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Inflammation in embryology: A review of neuroinflammation in spina bifida

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Abstract: The occurrence of neuroinflammation after the failure of neural tube closure, resulting in spina bifida aperta, is well established but whether or not neuroinflammation contributes to damage to the neuroepithelium prior to and during closure is not known. Neuroinflammation may occur at different time periods after perturbation to the developing spinal cord. Evidence suggests that early neuroinflammation is detrimental, whereas the later chronic phase of neuroinflammation may have useful roles. The role of neuroinflammation in neural tube defects is complex. It is important to make the distinction of whether neuroinflammation is important for neuroprotection or detrimental to the neural tissue. This may directly be influenced by the location, magnitude and duration of the insult, as well as the expression of neurotrophic or neurotoxic molecules. The current understanding remains that the chronic damage to the developing spinal cord is likely due to the chemical and mechanical damage of the exposed neural tissue owing to the aggressive intrauterine environment, described as the “two-hit mechanism”. Astrogliosis in the exposed spinal cord has been described in animal models of spina bifida after the failure of closure during embryonic life. Still, its association with neuroinflammatory processes is poorly understood. In this review, we will discuss the current understanding of neuroinflammation in neural tube defects, specifically spina bifida, and highlight inflammation-targeted strategies that may potentially be used to treat this pathophysiological condition.

Keywords: neuroinflammation; spina bifida; neural tube defects; haemangioma; cytokine;

1.0 INTRODUCTION

The most common birth defect of the central nervous system is neural tube defects (NTDs), which occur at a rate of 0.5–10 or more per 1,000 live births worldwide (Copp *et al.,* 2010). The human body employs numerous mechanisms to protect the foetus from the maternal immune system and the tissues involved in immunoregulation at the maternal and foetal interface (Guleria & Sayegh., 2007; Ander *et al.,* 2019). Cytokines play an important role in shaping the complex cellular organisation of the mammalian central nervous system (CNS) by acting as growth and survival factors for CNS-resident cells (Park *et al.,* 2018)**.** Therefore, maternal-foetal tolerance plays a delicate balance between two opposing immunological forces, whereby the semi-allogenic foetus learns to tolerate both self- and maternal-antigens, in which, it develops protective immunity during foetal development (Rackaityte & Halkias., 2020)

1.1 Interdependence between cytokine, neural tube defects and neuroinflammation

The formation of the central nervous system begins with the formation of the neural tube (NT), which is the precursor of the brain and spinal cord. This is a complex process that begins with a flat sheet of cells that undergoes sequential thickening, elevation, mediolateral convergence, rostro-caudal extension, and finally adhesion to form the neural tube. Disturbance of this sequence of events, can lead to neural tube defects (NTDs) which are common congenital malformations. NTDs are classified into two different types, open-lesion NTDs known as spina bifida (SB) aperta and closed-lesion NTDs such as spina bifida (SB) occulta (Mohd-Zin *et al.,* 2017). A pertinent question is whether a perturbed immune response may contribute to neurological deficits seen in spina bifida occulta (closed-defects). The cellular and molecular mechanisms underlying the initiation of inflammation as well as other pathophysiological disorder that occur in response to injury, are not fully understood.

In wild-type embryos, the onset of neuronal differentiation is closely linked with the timing of completion of cranial neural tube closure, which occurs at E9.5. Early neuronal cells can be detected by expression of β-tubulin type III (TuJ1; encoded by *Tubb3*), beginning in the midbrain neuroepithelium at the 13- to 15-somites stages, during the latter few hours of closure in this region. This is then followed by an increase in neuronal differentiation with completion of cranial neural tube closure which is achieved by the 16-somite stage in a $Pax^{Sp2H}(Sp^{2H};splotch)$ mice which provides a model to investigate the cause of NTDs (Sudiwala *et al.,* 2019).

Glial cells, which include astrocytes and microglia, play an important role in response to any CNS injury or disease (Kempuraj et al., 2017). Astrogliosis has previously been described in animal models with spina bifida at E16.5 to E18. It is not known if these cells affect the impaired neural tube, irrespective of whether it is the aperta-variety or the multitude of closed spinal dysraphism which encompass lipomyelomeningocele (which may sometimes be referred to as spina bifida occulta)(Mohd-Zin et al., 2017). These cells generate a variety of neurotrophic factors as well as cell-surface mediators with anti-inflammatory properties. On the other hand, inflammation in tissue can result in the production of neurotoxic factors such as cytokines and interleukins, which worsens the diseased state. Prolonged and chronic inflammatory responses can be harmful, affecting immune cells, CNS-resident cells, and signalling molecules (Kempuraj et al., 2017). Inflammatory stimuli produced by stressed or damaged neurons can activate glial cells (Reemst et al., 2016). Normal rat astrogenesis begins at E18 and continues postnatally until day 7 (Reemst et al., 2016). Reactive astrogliosis **(Figure 1)** is not only a marker for neuropathology, but it also plays an important role in tissue repair by regulating neuroinflammation and repair (Oria et al., 2018).

