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IN VITRO EVIDENCE OF COW'S MILK PROTEIN SENSITISATION IN NEONATAL NECROTISING ENTEROCOLITIS

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Background: Enteral feeding, particularly with formula feeds, has been implicated in the pathogenesis of necrotising enterocolitis (NEC). The direct role of milk antigens on mucosal and systemic inflammatory cascade in NEC has not been studied. The aim of the study is to characterise, in vitro, mucosal and systemic T-helper type 1/ type 2 (Th1/Th2) cytokine responses in NEC to mitogen and cow's milk protein (CMP) stimulation .

Methods: 11 neonates Bell's stage 2–3 NEC [median post-conceptional age (range): 31(27–41) weeks] and 36 neonatal (non-inflamed) controls [preterm blood controls (n=21): 33(28–40) weeks; gut controls (n=15): 39(34–42) weeks] were studied. Mucosal specimens were obtained from surgical resection margins in 9/11 NEC and non-inflamed resections or biopsies in gut controls. Cytokine profile, interferon-gamma (IFNg), interleukin-4 (IL-4) and IL-5, was enumerated using the enzyme-linked immunospot (ELISPOT) assay, for peripheral blood (PBMC) and lamina propria mononuclear cells (LPMC), at rest and following stimulation with mitogens [PHA, ConA] and CMP [â-lactoglobulin (BLG), á-casein].

Results: PBMC: Compared to preterm blood controls, NEC infants had increased baseline secretion, vigorous responses to mitogens for all 3 cytokines (by 20–120 fold), a strong BLG response (IFNg > IL-4 > IL-5) and a smaller casein response (Table 1).

Table 1: PBMC ELISPOT RESPONSE (spot forming cells/ 100 000 cells) * p<0.01; **p<0.001

Median (25th-75th)	Spontaneous	PHA	BLG	Casein
NEC IFNg (n=11)	19(9–53)**	412(285–717)**	125(81–281)**	39(17–62)**
NEC IL-4 (n=11)	17(9–59)**	484(237–748)**	81(58–206)**	23(7–206)**
NEC IL-5 (n=10)	23(4–69)**	353(189–463)**	74(49–328)**	14(3–82)*
Control IFNg (n=21)	0 (0–2)	7 (3–47)	1 (0–3)	1(0–4)
Control IL-4 (n=21)	0 (0–2)	4 (1–14)	1 (0–4)	1 (0–5)
Control IL-5 (n=21)	1 (0–3)	8 (1–14)	1 (0–4)	2 (0–5)

LPMC: Gut controls had no detectable cytokine production at rest or with mitogen/antigen stimulation. NEC infants had only a small but significant increase in baseline cytokine secretion [median (25th-75th), IFNg: 3(0–15), p 0.003; IL-4: 2(1–9), p 0.002; IL-5: 1(0–4), p 0.13]. PHA and ConA produced an increase in mainly IFNg and IL-4 positive cells respectively [IFNg: 11(5–41), p< 0.05; IL-4: 13(4–20), p 0.01]. Only 3/9 NEC infants showed positive response to BLG (IFNg) and none to casein.

Conclusion: Our study has demonstrated for the first time concomittant Th1/Th2 cytokine activation in NEC. We have also demonstrated significant CMP sensitisation primarily in the systemic compartment, without comparable mucosal activation, possibly suggesting a secondary event related to the alterations in mucosal barrier. These findings provide a novel mechanism for a potential direct contributory role of CMP in NEC inflammatory cascade. Clinical relevance with respect to e.g. post-NEC feeding regime management requires further investigation.

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INFANTILE ALLERGIC COLITIS: A HETEROGENOUS T-HELPER TYPE 1/ T-HELPER TYPE 2 FOOD MEDIATED MUCOSAL INFLAMMATION?

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Background: Allergic colitis (AC) is a well recognised cause of rectal bleeding in infancy. Dietary antigens have been clinically implicated, in particular, cow's milk protein (CMP). The underlying immune mechanism remains elusive. The aim of the study is to characterise the systemic and gut mucosal T-helper type1/ type 2 (Th1/Th2) cytokine profile in infants with allergic colitis and, to correlate the clinical CMP sensitivity with in vitro response.

