

Conclusions and Future Directions

The literature in the past decade underlines the central role of ETs in homeostasis and disease. ETs formation helps in clearing the pathogen but dysregulated and persistent ETs in different parts of the body may contribute to disease pathology. Cytoplasmic proteins including antimicrobial proteins and proteases present on traps help in killing the trapped bacteria and also serve as auto antigen leading to inflammation (Goldmann and Medina 2012, Jorch and Kubes 2017, Papayannopoulos 2017, Daniel, Leppkes et al. 2019, Yousefi, Stojkov et al. 2019). The aim of this thesis was to confirm the induction of microglia ETs by DA, to investigate the functionality and basic mechanism behind DA induced trap formation and to check the presence of microglia ETs in GBM tissues.

ETs are induced by cells of innate immune system when induced by different stimuli (Papayannopoulos 2018, Daniel, Leppkes et al. 2019, Yousefi, Stojkov et al. 2019). We confirmed the formation of ETs by microglia when stimulated with DA. The ability of DA to induce ETs points towards the role of DA in immune regulation and sterile inflammation. DA receptors are present on most of the immune cells. Lymphocytes and dendritic cells can produce DA that can act in an autocrine and/or paracrine manner (McKenna, McLaughlin et al. 2002, Cosentino, Fietta et al. 2007, Nakano, Higashi et al. 2009). This indicates that DA may play crucial role in regulating immune system. Our results provide a pivotal link between DA and neuro-inflammation.

To further look into the mechanism we performed MTT assay to investigate the toxicity of DA on BV2 microglia and established that DA is not cytotoxic. This implies that DA induced ETs formation in BV2 microglia does not lead to immediate cell death. Another aspect of the mechanism we observed was that DA induced ETs follow a ROS independent pathway. We pretreated cells with the ROS scavenger NAC and then with DA to confirm no significant increase in ROS levels. These results proposed that DA induced ETs formation is independent of ROS. We confirmed this by observing the formation of traps even when ROS was inhibited. Actin dynamics may also play role in ETs formation (Metzler, Goosmann et al. 2014, Stojkov, Amini et al. 2017). We observed that DA induces ETs in an actin polymerization independent manner in BV2 cells. It was evident that CytoD does not affect ETs formation, but it was cytotoxic as evident from our MTT assay. Inhibiting actin polymerization may induce lytic ETosis in BV2 cells.

To check whether DA induced ETs are functional we incubated BV2 cells with FITC tagged *E. coli*. DA induced ETs in BV2 cells successfully trapped FITC tagged *E. coli*. Even the ETs formed in presence of NAC or CytoD trapped *E. coli*. Thus inhibition of ROS did not affect functionality of traps. But functionality of traps was compromised in CytoD treated cells. How CytoD affects ETs formation and functionality in microglia needs further investigation. These results established trap formation as an important mechanism of microglial response to counter pathogens.

Encouraged by the results from BV2 microglia, we treated primary human adult microglia with DA, PMA or LPS and observed the formation of ETs. We also checked the presence of ETs in GBM tissues. ETs are protective and inflammatory (Daniel, Leppkes et al. 2019). They play role in pathogenesis of many diseases including cancer (Papayannopoulos 2017, Daniel, Leppkes et

al. 2019). A recent report by Caragher *et al.* shows that GBM cells express DR2 and they can synthesize and secrete DA (Caragher, Shireman et al. 2019). We found that microglia present in the GBM microenvironment form ETs. Inflammation caused by ETs might be involved in progression of GBM.

The role of ETs is evident in diseases like SLE, RA, AAV, cancer gout (Jorch and Kubes 2017, Papayannopoulos 2018, Daniel, Leppkes et al. 2019). Its role in other diseases related to sterile inflammation needs to be investigated. There has also been evidence suggesting that ETs may play a role in the pathology of COVID-19 (Middleton, He et al. 2020). Since microglia extracellular traps have been reported recently (Wang, Wang et al. 2019), not much is known regarding their contribution to sterile CNS inflammation and the mechanism thereof. While the presence of NETs in CNS have been reported in case of infection and TBI, induction of microglia ETs has only been reported when induced by *Listeria monocytogenes* (Appelgren and Enocsson 2019, Mohanty, Fisher et al. 2019, Wang, Wang et al. 2019, Vaibhav, Braun et al. 2020). One of the major future goals is to characterize microglia ETs and proteins present on them. Identifying the proteins present on microglia ETs will provide a better understanding of their role in sterile inflammation, autoimmune diseases and neurodegenerative diseases. ETs play an important role in autoimmune diseases such as lupus and arthritis (Garcia-Romo, Caielli et al. 2011, Khandpur, Carmona-Rivera et al. 2013, Yu and Su 2013). Our findings that DA can induce ETs, point towards a role of dopamine in other inflammatory diseases in the brain. In diseases like schizophrenia, Tourette syndrome, Parkinson disease and multiple sclerosis (MS) DA plays a substantial role in the pathophysiology (Denys, de Vries et al. 2013, Brisch, Saniotis et al. 2014, Arreola, Alvarez-Herrera et al. 2016). Studying DA induced microglial ETs may lead to interventions that may help in better management of these diseases. The presence of microglia ETs in CNS disorders is an interesting topic to be explored. We reported the presence of microglia ETs in GBM. It is further necessary to explore whether they are playing any role in GBM progression and metastasis.

The mechanism of ETs formation and characteristics of ETs varies with stimuli and the cells (Yousefi, Mihalache et al. 2009, Yousefi, Morshed et al. 2015, Jorch and Kubes 2017, Papayannopoulos 2018, Yousefi, Stojkov et al. 2019). The mechanism of trap formation is still unfolding even in neutrophils, in which the ETs are most studied. In our studies we have observed that inhibiting NLRP3 is increasing ETs formation by DA induced microglia. Until very recently it was reported that LPS induces NETs formation by activating NLRP3 via non canonical pathway (Chen, Monteleone et al. 2018). Our results show that NLRP3 might be regulating sterile inflammation by controlling microglia ETs formation. We will be further exploring the mechanism of microglia ETs formation and the role of inflammasomes and its components in the process. It is important to investigate the precise mechanism of microglia ETs formation. Studying the molecules involved in the pathway may help us in regulating the formation of microglia ETs. It may also help in better understanding of the mechanism of trap formation and its regulation by other innate immune cells.