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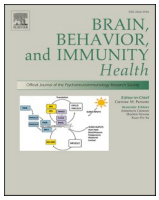

Neurodevelopmental impairment associated with neonatal invasive group B *Streptococcus* disease: Are animal models on track in understanding the mechanisms at play?

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ABSTRACT

Invasive Group B *Streptococcus* (iGBS) disease is a prominent cause of neurodevelopmental impairment (NDI) in neonates. While the clinical manifestation of iGBS disease in neonates may include pneumonia and meningitis, generalised sepsis without focus is the most frequent manifestation of iGBS disease in neonates. Though recent human based studies highlighted meningitis as an important manifestation in infants with NDI following iGBS disease, they also noted that ~18% of neonates present with NDI following iGBS related sepsis. Thus, it is important to not only understand the long-term pathophysiological changes associated with NDI in iGBS meningitis survivors, but so too for iGBS sepsis survivors. Since the late 1970's animal models have been used to unravel the pathophysiology of neonatal iGBS disease. These studies have inoculated neonatal or pregnant animals with GBS via various peripheral or central routes. The greatest challenge with using animal models to study NDI associated with neonatal iGBS disease, is effectively mimicking the clinical presentations of pneumonia, sepsis, and meningitis, while inducing relevant pathophysiological changes and ensuring animals survival, so as to test the neurodevelopment of the animals. This review aims to evaluate the validity of neonatal rodent models, specifically in studying NDI associated with neonatal iGBS disease and explore possible future avenues of research in addressing long-term NDI in the clinical setting.

1. Introduction

Streptococcus agalactiae, commonly known as Group B *Streptococcus* (GBS) is a gram-positive bacterium causing significant global neonatal morbidity and mortality (Gonçalves et al., 2022). Globally in 2020, ~19.7 million pregnant women were estimated to be colonised with GBS with regional differences ranging from ~6.1 million observed in Sub-Saharan Africa to ~120 000 in Oceania (Gonçalves et al., 2022). Neonatal invasive GBS (iGBS) disease is defined as early-onset GBS (EOGBS), with clinical symptoms presenting during 0–7 days of life, and late-onset GBS (LOGBS), with clinical symptoms presenting during 7–90 days of life (Miselli et al., 2022). In 2020, it was estimated that globally ~231 800 neonates had EOGBS, ~162 200 neonates experienced LOGBS and 58 3000–91 000 neonates died from iGBS, with the highest numbers in Sub-Saharan Africa (Gonçalves et al., 2022). Maternal vagino-rectal colonization leads to EOGBS via vertical transmission of GBS either through ascending spread leading to chorioamnionitis, or during parturition (Eickhoff et al., 1964; Baker and Barrett, 1973). In contrast, the mechanisms underlying the transmission of GBS for LOGBS is not very well understood with cases showing high rectovaginal and breast milk colonization rates, indicating a possible vertical transmission (Berardi et al., 2013). Additionally, LOGBS may occur via horizontal transmission via nosocomial sources and nonmaternal caregivers (Anthony et al., 1979; MacFarquhar et al., 2010).

The pathophysiology and clinical manifestations differ for EOGBS and LOGBS. Neonates with EOGBS usually present with respiratory distress, followed by septicaemia and pneumonia with less likelihood of meningitis (Stoll et al., 2011; Nanduri et al., 2019; Paul et al., 2022). In contrast, LOGBS manifests clinically as bacteraemia without focus and meningitis (Berardi et al., 2013; Nanduri et al., 2019; Paul et al., 2022). Studies conducted across multiple geographical regions have reported sepsis as the predominant symptom associated with neonatal iGBS disease, manifesting in 70%–100% of iGBS cases, with meningitis occurring in 9%–30% of iGBS cases (Bramugy et al., 2022; Harden et al., 2022; John et al., 2022; Paul et al., 2022; Van Kassel et al., 2022). Survivors of iGBS meningitis have a higher risk of NDI than iGBS sepsis survivors (Horváth-Puhó et al., 2021; Paul et al., 2022). Furthermore, children with a history of GBS meningitis and GBS sepsis have a higher NDI risk

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compared to children with no history of GBS exposure (Horváth-Puhó et al., 2021; Paul et al., 2022). Although the risk of NDI in iGBS sepsis survivors is lower than the risk of NDI in iGBS meningitis survivors, sepsis is a more common manifestation, and thus more likely to contribute to the total GBS-associated NDI burden (Gonçalves et al., 2022; Paul et al., 2022). Interestingly, iGBS exposed males had a greater chance of developing NDI when compared to iGBS exposed females at ages 5–10 years old (Van Kassel et al., 2022).

