

# ORAL COMMUNICATIONS

## CO-01 MATERNAL TRANSMISSION OF ASTHMA RESISTANCE

A.P. Matson<sup>1,2</sup>, E.G. Lingenheld<sup>1</sup>, L. Zhu<sup>1</sup>, E. Rafti<sup>1</sup>,  
L. Puddington<sup>1</sup>

<sup>1</sup>Department of Immunology, University of Connecticut Health Center, Farmington, CT, USA, Department of Pediatrics, <sup>2</sup>Division of Neonatology, Connecticut Children's Medical Center, Hartford, CT, USA

Parental phenotype is known to influence the inheritance of atopic diseases, such as allergic asthma, with a maternal history being a more significant risk factor for progeny than paternal history. The mechanisms that explain parent of origin effects are not known, but could be the result of immune interactions between mothers and their offspring in utero via the placenta or in early post-natal life via breast milk. Upon initiation of this study, our hypothesis was that modulation of the maternal immune environment would affect development of immune responsiveness in offspring via transfer of factors to influence susceptibility or resistance to development of allergic airway disease. We developed murine models to elucidate the contribution of maternal derived Th1- or Th2-type immune responses during pregnancy on the subsequent ability of offspring to develop allergic airway disease. Female C57BL/6J mice were immunized with ovalbumin (OVA) in combination with different adjuvants to generate antigen-specific memory T cells capable of producing predominantly Th1- or Th2-type cytokines. Sensitized mice were subjected to primary challenge with aerosolized OVA (Aer) and 7-8 wk later rechallenged with OVA Aer during pregnancy. Susceptibility or resistance to development of OVA-induced allergic airway disease was then assessed in offspring with different histories of exposure to maternal Th1- or Th2-biased immune responses. Starting at 1 month of age, progeny were immunized with OVA under Th2 inducing conditions and subjected to OVA aerosol challenge. Offspring of Th1-type OVA immune mothers were more protected from the development of allergic airway inflammation and antigen-specific IgE responses after challenge than offspring of Th2-type OVA immune mothers or naive mothers. To evaluate the "environmental" effects of maternal derived Th1-type immune responses on the innate immune system of offspring, a parallel experiment was performed using mothers immunized and aerosol challenged as described above except using a heterologous antigen, bovine serum albumin (BSA). In contrast to the postnatal protection against Th2-type immune responses observed in offspring of OVA immune mothers, Th1-type maternal immunity directed against BSA provided no protection from OVA-induced allergic airway disease. We determined that maternal antibody was necessary for transmission of antigen-dependent resistance to postnatal allergic challenge. Immunocompetent offspring nursed on B-cell deficient mothers with allergic airway disease demonstrated no alteration in immune response to postnatal allergic challenge, as opposed to similar offspring nursed on immune competent mothers. These data suggest that maternal immunity to cognate antigen impacted the development of OVA-induced allergic airway disease during postnatal life, and maternal antibody transmission was a significant factor in resistance to disease in offspring.

## CO-02 PLACENTAL DYSFUNCTION, ANNEXIN V AND CONSEQUENCES ON THE NEONATAL HEMATOLOGIC STATUS

C. Farina, F. Sala, V. Dell'Orto, S. Malguzzi, G. Urban,  
P. Vergani

Dipartimento materno-infantile, Ospedale San Gerardo, Monza, Università degli Studi - Milano Bicocca, Italy

**Background:** The activation of platelets in the placental circulation is thought to be associated with pathogenesis in intrauterine growth restriction (IUGR). Thrombin formation on damaged endothelium in antiphospholipid syndrome (APS) and fluid shear stress by vasospasm of the uterine artery in pre-eclampsia and the haemolysis, elevated liver enzyme and low platelet count (HELLP) syndrome leads to the activation of platelets in maternal circulation. The immunohistochemical detection of annexin V in syncytiotrophoblasts lining the placental villi suggests that annexin V may play a role in the maintenance of intervillous blood fluidity in the intervillous space. **Objective:** Evaluate the relationship between pre-eclampsia, amount of annexin V and neonatal hematologic parameters. **Study design:** Preliminary results in the context of a larger pilot study on the role of annexin V (immunohistochemically detected) on the pathogenesis of preeclampsia. The neonates were also analyzed in two groups based on umbilical artery Doppler status. At birth, the groups were compared for anemia and thrombocytopenia. **Results:** These are reported in the following table. The newborns with AEDV were delivered 4 weeks earlier and were smaller. AEDV neonates were significantly thrombocytopenic at birth as preterm born from eclamptic mothers compared to control mothers newborns. Though annexin V was significantly higher in the control group, no significant correlations were found between its quantified assay and the hematologic variables of the mother and the newborn.