Spina bifida is a congenital malformation characterised by failure of neural tube closure, leaving the foetal spinal cord unprotected. The foetal spinal cord is exposed to the enzymatic action of the amniotic fluid in spina bifida. Both chemical and physical erosion cause neural tissue loss, aggravating the primary lesion and altering the cytoarchitecture of the spinal cord which are not seen in healthy pregnancies. This pro-inflammatory environment dominates the first 12 weeks of pregnancy. During the next 15 weeks, the developing foetus experiences rapid growth and development. Anti-inflammatory cells and molecules are in charge (Aghaeepour *et al.,* 2017) whereas systemic maternal immune response can initiate as early as E8.5 which can lead to neural tube defects, including exencephaly in mice. Animal models show that a systemic maternal immune response is sufficient to cause neural tube defects, but the exact mechanisms and contributions of cytokines, oxidative damage, and nutrient imbalances to these phenotypes remain unknown (Yockey & Iwasaki., 2018). Reactive astrocytes proliferate and invade neural tissue to protect it from the aggressive intrauterine environment. Microglia

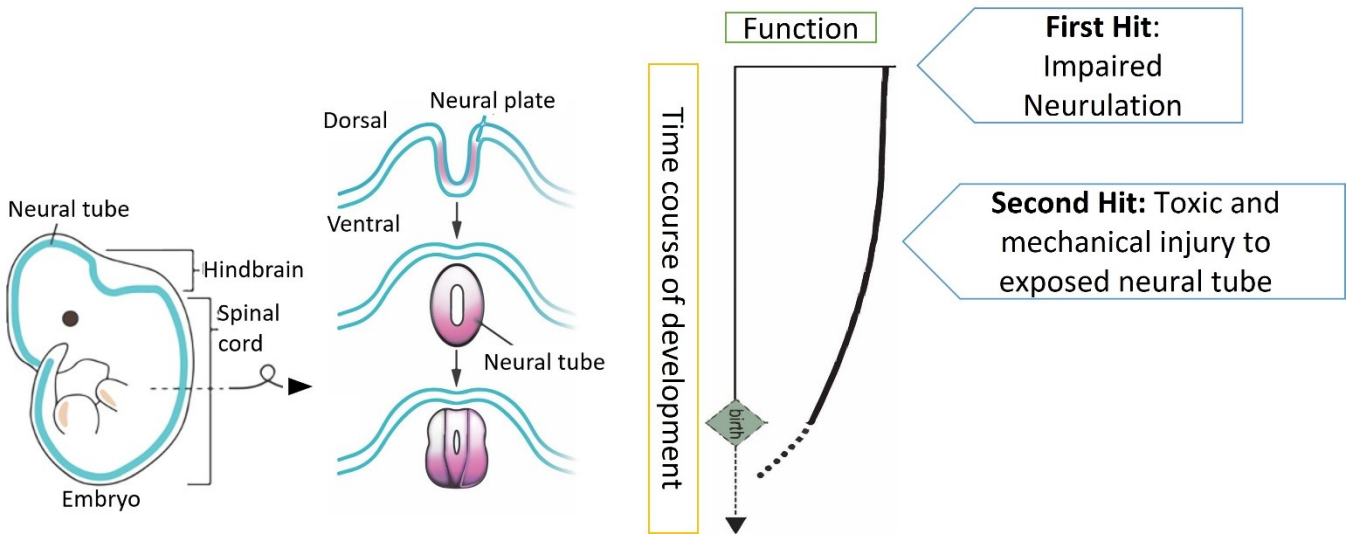

Figure 1: "Two-hit" mechanism of consecutive damaging process in neural placodes. The "first hit" denotes structural defects resulting from incomplete neurulation of the respective spinal cord segment. Subsequently, chronic exposure of the spinal cord to the aggressive intrauterine environment throughout gestation which represents as second hit. This results in an acquired injury to the exposed segment of the spinal cord.

migrate and interact with astrocytes, resulting in an inflammatory response to injury (Oria *et al.,* 2018). Microglia are CNS resident macrophages that maintain homeostasis for normal function during foetal development, modulate synaptic transmission, and promote angiogenesis (Oria *et al.,* 2018; Reemst *et al.,* 2016). The dysfunction of microglia and astrocytes during brain development contributes to neural degeneration in the injured tissues, which increased MHCII expression and the production of pro-inflammatory cytokines (IL1β, IL6, and IFNγ) as was described in Oria *et al.,* (2018). Chronic microglial activation is an important element in neurodegenerative diseases and the chronic neuroinflammatory response most likely contributes to the pathogenesis of neurological deficit in spina bifida.

IL-1 family cytokines include pro-inflammatory cytokines IL-1, IL-18, IL-33, and IL-36, as well as anti-inflammatory cytokines IL-1 receptor antagonist (IL-1Ra), IL-36Ra, IL-37, and IL-38 (Rudloff et al., 2020). The production of IL-1b contributes to the initiation of inflammatory signal 1. The inflammatory signal 1 initiates transcription and the production of pro-IL-1beta and pro-IL-18 precursors. Cytokine signalling, activation of innate pattern-recognition receptors (PRR) by pathogen-associated molecular patterns (PAMP), such as lipopolysaccharides (LPS), are examples of signal 1 events (Rudloff *et al.,* 2020).