Methods: AC infants (6/9 breast fed, onset 2 days-7 months) and 11 (non-inflamed) normal infant controls were studied. AC infants were classified as CMP sensitive (CMP+ve; n=6) or insensitive (CMP-ve; n=3) according to their clinical responses to dietary manipulations +/- double blind challenge. 8/9 AC infants underwent rectal biopsy. Cytokine profile, interferon-â (IFNâ), interleukin-4 (IL-4) and IL-5, was enumerated by ELISPOT (enzyme-linked immunospot) assay for peripheral blood (PBMC) and lamina propria (LPMC) mononuclear cells, at rest and following stimulation with mitogens [phytohaemagglutinin (PHA), concanavalin A (ConA)] and CMP [â-lactoglobulin (BLG), á-casein].

Results: PBMC: All controls and 7/9 AC infants showed no detectable spontaneous cytokine secretion. Mitogen stimulation produced a marked increase in cytokine production in AC infants, IFNâ IL-4 or IL-5, compared to controls (Table 1). LPMC from both controls and AC infants also showed no resting cytokine activation. ConA elicited a significant mucosal response in AC infants (IFNâ: p=0.01 and IL-4: p=0.01) but none in controls. In vitro CMP stimulation had no effect on PBMC or LPMC in controls and CMP-ve AC infants. In contrast, 3/6 CMP+ve AC infants had increased PBMC response to BLG and/or casein, albeit in no polarised Th1 or Th2 pattern. LPMC from 2/6 CMP+ve AC infants were milk antigen reactive (predominant BLG induced IL-5 secretion).

Table 1: PBMC ELISPOT RESPONSE (spot forming cells/ 100 000 cells) * p<0.05; ** p<0.01

Median (25th-75th)	Spontaneous	PHA	ConA
AC IFNâ(n=9)	0 (0–7)	51(43–247)**	147(57–336)**
AC IL-4 (n=9)	2(0–12)	15(2–26)*	26 (5–48)
AC IL-5 (n=7)	1(0–13)	16(4–36)*	23(7–110)*
Control IFNâ(n=11)	0 (0–1)	15 (7–50)	21 (5–65)
Control IL-4 (n=11)	0 (0–1)	2 (1–4)	6 (0–17)
Control IL-5 (n=8)	0 (0–1)	1 (0–5)	3 (0–8)

Conclusion: This is the first study that has attempted to correlate the in vivo with in vitro dietary antigen response in allergic colitis. Results following PHA/ConA stimulation illustrate Th1/Th2 immune activation in AC. In vitro CMP sensitisation could only be demonstrated in a minority of AC infants although all the in vitro responders had clinical evidence of CMP sensitisation. The differences may reflect limitations in assay sensitivity and the complexity of mucosal immune regulatory network.

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VITAMIN K STATUS IN PRETERM INFANTS: A RANDOMISED CONTROLLED TRIAL TO COMPARE THREE REGIMES OF PROPHYLAXIS

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Background: Neonatal vitamin K stores are precarious. Without adequate vitamin K prophylaxis preterm infants may be at particular risk of vitamin K deficiency bleeding (VKDB), but the optimal dose and route is unclear. Objective: To compare the vitamin K status of preterm infants during the first week of life and when on full enteral feeds, following three regimes of vitamin K prophylaxis after delivery. **Design/Methods:** Infants born at < 32 weeks gestation were randomised to receive vitamin K_1 0.5 mg intramuscularly (IM) (group 1: control), 0.2 mg IM (group 2) or 0.2 mg intravenously (IV) (group 3) on day 1. Cord blood was obtained; venous blood samples at 5 days postnatal and 2 weeks after establishment of full enteral feeds were analysed for serum vitamin K_1, vitamin K_1 2,3-epoxide, descarboxyprothrombin (PIVKA-II), and prothrombin time.