Globally, serotypes Ia, Ib, III and V are the most prevalent in causing infections with serotype III accounting for approximately half EOGBS cases and approximately three-quarters of LOGBS cases (Madrid et al., 2017; Shabayek et al., 2021). Multi-locus sequence typing of GBS isolated from different countries showed that most human carriage and clinical isolates cluster into five major clonal complexes (CC) (CC1, CC10, CC17, CC19 and CC23) (Jones et al., 2003), however CC26 is most common in African countries (Brochet et al., 2009). Serotype III, considered a hypervirulent strain of GBS, is primarily composed of CC17 (Bekker et al., 2014; Seale et al., 2016). Studies have shown that GBS adheres to a variety of epithelial cells including chorion, amnion, vaginal, respiratory, intestinal, blood brain barrier (BBB) and choroid plexus (Tamura et al., 1994; Tazi et al., 2010). Adherence of GBS to chorion and amnion epithelial cells of the amniotic membrane during pregnancy is required for *in-utero* infection, which has been demonstrated in chorion and amnion cell lines using serotypes Ia and III (Winram et al., 1998). Furthermore, transcytosis of GBS between chorion cells has been shown to occur without damage to the intracellular junctions (Winram et al., 1998). Once GBS enters the amniotic cavity, proliferation of GBS can occur at a high rate, as shown in a study using serotype III GBS within sterile amniotic fluid samples (Hemming et al., 1985). In neonates GBS can colonise the lungs or the gastrointestinal tract (GIT) by adhering to epithelial cells using multiple virulence factors some of which include GBS immunogenic bacterial adhesin (BibA), (Tazi et al., 2010) and two CC17 specific factors, serine-rich repeat (Srr) glycoprotein 2 and hypervirulent GBS adhesin (HvgA) (Tazi et al., 2010; Hays et al., 2019; de Cambronne et al., 2021). Srr transmembrane receptors α5β1 and αvβ3 integrins serve as ligands for GBS adhesion to epithelial layers (de Cambronne et al., 2021). Following adhesion to either lung or GIT epithelial cells, intracellular invasion is an important step in GBS pathogenesis leading to sepsis (Rubens et al., 1992; Hays et al., 2019). The pore forming β-hemolysin/cytolysin (β-h/c) toxin has been shown to cause cellular damage which could also facilitate the entry of GBS into the bloodstream (Nizet et al., 1996; Doran et al., 2002). *In vitro* studies using neonatal and adult sera have shown that neonates may be prone to iGBS sepsis because of deficiencies in innate immunity, particularly complement activation and recruitment of phagocytic cells such as neutrophils (Anderson et al., 1983). Furthermore, neutropenia is a common observation in animal studies of neonatal iGBS disease induced by serotype III (Christensen et al., 1980, 1982, 1983; Harper et al., 1986; Weisman et al., 1990; Chang et al., 1996; Khan et al., 2024).

For GBS to cause meningitis after bacteraemia, it needs to gain access into the central nervous system (CNS) by passing the BBB. As with lung and GIT epithelial cells, CC17 GBS uses multiple virulence factors some of which include Srr and HvgA in binding to BBB epithelial cells allowing for transcellular (endocytosis and transcytosis) or paracellular invasion of the BBB epithelial cells (Nizet et al., 1997; Tazi et al., 2010; de Cambronne et al., 2021). Using an *in vitro* model of brain microvascular endothelial cells isolated from a human, it has been shown that serotype III is more efficient in intracellular invasion and transcytosis when compare to other serotypes (Nizet et al., 1997). Interestingly, in the same study β-h/c was shown to be involved in cellular injury of brain microvascular endothelial cells, thus providing another mechanism by which circulating bacteria can access the CNS (Nizet et al., 1997). Results obtained from animal studies have shown that iGBS disease in neonatal rat pups is associated with a pronounced increase in pro and anti-inflammatory cytokines in the blood and brain (Teti et al., 1993; Mancuso et al., 1994a, 1994b, 1994c, 1997, 2004; Givner et al., 1995; Cusumano et al., 1996a, 2004; Kim et al., 1997; Kenzel et al., 2006; Barichello et al., 2011; Andrade et al., 2013; Bergeron et al., 2016; Chiarot et al., 2021; Khan et al., 2024). Peripherally and centrally released cytokines can also contribute towards circulating bacteria gaining access to the CNS by increasing the permeability of the BBB (De Vries et al., 1996; Barichello et al., 2011). Neonatal animals studies where GBS has been injected into the cerebrospinal fluid via the cisterna magna, show evidence of necrotic and apoptotic neuronal injury (Kim et al., 1995; Leib et al., 1996a, 1996b, 2003; Bogdan et al., 1997; Bifrare et al., 2005; Reiβ et al., 2011). Reactive oxygen intermediates have been indicated as a major contributor of the neuronal injury in animal models of neonatal GBS meningitis (Leib et al., 1996a; Barichello et al., 2011).

