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BASIC SCIENCE

Investigating caspases and other markers of apoptosis in ITP

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Apoptosis is the main mechanism that regulates cell life span and the elimination of damaged or infected nucleated cells. Although platelets are enucleate cells, they express at least part of the apoptotic machinery known from nucleated cells. In addition, they have been observed to undergo apoptotic-like events. This has been demonstrated by the activation and translocation of Bcl-2 family members, cytochrome *c* release, activation of caspase-9 and of caspase-3 [1–10]. These observations indicate that platelets contain the essential components needed to undergo programmed cell death executed in the cytoplasm. Recently, in a murine model of ITP, it has been shown that apoptotic-like processes can be induced by anti-platelet antibodies: injection of anti-GPIIb antibodies caused apoptotic-like events as well as profound thrombocytopenia [7, 11–14].In this murine ITP model, IVIg treatment was able to decrease the apoptotic-like events and to improve the low platelet count [7, 11–13].

As no such study has been performed in paediatric ITP so far, we started to investigate whether in children with primary ITP, an enhanced expression of markers of apoptotic processes can be found. Furthermore, we are interested to see if such markers correlate to platelet count and most important to clinical symptoms and if they decrease after IVIg treatment.

So far, we have shown in a limited number of patients that caspase-3 is activated in 40% of the platelets in patients with primary ITP and decreases to 10% after IVIg treatment ([15], manuscript in preparation). In parallel to caspase-3 activation also bleeding signs decreased after IVIg therapy. In ongoing studies, we investigate caspase-3, caspase-9, caspase-8, PS-exposure and mitochondrial membrane potential by flow cytometry and caspases by Western blot. We further will develop an immuno-fluorescence microscopy protocol for the detection and quantification of activated caspases in bone marrow smears. Investigating and comparing levels of activated caspases and other signs of apoptosis in platelets and bone marrow samples is important to understand the initiation of apoptotic processes in platelets, whether apoptosis is already triggered in bone marrow or only in platelets in ITP patients. Furthermore, it is important to understand whether apoptosis might contribute to the destruction of platelets in ITP patients.

To increase patient numbers, we invite other clinicians or laboratories treating respectively investigating ITP to collaborate with us.

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