Myelomeningocele or open spina bifida is a non-lethal but highly morbid form of open neural tube defect in which the meninges and neural tissue are exposed to the intrauterine environment. This defect occurs in ~1 in 2000 live births, even in countries where maternal folic acid supplementation is available. Myelomeningocele results in lifelong lower-extremity neurological deficiencies, orthopedic disabilities, incontinence, and sexual dysfunction. In addition to motor and sensory deficits caused by the spinal cord lesion, significant complications in MMC are associated with Chiari malformations Type II and ventriculomegaly.

Abbreviations used in this paper: fMMC = fetal myelomeningocele; MMC = myelomeningocele; MPH = meconium-positive histiocyte; pMMC = postnatally repaired MMC; SCI = spinal cord injury.

Although extensive research into the causes of MMC has elucidated both genetic and environmental causes, the pathophysiology of neural damage in MMC is incompletely understood. Based on compelling experimental and clinical evidence, it has been suggested that the neurological deficits associated with MMCs occur in stages (2-hit pathophysiology). This hypothesis states that the primary congenital abnormalities in anatomic development allow a relatively normal spinal cord to become secondarily and progressively damaged by amniotic fluid toxicity, mechanical trauma, hydrodynamic pressure, or a combination of these factors. Fetal surgical coverage of the exposed spinal cord was recently initiated as...
a potential treatment for MMC with the goal of protecting the neural placode from the intrauterine environment. Although early clinical experience with in utero MMC closure suggests that midgestational fetal surgery in this highly selected population of children with MMCs may reduce disability related to Chiari malformations Type II and hydrocephalus by reversing hindbrain herniation and reducing the need for ventriculoperitoneal shunting, fetal intervention for MMC remains highly controversial. One of the primary controversies relates to the relative role of each of the 2 “hits” in the pathogenesis of the neurological damage observed. Fetal surgical coverage for protection of the cord might prevent the acquired secondary component of the damage but would not prevent damage related to the primary defect. Thus, the rationale for fetal intervention depends on how much of the damage is acquired after the primary event, and from a practical standpoint, when during fetal life the damage is acquired.

One factor that has been suggested to play an important role in the pathophysiology of SCI is meconium. It is now evident that anal sphincter dysfunction in human fetuses with MMC may result in continuous in utero defecation and meconium leakage late in gestation, which subsequently results in an increased chemical toxicity of the amniotic fluid. In addition, extensive prenatal meconium exposure has been shown to increase the severity of SCI in MMC in experimental studies. It is evident that ifMMC coverage prevents in utero meconium exposure to the neural placode. Using established microscopic techniques, we analyzed the MMC sacs obtained from fetuses that underwent fMMC closure and compared the presence and distribution of meconium histochemistry in MMC sacs obtained from newborns who underwent standard postnatal neurosurgical closure at our institution. The cases were treated before the National Institutes of Health–sponsored Management of Myelomeningocele Study (MOMS).

Methods
This study was approved by the Committee for Protection of Human Subjects Institutional Review Board (IRB# 2000–11–2081 and IRB# 2007–3–5253) of the Children’s Hospital of Philadelphia.

Patient Population
Between January 1998 and February 2003, 54 patients met our inclusion criteria and underwent fMMC closure. Details of the preoperative evaluation, surgical approach, and postnatal management have been described previously. Briefly, after induction of general anesthesia, a maternal hysterotomy was performed, and the lumbosacral area of the fetal spine was exposed. After a circumferential skin incision, the MMC sac was mobilized and carefully excised from the placode. Primary dural closure was attempted, and a layered closure of the defect was performed. After completion of fetal surgery, amniotic fluid was replaced with warmed, sterile, lactated Ringer’s solution. Tocolysis was initiated and maintained postoperatively to prevent preterm labor.

Myelomeningocele sacs obtained in 46 (85%) of 54 fetuses were available for further analysis. The control group consisted of 53 postnatally resected MMC sac specimens that were identified in the surgical and pathology files of The Children’s Hospital of Philadelphia from 1988 to 2006, which represents approximately one-third of the postnatally repaired MMCs during the study period. Clinical information was obtained by standard chart review. For comparison purposes, the lesion levels were grouped into high- (T8–L2), mid- (L3–4), and low-level lesions (L5–S). To evaluate whether the gestational age of the fetus at the time of repair influenced the distribution of meconium, fMMC sacs were divided into 3 groups: obtained before 22 weeks of gestation (early repair), between 22 and 24 weeks (intermediate repair), and 24–26 weeks (late repair).