These findings would lend support to the observations that neonates who survive iGBS meningitis present with severe NDI (Gonçalves et al., 2022; Harden et al., 2022; Paul et al., 2022). However, studies using animal iGBS meningitis models, where GBS is injected via the cisterna magna, may not be as valuable in understanding the pathophysiological changes associated with the less severe NDI noted in iGBS sepsis survivors. Thus, the aim of this review is to discuss the pathophysiology of NDI related to iGBS disease in neonates and assess the usefulness of neonatal rodent models, specifically in understanding the potential variations in NDI severity among children who have experienced either sepsis alone or combined sepsis and meningitis.

1.1. iGBS neurodevelopmental impairments – evidence from human studies

A recent series of studies published in the journal of Clinical Infectious Diseases in 2022, have aimed at establishing global statistics related to the development of NDI in iGBS survivors (Bramugy et al., 2022; Harden et al., 2022; Horváth-Puhó et al., 2021; John et al., 2022; Paul et al., 2022; Van Kassel et al., 2022). Study populations in both low- and high-income countries have shown development of moderate to severe NDI to be greater in iGBS survivors who had neonatal iGBS meningitis compared to neonatal iGBS sepsis alone (Bramugy et al., 2022; Harden et al., 2022; Horváth-Puhó et al., 2021; Paul et al., 2022; Van Kassel et al., 2022). While there is a lower prevalence of severe NDI in iGBS sepsis survivors, they have a greater prevalence of mild NDI compared to iGBS meningitis survivors, particularly under the age of five years old (Paul et al., 2022). Fig. 1 shows the domain specific impairment for survivors of iGBS. The frequency of impairment varies across the different neurodevelopmental domains, with a greater prevalence of impairment within the motor, cognitive and social/-behavioural domains compared to the vision and hearing domains (Paul et al., 2022; van Kassel et al., 2022). Children with a history of iGBS disease have greater impairment in the motor domain compared to the cognitive and social domains at five years of age, while at ten years of age a greater impairment is noted in the cognitive and social domains compared to the motor domain (van Kassel et al., 2022). Interestingly, boys are at a higher risk of NDI following iGBS disease compared to girls (Van Kassel et al., 2022). Overall, these findings not only highlight the importance of meningitis in the development of NDI related to neonatal iGBS disease, but importantly also identify sepsis as an overlooked role player in NDI related to neonatal iGBS disease. Taken together it is clear that low-income countries struggle not only with the overall neonatal burden of iGBS disease, but also with the long-term NDI complications in iGBS survivors (Bramugy et al., 2022; Harden et al., 2022). Thus, the need to study the pathophysiology, particularly related to both sepsis and meningitis, leading to NDI holds great value in understanding, treating and ultimately preventing the neurological deficits associated with neonatal iGBS disease.