In several mouse models of inflammatory diseases, IL-37, a member of the IL-1 family, has broad anti-inflammatory effects. Studies have shown that IL-37 reduces inflammation and protects against neurological deficits and myelin loss in experimental autoimmune encephalomyelitis (EAE) mice (Sánchez-Fernández *et al.,* 2021). As a result, IL-37 may represent a novel therapeutic avenue for treatment with great promising potential. In its precursor protein form, IL-37 has a caspase-1 cleavage site similar to IL-1b and IL-18. However, IL-37 can be secreted even when caspase-1 is not activated. In a Smad3-dependent manner, intracellular IL-37 can translocate into the nucleus and inhibit inflammation (Zhao et al., 2018; Rudloff et al., 2020). Furthermore, IL-37 binds to its receptor complex, including IL-18Ra and IL-1R8, activating various anti-inflammatory pathways (Zhang et al., 2017). Inflammasome inhibition is one of the pathways IL-37 uses to mediate its anti-inflammatory properties.

Multiple molecules involved in the inflammatory pathway, such as IL-6, TNF-alpha, IL-1 beta, and others, have been linked to neuroinflammation in neural tube defects models **(Table 1)**. Some of these chemokine/ cytokines/ molecules are also implicated in primary neurulation, whose failure results in open spina bifida. The exposed tissue may then be subject to damage. Hence, certain molecules may be involved in both 'hits' of the "two-hit" mechanism that has been proposed to cause physical and chemical perturbation to the developing brain and spinal cord if left exposed to the

uterine environment **(Figure 2)** (Janik *et al.,* 2020). Anti-inflammatory cytokines regulate macrophage activation, differentiation, and incur macrophage polarisation. However, the potential of inflammation causing denigration to the developing spinal cord and brain before primary neurulation ends (E10.5) has never been studied although we know that inflammation does indeed occur in a model of closed spina bifida such as the *EphA2*$^{+/tm1Jrui}$*Epha4*$^{+/rb-2j}$ double heterozygous mice (E11.5). Individual mouse knockouts of *EphA2* and *EphA4* do not exhibit neural tube defects (NTDs) per se. The embryos generated as double heterozygotes *EphA2*$^{tm1Jrui/+}$*EphA4*$^{rb-2j/+}$ results in NTDs of varying severity, such as close exencephaly and close spina bifida (spina bifida occulta) (Abdullah *et al*. 2017).