Pathological Characteristics of MMC Sacs
According to standard protocols, all MMC sac specimens were fixed in 10% formalin, embedded in paraffin, and sectioned in 4-μm-thick slices. Hematoxylin and eosin–stained sections were reviewed under light microscopy (Leica DMRD) by a perinatal pathologist (L.M.E.) who was blinded to specimen identification. Magnification of 400 was used. The entire slide was examined for the presence of macrophages containing pale brown pigment. Only cells with a nucleus were counted. Because there is no reliable histochemical technique for demonstrating meconium, we used Hall’s bile stain to determine whether the pigment-laden histiocytes contained bile, which would suggest a digestive origin of the brown pigment. Meconium was differentiated from hemosiderin by the histomorphological appearance of the pigmentation within the macrophages (meconium has a smooth brown pigmentation and hemosiderin has a diffusely distributed, granulated yellow-brown pigmentation) and by the absence of Prussian blue staining. A Fontana Masson stain was also used to exclude melanin.

We developed a grading system for the presence of meconium, defined as follows: absent (none present), mild (< 10 MPHs/hpf), moderate (10–25 MPHs/hpf), and severe (≥ 25 MPHs/hpf). In specimens with different counts of MPHs between hpf, the highest number of positive cells (worst) was used to compare between fMMC and pMMC groups.

Data Analysis
Statistical analysis was performed using JMP 7 statistical discovery software (SAS Inc.). The median test, chi-square test, and 1-way analysis of variance test were used for statistical comparisons as appropriate. Probability values < 0.05 were considered significant.

Results
Patient Population
Median gestational age at fMMC surgery was 23 weeks (mean 23.1 ± 1.3 weeks; range 20–25.8 weeks). All newborns who underwent MMC repair postnatally were delivered after 37 weeks of gestation. In accordance with our institutional guidelines, spinal lesion closure was per-
formed within 48 hours of birth. The distribution of the lesion level was similar between groups: the median lesion level of fMMCs was L-4 (range T8–S1), and the median lesion level of pMMCs was L-4 (range T8–S; Table 1).

Pathological Characteristics of MMC Sacs

The histological constituents of both the fMMC and pMMC sacs were typically skin cells with associated underlying subcutaneous tissue. Frequently disorganized nodules of strips of neural glial tissue were present in the subcutaneous tissue. Rarely, more well-formed segments of spinal cord and nerve roots were seen. Meconium-positive histiocytes that stained positive for Hall’s bile stain and pigmented macrophages negative for Prussian blue and Fontana stain were most frequently seen in the connective tissue in areas where the skin showed epidermal loss, most likely representing the area of the “true” MMC sac at its thinnest component (Fig. 1). Occasionally, meconium was also found deeper in close contact with the neurological elements.

Meconium histiocytosis was significantly less often detected in fMMC (in 42 [79%] of 53) than pMMC sacs (in 26 [57%] of 46; p = 0.017). Meconium staining was completely absent in 20 fMMC (43%) and 11 pMMC (21%) sacs. Mild meconium histiocytosis was found in 9 fMMC sacs (35%) and 25 pMMC sacs (61%; p = 0.035). There was no statistically significant difference between groups with moderate (fMMC, 11 [42%] vs pMMC, 9 [22%; p = 0.07]) and severe meconium histiocytosis (fMMC, 6 [23%] vs pMMC, 7 [17%; p = 0.54; Fig. 2).

Although meconium staining was significantly more often present in fMMC sacs obtained from high-level lesions compared to mid- and low-level lesions (p = 0.01), distribution of meconium was similar between lesion levels in the pMMC group. Comparison between groups and lesion levels demonstrated that, among fMMC sacs obtained from mid- and low-level lesions, meconium was detected significantly less often than in pMMC sacs (p = 0.001). No difference in presence or absence of meconium staining was found between high-level lesions in the groups (p = 0.35; Table 2).