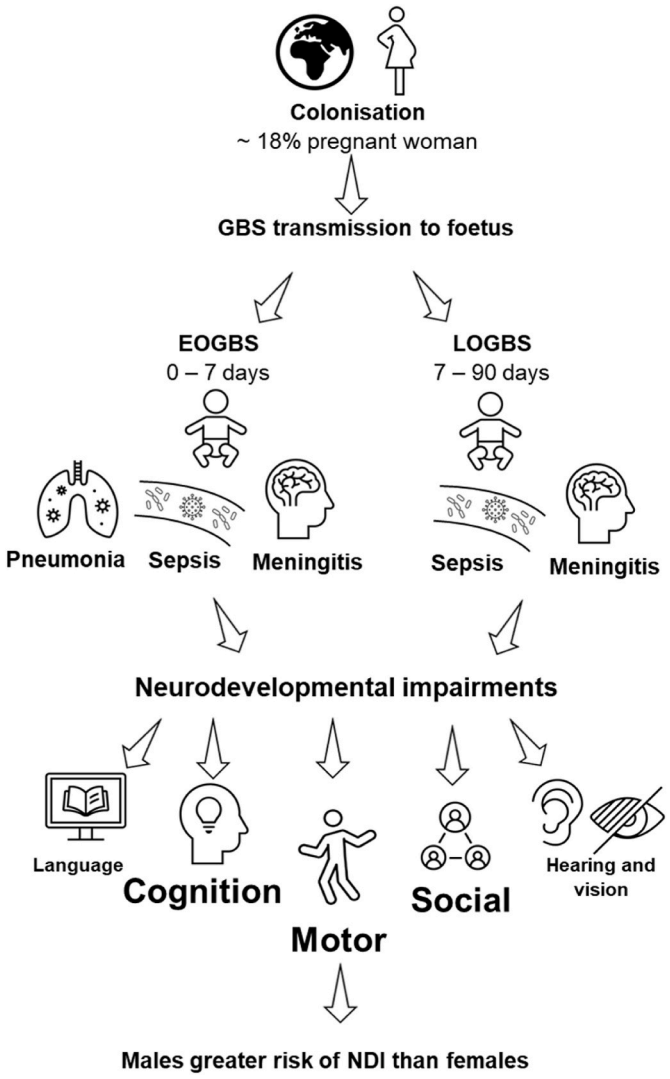

Fig. 1. Schematic representation of human neonatal invasive Group B *Streptococcus* disease leading to neurodevelopmental impairment. Global prevalence of colonization in pregnant women (~18%) leads to GBS transmission to foetus. Neonates that develop EOGBS present with respiratory distress leads to sepsis with less likelihood of meningitis, while neonates that develop LOGBS present with sepsis without focus and meningitis. Additionally, NDI may develop with the impairment frequency being greater within the motor, cognitive and social/behavioural domains compared to the vision and hearing domains. At five years of age children present with greater motor domain deficits compared to the cognitive and social domains deficits, while at ten years of age a greater impairment is noted in the cognitive and social domains compared to the motor domain. Furthermore, boys are at a higher risk of NDI following iGBS disease compared to girls.

2. iGBS disease neurodevelopmental impairment – evidence from animal studies

Since the late 1970's animal models have been used to unravel the pathophysiology of iGBS disease. In this review I will reflect on published studies from 1976 to 2024 using neonatal rodent models, where the animals were less than three weeks old and inoculated with GBS via one of the following routes: intraperitoneal (i.p.), subcutaneous (s.c.), intracardiac (i.c.), intravenous (i.v.) intracisternal magna (i.c.m.), intranasal (i.n.), intrathoracic (i.t.), orally or intravaginal (i.vag.). Of the published studies using neonatal rodent models, 40% used the i.p. route for GBS inoculation, 25% the s.c. route, 12% the i.c.m. route, 7% the oral route, 4% the i.n. and i.t. route, 3%the i.vag route, while 2% of studies used the i.c. and i.v. routes of GBS inoculation.

2.1. iGBS sepsis and pneumonia models

Peripheral GBS inoculation models offer varying levels of effectiveness at expressing the clinical syndromes associated with iGBS disease, namely bacteremia without a defined focus of infection, pneumonia, and meningitis. Furthermore, peripheral routes of GBS inoculation (i.p., s.c., i.t. and i.n.) induces 0–100% mortality between 24 and 168h after inoculation, with the extent of mortality not being linked to dose or age of pups. Among the various peripheral GBS inoculation sites in neonatal rodent models, the two least effective routes at inducing iGBS disease included administering GBS i.n. and orally. Administering GBS serotypes Ia or III i.n. to rat pups on PND 5 or mice pups younger than PND 2 resulted in inconclusive outcomes, with studies reporting neither illness nor mortality (Ferrieri et al., 1980; Chiarot et al., 2021). Oral administration of high doses of GBS serotype III (10^8 CFU), proved largely ineffective in inducing infection in rats between PND 2–7 and mice between PND 12–14 (Ferrieri et al., 1980; Ascher et al., 1991; Vaz et al., 2020). Illness seemed to be induced through oral administration of GBS serotypes II and III using high doses over consecutive days with an incubation period lasting between 2 and 4 days post-inoculation (Furtado, 1976; Kim et al., 1984). Despite the convenience and clinical relevance of using inhalation or oral gavage as a route of inoculation, the low and inconsistent incidence of illness induced using these two routes does not make them suitable for studying NDI related to iGBS sepsis.

Findings from studies using peripheral inoculation sites (i.p., s.c., i.t. and i.n.) and various serotypes (Ia, II and III) of GBS in neonatal rats have expanded our understanding of the neonatal immune responses to GBS. In particular they have shown early phase neutrophilia, late phase neutropenia and increases in monocytes/macrophages in the blood, liver and spleen (Christensen et al., 1980, 1982, 1983; Harper et al., 1986; Martin et al., 1988; Weisman et al., 1990; Chang et al., 1996; Khan et al., 2024). In contrast, the lungs of neonatal rats aged 6h to PND10 inoculated with serotype III GBS via the i.t. or i.p. route exhibit neutrophilia and an increase in alveolar macrophages (Harper et al., 1986; Martin et al., 1992; Khan et al., 2024) indicative of a lung-specific innate immune response to GBS with some variability based on serotype and duration of infection. Additionally, studies using peripheral GBS serotype III inoculation sites document bacteraemia with elevated pro and anti-inflammatory cytokines (interleukin (IL)-6, IL1-β, tumour necrosis factor (TNF)-α, interferon (IFN)-γ and IL-10) within blood plasma (Teti et al., 1993; Mancuso et al., 1994a, 1994b, 1994c; Givner et al., 1995; Cusumano et al., 1996a, 1996b, 2004; Andrade et al., 2013; Khan et al., 2024) and the brain, in particular the hippocampus (Khan et al., 2024).

