Myelomeningocele (MMC) is a severe form of spinal dysraphism. Due to the failure of neural tube closure during early embryonic development, the affected part of the spinal cord is left open like a book at the back of the affected child. This malformed part of the spinal cord is not covered by its protective mesodermal and ectodermal layers. Consequently, the exposed neural tissue (i.e., the neural placode) is prone to injury during further intra-uterine development. Former investigations in sheep MMC models and ultrasound examinations in human fetuses demonstrated progressive decreased limb function during the later fetal course (StiefeI and Meuli, 2007). As a possible morphological correlate, StiefeI and Meuli (2007) demonstrated progressive tissue destruction of the initially intact appearing unfolded neural placode in curly tail/looptail mouse fetuses. These observations were consistent with the hypothesis of secondary damage of the neural placode (so-called “second hit hypothesis”) (Heffez et al., 1990). According to this hypothesis, the “first hit” is considered the primary structural defect, which is due to faulty developmental processes. The size and location of the spinal cord abnormality within the spinal axis are important in determining the initial functional status. The assumed ongoing toxic and mechanical damaging impacts on the exposed neural placode are viewed as the “second hit”. The second hit presumably leads to additional deficits at and below the lesion level. These processes might also be responsible for further sequelae, like the development of secondary tethered cord syndrome (TCS), that typically occur during the later clinical course of the affected child. As in spinal cord injury (SCI), the second hit presumably induces further cellular and molecular lesion cascades in the placode, which are summarized under the term “third hit” (Figure 1A).

This perspective article—which is based on recent investigations in human and rat-derived MMC specimens—focuses on potential molecular inflammatory mediators of such hypothesized “third hit” lesion cascades in the placode. In the first approach, pro-inflammatory cytokines were investigated in human MMC placode fragments which were obtained during the initial surgical reconstruction of the spinal cord and its surrounding tissue layers in the first days after birth (Kowitze et al., 2016). In these studies, the ligand/receptor pairs interleukin (IL)-1β/IL-1R1 and tumor necrosis factor-a (TNF-a)/TNF-R1 were found to be elevated. To investigate if these mediators became detectable during the fetal development, we established the retinoic acid MMC (RATMMC) model according to Danzer et al. (2011). Briefly, time-dated Sprague-Dawley rats were gavage-fed with all-trans retinoic acid (60 mg/kg) dissolved in olive oil at £10. Control animals received olive oil only. Fetuses from both groups were obtained and investigated at £16, £18, and £22 (Cohrs et al., 2021). (Figure 1B–Exemplarily depicts immunohistochemical staining for IL-1β in fetal rat spinal cord and MMC-placodes at £16 and £22. In a further approach, we examined the expression of pro-inflammatory mediators in spinal tissue which was obtained during untheatering surgery of patients who developed a secondary TCS in their late fetal or early postnatal life being operated for MMC early after birth (Cohrs et al., 2019).

To summarize the results, cellular gliosis with significantly elevated gial fibrillary acidic protein (GFAP) and Vimentin-immunoreactivity became evident in human neuroepithelial MMC placode tissues that were obtained during repair surgeries early after birth (Kowitze et al., 2016). Also, round, partially clustered CD3-, CD11b-, and CD68-positive cells were detectable in MMC cases indicating the presence of cellular inflammatory reactions (these cell types were not detectable in respective control specimens). All investigated MMC specimens exhibited significantly higher IL-1β, IL-1R1, and TNF-α immunoreactivities compared to the immunoreactivity level in normal spinal cord controls (Figure 1F and G). As in the immunohistochemical analyses, IL-1β-, IL-1R1-, and TNF-α-mRNA levels were found to be higher in the placentes of the MMC-group compared to expression level in control SC tissues. TNF-R1 was also induced in the MMC placode specimens, but this did not reach statistical significance. Immunofluorescence staining confirmed that these cytokines were co-expressed with cellular markers of glial, inflammatory, and neuronal cells (as shown for IL-1β and IL-1R1 in Figure 1H and I).

In rat fetal MMC tissues, there was strong immunoreactivity for Nestin and Vimentin at £16 and £18 in cells exhibiting long immunoreactive processes that traverse through the neuroepithelium. At £22 there was a shift with less nestin- and more GFAF-immunoreactive cells appearing in the placentes. GAP reactive astroglia thereby appeared in a morphologically active state with thickened immunoreactive processes compared to non-reactive astroglia in control specimens. In addition to astrogliosis,round inflammatory cells became detectable at £18 and £22. IL-1β was significantly elevated on mRNA-level on £22 in MMC fetuses, while its receptor was found induced on day £16 and £22 in MMC placodes. The respective immunostaining exhibited strong expression in distinct regions of the placode with co-staining of inflammatory and neuronal markers. TNF-α and TNF-R1 exhibited similar expression profiles on £22. This inflammatory cytokine receptor/effect pair became detectable at £16, £18, and £22, but reached a significantly elevated level only at the perinatal day £22. To summarize these new insights, pro-inflammatory molecules became detectable at a significantly elevated level in the late fetal and perinatal time-course in MMC rats (Cohrs et al., 2021).

To cover another aspect of open spinal dysraphism, the same inflammatory markers were investigated in neural tissue obtained from patients who suffered from secondary TCS some time after being operated for MMC defects soon after birth. These specimens exhibited strong astrogliosis with morphological signs of cellular reactivity and CD3, CD68, and CD11b immunoreactivities. Thus, similar to the findings in MMC specimens, there were signs of a distinct inflammatory cellular reaction. Further, TUNEL and PARP-positive cells indicated the appearance of apoptotic cell death in the examined materials. Along with these findings, TNF-α and IL-1β and their main receptors exhibited strong immunostaining in reactive neuronal and glial cells, which was co-expressed with GFAP, CD68, and NeuN (Cohrs et al., 2019) (Figure 1J and K).

