40.2 MATERNAL IMMUNE ACTIVATION LEADS TO INCREASED LEVELS OF INFLAMMATORY CYTOKINES IN THE ABSENCE OF OVERT MICROGLIA ANOMALIES IN THE MIDBRAIN

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Background: Inflammatory theories in schizophrenia have gained increasing recognition and acceptance in recent years. The evidence supporting a role of altered inflammatory processes in the etiology and pathophysiology of schizophrenia involves early-life exposure to infectious pathogens or inflammatory stimuli, increased expression of cytokines and other mediators of inflammation in the adult central nervous system (CNS) and periphery, as well as signs of glial anomalies. Given the role of dopaminergic deregulation in the pathophysiology of schizophrenia, inflammatory processes in the midbrain may contribute to dopamine abnormalities in the midbrain and its subcortical and cortical output regions. Here, we tested this hypothesis using an established neurodevelopmental mouse model with relevance to schizophrenia, namely the maternal immune activation (MIA) model.
Methods: Pregnant C57BL6/N mice on gestation day 17 were treated with the viral mimetic polyriboinosinic-polyribocytidilic acid (poly(I:C)) or vehicle control solution. We then quantified the gene transcripts of an array of pro-inflammatory cytokines, acute phase proteins, and dopaminergic markers in the midbrain of MIA offspring (N=32) and control offspring (N= 32) at adult age. We also assessed the cell density of microglial cells expressing Iba1 and CD68 by immunohistochemistry to ascertain whether putative inflammatory changes are accompanied by microglia anomalies. Given the large sample sizes, we performed two-step recursive cluster analyses in order to identify possible subgroups of offspring that are characterized by "high" and "low" inflammatory profiles.
Results: When considering the entire treatment group, MIA-exposed offspring displayed significantly increased expression of several inflammatory cytokines in the ventral midbrain, including IL-1b ($p < 0.01$), TNF-a ($p < 0.01$) and SERPINA3 ($p < 0.01$). These inflammatory changes occurred in the absence of overt microglia anomalies but were paralleled by changes in dopaminergic markers. The two-step cluster analyses further identified subgroups of MIA-exposed offspring that are characterized by a "high" (41 %, N = 13) and "low" (59 %, N = 19) inflammatory profiles. The "high" inflammatory subgroup of MIA-exposed offspring was defined by marked elevations of SERPINA3, IL-1β, IL-6, and TNFα mRNA levels (all p's < 0.01).
Discussion: Maternal immune activation during pregnancy causes persistent signs of inflammation in the offspring's midbrain. In agreement with post-mortem studies in schizophrenia, these inflammatory abnormalities are clearly noticeable in a subgroup of MIA-exposed offspring only. Hence, prenatal immune activation may be one of the factors inducing lasting inflammatory changes relevant to (some cases of) schizophrenia and may contribute to dopaminergic dysfunctions in this disorder.

40.3 MATERNAL IMMUNE ACTIVATION AND CHRONIC HALOPERIDOL INTERACT TO INCREASE MICROGLIAL ACTIVATION IN VIVO: DO ANTIPSYCHOTICS INFLAME THE BRAIN?