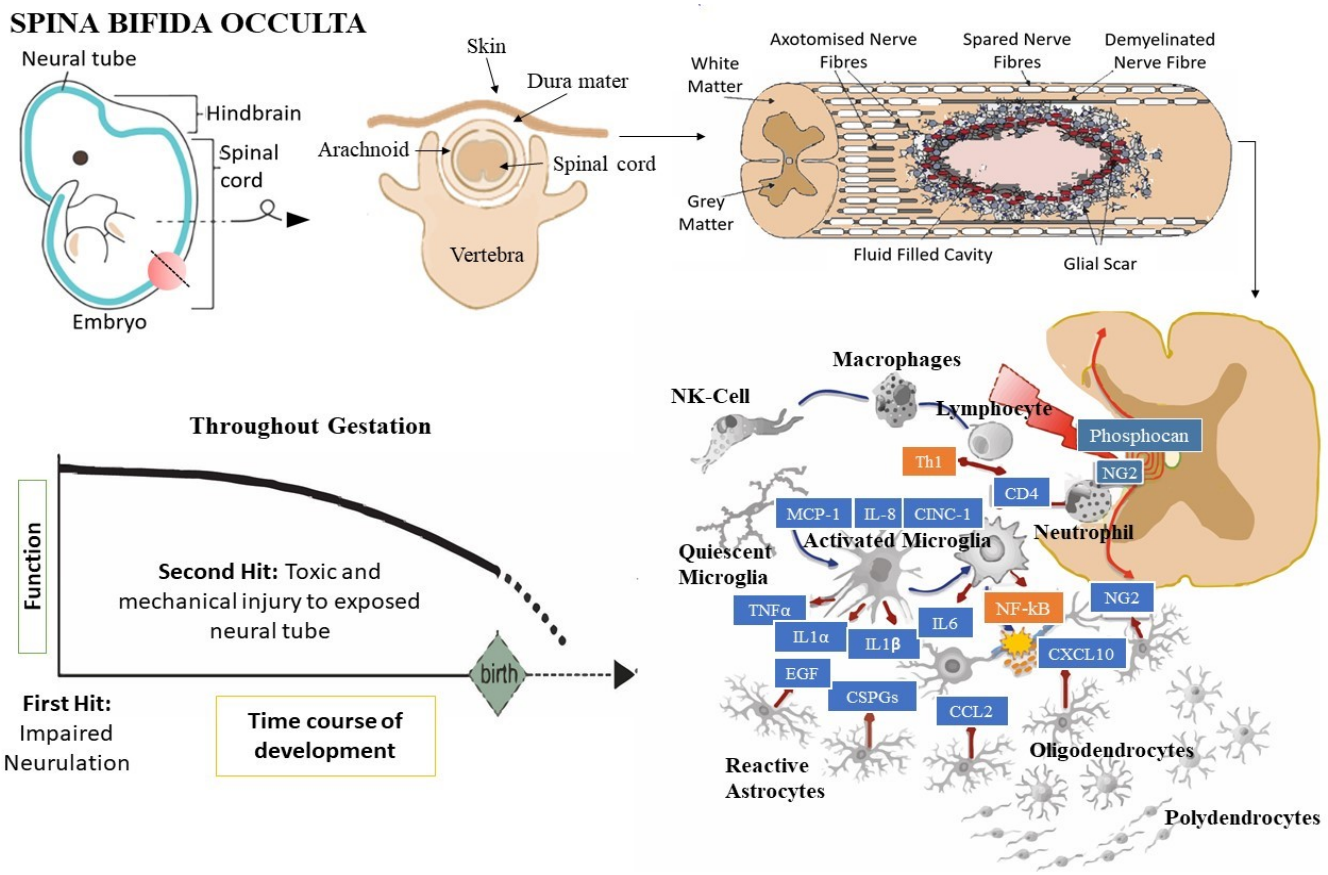

Figure 2: The development of spinal cord disruption is followed by a cascade of biochemical events (secondary injury) that are thought to enlarge the area of cell death via necrosis and apoptosis in spina bifida occulta (closed spina bifida). Mechanical impact on cord tissue, along with blood vessel rupture and extravasation of peripheral blood elements, introduces tumour necrosis factor alpha (TNF) and other inflammatory molecules into the lesion. Neuroinflammation-related damage is also seen in spina bifida occulta (closed spina bifida), as opposed to the 'two-hit' mechanism seen in spina bifida aperta.

This review gives the understanding that inflammation-related genes do contribute to both aperta and occulta type NTDs, cutting across the spinal dysraphism spectrum (Netto *et al.,* 2009; Mohd-Zin *et al.,* 2017). This however, does not mean that inflammation-related genes are solely to blame for NTDs. Still, they contribute to increased inflammatory cytokine expression due to a cascade of biochemical and physical events that are thought to further damage the neural tissue **(Figure 2)**.

Epigenetic modifications in human NTDs have been indicated previously by past studies. Wan *et al.,* (2018) reported the occurrence of epigenetic modifications of apoptosis-related genes in a retinoic-acid (RA) induced NTD mouse model. Among the 84 key genes involved in programmed cell death, 13 genes, including those for tumour necrosis factor (TNF), annexin alpha 5, apoptosis inhibitor 5, bcl2-associated-anthanogene 3, baculoviral IAP repeat containing 3, caspase 12, casp4,

casp8, TNF-1α, TNF superfamily (Tnfs)f10 and Tnfsf12 were downregulated. Furthermore, the genes reviewed **(Table 1)** are associated with inflammation in the respective knockouts.

Apaf1's (Apoptosis protease activating factor 1) role in T-cells during antigen-induced immune response was investigated (Tong *et al.,* 2018). The recruitment of T cells into tissues to be activated by antigen-presenting cells to produce cytokines that mediate local inflammation is referred to as delayed-type hypersensitivity (DTH). DTH responses were dramatically exacerbated in Lck-Cre-Apaf1f/f-OTII mice, including footpad swelling, cell infiltration, and inflammatory cytokine production. TNF- and IL-6 levels in supernatants of alveolar type I (AT-I) epithelial cells treated with LPS were significantly reduced by Axin-1 knockdown. Using CRISPR/Cas9 technology to disrupt the interaction between Caveolin-1 and Axin-1 resulted in a significant increase in TNF- and IL-6 production from AT-I cells, as well as a significant decrease in -catenin expression (Zhang *et al.,* 2019). The NF-kappaB transcription factor family is activated in response to various stimuli, including pro-inflammatory cytokines, environmental stresses, and antigenic stimulation in B and T lymphocytes (Zhang *et al.,* 2019). Bcl10 is required for T- and B-cell receptor activation of NF-kappaB. T and B lymphocytes from Bcl10-deficient mice cannot proliferate because they fail to activate NF-kappaB in response to antigen-receptor stimulation (Zhou *et al.,* 2004).

Bardet-Biedl syndrome (BBS) is a rare hereditary autosomal recessive disease characterised by obesity, hypertension, and renal abnormalities. Obese hypertensive Bbs4$^{-/-}$ mice developed inflammatory infiltration and renal cysts, whereas obese normotensive Bbs2$^{-/-}$ mice developed only minor inflammatory infiltration (Guo *et al.,* 2011). Caspase-3 is thought to play a role in disuse muscle atrophy (DMA) and associated decreased tension by acting on the apoptosis and inflammation pathways. On embryonic day 14, Caspase-3 deficiency displayed significantly reduced muscle mass loss and gastrocnemius twitch tension. Macrophages are distributed strategically in mammalian tissues and play an important role in priming the immune response. To avoid a futile inflammatory response, macrophages must constantly strike a balance between activation and inhibition states (Zhu *et al.,* 2013). Cited2-deficiency significantly increased proinflammatory gene expression in macrophages by stabilising the hypoxia-inducible factor 1 alpha (HIF1) protein (Kim *et al.,* 2018). Cre-Pax3 (P3Pro-Cre) conditional gene deletion and/or lineage tracing in the neural crest, neural tube, metanephric mesenchyme, and ureteric mesenchyme derivatives have been performed using transgenic mice (Zhao *et al.,* 2014). Histology examination reveals typical muscular dystrophy tissue alterations only in mutant caudal muscles. Mutant caudal muscles were found to be inflamed (Zhao *et al.,* 2014).