Therefore, the question whether the inflammatory mediators–which are summarized under the term “third hit”–are involved in the development of secondary tethered cord syndrome (TCS) is captured in the scheme. (Figure 1). Perspective on inflammatory cytokines in open spinal dysraphism.
The presented studies demonstrated a potential role of pro-inflammatory cytokines such as IL-1β and TNF-α in the late fetal, perinatal, and further post-natal time-points in MMC neural placodes of rat and human origin. As factors, these cytokines are well-known molecular mediators of secondary lesion cascades, which are induced after traumatic SCI. Under pathological conditions, these mediators exceed their normal expression level and become involved in pro-inflammatory and pro-apoptotic cascades, which have the potential to further damage primarily intact tissue (summarized under the term “second lesion” in the neurotrauma literature).

Because mechanical injury to the neural placode is considered one crucial factor of the “second hit”, one can assume that some processes are induced by the neural placode. Strong gliosis and the appearance of inflammatory cells in the neuroepithelia of fetal rat and newborn human MMC specimens indicate that such inflammatory processes take place in open spinal dysraphism. It may be that limited intra-uterine space in addition to changes of amniotic fluid consistency increases the probability of damaging insults to the developing CNS. This may explain the elevated cytokine expression level at later fetal and perinatal time-points in our studies. With their pro-inflammatory properties, increases of TNF-α and IL-1β may promote further neural damage and thus decline neural function.

To date, the therapeutic options for open spinal dysraphism involve surgical repair of the dysraphic defect in fetal, perinatal, birth or during further fetal surgery in specialized centers. In order to improve the long-term outcome of children born with MMC, special attention has been directed to refining surgical techniques to meticulously reconstruct a malformed layer. Especially in fetal surgery handling the very delicate and fragile fetal neural tissue remains a challenge under the already constrained time frame of fetal and perinatal intraoperative needs. Careful reconstruction of the placode and its meninges protects the placode from mechanical injury to the neural placode. However, recent publications indicate that even after such careful surgical approaches during intrauterine closure, problems related to meningocoeles may remain as well as the development of early TCS (Weaver et al., 2021).

An alternative approach to surgical reconstruction of the anatomical defect in utero is the application of patches on the fetal placode to protect the exposed neural tissue from outer harmful impacts and to prevent re-repulsion during further development. Ongoing research has attempted to optimize the regenerative capacity of the patches by altering cellular composition and consistency of the grafts. For example, Mann et al. (2019) demonstrated different properties of patches, which were derived from acellular dermal matrix versus cryopreserved human umbilical cord matrix. They observed improved cellular outgrowth of cells into the outer surface of the graft (i.e., promoted “meningeal cell tropism”) and reduced the acute inflammatory reaction between the placode and repair site. The acellular dermal matrix graft was associated with a more inflammatory and astroglial reaction to the inner and outer surfaces of the patch, which could potentially contribute to preventing further re-repulsion (Weaver et al., 2021). In the light of our findings of cytokine expression and astroglial inclusions in fetal placodes, it would be interesting to investigate the influence of both patch types on the underlying placode, which was not addressed by the cited group so far. In this context, findings of another group were of interest as they demonstrated a reduction in caspase-3 (a marker for apoptotic processes), iiba (a cellular inflammatory marker), and GFAP expression in fetal ovine MMC placodes after applying a reverse thermal gel patch (Bardill et al., 2022). This type of patch, however, the capability to undergo a spontaneous temperature-induced phase transition from liquid to gel consistency, which provides advantages in regard to potential application during in-utero MMC repair surgery.

Considering the presented data of our studies and those in the current literature, application of specifically TNF-α-blocker or IL-1R1-antagonist proteins during the initial repair surgery for MMC or patch application for further refinement of the approach to potentially prevent further damaging of the placode and prevent long-term complications such as secondary TCS. Therefore, specifically anti-TNF strategies have been evaluated in preclinical candidates for therapeutic trials: Activated TNF-α initiates an induction of further cytokines and associated intracellular signaling pathways. Versa versa blockage of TNF-α reduces activation of diverse other cytokines and their potentially detrimental activities. Also, anti-TNF drugs have been already Food and Drug Administration-approved in chronic inflammatory diseases, though with sometimes critical side effects (Esposito and Cuzzocrea, 2011). Considering SCI, peripheral application of the TNF-α-antagonist etanercept (given early time-course after injury resulted in interruption of proapoptotic signaling cascades (Esposito and Cuzzocrea, 2011)). Interestingly, peripheral administration of IL-1R1 antagonists after SCI improves reparative response after contusive SCI in mice (Yates et al., 2021). Considering the presented findings on cytokine expression in fetal and human MMC placodid tissue, there would be interesting to examine the actual effects of such biopharmaceutical agents on the tertiary lesion cascades under the controlled settings of an MMC animal model. Therefore, the role of the available data on fetal cytokine expression in the murine MMC model has to be still confirmed in respective age-matched material of human origin.

Despite the current lack of functional data, our studies have identified specific pro-inflammatory cytokines as crucial mediators of proposed tertiary lesion cascades in MMC placodes. These aspects should be considered in future approaches to protect neural tissue, the placode and its meninges during further surgery handling the very delicate and fragile fetal tissue, but also the repair surgery. Problems related to meningocoeles may remain as well as the development of early TCS. This could be in form of combining surgical approaches with an adjunction of targeted antagonists to cytokine receptors and neuroprotective substrates. Considering the latter, placental mesenchymal stem cells have been shown to promote anti-inflammatory and neuroprotective effects in SCI treatment and fetal MMC repair (Kuluba et al., 2021), making them interesting candidates for future multimodal therapeutic approaches to open spinal dysraphism.

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