Details of Research work

The focus of Anirban's laboratory is to understand the patho-physiology of infection and inflammation in central nervous system (CNS). The pioneering research has dissected the crucial regulatory role of neuroinflammation in the pathogenesis of Japanese encephalitis (JE), the leading cause of viral encephalitis in India and entire Asia-Pacific region. JE virus infection generates a rapid inflammatory response including peripheral cell infiltration into the central nervous system. As level of inflammation correlates well with the clinical outcome in JE patients, it is likely that therapies that target "inflammation process" selectively may be highly effective. So far research from Anirban's laboratory unravelled in great details the molecular mechanism of neuro-inflammation that contributes to neuro-degeneration in JE. (1, 2)

By exploring the pathways which are involved in inflammation, Anirban's laboratory has identified several anti-inflammatory compounds with therapeutic potential in an experimental model of JE. Based upon pre-clinical study undertaken in Anirban's laboratory at National Brain Research Center, a Phase II clinical trial has been conducted at King George Medical University (KGMU), Lucknow, where minocycline has been used as a therapy for Japanese Encephalitis patients and the patients with Acute Encephalitis Syndrome (AES). (3,4)

The commonly encountered sequelae in JE survivors are mental retardation, learning disabilities, behavioral abnormalities, and motor paralysis, speech and movement disorders. Research from their group conspicuously documented the involvement of Neural Progenitor Cells (NPC) in the pathogenesis of JE. JEV infect NPC and harbor in them. Interestingly, the virus does not induce robust NPC death, but with progressive infection arrests their proliferative ability. This eventually culminates in depletion of NPC pool upon JEV infection, which could lead to long-term neurological sequel in JE. (5, 6, 7, 8, 9, 10)

Few years back Anirban and his group have demonstrated that the expression of miR-301a is increased in JEV-infected microglial cells and human brain. Overexpression of miR-301a augments the JEV-induced inflammatory response, whereas inhibition of miR-301a completely reverses the effects. Mechanistically, NF-κB repressing factor (NKRF), functioning as inhibitor of NF-κB activation is identified as a potential target of miR-301a in JEV infection. Consequently, miR-301a mediated inhibition of NKRF enhances nuclear translocation of NF-κB, which in turn resulted in amplified inflammatory response. Conversely, NKRF overexpression in miR-301a inhibited condition restores nuclear accumulation of NF-κB to a basal level. (11)

They also observed that JEV infection induces classical activation (M1) of microglia that drive the production of pro-inflammatory cytokines, while suppressing alternative activation (M2) that could serve to dampen the inflammatory response. Furthermore, in vivo neutralization of miR-301a in mouse brain restores NKRF expression, thereby reducing inflammatory response, microglial activation and neuronal apoptosis. This study suggests that the JEV-induced expression of miR-301a positively regulates inflammatory response by suppressing NKRF production, which might be targeted to manage viral-induced neuro-inflammation. (12)

Alongside with neurobiology of JE, Anirban is also deeply engaged in basic research to understand the pro-inflammatory cytokine mediated transcriptional regulation of microglial activation. They have shown a novel transcription factor Krüppel-like factor 4, which regulates microglial activation and subsequent neuro-inflammation. This transcription factor promises to be a potent target for therapeutic agents aiming to alleviate inflammation in brain. As a continuation of the previous they have further demonstrated that therapeutic targeting of KLF4 abrogates microglial activation and subsequent inflammation. (13)

Microglial activation resulted from various stress signals (including pathogenic invasion and neurodegeneration) is responsible for forming the first line of defense in the CNS and results in the secretion of various cyto-chemokines. IL-1β is the most important cytokine that initiates a vicious cycle of inflammation by inducing the expression of other pro-inflammatory cytokines along with its own production. Work from Anirban's laboratory has showed that microglia mediated IL-1β production is a tightly regulated mechanism which involves the activation of nucleotide binding oligomerization domain leucine rich repeat and pyrin domain containing 3 (NLRP3) inflammasome pathway. Their results identify an important signaling mechanism underlying microglial inflammation and suggest HSP60 as a novel molecule which can be targeted to regulate IL-1β production to ameliorate inflammatory conditions in CNS. (14)

As a conclusive remark I can say that work done by Anirban and his associates in last eighteen years at NBRC, not only provides a new and general principle for cure of the whole spectrum of neuroinflammatory diseases that include infection of the CNS, but also other neurodegenerative disorders that affects millions of people worldwide.