Neonatal animal-based studies using peripheral GBS inoculation routes have not proved to be a good model for studying the effects of iGBS on neurodevelopment, as they document fatalities between 48 and 72 h after inoculation, which impacts the long-term follow-up of animals neurodevelopment. They are, however, a good model for improving our understanding of how peripherally released cytokines may affect the brain and contribute to the NDI observed in iGBS sepsis survivors. Two proposed mechanisms by which blood-borne cytokines may affect the functioning of the CNS is by carrier-mediated transport across the BBB or access through the circumventricular organs (Banks et al., 1995). A study using a rat model of neonatal encephalopathy, has shown that blocking the effects of peripherally released IL-1β reduced the extent of brain lesions confirming the involvement of circulating IL-1β in mediating CNS related pathophysiological responses (Savard et al., 2013). Interestingly, neonatal rat pups injected i.p. with serotype III GBS and presenting with signs of sepsis show an increase in circulating IL-1β (Khan et al., 2024). Thus, using peripheral models of GBS administration, which results in sepsis, we can study the pathophysiological changes likely contributing to the CNS associated changes which lead to the mild NDI observed in iGBS sepsis survivors.

2.2. iGBS meningitis models

Central GBS inoculation models, using i.c.m. delivery, are effective at expressing the clinical syndrome of meningitis, but are not effective at expressing the other two clinical syndromes of iGBS disease, namely bacteremia without a defined focus of infection and pneumonia. As with the peripheral routes of GBS inoculation, the i.c.m. delivery of GBS induces 50–100% mortality between 24 and 96h after inoculation, with the extent of mortality being dose dependent (Barichello et al., 2011; Bogdan et al., 1997; Woltjes and de Graaff, 1983). Findings from studies using central GBS inoculation sites (i.c.m.) have expanded our understanding of the pathophysiology of GBS meningitis and its risk of severe NDI in iGBS survivors. Neonatal rats (PND 3–4) had increased levels of cytokine levels (TNF-α, IL-6, IL-1β, IL-10 and CINC-1) in the cerebrospinal fluid (CSF), cortex and hippocampus between 6 and 24h after i.c.m. administration of GBS serotype III (Barichello et al., 2011). Other studies using GBS serotype III described histological levels of inflammation in the subarachnoid space and ventricles of the CNS (Kim et al., 1995; Leib et al., 1996a, 1996b). Furthermore, neonatal animal studies using i.c.m. delivery of GBS serotype III have shown histological neuronal damage particularly cortical necrosis (Kim et al., 1995; Leib et al., 1996a, 1996b; Bogdan et al., 1997; Bifrare et al., 2005) and hippocampal apoptosis (Leib et al., 1996a, 1996b; Bogdan et al., 1997; Bifrare et al., 2003; Reiβ et al., 2011). Interestingly, only one study, having used i.c.m. delivery of GBS to induce meningitis in PND 3–4 rats, investigated NDI (Barichello et al., 2013). Three months after the initial infection, when the animals were adults, their motor function and learning and memory functions were assessed using the open-field test and the step-down inhibitory avoidance task (Barichello et al., 2013). Explorative behaviour and motor function was not affected, however, although short-term memory to aversion behaviour remained intact, long-term memory to aversion behaviour seemed to be impaired (Barichello et al., 2013). Furthermore, brain-derived neurotropic factor and neuron growth factor was decreased in the hippocampus, suggesting that neuronal health was affected by GBS meningitis induced when the rats were neonates (Barichello et al., 2013). While this study provides some insight into the potential effects of iGBS meningitis, its scope was confined to male rats, and it only included one cognitive behavioural assessment. Animal models that directly target the CNS, by using the cisterna magna as the route of GBS inoculation, allow insight into the pathophysiological changes associated with iGBS disease leading to NDI, however this model precludes the contribution made by the hosts peripheral immune system, in particular peripherally released cytokines. Thus, the meningitis model of i.c.m. GBS inoculation, may not fully translate into mimicking the NDI observed in iGBS sepsis survivors.