Results: Of 98 infants enrolled, 90 had a day 5 sample and 80 had a second sample obtained at a median (IQR) of 25 (22–31) days. Baseline characteristics (mean ±SD) for groups 1 (n=31), 2 (n=34) and 3 (n=33) were respectively: gestational age 28.3 ±2.5, 28.6 ±2.3, and 28.1 ±2.6 weeks; birthweight 1025 ±379, 1138 ±379, and 1060 ±371 g.

Serum vitamin K concentrations (ng/mL)

Prophylaxis	Day 5 (n=90)	After 2 weeks' enteral feeding (n=80)
0.5 mg IM (group 1: Control)	111.77(12.09–388.04)	2.52(0.53–33.17)
0.2 mg IM (group 2)	59.30(3.17–318.75)	1.57 (<0.05–6.79)
0.2 mg IV (group 3)	74.52(2.85–259.50)	1.28 (<0.05–6.23)

Values are median (range)

Compared with the control group, day 5 vitamin K concentrations were significantly lower in group 2 (p = 0.04), and at the time of established feeds they were lower in group 3 (p = 0.03). Three infants (one in group 2; two in group 3) had undetectable levels of vitamin K at the time of the second sample, however in each case PIVKA-II was undetected. Eleven of ninety (12%) infants (seven in group 1; four in group 3) had detectable concentrations of vitamin K epoxide on day 5 (p = 0.007).

Conclusions: Preterm infants given 0.2 mg or 0.5 mg vitamin K_1 at birth have very high serum concentrations during the first week of life. The presence of vitamin K epoxide is significantly associated with a higher dose (0.5 mg) of vitamin K given IM and with a reduced dose (0.2 mg) given IV, and may reflect vitamin K overload of the immature liver by these regimes of prophylaxis. With a reduced dose given IV or IM, vitamin K can fall to undetectable levels by as early as the fourth postnatal week. The risk of subsequent late VKDB may be increased in these infants unless further vitamin K supplements are given.

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RELATIONSHIP BETWEEN GASTROESOPHAGEAL REFLUX AND GASTRIC EMPTYING IN INFANTS

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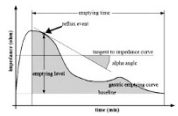
Background: Gastroesophageal reflux (GER) is a common and relevant clinical problem in infants. Relationships between GER and gastric emptying are still unclear. The multiple intraluminal impedance (MII) technique is based on the intraluminal measurement of electrical impedance between 6 electrodes during a bolus passage. On the basis of the temporal sequence of impedance changes, it can be identified whether a change in impedance is caused by a bolus moving in an antegrade (swallow) or a retrograde (reflux) direction. Epigastric impedance (EGI) is a technique to evaluate gastric emptying by measuring modifications in the impedance value in the gastric area by means of 4 skin electrodes. Aim of this study was to investigate the relationship between GER and gastric emptying by multiple intraluminal impedance and epigastric impedance in infants.

Methods: 9 term infants with GER simptoms were evaluated by MII (Sleuth Recorder Sandhill) connected to an intraluminal 6 channel probe and by EGI (Akern) simultaneously. Data were collected for 180 min after the ingestion of a milk meal for two time for each patient. Refluxate events were detected by MII as impedance changes in the distal channels proceeded sequentially to the more proximal channels. From the epigastric impedance emptying curve was calculated for each refluxate event the level of gastric emptying and the emptying velocity (figure). Data were compared by using Pearson correlation.

Results: 18 simultaneously analysis were performed for a total of 54 hours recording. 163 refluxate events were detected. The average emptying time and the number of reflux events were respectively 132±34(minutes), 9.1±3.3(events/analysis). The frequency of refluxate events was significantly correlated with the emptying velocity (r=0.93; p<0.05). No correlations were found between GER and gastric emptying time or level of gastric emptying.

Conclusion: Gastric emptying in GER infants has not a linear trend. Delayed emptying periods with an increasing of refluxate events are followed by fast emptying periods with decreasing of refluxate events. This variability suggests that a dismotility disorder interesting all the gastroesophageal tract might be relevant in infants affected by GER.