Survivin, a member of the apoptosis inhibitor family, has been proposed as a key intermediate in the signalling pathways that lead to T-cell development, proliferation, and expansion. OX40-deficient/survivin transgenic mice exhibited normal Th2 responses as well as significant lung inflammation. These findings suggest that OX40 co-stimulation is critical for activating *survivin* during antigen-mediated Th2 responses. These findings also support the idea that OX40 co-stimulation regulates allergic responses or lung inflammation by targeting *survivin*, resulting in increased T-cell proliferation and more differentiated Th2 cells in the allergic inflammatory response (Lei *et al.,* 2013). The bone morphogenetic protein (BMP)-SMAD signalling pathway is a key transcriptional regulator of hepcidin in response to tissue iron stores, serum iron, erythropoiesis, and inflammation to increase iron supply when needed for erythropoiesis while preventing iron toxicity. Chronic dietary iron loading, which increased liver iron, induced BMP2 expression, whereas acute oral iron gavage, which increased serum iron but had no effect on liver iron, had no effect on BMP2. However, hepcidin was still induced by both iron loading methods in the Bmp2 conditional knockout (CKO) mice, albeit to a lesser extent than in control mice. On the other hand, acute oral iron gavage failed to induce hepcidin in Bmp6 CKO mice. Thus, BMP2 plays a partially redundant role in regulating hepcidin by serum iron, tissue iron, inflammation, and erythropoietic drive (Wang *et al.,* 2019).

There is mounting evidence that the highly conserved serine/threonine kinase CK2 promotes Th17 cell differentiation. CK2 deficiency (CK2α$^{-/-}$) resulted in a significant decrease in CK2 kinase activity overall. Furthermore, in the context of autoimmune neuroinflammation, CK2 deficiency resulted in a significant defect in Th17 cell polarisation and a reciprocal increase in regulatory T cells (Tregs) both in vitro and in vivo (Gibson *et al.,* 2018). Systemic iron requirements are primarily met by macrophages recycling iron from senescent erythrocytes, a process in which the iron exporter ferroportin (Fpn1) is thought to be essential. Macrophage Fpn1 deletion mice were ostensibly normal; however, when fed a standard diet,

Table 1: Genes involved in mouse mutants of spina bifida or exencephaly and the involvement in inflammatory response.

Type of NTD	Gene	Function of Gene	Gene expression in neural folds	Other defects and structures affected	Involvement in Inflammatory Response	References
Exencephaly	*Apaf1* (Apoptosis protease activating factor 1)	Apoptosis	-	Severe craniofacial malformation, brain hyperplasia, alteration of limb, ear and eye structure	Higher production of inflammatory cytokine in Lck-*Cre-Apaf1*$^{f/f}$ mice	Cecconi *et al.,* (2008); Tong *et al.,* (2018)
Exencephaly or Spina Bifida Aperta	*Axin1* (Axis inhibitor 1)	Signalling (*Wnt* pathway)	GD 7.5-9.5	Duplication of caudal neural tube, vertebral, hindlimb, kidney, bladder, inner ear	Involved in inflammatory response in AT-1 cells induced by LPS	Zhang *et al.,* (2019); Zeng *et al.,* (1997)
Hindbrain Exencephaly	*Bcl10* (B-cell lymphoma/leukemia 10)	Apoptosis	-	Non-EX are immunodeficient	Essential for NF-kappaB activation by T- and B-cell receptors	Ruland *et al.,* (2001); Zhou *et al.,* (2004)
Exencephaly	*Bbs4* (Bardet-Beidl syndrome 4)	Cilia	-	Olfactory cilia	*Bbs4*$^{-/-}$ mice exhibited inflammatory infiltration and renal cysts	Ross *et al.,* (2005); Guo *et al.,* (2011)
Split face + rostral exencephaly or hindbrain exencephaly	*Casp 3* (Caspase 3)	Apoptosis	GD 8.5-9; forebrain, neuroepithelium	Retina	*Casp-3* knock out (KO) mice shows immobilization-induced inflammation was attenuated by the absence of caspase-3	Houde *et al.,* (2004); Zhu *et al.,* (2013)
Hindbrain Exencephaly	*Casp 9* (Caspase 9)	Apoptosis	-	Forebrain protrusion	-	Hakem *et al.,* (1998)
Spina Bifida or Exencephaly	*Cited 2* (Cbp/P300 Interacting Transactivator with Glu/Asp Rich Carboxy Terminal Domain 2)	Transcription	GD 8.5; cranial neuroectoderm and mesenchyme	Heart, adrenal, cranial, ganglia	Deficiency of *cited2* resulted in heightened pro-inflammatory gene expression	Bamforth *et al.,* (2001); Barbera *et al.,* (2002); Kim *et al.,* (2018)
Exencephaly or Spina Bifida Aperta	*Pax3* (Paired box 3)	Wnt/β-catenin signalling	GD 9.5; caudal neural tube closure	-	Inflammation in mutant caudal muscle	Zhao *et al.,* (2014); Jarad & Miner (2009)
Exencephaly or Spina Bifida	*Ambra1* (Autophagy And Beclin 1 Regulator 1)	Autophagy in influencing embryogenesis	-	-	-	Cecconi *et al.,* (2009)