2.3. iGBS maternal colonization models

Maternal colonization may be viewed as an ideal model to mimic the bacterial transmission from mother to foetus seen in the clinical setting (Andrade et al., 2018). In this regard, studies have aimed to colonise the dam with GBS by either administering GBS into the vagina or the peritoneum. Using maternal inoculation via i.p. GBS serotype III administration on gestational day 20–21, mortality of the dams was between 0 and 67% with higher doses (10^{10} CFU) inducing greater mortality (Noel et al., 1985). Furthermore, there was a low percentage of still births (8%) and mortality of the pups (6%). The transmission of GBS from the dam to the foetus appeared to be low with maternal i.p. GBS administration, due to bacteria only being detected in the blood, brain, liver and spleen of 13–33% of the pups (Noel et al., 1985). As with i.p. maternal GBS administration there is also varying mortality rates with GBS serotype III CC17 maternal i.vag. inoculation. In the first study maternal i.vag. inoculation (10^4 CFU) occurred on G17 and G18 with 14% of still births and 39% of pups dying within the first 48h of life reaching a peak of 40% pup death by day 7 (Andrade et al., 2018). In the second study, similar to the first study, maternal i.vag. inoculation of GBS serotype III (10^8 CFU) occurred between G18 and G19 with 31% of pups dying within the first 48h of life (Chiarot et al., 2018). Contrastingly, in the third study, inoculation with GBS serotype III (10^6 CFU) occurred on G17 with only 5% of pups dying within 48h of life. However, similar to the first study, 50% of pups died by day 7 (Vaz et al., 2020). Even though dosages varied greatly between study one and three, similar outcomes were observed by PND 7 showcasing the virulence of GBS serotype III CC17 and the mother to foetus transmission. Swabbing GBS serotype III around the vaginal area of dams proved entirely ineffective at transmitting GBS to the pups (Furtado, 1976). A study aiming to expose neonatal mice pups to GBS serotype III through simultaneous maternal sources, including intravaginal, oral, and nipple swabbing, resulted in 40% of the pups being colonized with GBS (Cox, 1982). In terms of cytokine responses, two studies using maternal GBS serotype III i.p. inoculation have shown increased IL-1β and TNF-α in fetal sera 72 h after maternal inoculation (Bergeron et al., 2016; Allard et al., 2019a). Interestingly, in the one study they also noted a sexually dichotomous profile in terms of the cytokine responses, with a more prominent increase in cytokines in fetal sera from males compared to females (Allard et al., 2019a). Only one study has investigated cytokine responses in pups following maternal i.vag. GBS inoculation on G17 and G18 (Andrade et al., 2018). In this study they found there was a decrease in the plasma cytokine levels and no change in brain cytokine levels at PND 5 in pups born to dams colonised with GBS serotype III compared to those born from dams not colonised with GBS (Andrade et al., 2018). The failure to observe changes in cytokines in the study may have been related to the use of only one timepoint (PND 5) and the study did not report on cytokine changes which could have occurred *in utero*, as noted in other studies. Notably, five maternal GBS inoculation studies have delved into the long-term outcomes of neonatal iGBS disease. In the initial study, conducted on adolescent rats five weeks after neonatal GBS serotype Ia exposure via maternal i.p. inoculation, male animals exhibited enlarged lateral ventricles in the brain, along with deficits in social behaviour, olfactory discrimination, startle reflex, and motor function, suggestive of autistic traits (Bergeron et al., 2013). The second study, GBS serotype Ia via maternal i.p. inoculation histologically showed thickening of the white matter in the corpus callosum in males and not females, which is important for integrating information between brain hemispheres in motor, cognitive and behavioural functions (Allard et al., 2017). Additionally, males animals at various ages where shown to have reduced ultrasonic vocalization (PND 7) indicative of autism, impaired nesting behaviour (PND 9), disorganised exploratory behaviour (PND 20) and social impairments (PND 40) indicative of autism-like behaviour (Allard et al., 2017). The next two studies shared methods of maternal i.p. inoculation of GBS serotype Ia or III. Although serotype Ia showed no behavioural effects pre-puberty, post-puberty females showed decreased motor function, which suggests that NDI should be observed well into puberty (Allard et al., 2018). When serotype III was used, male animals showed thinner primary motor cortices, reduced microglia and reduced travel in open field test, indicative of anxiety (Allard et al., 2019b). In the last study, conducted on adult male rats three months after neonatal GBS exposure via maternal vaginal inoculation, animals exhibited motor dysfunction, impaired learning and memory in the 8-arm radial test and a reduced travel distance in the open field test, indicating potential anxiety (Andrade et al., 2018). Fig. 2 provides a summary of rodent models that use various routes of neonatal and maternal inoculation (i.c.m., i.p., s.c. and i.vag.) of GBS and the subsequent outcomes with regards to pathophysiology changes and possible mechanisms of NDI.

