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Introduction

Our immune system protects us from various disease and helps regulate homeostasis. Immune cells are integral part of the immune system. They protect us from various pathogens and intrinsic dangers with the help of vast armamentarium of defense strategies. Formation of extracellular traps (ETs) is one of the mechanisms which plays a significant role in slowing down the dissemination of pathogens and killing them over time (Brinkmann, Reichard et al. 2004, Daniel, Leppkes et al. 2019). ETs were discovered in 2004 by Zychklinsky *et al*. in neutrophils and were initially known as neutrophil extracellular traps (NETs) (Brinkmann, Reichard et al. 2004). The authors found that activating neutrophils with phorbol 12-myristate 13-acetate (PMA), interleukin-8 (IL-8), *Staphylococcus aureus*, *Salmonella typhimurium* or *Shigella flexneri* lead to the formation of NETs composed of complex network of decondensed chromatin (Brinkmann, Reichard et al. 2004). NETs are embedded with proteins like neutrophil elastase (NE), myeloperoxidase (MPO), cathepsin G, gelatinase, lactoferrin, histone and others (Papayannopoulos 2018, Daniel, Leppkes et al. 2019). It was subsequently discovered that macrophages (Chow, von Köckritz-Blickwede et al. 2010), monocytes (Webster, Daigneault et al. 2010), eosinophils (Yousefi, Gold et al. 2008), basophils (Schorn, Janko et al. 2012, Morshed, Hlushchuk et al. 2014) and mast cells (Schorn, Janko et al. 2012) can also form ETs . ETs are an evolutionary conserved mechanism to counter pathogens as cells from plants and different mammalian and non-mammalian species can also form traps (Neumann, Brogden et al. 2020). ETs are formed in response to many microbial and chemical stimuli and are embedded with proteins some of which have antimicrobial property. Some ETs embedded proteins are responsible for sensitizing immune cells by the exposure of self-antigens resulting in autoimmunity. Unregulated formation of ETs leads to aggravated inflammation initiating further damage (Papayannopoulos 2018, Daniel, Leppkes et al. 2019, Neubert, Meyer et al. 2020). A proper balance in ETs formation and clearance is critical for homeostasis within a biological system. Until recently formation of ETs by microglia was not reported (Wang, Wang et al. 2019).

Microglia are innate immune cells of central nervous system (CNS). They play a central role in fighting pathogens, clearing debris and maintaining homeostasis in the CNS (Li and Barres 2018). Microglia are critical for development and control of neurodegenerative disease and cancers of the CNS (Salter and Stevens 2017, Gutmann and Kettenmann 2019). Microglia are regulated by various physiological and chemical factors including neurotransmitters (Pocock and Kettenmann 2007, Liu, Leak et al. 2016, Fan, Chen et al. 2018). Dopamine (DA) is one of the monoamine neurotransmitters of the CNS which regulate motor activities, emotion, reward, cognition and addiction (Beninger 1983, Ferreri, Mas-Herrero et al. 2019, Ott and Nieder 2019). DA mediated immunoregulatory effects have been demonstrated within and outside the CNS, however further cellular mechanistic insights remain undiscovered (Yan, Jiang et al. 2015, Pinoli and Marino 2017). Recently the expression of dopamine receptor 2 (DR2) on *Glioblastoma multiforme* (GBM) cells was reported (Caragher, Shireman et al. 2019). GBM cells are capable of synthesizing and secreting DA (Caragher, Shireman et al. 2019). DR2 was also observed to regulate spheroid forming capability of GBM cells (Caragher, Shireman et al. 2019, Weissenrieder, Reed et al. 2020). These findings signify the role of DA in regulation of disease and homeostasis.

