RPE cells may reduce inflammation in the retina (5), however, the precise mechanisms, and the role of EVs in this process, are unknown.

In the current paper, Knickelbein *et al.*, explored the notion that EVs, such as exosomes, released from RPE cells play in the immunosuppressive environment required to protect the cells from immune-mediated degeneration. These authors mimicked an inflammatory environment in an *in vivo* setting by stimulating the cells with inflammatory cytokines, including IL-1 $\beta$ , IFN- $\gamma$ , TNF- $\alpha$ , then they characterized the EVs released by the RPE cells under those conditions and compared to the untreated controls. These

authors found that although the size of the vesicles released by the activated cells did not change (~100 nm in diameter), there was an increase in the amount of the CD81 protein, which, the authors believe, suggests an increase in the quantity of exosomes released from the activated RPE cells under inflammatory conditions. These results propose an increase in the release of EVs from these RPE cells under inflammatory conditions, which is consistent with increased exosome release under oxidative stress conditions shown previously (6).

Although the data suggested an increase in CD81 protein in exosomes under inflammatory conditions, it was important to determine the effect these EVs have on the immune system. The authors specifically investigate T-cell and monocyte response to the EVs released by activated RPEs. T-cell proliferation was reduced when exposed to both stimulated and untreated RPE cell exosomes, consistent with previous data implicating the role of RPEs in the suppression of T-cell activation. However, neither the exosomal samples from stimulated or non-stimulated RPEs resulted in T-cell death, which is a function of RPE cells under normal conditions. Taken together, these findings show that exosomes may play a role in the reduction of T-cell proliferation, however, despite one of the mechanisms of immune suppression by RPE cells being the induction of T-cell apoptosis, it is unlikely to be a result of exosome release.

These authors next explored the effects of RPE EVs have on monocyte population. Monocyte phenotype is based on surface expression of CD14 and CD16, and an increase in the population classified as intermediate phenotype (CD14<sup>+</sup>CD16<sup>+</sup>) was detected when monocytes were incubated with unstimulated RPE EVs when compared to untreated monocytes. This shift of population phenotype suggests an increase in the immunoregulatory functions in the monocytes. In addition, when monocytes were incubated with cytokine-induced RPE-derived EVs, an induction in monocyte death correlated with the volume of EVs added to the monocytes. This cell death was not seen with EVs from non-cytokine stimulated RPE cells. Furthermore, a greater amount of TGF- $\beta$ 1 (which has immunosuppression functions) was released by monocytes

exposed to the non-stimulated RPE EVs. In contrast to those monocytes exposed to cytokine-stimulated RPE-derived EVs, an increased amount of inflammatory cytokines such as TNF- $\alpha$ , IL-6 and IL-8 was released.

Overall, this manuscript suggests that EVs from RPE cells mediate changes in T-cell proliferation and monocyte phenotype depending on the inflammatory environment of the RPE cells. Importantly, results from the current studies implicate EVs of the RPE cells as a mechanism of immunomodulatory effects of the cells by way of suppressing T-cell proliferation.

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None.

### Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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