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Background: Evidence-based medicine suggests that a subset of schizophrenia is associated with an inflammatory syndrome. To fully harness the potential of novel immunomodulatory therapeutics, it is critical to first determine the impact of antipsychotics on microglial function in vivo. Evidence suggests antipsychotics are anti-inflammatory; however, this is based on in vitro models and non-clinical doses of antipsychotics in vivo. It therefore remains unknown if antipsychotics promote detrimental neuroinflammation or beneficial, homeostatic changes in the brain. To address this question, we explored the effects of chronic haloperidol treatment on microglia in a rat maternal immune activation (MIA) model, representative of schizophrenia pathology.
Methods: Pregnant SD rat dams were exposed to poly (I:C) on GD15 (4 mg/kg, i.v.; n=5; POL) to induce MIA, or saline (n=5; CON) as a control. At 4 months of age, male offspring from CON and POL (n=2 per litter), were randomly allocated to treatment with either haloperidol (0.5 mg/kg/d s.c.) or vehicle for 28 days by osmotic minipumps, giving four groups: CON/vehicle; CON/haloperidol; POL/vehicle and POL/haloperidol (all n=10). After 28d treatment, animals were culled and perfused transcardially with 4% PFA. Fixed brain tissues were dissected, cryoprotected and microtome sectioned (1 in 12 series, 40 µm thick). Serial sections were stained for Iba1 as a marker of microglia using an immunoperoxidase protocol. The density and morphology (soma size) of Iba1+ microglia were then assessed in the corpus striatum (CS) and anterior cingulate cortex (ACC) using unbiased stereology. Data were analysed using 2x2 ANOVA in SPSS with main effects of prenatal, postnatal and pre x post-natal interactions.
Results: There were significant main effects of prenatal exposure to POL on Iba1+ microglia density in the CS ($F(1,32)=18.09$; $p<0.001$) and the ACC ($F(1,32)=5.04$; $p<0.05$) and for Iba1+ microglia soma sizes (increased) in POL offspring in both the CS ($F(1,32)=88.5$; $p<0.001$) and ACC ($F(1,32)=45.06$; $p<0.001$). There were no main effects of postnatal treatment (vehicle or haloperidol) on Iba1+ microglia density in either the CS or ACC, but there were main effects of postnatal treatment for Iba1+ microglia soma size in both CS ($F(1,32)=17.3$; $p<0.001$) and ACC ($F(1,32)=7.69$; $p<0.01$). Strikingly, there were significant interactions between pre- and post-natal treatments for both Iba1+ density in the CS ($F(1,32)=5.15$; $p<0.05$) and PFC ($F(1,32)=9.43$; $p<0.01$) as well as soma size in the CS ($F(1,32)=11.6$; $p<0.01$) and ACC ($F(1,32)=11.7$; $p<0.01$). Post-hoc testing on this interaction confirmed a significant increase in both Iba1+ density and soma size in poly(I:C)-exposed offspring treated with haloperidol, relative to all other groups in both the CS ($p<0.01$) and ACC ($p<0.01$).
Discussion: Our data suggest increased microglial density and activation in the CS and PFC of rats exposed to POL in utero. Haloperidol treatment for 28 days replicating clinically comparable dosing and pharmacokinetics did not affect microglia density in saline-exposed offspring, but increased Iba1+ soma size in both CS and ACC, also suggestive of microglial activation. Strikingly, there were significant interactions between prenatal POL exposure and post-natal haloperidol treatment, leading to significantly increased microglia density and soma size in both CS and ACC. Taken together, these preliminary data suggest adult haloperidol treatment may interact with prenatal immune activation to worsen neuroinflammation.

40.4 LOW-DOSE RISPERIDONE TREATMENT IN ADOLESCENCE PREVENTS THE DEVELOPMENT OF NEUROINFLAMMATION IN THE MATERNAL IMMUNE ACTIVATION MODEL

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Background: Postnatal consequences of prenatal immune activation mimic a broad spectrum of neuro-psycho-pathological features phenotypic of schizophrenia (SCZ). We previously showed that SCZ-relevant behavioral

and brain structural abnormalities emerging in adult offspring of moms exposed to the viral mimic polyI:C, are prevented by treatment with the atypical APD risperidone (RIS) in adolescence, prior to the emergence of structural and behavioral abnormalities. Given the increasing centrality of neuroinflammation in SCZ and its treatment and/or prevention, here we assessed whether adolescent RIS is able to prevent neuroinflammation in the polyI:C offspring.

Methods: On gestation day 15, pregnant Wistar rats were injected IV with polyI:C (4 mg/kg/ml) or saline. Pups were weaned on postnatal day (PND) 21. Preventive treatment with RIS (Janssen, Belgium; 0.045 mg/kg) was administered daily on PNDs 34–47. Offspring were sacrificed on PND48, prior to full spectrum of structural and behavioral abnormalities, or on PND90, after the emergence of structural and behavioral abnormalities. Microglial activation was assessed in ten regions (nucleus accumbens, striatum, substantia nigra, frontal, anterior cingulate and occipital cortices, dorsal hippocampus (sub-regions CA1, CA3 and dentate gyrus [DG]) and ventral hippocampus (vHPC), using quantitative [3H]PK11195 autoradiography. Another cohort of offspring underwent behavioral testing and imaging.

Results: ANOVAs of [3H]PK11195 binding in offspring sacrificed on PND48 revealed no significant effects of prenatal polyI:C in any of the regions assessed. In adult male offspring, [3H]PK11195 binding was significantly increased in the CA1, CA3 and DG hippocampal subfields as well as in the frontal and occipital cortices, compared to controls. No such increases were observed in polyI:C offspring treated with RIS in adolescence (significant prenatal x preventive treatment interactions, and significant difference in [3H]PK11195 binding between polyI:C-VEH and saline-VEH but not between polyI:C-RIS and saline-VEH offspring in post-hoc analyses, in each of the regions). In females, [3H]PK11195 binding was significantly increased only in the vHPC, occipital cortex, and nucleus accumbens. Such increases were not observed in polyI:C female offspring treated with RIS in adolescence. In a second cohort of offspring, prenatal poly-I:C led to structural abnormalities in the hippocampus, striatum, prefrontal cortex and lateral ventricles, as well to deficits in selective attention, executive function, working memory and social interaction, all of which were prevented by RIS.

