# Alpha B-crystallin activates an immune-regulatory response of microglia in preactive multiple sclerosis lesions.

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## Research question and background

In normal-appearing white matter (NAWM) of multiple sclerosis (MS) patients, small clusters of activated microglia are frequently observed. Pathological descriptions of these clusters, which we term pre-active lesions, have been supported by in vivo imaging studies, which have additionally shown that pre-active lesions can precede the appearance of actively demyelinating MS lesions by several months. An endogenous trigger for pre-active MS lesions is strongly suggested by the fact that microglial activation occurs in non-demyelinated tissue in the absence of overt blood-brain barrier breakdown or lymphocyte infiltration. Consistently associated with pre-active lesions are stressed oligodendrocytes that express markedly elevated levels of the small stress protein alpha B-crystallin. In the present study, we examined the idea that oligodendroglial alpha B-crystallin activates microglia to cluster into pre-active MS lesions.

## Tissues and methods used

Tissue samples from subcortical white matter were obtained from control cases to isolate and culture microglia. Cultured microglia were used for microarray profiling of the alpha B-crystallin-induced transcript response at 1 and 4 h after activation. RT-PCR, immunocytochemical staining, and ELISA-based analyses of soluble mediators in the culture medium was used for validation of microarray data.
For pathology studies, the tissues from MS patients were either snap-frozen in cooled isopentane and stored in liquid nitrogen, or fixed in 10% formalin and paraffin-embedded. Classification of lesions stages was based on immunohistochemical detection of major histocompatibility complex (MHC) class II/HLA-DR and the presence of proteolipid protein (PLP) to reveal areas of NAWM. Preactive lesions were stained with monoclonal antibodies against various mediators identified by microarray analyses as alpha B-crystallin-induced products.

## Results and conclusion

By genome-wide microarray profiling, we analyzed the transcript response of twelve primary human microglial cultures after activation by alpha B-crystallin. These response profiles revealed that alpha B-crystallin activates an array of chemokines, immune-regulatory and anti-inflammatory mediators, and a striking number of antiviral genes that are generally inducible by type I interferons (IFNs). This not only clarifies the molecular basis for the beneficial, anti-inflammatory effects of extracellular alpha B-crystallin during neuroinflammation *in vivo*, it also provides molecular markers that can be used to trace the microglial response to alpha B-crystallin *in vivo*. Immunohistochemistry revealed these markers to be consistently expressed by microglia in pre-active MS lesions, along with CD14, TLR1 and TLR2, the receptors required for recognition of alpha B-crystallin. Together, our findings suggest that pre-active MS lesions reflect a microglial response to alpha B-crystallin-expressing, stressed oligodendrocytes. They also indicate that this response is aimed at defending the tissue against viral infection, at restoring tissue homeostasis.