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Possible Autophagy induction in Vernal Keratoconjunctivitis via Tumor Necrosis Factor Alpha Stimulation

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Tumor necrosis factor alpha (TNFα) is one of the main mediators of inflammatory response in many pathological diseases, involved in a widespread biological functions, including autophagy. Previous data obtained in our laboratory demonstrated that TNFα and some autophagy markers (which markers please indicate) are overexpressed in a severe inflammatory disease such as vernal keratoconjunctivitis (VKC).

In the present study we explored the role of TNFα in the induction of autophagy in VKC, using an *in vitro* model.

Primary conjunctival cell cultures were treated with TNFa and analysed by qPCR and western blotting for expression of some autophagy and lysosomial markers at 4, 10 and 24 hours after exposure. qPCR results demonstrated that LC3B, Beclin-1, LAMP1 and p62 strongly increased from 4 to 24 hours, whereas the expression of Catepsin D, a protein implicated in lysosomial apototic pathway, was comparable to that of untreated control. Western blotting analysis revealed lipidation of LC3B quantified as an increased LC3BII/LC3BI ratio. Moreover, double immunofluorescence for Cathepsin D and LAMP1 showed that Cathepsin D was localized within the lysosomes at 4, 10, 24 hours after cell exposure to inflammatory stimuli.

In conclusion, our data demonstrated that TNFα significantly induce in VKC LC3B lipidation, LC3BII/LC3BI ratio and p62 (qPCR) in the cells exposed to inflammatory stimuli which shows possible activation of autophagy pathway.

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