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The Role of FLIP Long in C5a-Modulated Spontaneous Apoptosis of Neutrophils

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The complement system is a very important part of the innate immune system and is well-known to induce cell death through the production of the membrane attack complex (MAC), as well as also leading to necrosis, a type of cell death that results in inflammation. Recent data shows that the complement system is involved in apoptosis, another type of cell death that produces a less inflammatory response. Complement also participates in the clearance of apoptotic cells. This is important to prevent these cells remaining in inflamed tissue and perpetuating it, eventually leading to autoimmunity or chronification of inflammation.
Neutrophils, important cells of the innate immune system, play a decisive role in inflammation. These immune cells have an interesting characteristic, a short life span (8-20 hours), and in the absence of survival factors such as IL-2, IL-4, and IL-15 and other agents such as GM-CSF or LPS, undergo spontaneous apoptosis. Neutrophil apoptosis and subsequent ingestion by macrophages are the major mechanisms for clearing neutrophils that have been recruited to the inflamed sites and thus for promoting resolution of the inflammation. When these regulatory mechanisms are impaired or when the acute insult cannot be resolved, neutrophils become the predominant contributor to tissue injury. Understanding the processes that regulate survival and spontaneous death of neutrophils is very important for the treatment of acute and chronic inflammations.
The complement-derived anaphylatoxin C5a has been described to delay neutrophil apoptosis through the BAD-mediated signaling pathway in mitochondria (intrinsic pathway). However, the role of the death receptor pathway (extrinsic pathway) in the apoptosis of neutrophils is still controversial.
Aim of this study was to evaluate whether C5a modulates the spontaneous apoptosis of neutrophils through analysis of the extrinsic pathway regulator $FLIP_L$.
Neutrophils were isolated from 20 healthy adult donors. FACS analysis was carried out to evaluate apoptosis. Activity of caspases 9, 8, and 3 was measured by substrate cleavage using AFC fluorescence. $FLIP_L$ and β-actin were quantified using Light Cycler-RT-PCR. For all experiments, neutrophils were analysed after 1 hour or 21 hours of incubation with or without C5a. After 21 hours of incubation, no statistically significant difference ($p < 0.3$) in spontaneous apoptosis of neutrophils was observed if treated with C5a (median = 71.5%) or without C5a (median = 73, 5%). No significant differences were found in caspases activities. However, C5a delayed spontaneous apoptosis in a subgroup of donors tentatively named “responders“ (n = 10). A significant reduction of spontaneous apoptosis of neutrophils upon C5a treatment was observed in the “responders“group ($p < 0.005$) going along with an upregulation of $FLIP_L$ ($p < 0.05$) in neutrophils (median FLIP/β-actin = 4, 62 ag vs. 3.92 ag).
In our study we demonstrated for the first time the expression of $FLIP_L$ in neutrophils and its upregulation in cases where C5a delayed neutrophil apoptosis. $FLIP_L$ is known as a regulator of the extrinsic apoptosis pathway and has for a long time, exclusively described as an anti-apoptotic molecule. Recent evidence supports the notion that $FLIP_L$ exerts pro-apoptotic function under certain conditions.

Summarizing the results of our study we propose the potential involvement of C5a in neutrophil survival or apoptosis pathways. C5a may promote survival of neutrophils by three different means: through Bad-phosphorylation and inhibition of caspase 9 activation, through stimulation of NF-κB associated with expression of $FLIP_L$ or through inhibition of ROS production.
C5a may induce apoptosis of neutrophils by either production of ROS or induction of IκBα, which is an NF-κB inhibitory protein.
To address these questions, further analysis of NF-κB signaling pathway as well as ROS production in neutrophils exposed to C5a is needed. The complex role of $FLIP_L$ has to be further characterized to better understand how C5a influences apoptosis.