3. Conclusion and future direction

Neonatal iGBS disease is classified into EOGBS and LOGBS, with neonates having EOGBS often presenting with respiratory distress, leading to sepsis and pneumonia with less likelihood of meningitis, while neonates having LOGBS typically presents with bacteraemia and

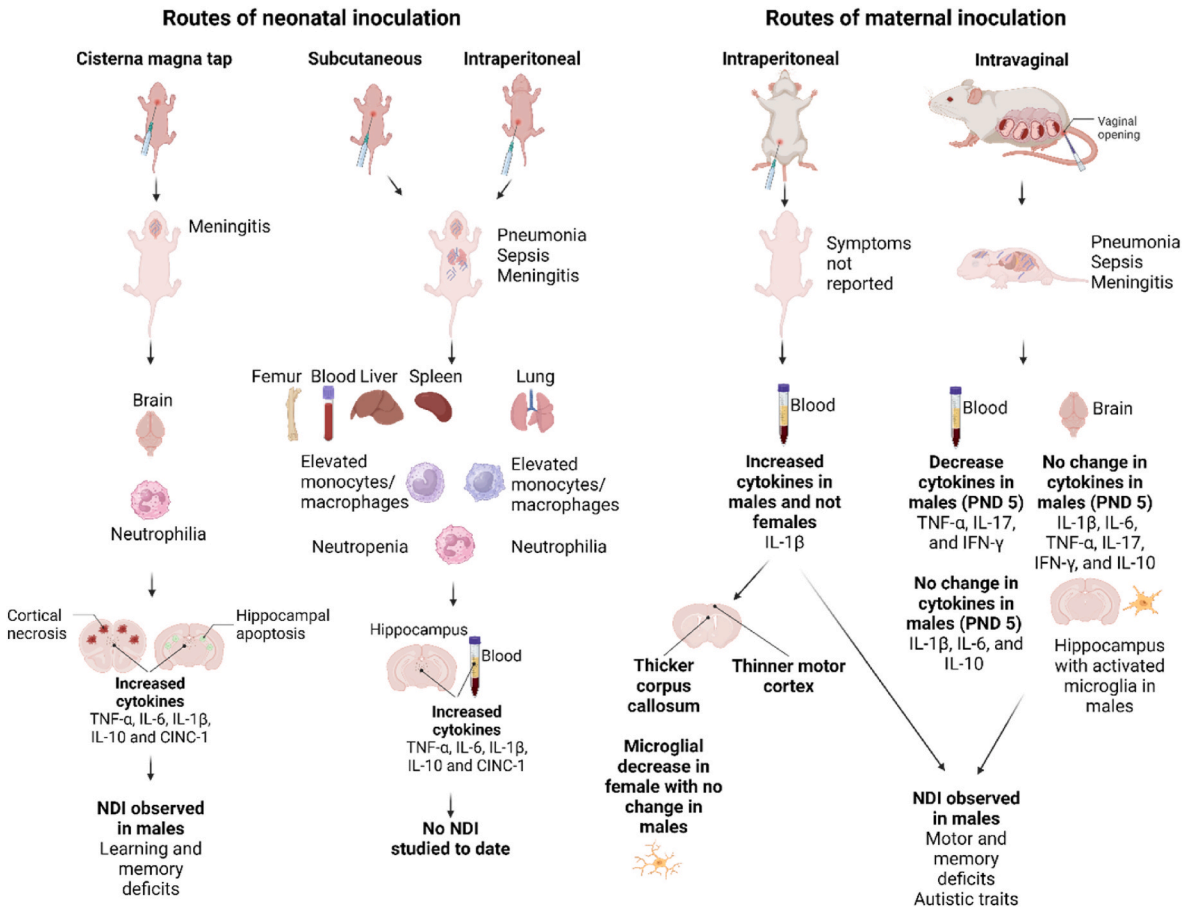

Fig. 2. Schematic representation of models that have provided major contributions in understanding the pathophysiology of invasive Group B *Streptococcus* disease with possible mechanism leading to neurodevelopmental impairment. Common inoculation routes include delivery of GBS to neonates via the cisterna magna, introducing GBS directly into the CNS mimicking meningitis, intraperitoneal and subcutaneous delivery, introducing GBS to the peripheral system to mimic sepsis and meningitis. Maternal inoculation routes include intravaginal and intraperitoneal delivery, introducing GBS to a pregnant dam with the aim of mimicking vertical transmission leading to pneumonia, sepsis and meningitis. Delivery of GBS via the cisterna magna has provided insight into neurological damage associated with iGBS meningitis, including cortical necrosis and hippocampal apoptosis and shown learning and memory deficits in pups surviving iGBS meningitis. Intraperitoneal and subcutaneous delivery of GBS in neonatal pups are the most used peripheral routes of GBS administration and have provided insight into systemic inflammation associated with iGBS sepsis leading to meningitis. No NDI studies have been conducted to date using these two methods of GBS delivery. Intravaginal and peripheral maternal inoculation allows for the clinical mimicry of maternal to fetal GBS transmission leading to clinical syndromes of pneumonia, sepsis and meningitis noted with EOGBS. Neonatal and fetal cytokine responses are poorly understood in intravaginal and intraperitoneal models however, sex-dependent motor and memory deficits and autistic traits have been reported in males. Illustration created using Biorender.com.