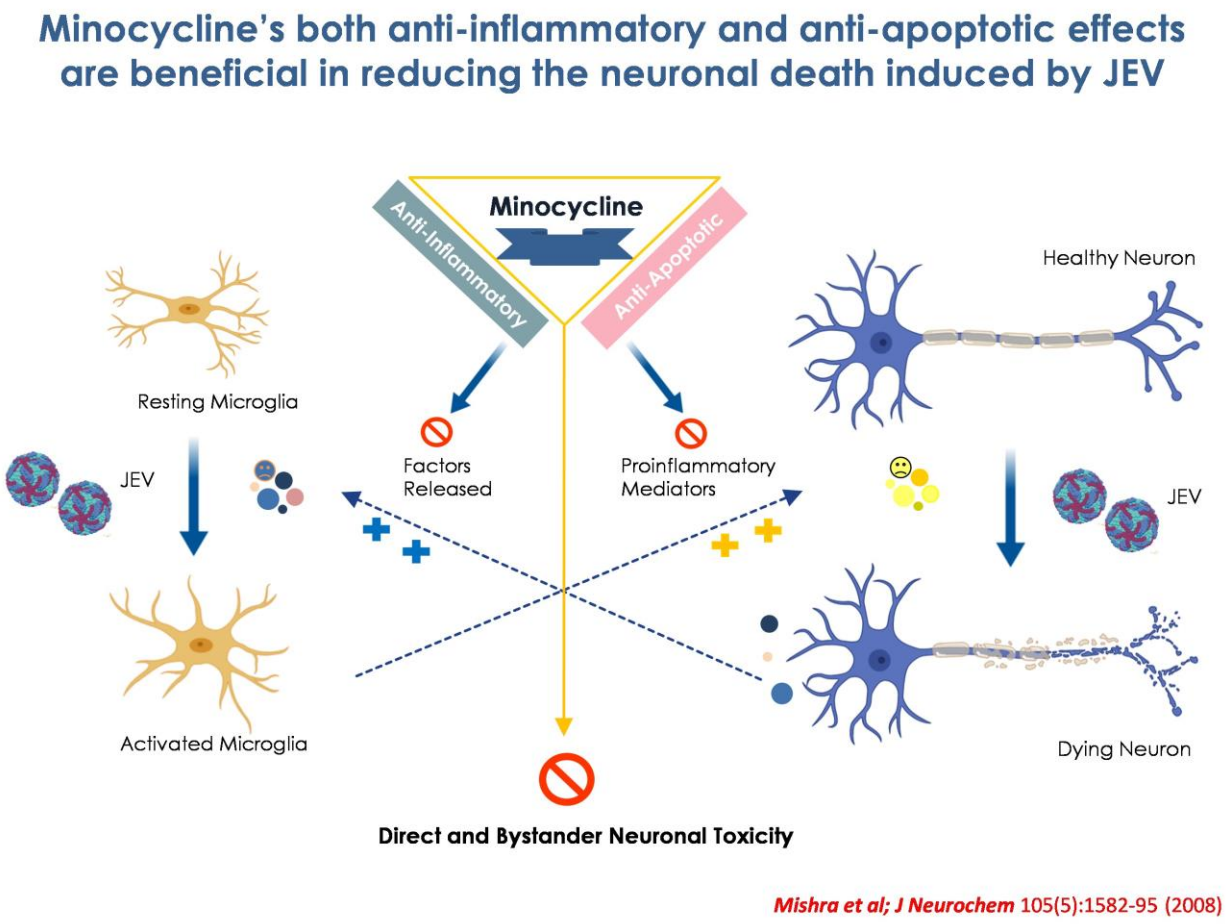

Figure 1

Absence of safe, efficient as well as cost effective vaccine and anti-viral drug prompts us to explore the potential of neuroprotective and/or anti –inflammatory/and or anti-viral compounds as a therapeutic strategy for JE. By exploring the pathways which are involved in inflammation, Anirban's laboratory has identified several anti-inflammatory compounds with therapeutic potential in an experimental model of JE. One of these compounds is Minocycline. Minocycline is an approved drug with a long standing record of acceptable safety and has a similar spectrum to Doxycycline, both for bacterial infections as well as for Rickettsia.