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CORD BLOOD IGF-2: RELATIONSHIP TO MACROSOMIA IN INFANTS OF GESTATIONAL DIABETIC AND NON-DIABETIC MOTHERS

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Background: Insulin and IGF-1 are considered as the main growth factors during fetal life. Both these factors were found accelerated in cord blood of LGA infants born to diabetic (IDM) and non-diabetic mothers. There is a few and not precise data about significance of IGF-2 in promoting of LGA. **Design:** to evaluate whether there is an association between occurrence of macrosomia and cord blood IGF-2 levels in infants of gestational diabetic and non-diabetic mothers. **Material and Methods:** The study material consisted of 74 LGA newborns (35 IDM and 39 non-IDM). 79 AGA neonates (37 IDM and 42 non-IDM) served as control groups. Gestational diabetes was recognized on basis of abnormal OGTT performed between 24–28 weeks of pregnancy. Macrosomia was defined as birthweight above 90th percentile. Gestational age (GA) in LGA groups were between 30 - 40 weeks while in AGA groups between 31 - 43 weeks. There was no signifant difference in mean GA between LGA and appropriate AGA group. The anthropometric parameters such as: birthweight, body length, head and chest circumference were measured at delivery and compared between the groups. Cord blood was sampled and IGF-2 levels were estimated using specific ELISA.

Results: Mean birth weight (4318g v. 4097g), length (59,2 v. 57,7 cm) and chest circumference (36,1 v. 35,7 cm) in non-diabetic LGA were significantly higher than in diabetic LGA, but there were no significant difference in head circumference (36,3 v. 36,0 cm). Cord blood IGF-2 levels in both LGA groups was not significantly different (diabetic – 383,3 versus 362,3 ng/ml in non-diabetic). A significant correlation (r=0,32; p <0,05) between birthweight and IGF-2 levels in both LGA groups but not in AGA groups was found. The significant correlation between head circumference and IGF-2 in diabetic (r= 0,37; p < 0,03) but not in non-diabetic LGA was also found. There was no significant correlation between cord blood IGF-2 levels and other anthropometric parameters in all groups. There was also no significant correlation between cord blood IGF-2 levels and gestational age.

Conclusion: There is an association between occurrence of macrosomia and cord blood IGF-2 levels independently of maternal diabetes.

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PROPHYLACTIC IBUPROFEN FOR THE PREVENTION OF INTRAVENTRICULAR HEMORRHAGE IN PRETERM INFANTS : A MULTICENTER RANDOMIZED STUDY

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Background: Intraventricular hemorrhage (IVH) remains a frequent complication in preterm infants and this point is crucial because the most severe IVH are related to a high risk of neurodevelopmental handicaps. Ibuprofen enhances cerebral blood flow autoregulation and has been shown to protect neurological functions following oxidative stresses in an animal model. For these reasons we hypothesized that the prophylactic use of ibuprofen would reduce the occurrence of IVH.

Methods: We studied 155 infants with gestational age less than 28 weeks . The infants were randomly assigned at seven neonatal care units to receive ibuprofen (10 mg/kg, within 6 hours of life, followed by 5 mg/kg after 24 and 48 hours) or placebo. Serial echoencephalography was performed 24 and 48 hours after the initial cerebral ultrosound, on postnatal day 7, 15, 30 and at 40 weeks postconceptional age. Grade 1 IVH or no IVH was considered a successful outcome, while grade 2 to 4 represented failure. The rate of ductal closure, side effects, and complications were recorded.

Results: Grades 2 to 4 IVH developed in 16% of the ibuprofen-treated infants and in 13% of the placebo group (p0.05). The occurrence of PDA was less frequent only on the 3rd day of life in ibuprofen group. There were no significant differences with respect to other complications or adverse effects.

Conclusions: Our study demonstrated that prophylactic ibuprofen is ineffective in preventing grade 2–4 IVH and that its use cannot be recommended for this indication. á