Discussion: Increased [3H]PK11195 binding in the brains of adult poly-I:C offspring is consistent with increased uptake of [11C]PK11195 in patients with SCZ, measured in-vivo by PET. Microglial activation emerged in adulthood, with no such activation in young (PND48) offspring. Late emergence of microglial activation parallels the developmental course of behavioral and brain structural abnormalities in poly-I:C offspring (Piontkewitz et al, 2011a, 2012a; Piontkewitz et al, 2009), suggesting that these late-emerging abnormalities are linked. The latter is supported by the fact that RIS in adolescence prevented the emergence of behavioral and brain structural abnormalities as well as microgliosis in the adult offspring. These data suggest that prevention of adult microgliosis is one of the mechanisms underlying RIS capacity to prevent polyI:C-induced behavioral and neuroanatomical deficits, however, a causal relationship remains to be established.

41. RECONSIDERING THE EVIDENCE FOR CLOZAPINE FOR TREATMENT REFRACTORY SCHIZOPHRENIA

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Overall Abstract: The superiority of clozapine for treatment refractory schizophrenia was, until very recently, seen as one of the few unshakeable truths in psychiatry. But the pre-eminence of clozapine has recently been called into question by meta-analyses. What are we to make of the fact that meta-analyses of clinical trials, supposedly the pinnacle of evidence based medicine, fail to show an effect which seems clearly evident to most clinicians, and on which many of our guidelines are based? Have we believed in a fairytale for the past three decades? Or do biases in RCTs and methodological limitations of meta-analyses explain the results? These questions will be discussed by Dan Siskind.

Another way to address the question of efficacy is to examine the pharmaco-epidemiological evidence using population-based registers. Jari Tiihonen will present data from his seminal studies of mortality and readmission rates under clozapine treatment versus other antipsychotics, as well as other data. These data seem to show powerful positive effects of clozapine at the population level. Furthermore, more recent evidence suggests a role for clozapine in reducing rates of violent offending, with new data presented for the first time by Vishal Bhavsar.

Finally, despite clinical guidelines recommending the use of clozapine, the actual rates of clozapine use are much lower than expected, with large regional and international variations. There is evidence that the burden of blood monitoring deters physicians from prescribing clozapine. Yvonne van der Zalm will present new data from a cluster randomised trial testing the efficacy and safety of an intervention to increase rates of clozapine prescribing by employing nurse practitioners trained in the initiation and monitoring of clozapine.

John Kane, author of the first, seminal RCT of clozapine in 1988, will lead the discussion.

41.1 WHAT DO META-ANALYSES TELL US ABOUT CLOZAPINE'S EFFICACY AND EFFECTIVENESS FOR TREATMENT REFRACTORY SCHIZOPHRENIA?

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Background: Clozapine has long been considered the gold standard antipsychotic for treatment refractory schizophrenia (TRS). There have been a number of recent meta-analyses of efficacy of clozapine on psychotic symptoms and effectiveness in reducing hospitalisations that have sparked debate on the role of clozapine.

Methods: Current literature regarding the efficacy of clozapine for TRS, including pair-wise and network meta-analyses of RCTs with reported outcomes of total psychotic symptoms, positive symptoms and negative symptoms were reviewed. We also examined the results of a meta-analysis of the effectiveness of clozapine on reducing hospitalisations based in RCTs and observational studies.

Results: Two recent meta-analyses: Samara et al (2016), a network meta-analysis in JAMA Psychiatry; and Siskind et al (2016) a pairwise meta-analysis in BJPsych, found similar equivocal results for total psychotic symptoms. However, Siskind et al (2016) found clozapine to be superior to other anti-psychotics for positive symptoms. Factors influencing the difference in results included pair-wise vs network methodology and sensitivity analyses of pharmaceutical industry support. Of note, only 40% of people with TRS responded to clozapine. Clozapine's effectiveness for reducing hospitalisations was significant, with a relative risk of 0.74 (95%CI 0.69–0.80).

Discussion: There are a lack of recent non-industry funded randomised control trials of clozapine compared to SGAs, which hinders an equivocal statement about the superiority of clozapine for total psychotic symptoms. However, there is evidence to suggest that clozapine is superior to other antipsychotics, including SGAs, for positive symptoms. In terms of effectiveness, initiation of clozapine can reduce the proportion of people hospitalised and reduce bed days. Use of clozapine needs to be balanced against its adverse drug reaction profile. There remains a need for more effective treatments for TRS, and biomarkers to identify TRS.