Table 1. Fetal positive end diastolic velocities (PEDV), absent or reversed end-diastolic velocities (AEDV)

ECLAMPTIC	AEDV			PEDV			t student	p
	mean	SD	n	mean	SD	n		
<b>ALL newborns</b>								
GA (wks)	30,4	3,5	15	34,4	3,9	23	3,2	0,0028
Peso (g)	1195	794	15	1953	930	23	2,5	0,0136
Hb (g/dl)	15,7	1,76	12	15,7	2,6	18		ns
PTL (10 <sup>3</sup> )	151	49,7	12	217,5	80,6	18	2,54	0,0166
PA (%)	60,43	10,3	7	57,2	22,4	13		ns
PTT (sec)	42	3,74	7	42,3	5	11		ns
Fibrinogen (mg/dl)	162,2	40,9	6	193,5	70	11		ns
	ECLAMPTIC			CONTROL				
<b>newborn &lt; 34 wks</b>	mean	SD	n	mean	SD	n	t student	p
<b>MOTHERS</b>								
Hb	12,3	1,2	22	11,6	0,7	19		ns
PTL (10 <sup>3</sup> )	168	86	22	190	67	19	0,9	0,37
PT (sec)	28,4	2,8	22	28,3	2,35	19		ns
PTT ratio	0,96	0,23	22	1	0,08	19		ns
INR	0,84	0,07	22	0,91	0,08	19		ns
Fibrinogen (mg/dl)	435	78	22	433	59,5	19		ns
<b>NEWBORNS</b>								
Hb (g/dl)	15,3	1,8	21	15,7	2,07	11		ns
PTL (10 <sup>3</sup> )	161,3	45	21	233	84,24	11	3,18	0,0034
PA (%)	60	18	13	49,6	4,1	3		ns
PTT (sec)	42	5,3	13	43,3	3,05	3		ns
Fibrinogen (mg/dl)	147	1,26	9	110	14,1	2		ns
Annexin V	11,04	3,4	20	16,71	5,9	7	2,92	0,0073

*Conclusions:* hematologic status of the newborn at birth is proportional to the degree of placental dysfunction and is related to the fetal Doppler pattern. Although annexin V was higher in the control group, suggesting a role in the regulation of the coagulatory cascade, its clinical effects have to be defined.

**CO-03**  
**EXPOSURE OF MURINE NEONATES TO NIMA-LIKE ALLOANTIGENS INDUCES VIGOROUS T HELPER RESPONSES**

S.J. Opiela, B. Adkins

*Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL, USA*

Neonates are thought to be uniquely susceptible to tolerance induction, particularly to alloantigens. Indeed, there is a growing body of evidence that early life exposure to non-inherited maternal alloantigens (NIMA) may lead to life-long tolerance to NIMA. However, there is also evidence that exposure to NIMA during fetal or neonatal life may lead to cytotoxic priming against maternal antigens. The available data suggests that the level of NIMA exposure during early life may determine the immunological outcome. While high doses of NIMA-like cells can lead to tolerance, we recently reported that murine neonates mount vigorous cytotoxic responses to low doses of NIMA-like alloantigens. This finding is particularly important, as many studies have demonstrated that neonates typically mount poor cytotoxic responses unless strong Th1-inducing agents are also administered. In addition to poor cytotoxic responses, neonates typically exhibit Th2-biased memory CD4<sup>+</sup> cytokine responses compared to adults. This Th2-bias develops to a wide variety of antigens, and is characterized by high level IL-4 (Th2) and deficient IFN $\gamma$  (Th1) production. As cytotoxic responses are usually accompanied by vigorous Th1 responses in adults, we hypothesized that the development of cytotoxicity to donor NIMA-like alloantigens in neonates would be accompanied by adult level Th1 function. In fact, neonates developed cytokine responses to NIMA-like alloantigens that were even greater than those that developed in adults. The neonatal Th1 memory response was 3-fold greater than in adults, and neonates produced a striking ~70-fold more IL-4 than adults. These cytokine responses were accompanied by greater frequencies of both IFN $\gamma$  and IL-4 secreting neonatal CD4<sup>+</sup> T cells compared with adult cells. Based on previous studies, we hypothesized that a molecule expressed by the NIMA-like cells was necessary for the development of these neonatal cytotoxicity and Th1 responses. One candidate molecule is CD40L, which is poorly upregulated by neonatal CD4<sup>+</sup> T cells. To test the importance of this molecule in eliciting neonatal T cell responses, neonates were exposed to NIMA-like cells deficient in CD40L. Under these conditions, neonates exhibited vigorous Th1 and Th2 cytokine responses, similar to those observed with wild-type cells. However, neonates were unable to develop cytotoxic function against NIMA-like alloantigens. Therefore, the development of specific cytotoxicity, but not Th1/Th2 responses, is dependent on CD40L expressed on donor cells. Overall, we conclude that exposure to low doses of NIMA-like alloantigens induces robust Th1/Th2 and cytotoxic responses in neonates. This suggests that under specific conditions (i.e. low dose), early exposure to NIMA may indeed lead to immunological priming of all arms of T cell adaptive immunity, rather than tolerance.