Based upon pre-clinical study undertaken in Anirban's laboratory at NBRC, few years back a Phase II clinical trial has been conducted at King George Medical University (KGMU), Lucknow, where minocycline has been used as a therapy for JE patients and the patients with

Acute Encephalitis Syndrome (AES). It has been observed in this trial that Minocycline has some beneficial effect in patients especially in those patients who survive the initial days in hospital. These findings could form the basis for planning a larger study and possibly including minocycline in the management of AES and JE. Although clinical research in JE had been actively pursued by different investigators in India prior to Anirban, it was the neuro-immunological approach that their group has introduced that gave an altogether new dimension to this field. (3,4)

More recently, Anirban and his associates have shown the therapeutic potential of AMG487, an antagonist of CXCR3, in Dengue virus (DV) as well as in JEV infection. They have reported the crucial role platelet cytokine PF4 plays in enhancing replication and propagation of both DV and JEV in host cells including monocytes by inhibiting IFN response of these immune cells. Definitely the PF4-CXCR3-IFN axis acts as a potential target for developing treatment regimens against viruses including DV and JEV. (15)

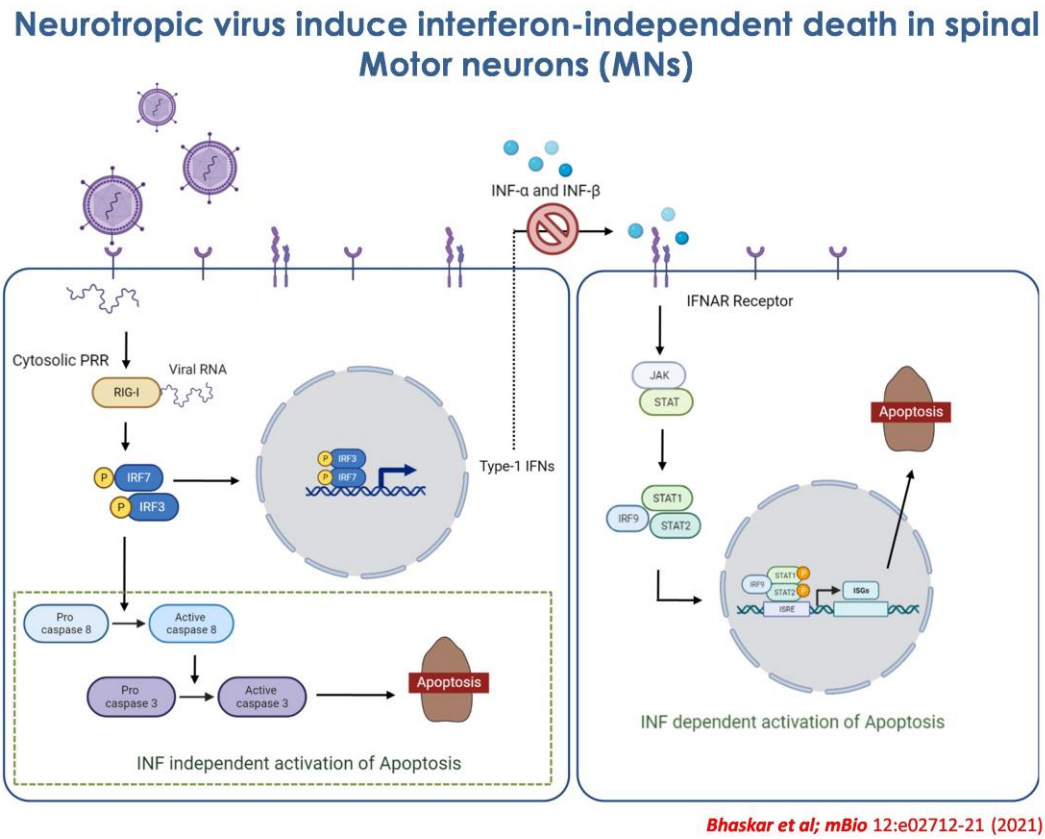

Figure 2:

Besides identifying repurposed drug for new use in viral diseases, Anirban's group is also keen to develop newer generation of therapy for JE and other viral encephalitis. They have shown that administration of Vivo-Morpholino effectively resulted in increased survival of animals and neuroprotection in a murine model of JE. Vivo-Morpholinos are also cost effective, non-immunogenic, and stable under physiological conditions as compared to other types of Morpholinos. Hence, these oligomers represent a potential antiviral agent that merits further evaluation. In a recently published work, they have elucidated the mechanism responsible for limb paralysis by studying clinical isolates of JEV and Chandipura virus (CHPV) causing clinical-AFP (Acute flaccid paralysis) in vast region of south-east Asia including Indian subcontinent. During his tenure as an independent investigator at NBRC, Anirban has successfully built up a strong research group in the area of drug discovery and molecular medicine particularly encompassing Viral infection of brain. (16, 17)

References:

1. D Ghosh and **A Basu** (2009) Japanese Encephalitis - A Pathological and Clinical Perspective PLoS Neglected Tropical Diseases 3(9) e437
2. A Ghoshal, S Das, S Ghosh, M K Mishra, V Sharma, P Koli, E Sen and **A Basu** (2007) Proinflammatory mediators released by activated microglia induces neuronal death in Japanese Encephalitis. ***Glia*** 55:483-496.
3. M K Mishra and **A Basu** (2008) Minocycline neuroprotects, reduces microglial activation, inhibits caspase-3 induction, and viral replication following Japanese Encephalitis. J Neurochem 105(5):1582-95
4. R Kumar, **A Basu**, S Sinha, Das M, Tripathi P, Jain A, Kumar C, Atam V, Khan S, Singh AS (2016) Role of oral Minocycline in acute encephalitis syndrome in India - a randomized controlled trial. ***BMC Infect Dis***. 2016 Feb 4;16(1):67.
5. S Das and **A Basu** (2008) Japanese Encephalitis virus infects neural progenitor cells and decreases their proliferation. ***J Neurochem*** 106(4):1624-36
6. S Das, S Chakraborty and **A Basu** (2010) Critical role of lipid rafts in virus entry and activation of Phosphoinositide 3' Kinase / Akt signaling during early stages of Japanese Encephalitis Virus infection in neural stem/progenitor cells. ***J Neurochem*** 115 (2): 537-549
7. S Das, K Dutta, K L Kumawat, A Ghoshal, D Adhya and **A Basu** (2011) Abrogated Inflammatory Response Promotes Neurogenesis in a Murine Model of Japanese Encephalitis. ***PLoS One*** 6(3); e17225
8. S Mukherjee, N Singh, N Sengupta, M Fatima, P Seth, A Mahadevan, S K Shankar, A Bhattacharyya, **A Basu** (2017) Japanese encephalitis virus induces human neural stem/progenitor cell death by elevating GRP78, PHB and hnRNPC through ER stress. ***Cell Death & Disease*****;** 8(1):e2556

9. S Mukherjee, I Akbar, R Bhagat, B Hazra, A Bhattacharyya, P Seth, D Roy, **A Basu** (2019) Identification and classification of hubs in miRNA target gene networks in human neural stem/progenitor cells following Japanese encephalitis virus infection. ***mSphere*** 4(5). pii: e00588-19
10. I M Ariff, M C Thounajam, S Das and **A Basu** (2013) Japanese Encephalitis virus infection alters both neuronal and astrocytic differentiation of Neural Stem/progenitor Cells. ***J Neuroimmuno Pharmacology***; 8(3):664-76
11. B Hazra, K L Kumawat, **A Basu** (2017) The host microRNA miR-301a blocks the IRF1-mediated neuronal innate immune response to Japanese encephalitis virus infection. ***Science Signaling*** 10(466):eaaf5185. (***Cover page article***)
12. B Hazra, S Chakraborty, M Bhaskar, S Mukherjee, A Mahadevan, **A Basu** (2019) miR-301a regulates inflammatory response to Japanese Encephalitis Virus infection via suppression of NKRF activity. ***Journal of Immunology*** 5;203(8):2222-2238
13. D K Kaushik, R Mukhopadhyay, K L Kumawat, M Gupta and **A Basu** (2012) Therapeutic targeting of Kruppel like factor 4 abrogates microglial activation. ***Journal of Neuroinflammation*** 9(1), 2012:57 (*Recommended article by Faculty of 1000*)
14. D K Kaushik, M Gupta, K L Kumawat and **A Basu** (2012) NLRP3 inflammasome: Key mediator of neuroinflammation in murine Japanese Encephalitis. ***PLoS One*** 7(2); 2012:e32270.
15. A Ojha, A Bhasym[$], S Mukherjee[$], G K Annarapu, T Bhakuni, I Akbar, T Seth, N K. Vikram, S Vrati, **A Basu**[#], S Bhattacharyya, P Guchhait (2019) Platelet factor 4 promotes rapid replication and propagation of Dengue and Japanese encephalitis viruses. ***EBioMedicine*** 39:332-347
16. A Nazmi, K Dutta and **A Basu** (2010) Antiviral and Neuroprotective Role of Octaguanidinium Dendrimer-Conjugated Morpholino Oligomers in Japanese Encephalitis. ***PLoS Neglected Tropical Diseases*** 4(11) e892
17. M Bhaskar, S Mukherjee, and **A Basu** (2021) Involvement of RIG-I Pathway in Neurotropic Virus-Induced Acute Flaccid Paralysis and Subsequent Spinal Motor Neuron Death. ***mBio*** 12(6):e0271221

ANIRBAN BASU;

Dated: 12th July