Exencephaly	*Birc5(survivin)* (Baculovirol IAP Repeat Containing 5)	Anti-apoptotic molecule from inhibitor of apoptosis protein (IAP) family; Transcription	Diffuse haemorrhage at GD 9.5	Abnormal generation of the heart during gestation, diffuse haemorrhage. Abnormal EMT causing hypoplastic endocardial cushions and in utero heart failure; Mutant die at E13.5	*Survivin* transgenic mice exhibit an increased antigen-driven Th2 lung inflammation and that constitutive expression of *survivin* reversed the defective lung inflammation even in the absence of OX40 co-stimulation	Assadiasl *et al.,* (2018); Zwerts *et al.,* (2007); Lei *et al.,* (2013)
Exencephaly	*Bmp2* (Bone morphogenetic protein 2)	Transcriptional regulator of hepcidin; BMP2 as a unique player in the developing NT for dorsal patterning and identity and normal cephalic neural tube closure	GD 8, cranial neuroepithelium	-	*BMP2* has at least a partially redundant role in hepcidin regulation by serum iron, tissue iron, inflammation and erythropoietic drive	Wang *et al.,* (2019);
Exencephaly	*C2cd3* (C2 Domain Containing 3 Centriole Elongation Regulator)	Cilia formation; Hedgehog signalling in mouse	GD 8.5 – 10.5	Heart laterality defects	-	Hoover *et al.,* (2008)
Exencephaly	*Csnk2a1* (Casein Kinase 2 Subunit Alpha 1)	Serine/threonine kinase, regulator of Wnt signalling	GD 8.5	Homozygous null *CK2α*$^{-/-}$mice male mice are infertile, mice lacking CK2α die in mid-gestation, Structural defects in the heart, craniorachischisis	*CK2α* deficiency resulted in a significant defect in Th17 cell polarization and increase in Tregs both in vitro and in vivo in the context of autoimmune neuroinflammation.	Lou *et al.,* (2008); Gibson *et al.,* (2018)
Exencephaly	*Fpn1* (Ferroportin 1)	Iron transport	GD 8.5 – 10.5, essential for delivery of nutrients to the embryo	*Fpn1*^{ffe} is a model of the iron overload disorder Hemochromatosis Type IV (HFE4)	Hepcidin expression is induced during inflammation, commonly by bacterial products acting through Toll-like receptors. Inflammation can also affect transcription of Fpn.	Stokes *et al.,* (2017); Ward & Kaplan (2012); Zhang *et al.,* (2011)
Exencephaly	*Fuz* (Fuzzy)	PCP pathway, ciliogenesis	-	Ocular defects, polydactyly on all limbs, skeletal development defects and organogenesis, malformed sternum, ribs and long bones and hypoplastic lungs	-	Gray *et al.,* (2009)

Exencephaly or Spina Bifida Aperta	*F2r (Par1)* (Coagulation Factor 2 Thrombin Receptor)		GD 8.5 – 9.5, *Fr2* in surface ectoderm adjacent to neuroepithelium at tips of closing neural folds	Heart defects, curly tail	Activation of PAR2 produces microvascular inflammation by rapid induction of P-selectin-mediated leukocyte rolling. In the absence of PAR2, the onset of inflammation is delayed.	Camerer *et al.,* (2010); Lindner *et al.,* (2000)
Exencephaly or Spina Bifida Aperta	*Tulp3 (hhkr mutant)* (TUBBY-Like Protein 3 – Hitchhiker mutants)	Shh signalling pathway	-	Mutants exhibited oedema	-	Patterson *et al.,* (2009)
Exencephaly or Spina Bifida Aperta	*Fzd6/Fzd3* (Frizzled 6/ Frizzled 3)	Wnt signalling	-	Focal disruption of the ventricular basal lamina, $Fzd3^{-/-}$; $Fzd6^{+/-}$ mutants dissected at E10.5 and E12.5 had a curly tail and mid-/hindbrain bulge in the double mutant embryo	-	Stuebner *et al.,* (2010)
Exencephaly or Spina Bifida Aperta	$Rac1^{-/-}Grhl3^{Cre/+}$ (Rac Family Small GTPase 1/ Grainyhead-like Protein 3)	Disruption of GPCR links, cell adhesion	-	-	$Grhl1^{-/-}$ mice induces tumour-promoting mild chronic inflammatory microenvironment in the skin	Camerer *et al.,* (2010); Kikulska *et al.,* (2018)
Exencephaly	*Gnb1 (Gβ1)* (Guanine nucleotide-binding Protein Subunit Beta 1)	GPCR pathway	GD 8.5, Cranial neuroepithelium	-	NLRP3 inflammasome was enhanced in GNB1-knockdown or GNB1-deficient murine macrophages	Okae & Iwakura., (2010); Murakami *et al.,* (2019)
Exencephaly	*Intu* (Inturned Planar Cell Polarity Protein)	PCP pathway	GD 8.5-9.5	-	-	Zeng *et al.,* (2010)
Exencephaly	*Pip5klc* (Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma)	Inositol metabolism, actin dynamics	-	Growth defects and heart retardation	-	Wang *et al.,* (2007)

they developed mild anaemia and iron accumulation in splenic, hepatic, and bone marrow macrophages. When Fpn1(LysM/LysM) mice were fed an iron-deficient diet, they developed severe anaemia as well as significantly higher splenic iron levels than control mice, indicating significantly impaired iron mobilisation from macrophages. M1 macrophages are known to be able to take up and release iron when challenged with a high concentration of the metal, confirming M2 macrophages ability to recycle iron (Corna *et al.,* 2010). Iron status can influence immune responses, TNF- and IL-6 expression levels were significantly increased in Fpn1(LysM/LysM) macrophages lacking Fpn1 (Zhang *et al.,* 2011). Mast cell-derived histamine and thrombin binding to protease-activated receptor-1 can induce endothelial surface expression of P-selectin and subsequent leukocyte rolling in venules (PAR1). The defect in leukocyte rolling in PAR2-deficient mice did not last more than 30 minutes after surgical trauma. These findings suggest that PAR2 activation causes microvascular inflammation through the rapid induction of P-selectin-mediated leukocyte rolling. The onset of inflammation is delayed in the absence of PAR2 (Lindner *et al.,* 2000).