Of the 46 fetuses in the fMMC group, 17% underwent surgical repair before 22 weeks of gestation, 52% at 22–24 weeks, and 31% at 24–26 weeks. As summarized in Table 3, the gestational age at fetal intervention did not correlate with the presence and absence of meconium histiocytosis. Acute inflammation, tissue destruction, and superficial ulcerative changes were found significantly more often in sacs obtained from pMMC newborns (27 [51%] of 53) compared to fMMC specimens (0; p = 0.001). The development of these additional pathologies in the pMMC group correlated with the presence of meconium within these specimens. In pMMC sacs without meconium macrophages, only 1 (9%) of 11 demonstrated superficial ulcerations, whereas in the pMMC specimens with meconium staining, 26 (62%) of 42 had additional pathological changes (p = 0.002).

Table 1: Distribution of lesion level in 46 patients with fMMCs and 53 with pMMCs

<table>
<thead>
<tr>
<th>Lesion Level</th>
<th>No. of fMMCs (%)</th>
<th>No. of pMMCs (%)</th>
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</thead>
<tbody>
<tr>
<td>high (T8–L2)</td>
<td>11 (24)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>mid (L3–4)</td>
<td>18 (39)</td>
<td>21 (40)</td>
</tr>
<tr>
<td>low (L5–S)</td>
<td>17 (37)</td>
<td>25 (47)</td>
</tr>
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</table>

Discussion

Continuous in utero damage to the exposed spinal cord has been postulated to be the primary cause of disability associated with MMCs. Continuous in utero damage to the exposed spinal cord has been postulated to be the primary cause of disability associated with MMCs. Continuous in utero damage to the exposed spinal cord has been postulated to be the primary cause of disability associated with MMCs. Continuous in utero damage to the exposed spinal cord has been postulated to be the primary cause of disability associated with MMCs. Continuous in utero damage to the exposed spinal cord has been postulated to be the primary cause of disability associated with MMCs.

We therefore analyzed MMC sacs obtained in patients with spina bifida who underwent either fetal or standard postnatal neurosurgical repair at our institution to determine whether midgestational maternal-fetal surgery for MMC prevents intrauterine meconium expo-
sure. In accordance with our hypothesis, meconium was significantly less often detected within fMMC sacs compared with pMMC specimens (57 vs 79%). However, we also observed that by the time of fetal surgery (median 23 weeks of gestation), meconium-positive histiocytes were found in more than half of the fMMC sacs, suggesting that meconium passage in MMC already occurs during the second trimester and that the acquired component of neurological injury to the exposed spinal cord might occur earlier in gestation than previously believed.9,13,18,33

Anal sphincter maturation occurs in several stages, the first being perforation of the anal membrane at 12 weeks, followed by the progressive development of the anal sphincter muscles (external, superficial, and deep) from 15 to 28–30 weeks.3,17 We postulate that the presence of MPHs within the fMMC sacs reflects fecal incontinence as a result of defective anal sphincter innervation associated with MMC. This hypothesis is supported by a study of Shapiro and associates30 that showed that midgestational human fetuses with MMCs present with decreased innervation and defective smooth muscle development of the lower gastrointestinal tract and anorectum. Also in agreement with our hypothesis of early-onset meconium passage in MMCs is a recent report by Talabani and colleagues,32 who analyzed the digestive enzyme profile of amniotic fluid in fetuses with MMCs throughout pregnancy. Although at < 20 weeks of gestation fetuses with MMCs demonstrated normal amniotic fluid profiles, the amniotic fluid samples obtained between 20 and 26 weeks showed a significant increase in digestive enzymes compared with controls. The authors concluded that the abnormally high concentration of digestive enzymes in the amniotic fluid of midgestation fetuses with MMCs might reflect neurological injury resulting in alteration of anal sphincter muscle function.