meningitis. Recent studies reveal that moderate to severe NDI is more common in survivors of iGBS meningitis compared to those with iGBS sepsis, while mild NDI is more prevalent among sepsis survivors, especially under five years of age. Impairments are most frequent in motor, cognitive, and social/behavioural domains, with boys surviving iGBS being at higher risk of NDI than girls. These findings highlight the need for further research on the pathophysiology of both sepsis and meningitis to address and prevent long-term neurological deficits in iGBS survivors, particularly in low-income countries. Animal models used to study the pathophysiological changes associated with the clinical syndromes of neonatal iGBS, namely pneumonia, sepsis and meningitis have used various routes of maternal (i.p., i.vag.) or neonatal (i.p., s.c. i.c., i.v., i.c.m., i.n., i.t., orally) GBS delivery. When assessing the validity of a given animal disease model, three criteria need to be considered, namely face validity (i.e., does the model exhibit the salient features of the condition in humans), construct validity (i.e., is the condition arising from the same biological background) and predictive validity (i.e., will the model respond to well-established treatments) (Tadenev and Burgess, 2019). Delivery of GBS via maternal inoculation exhibits good face, construct and predictive validity as neonatal animals show the key clinical salient features (pneumonia, sepsis and meningitis) and pathophysiology of iGBS disease in humans and can be used for testing the effectiveness of maternal vaccines and for the treatment of the various clinical conditions present in the neonate. However, outcomes such as autism (Bergeron et al., 2013; Allard et al., 2017) in maternal inoculation studies have not been observed in human clinical setting. While studies using peripheral (i.p. and s.c.) delivery of GBS in neonates do not have as good construct validly compared to maternal GBS inoculation models, they do have similar face and predictive validity, in that they show key pathophysiological changes associated with iGBS in neonates and they could be used to investigate treatment of the different clinical syndromes associated with iGBS disease in neonates. Of all the GBS delivery routes the central delivery route via the cisterna magna tends to have the lowest face, construct and predictive validity as it only mimics the clinical syndrome of meningitis. Using different routes of GBS delivery, we have managed to gain a good understanding of the pathophysiological changes associated with neonatal iGBS disease, but the next major area which needs to be explored further in animal-based studies is the long-term consequences of neonatal iGBS disease, in particular iGBS sepsis. To do this, future studies should target using peripheral delivery (i.p. and s.c.) of GBS in neonatal animals, as well as maternal GBS delivery (i.vag. and i.p.), combined with treatment

through antibiotic therapies to ensure survival, to conduct long-term behavioural assessments one month until puberty after infection.

In terms of human-based studies, while there has been an increase in the number of studies investigating NDI which has provided a clearer picture of the NDI profile for iGBS survivors, there has not been a similar increase in longitudinal studies documenting the acute immune response of neonates with iGBS disease followed by long-term follow-up of iGBS survivors. Most studies investigating NDI have solely relied on behavioural screening tools to identify domains of impairment but have not explored the associated morphological changes in the brain. Advances in imaging techniques have enhanced our understanding of normal developmental trajectories in the brain and could be a useful tool to add to studies investigating NDI in iGBS sepsis survivors (Giedd and Rapoport, 2010). Critical to these studies would be the inclusion of a counter-factual group of children not exposed to GBS in early life. Furthermore, there has not been any studies aimed at investigating how therapeutic interventions early in life could alter the long-term outcomes of iGBS survivors, in particular children presenting with milder impairment. With studies showing an increased NDI frequency as iGBS survivors grow up, identifying early windows for therapeutic interventions is very important to altering the neurodevelopmental trajectory (Horváth-Puhó et al., 2021). Moreover, the finding that boys with a history of iGBS are at higher risk of NDIs and more often require special educational support than girls who had iGBS (Horváth-Puhó et al., 2021), highlights the need for animal and human-based studies investigating the underlying physiological mechanisms accounting for this sex-dependent effect.

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Declaration of Competing interest

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Data availability

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