**CO-04**  
**MYOCARDIAL PERFORMANCE INDEX PROVIDES PRELIMINARY EVIDENCE OF PROLONGED HEMODYNAMIC EFFECT OF TRANSFUSION IN PREMATURE NEWBORNS**

M. Radicioni, D. Mezzetti

*UTIN Azienda Ospedaliera Perugia, Italy*

Our knowledge of the impact of red blood cells transfusions (RBCT) on premature newborn hemodynamics is incomplete. We studied the myocardial performance index (MPI) of left ventricle in prematures before and up to 200 hours after a transfusion. This index has been used in adults and children as a reliable indicator of global ventricular function. Our results are a subset of a larger data set obtained in newborn from 22 to 40 weeks of gestational age (GA). We studied 18 RBCT in 15 prematures without significant disease other than respiratory distress of prematurity (mean GA 26.1 $\pm$ 3.3 weeks, 15/18 ventilated, 4/18 patent ductus arteriosus; mean transfusion volume 14.6 $\pm$ 3.1 mL/Kg) and calculated the MPI before transfusion (baseline), within 48 hours and from 60 to 200 hours after the transfusion. The index was obtained with standard technique averaging three or more cardiac cycles. Our results also show that two different MPI behaviours are recognizable: babies with a high baseline MPI (> 0.55) and of lower corrected GA show a tendency toward decrease (e.g. improvement) of MPI with a nadir at 40-80 hours post-transfusion, while babies with low baseline MPI (< 0.55) and of more advanced corrected GA show the opposite, displaying an increase of MPI that peaks at 40-60 hours post-transfusion (MPI at 40-90 hours post-transfusion T-test, baseline hi versus low: p < 0.01). Since baseline MPI is inversely correlated with corrected GA ( $\beta$  -0.37, p < 0.01) it difficult to analyze separately the two factors. The mean peak shift, both positive and negative, was 0.2 ratio units. These preliminary results show that the hemodynamic impact of RBCT in prematures is more far-reaching than suspected and has a complex relationship with the maturation of the cardiovascular system. Other factors that are likely to be involved in this hemodynamic response and that need a thorough investigation are ventilation status, ductus patency and transfusion volume. Further studies may elucidate this complex interplay and help the neonatologist to identify the baby most likely to benefit from the transfusion and the right timing and technique for RBCT.

**CO-05**  
**ROLE OF TLR4 AND CD14 IN LPS-INDUCED IL-8, TNF-ALPHA AND IL-10 RELEASE IN NEONATES**

E. Levi, A. Mouchtouri, K. Xini, G. Xanthou, S. Fotopoulos, M. Xanthou

*Neonatal Immunology Laboratory, Bā NICU "Aghia Sophia" Children's Hospital, Athens, Greece*

Sepsis related morbidity in neonates is mediated through inflammatory responses. Toll-like receptor (TLR)-4 and CD14 expressed on immune cells are important inflammatory mediators which bind to invading pathogens and trigger the release of cytokines. *Aims:* To investigate TLR4 and CD14 surface expression in healthy neonates and their role in IL-8, TNF- $\alpha$  and IL-10 release following LPS stimulation. *Materials and Methods.* Peripheral blood from 20 preterm, 20 fullterm neonates and 20 adults was cultured with LPS. TLR4 and CD14 surface expression was measured by flow cytometry. Cytokine levels were assayed by ELISA on culture supernatants. For blocking experiments, blood samples