Previous studies have shown that meconium increases amniotic fluid toxicity in other congenital malformations, such as the eviscerated intestine in experimental models of gastroschisis.2 Inflammation and induction of necrosis by meconium has also been shown in lungs with meco-

TABLE 2: Meconium presence or absence by lesion level and MMC group

<table>
<thead>
<tr>
<th>Lesion Level</th>
<th>No. of fMMCs (%)</th>
<th>No. of pMMCs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>high (T8–L2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>8 (73)</td>
<td>5 (71)</td>
</tr>
<tr>
<td>absent</td>
<td>3 (27)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>mid (L3–4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>10 (55)*</td>
<td>18 (86)</td>
</tr>
<tr>
<td>absent</td>
<td>8 (45)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>low (L5–S)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>8 (47)*</td>
<td>19 (76)</td>
</tr>
<tr>
<td>absent</td>
<td>9 (53)</td>
<td>6 (24)</td>
</tr>
</tbody>
</table>

* Different from high lesion level with the fMMC group (p = 0.01) and different from pMMC specimens (p = 0.001).

TABLE 3: Meconium presence or absence by gestational age at fetal surgery

<table>
<thead>
<tr>
<th>Gestational Age</th>
<th>No. of fMMCs (%)</th>
</tr>
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<tbody>
<tr>
<td>early (&lt;22 wks)</td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>5 (63)</td>
</tr>
<tr>
<td>absent</td>
<td>3 (37)</td>
</tr>
<tr>
<td>intermediate (22–24 wks)</td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>14 (58)</td>
</tr>
<tr>
<td>absent</td>
<td>10 (42)</td>
</tr>
<tr>
<td>late (24–26 wks)</td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>7 (50)</td>
</tr>
<tr>
<td>absent</td>
<td>7 (50)</td>
</tr>
</tbody>
</table>
Meconium in myelomeningocele

nium aspiration syndrome. It is therefore not surprising that excessive meconium exposure in fetal rats with surgically created MMCs increases the damage to the exposed neural elements. However, the exact pathophysiological mechanisms of meconium-induced SCI are yet to be determined. In vitro studies have shown that meconium or substances within meconium (such as bile acids) can stimulate vasoconstriction of the placenta, umbilical cord, and other fetal vessels, which subsequently results in hypoxic damage to fetal tissues. The microscopic features of the meconium-induced vascular changes within the fetoplacental unit include eosinophilic cytoplasmatic degeneration, nuclear pyknosis, discohesion, and rounding of peripheral vascular smooth muscle cells in large chorionic and umbilical fetal vessels. Whether prolonged meconium exposure in MMCs destroys the vulnerable neural elements via similar vasoreactive mechanisms or via direct cytotoxic mechanisms as suggested previously is unknown. It is noteworthy, however, that in > 60% of the meconium-positive pMMC sacs, meconium staining was accompanied by additional histopathological changes typical of meconium-associated vascular necrosis, such as inflammation, necrosis, and tissue ulceration. Because these additional pathological features were completely absent in all MMC sacs obtained at the time of fetal intervention, it seems reasonable to speculate that fetal MMC closure may reduce the duration of meconium exposure, thereby limiting the toxic injury to the vulnerable neural elements.

Although the role of fetal neurosurgical closure of MMCs is being established through a randomized-controlled prospective trial (www.spinabifidamoms.com), our data reinforce the theory that injury to the exposed spinal cord in MMCs is, at least in part, acquired during fetal generation, nuclear pyknosis, discohesion, and rounding of peripheral vascular smooth muscle cells in large chorionic and umbilical fetal vessels. Despite these intriguing results, the limitations of our study must be acknowledged. Our experimental design cannot exclude the contribution of other factors to the acquired spinal cord damage (such as trauma, osmotic pressure gradients, urea, and/or ammonia). Also, whether the additional ulcerative or inflammatory changes observed in the specimens obtained in children with postnatally repaired MMCs play a permissive role in meconium staining, or whether prolonged meconium exposure causes increased ulceration and inflammation within the MMC sacs remains unknown and warrants further evaluation.

Finally, due to the retrospective design, detailed long-term follow-up data, especially in the pMMC group, were not available to correlate morphological findings with neurofunctional outcomes. Nevertheless, to our knowledge, we provide for the first time direct evidence that meconium can be present in MMC sacs of human fetuses and newborns with spina bifida.

Conclusions

In the present study, we demonstrated that: 1) meconium passage in MMC appears to occur early in fetal life; 2) midgestational fetal MMC repair may not prevent meconium exposure completely but may reduce the duration of meconium exposure and thereby limit the toxic injury to the vulnerable neural elements; and 3) earlier prenatal management strategies may be needed to effectively prevent meconium exposure in MMC.

Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

References


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