All of the GRHLs have been linked to various cancers. Grainyhead-like (Grhl) is a conserved transcription factor (TF) that regulates epithelial differentiation and regeneration (Yao *et al.,* 2017). The underlying molecular mechanism in Grhl1$^{-/-}$ mice involves aberrant keratinocyte terminal differentiation and subacute skin barrier defects, increasing the risk of skin cancer by inducing a tumour-promoting mild chronic inflammatory microenvironment in the skin. The NLRP3 inflammasome plays a critical role in the pathogenesis of a variety of inflammatory diseases. G protein subunit 1 (GNB1) is a downstream molecule of G protein-coupled receptors (GPCRs) that regulates the activation of the NLRP3 inflammasome. GNB1 was physically linked to NLRP3 via NLRP3's pyrin domain. Activation of the NLRP3 inflammasome was increased in GNB1-knockdown or GNB1-deficient murine macrophages, whereas GNB1 deficiency had no effect on AIM2 inflammasome activation (Murakami *et al.,* 2019).

The role of tumour necrosis factor receptor-associated factor (*traf4*) has not yet been fully established in understanding the pathogenesis of NTDs during embryogenesis. Research done by Régnier *et al. (2002)* had generated a *traf4-deficient* mouse by gene disruption, which presents multiple developmental abnormalities. The surviving mutant mice exhibited a high incidence of spina bifida, a defect linked to NTDs that are a common congenital malformation in humans. Additional malformation concerns mainly the axial skeleton as the ribs, sternum and vertebral arches are affected thus leading to a respiratory disorder, and wheezing caused by tracheal ring disruption. It was postulated that there is a normal expression pattern of traf4 in neural tissue and also participates in neurulation in vivo (Régnier *et al.,* 2002).

The morphological and histological processes of murine neural tube maldevelopment (days 8–10) have not yet been closely examined in the majority of mutants and affected strains. The gene ‘‘knockout" mutations that cause NTDs, inactivate genes for proteins whose cellular biochemical functions are at least partially known. The role of these biochemical functions (inflammation) in neural tube closure is unknown. However, for some mutants and strains, a portion of the sequence of the morphological or biochemical event had been observed, each of which sheds light on one or more aspects of the mechanisms that lead to NTDs. **Table 1** lists mouse mutants and strains with exencephaly or spina bifida aperta (or both) that are developmentally and morphologically similar to the common human NTDs, anencephaly and spina bifida and are also associated with inflammation.

Genes that caused the failure of neural tube closure may also be involved in inflammation which causes further damage to the neural tube. It is clear that the intricate embryonic development that involves more than 300 genes previously identified as crucial for neural tube closure is a highly complex process and many of the associated mechanisms have yet to be elucidated (Wilde *et al.,* 2014). Therefore, the various genes studies involved in mouse mutants of spina bifida or exencephaly and the involvement of neuroinflammation are collectively a valuable resource for understanding human spina bifida **(Figure 1)**.

Several mutations involved in intercellular and intracellular signalling pathways are known to be involved in transcription-associated mechanisms. All the mouse mutants and strains with exencephaly and spina bifida have been examined developmentally and their involvement in inflammatory responses have been established (*Apaf1, Axin1, Bcl10, Bbs4, Casp3, Casp9, Cited2, Pax3, Ambra1, Birc5, Bmp2, C2cd3, Csnk2a1, Fpn1, Fuz, F2r, Tulp3, Fzd6/Fzd3, Rac1/Grlh3, Gnb1, Intu, Pip5klc*; references in **Table 1**). As a result, genes involved in failed neural tube closure may also be involved in neuroinflammation, which leads to later damage **(Figures 1 & 2)**.

1.2 Intervention and rescue of inflammation-based neural tube defects

In utero surgery has been chosen as the intervention of choice to repair neural tissue destruction during pregnancy. Neural tissue destruction is the primary cause of the functional loss of neurological function. According to the proposed "two-hit" mechanism, exposing the neural tissues of the spinal cord to amniotic fluid gradually destroys the neural tissue during pregnancy. The timely *in utero* repair of open spina bifida might rescue neurological function greatly although not fully (Janik *et al.,* 2020*;* Meuli *et al.,* 1995). This review paper shows the uniqueness of occurrence of chronic neuroinflammation-associated damage to the neural tissue leads to the worsening of neurological function irrespective of spina bifida occulta (closed spina bifida) or spina bifida aperta (open spina bifida) as deficit associated with spina bifida aperta are not caused entirely by defect in primary neurulation but are worsened by *in utero* spinal cord injury during pregnancy as opposed to spina bifida occulta (closed version spina bifida with skin covering) **(Figure 2)**.

To explain the pathogenesis of developing spinal cord insult and neurological deficits in myelomeningocele, the "two-hit" mechanism has been proposed (MMC). The first hit is impaired neurulation, which is the incomplete closure of the developing neural tube during early gestation. This causes spinal cord malformation and protrusion through the vertebral opening, musculature, and skin. The second hit is the chronic exposure of the spinal cord to the intrauterine environment throughout pregnancy. As a result, the exposed spinal cord sustains an acquired injury (Janik et al., 2020). This lesion-amplifying cascade can result in a continuous decline of neurological function.