were pre-treated with anti-TLR4 and anti-CD14 antibodies. **Results.** Neonates exhibited increased TLR4 and CD14 expression following LPS stimulation, as compared to adults. The LPS-induced release of IL-8 was higher in neonates compared to adults, whereas that of TNF- $\alpha$  and IL-10 was similar. Blocking of TLR4 inhibited LPS-induced IL-8 release significantly more in preterms (38%) than in adults (14%). CD14 blockade resulted in an even greater inhibition (80%) of IL-8 release, similar in all groups. Blocking of both TLR4 and CD14 did not result in a further increase in the inhibition of IL-8 release. TLR4 blockade inhibited (60%) LPS-induced TNF- $\alpha$  and IL-10 release, while CD14 blockade completely inhibited (100%) TNF- $\alpha$  and IL-10 release in all groups. **Conclusions.** Neonates have higher TLR4 surface expression and IL-8 release following LPS stimulation and thus may be more susceptible to bacterial infections. Preterms appear to depend more on TLR4 for IL-8 release than adults. CD14 is critical for LPS-induced IL-8, TNF- $\alpha$  and IL-10 release, whereas the role of TLR4 is less prominent. LPS-induced IL-8 release depends not only on CD14 and TLR4 signalling but also on other immune receptors.

#### CO-06

##### IMMUNE SYSTEM EVALUATION IN INFANTS FROM MOTHERS WITH AUTOIMMUNE DISEASES

L. Ciardelli<sup>1</sup>, M. Motta<sup>2</sup>, M. Marconi<sup>3</sup>, F. Garofoli<sup>1</sup>, A. Gasparoni<sup>2</sup>, C. Offer<sup>2</sup>, M. Stronati<sup>4</sup>, G. Chirico<sup>2</sup>

<sup>1</sup>Research Laboratories (Neonatal Immunology), IRCCS Policlinico San Matteo, Pavia, Italy; <sup>2</sup>Department of Neonatology and Neonatal Intensive Care, Spedali Civili, Brescia, Italy; <sup>3</sup>Department of Paediatrics, University of Pavia, Italy; <sup>4</sup>Neonatal Intensive Care Unit, IRCCS Policlinico San Matteo, Pavia, Italy

Rheumatic autoimmune diseases have a higher prevalence in women, particularly during their childbearing age. Many patients can carry out one or more pregnancies, but drugs administered to the mothers during pregnancy and lactation might affect the fetal and neonatal immune system development and function. We performed an open study to evaluate immune function of newborns, whose mothers received immunosuppressants during pregnancy for the treatment of their autoimmune disorders. Neonates enrolled in the study were assigned into 2 groups: 15 infants from mothers receiving low dose aspirin (100 mg/die) were included in group 1, and 19 infants from mothers receiving glucocorticoids alone or in combination with other immunosuppressive agents (azathioprine, cyclosporine A or hydroxychloroquine) in group 2. Mothers received low glucocorticoid dosage ranging from 15 to 125 mg, but one who received a high dosage of more than 1000 mg i.v. because of a lupus nephritis flare. Lymphocyte subpopulations and "in vitro" immunoglobulin production were evaluated at birth and after 1, 6 and 12 months of life. Peripheral blood lymphocytes (PBL) were stained to evaluate subpopulations of lymphocytes expressing CD3, CD4, CD8 (T lymphocytes), CD19+/CD45+ (B lymphocytes), CD20+/IgM+, CD5+/CD19+ (B-1 cells) and CD4+/CD25+ high (human circulating CD4+ regulatory T lymphocytes) by flow cytometry. The "in vitro" immunoglobulin production was evaluated culturing the infants PBL in resting and stimulated conditions (culture medium was supplemented with irradiated CDw32 L cells, anti-CD40

monoclonal antibody and rhIL-10). IgG, IgA and IgM concentrations in the culture supernatants were determined in both conditions by ELISA. No statistically significant differences were observed in the percentage of B, T and regulatory lymphocytes between the two studied groups. As well as the IgG, IgA and IgM "in vitro" production, in resting and stimulated condition, did not show a statistical difference between the two groups. This data underline that at birth, infants exposed "in utero" to immunosuppressive agents do not show differences in percentage of B and T lymphocytes in comparison to infants not exposed to immunosuppressants. During the 12 months follow-up, infants from the two studied groups showed normal and similar distribution of lymphocyte subsets with particular reference to the expression of T lymphocyte regulatory and activation subset and to the expression of B-1 cells. This suggests a regular immune function in both infants groups. Moreover, the "in vitro" Ig production, in resting and stimulated condition, were similar in group 1 and 2 at birth and during the follow-up period, thus confirming the normal function of B lymphocyte. In conclusion, our results show that neonate's immune system development and function is not affected from the prenatal exposure to mother's immunosuppressive therapy, at least with the dosages used in this study.