1.1 PURPOSE OF THE STUDY:

The aim of this study was to investigate the role of dopamine in inducing ETs in microglia. Dopamine is a well-established neurotransmitter. Its role has been intensively studied in controlling motor functions of the body, reward and addiction mechanisms (Färber, Pannasch et al. 2005, Ferreri, Mas-Herrero et al. 2019, Ott and Nieder 2019). DA receptors are present on immune cells and dopamine also regulates various aspects of central nervous system and peripheral immunity (Levite 2012, Pinoli and Marino 2017). However, the immunoregulatory role of DA is largely unknown. We wanted to investigate if DA can induce extracellular traps in microglia and the cellular and molecular mechanism underlying this phenomenon. This research will help gain insights into the regulatory role of DA and microglia in diseases and sterile inflammation.

1.2 BRIEF RESULTS, SCOPE AND FUTURE PROSPECTS OF THE WORK:

We observed that DA induced extracellular traps in BV2 mouse microglia cell line and primary adult human microglia at different concentrations. We confirmed the presence of extracellular traps by staining them with 4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI) and myeloperoxidase (MPO). ETs were also present in culture supernatants. ETs are formed through two mechanisms: 1) Reactive oxygen species (ROS) independent and 2) ROS dependent. ROS independent mechanism is generally cell death independent (Pilsczek, Salina et al. 2010, Yipp, Petri et al. 2012, Pieterse, Rother et al. 2016, Papayannopoulos 2017). We found that DA induced ETs in BV2 microglia by a ROS, cell death and actin polymerization independent mechanism. We further investigated and quantified the presence of ETs in GBM tissues.

The induction of ETs in microglia by DA highlights the role of DA and microglia in sterile inflammation. Further investigation of ETs in neuroinflammation is needed. Studying how DA regulates inflammation in case of CNS infection and other insults will help us better understand and manage neuroinflammation. As DA has also been reported to regulate the progression of glioblastoma (Caragher, Shireman et al. 2019), investigating the role of ETs in gliomas especially grade IV *glioblastoma multiforme* (GBM) is critical. This will help us better understand the inflammatory landscape of GBM. Further studying the intracellular signaling in ETs formation will provide mechanistic insights into ET induction. This may also help us regulate ET formation in case of inflammation and disease.

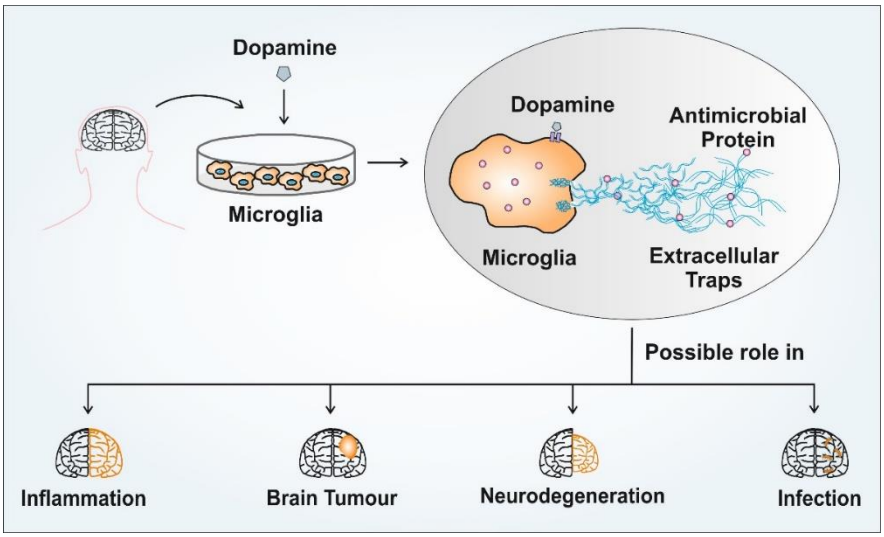

Figure 1.1 : Dopamine induces extracellular traps in BV2 and primary adult human microglia. Dopamine induced microglia extracellular traps are embedded with proteins like histone and myeloperoxidase. Microglia extracellular traps are also present in glioblastoma multiforme (GBM) tissues. The traps may play central role in sterile inflammation, GBM progression, neurodegeneration and regulating infection in central nervous system.