In the one and only spina bifida occulta mouse model arising from failure of primary neurulation, Ephs and ephrins play a critical role. Eph receptor tyrosine kinase members have previously been linked to cranial neural tube development (Abdullah *et al.* 2017). *EphA2* and *EphA4* are found at the tips of closing spinal neural folds prior to or during neural tube closure during the final stage of primary neurulation, adhesion and fusion. Embryos produced by crossing double heterozygous $EphA2^{tm1Jrui/+}EphA4^{rb\text{-}2j/+}$ have NTD with a high degree of severity, including close exencephaly and close spina bifida (spina bifida occulta). The phenotypes seen in $EphA2^{tm1Jrui/+}EphA4^{rb\text{-}2j/+}$ suggest that both genes play a compensatory role during neural tube adhesion and fusion (Abdullah *et al.* 2017). Previous research has suggested that exposing neural tissue of the spinal cord to the intrauterine environment affects neurological function, but this state of persistent inflammation was also observed in spina bifida occulta mouse models (spina bifida with skin covering). Therefore, the "two-hit" mechanism does not apply to spina bifida occulta, seeing that occurrence of inflammation is evident even in a close neural tube defect model without any exposure to the uterine environment. Furthermore, in that particular mouse model, inflammation was observed in the developing spinal cord and the brain (Abdullah *et al.,* 2017).

Clinical and experimental evidence suggests that the impairment of spinal and brain development and systemic activation of neuroinflammation, neurodegeneration, and neuronal repair in foetuses with myelomeningocele may influence the progressive impairments of neurological functioning, motor and cognitive function during pregnancy. A systems biology approach to disease analysis may be ideal for identifying common molecular pathways relevant to pathological/pathogenesis mechanisms. Tarui et al. (2017) reported differential gene regulation and diverse active molecular pathways in a transcriptomic analysis of myelomeningocele using human amniotic fluid samples collected between 22 and 27 weeks of gestation. Transcriptomic analysis of human amniotic fluid samples revealed 284 differentially regulated genes. Myelomeningocele is associated with known genes (*PRICKLE2, GLI3, RAB32, HES1, FOLR1*). Novel dysregulated genes in neurodevelopment and neuronal regeneration (up-regulated, *GAP43* and *ZEB1*), as well as axonal growth and guidance, were discovered (down-regulated, *ACAP1*). Pathway research revealed that inflammation plays a significant role in pathology and that Wnt signalling pathways have a broad effect (*Wnt1, Wnt5A, ITPR1*). However, the significance of the pathways that leads to secondary pathology, such as inflammation and neurodegeneration is important for foetal neuropathology which indicates spina bifida aperta (Tarui *et al.,* 2017).

1.3 The role of inflammation in mast cells and haemangioma

Examination of radiological images for 20 cases of lumbosacral infantile hemangioma with spina bifida occulta by Schumacher *et al.,* (2012) revealed that they have a very specific characteristic of the rapid phase of growth in the first 2-6 months of life followed by a plateau phase and natural involution that may leave a characteristic residual tissue. The invasion of the developing neural tube is enabled by the premature separation of the neuroectoderm from the cutaneous

ectoderm. Lipomyelomeningocele associated with a dural defect were caused by a failure of primary neurulation (Schumacher *et al*., 2012). Anomalies in secondary neurulation, which occurs under intact ectoderm, causing lipomyelomeningocele without an associated dural defect were also observed. Studies were done and there does not appear to be any evidence of a specific type of lipoma associated with lumbosacral infantile haemangioma. Lipomyelomeningocele (a lipoma associated with a dural defect) was found in three of the patients, with only one having an intraspinal haemangioma. Therefore, it is difficult to distinguish the occurrence of haemangioma during primary neurulation or secondary neurulation during embryogenesis (Schumacher *et al*., 2012).

Syncytial masses of rapidly dividing capillary endothelial cells with multi-lamination of basal membranes and accumulation of other cellular elements such as mast cells, macrophages, and pericytes characterise the proliferative phase of hemangioma. Endothelial turnover slows during the involuting phase, and the cellular parenchyma is gradually replaced by fibro-fatty tissue. Mast cells secrete a variety of cytokines and growth factors that modulate inflammatory and immune responses. Tumor necrosis factor (TNF), interleukin (IL)-1, IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, vascular endothelial growth factor (VEGF), and transforming growth factor (TGF-31,32) are all produced by mast cells (Tan et al., 2004).

2.0 CONCLUSION

Neural tube defects, are the second most common birth defects. Despite *in utero* surgery as the intervention of choice to repair neural tissue destruction during pregnancy, significant anomalies that have already been established persist throughout pregnancy and after birth. The pathophysiology of complex inflammatory activation is still unknown. Secondary pathologies of foetuses with closed myelomeningocele (with or without lipid) may include neuroinflammation, injuries, and regeneration, which warrant further investigation as potential therapeutic targets. We envisage that the development of targeted therapies to improve the neurological outcomes of spina bifida patients by elucidating the cellular and molecular mechanisms of neuroinflammation events regardless of open or closed myelomeningocele (MMC) is highly warranted.

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