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Immune factors in the Neuregulin-1 knockout mouse model of schizophrenia

P Snikeris
University of Wollongong, petas@uow.edu.au

Xu-Feng Huang
University of Wollongong, xhuang@uow.edu.au

Elisabeth T. Frank
University of Wollongong, efrank@uow.edu.au

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Centre for Translational Neuroscience

University of Wollongong

SCHIZOPHRENIA RESEARCH INSTITUTE

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Peta Snikeris[1,2], Xu-Feng Huang[1,2], Elisabeth Frank[1,2]

1: Centre for Translational Neuroscience, School of Health Sciences, University of Wollongong, Wollongong, 2522, NSW, Australia

2: Schizophrenia Research Institute, 384 Victoria Street, Darlinghurst, 2010, NSW, Australia

Contact: pas649@uow.edu.au

BACKGROUND

Schizophrenia

- Neurodevelopmental disorder affecting 1% of the population
- Complex gene x environment interaction
- Neuregulin 1 (Nrg1) gene mutation highly associated in genetic linkage studies
- Neuregulin-1 heterozygous knockout (Nrg1 KO) mice are an established model of Schziphrenia[1]

Schizophrenia and Cytokines

- Cytokines
  - modulate peripheral immune response
  - can penetrate the blood-brain-barrier (BBB)
  - regulate complex behaviours in healthy brain
- Altered cytokine levels in schizophrenia patients in both blood and CSF
- Cytokine levels correlate with symptom severity
- Epidemiological link between schizophrenia and lower incidence of autoimmune and inflammatory diseases

AIMS

- To determine the basal Nrg1 KO mouse peripheral cytokine profile as observed in schizophrenia patients
- To determine if the peripheral cytokine profile in Nrg1 KO animals is altered following a chronic immune stimulus compared to wild type litter mates

METHODS

Nrg1 KO mice with a heterozygous knockout of the Nrg1 transmembrane protein domain on C57Bl/6 background

1. Spleen samples obtained:
   a) Adulthood (PND161) (n=6-19)
2. Plasma samples obtained:
   a) Late adolescence/early adulthood (PND56) (n=3)
3. Immune challenge:
   a) B16F0 murine melanoma cell line injected subcutaneously ($3x10^5$ cells per mouse) (Nrg1 KO and WT littermates; n=8)
   b) Control = PBS injections (Nrg1 KO and WT littermates; n=8)
   c) Late adolescence/early adulthood (PND56)
   d) Sacrifice after 10 days (PND66)

- Spleen cells analysed used BD Biosciences fluorescent conjugated antibodies against T and B cell surface markers on LSRII flow cytometer
- Plasma analysed with multiplex flow cytometry bead array to determine levels of IFN-γ, TNF-α, IL-1α, IL-1β, IL-2, IL-4, IL-6, KC (murine IL-8), IL-10 and IL-12p70

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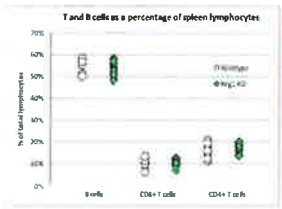
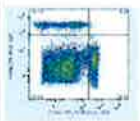
RESULTS Basal



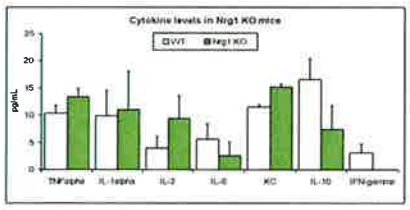


Representative flow cytometry dot plot of total spleen lymphocytes demonstrating 10.75% CD8+ cells in the first and 18.67% CD4+ cells in the forth quadrant

B and T cells and their subsets as a percentage of total spleen lymphocytes in Nrg1 KO mice compared to WT litter mates

No differences were found for total B or T-cells as a percentage of total spleen lymphocytes when comparing Nrg1 KO mice with wild type litter mates without an immune challenge (n=6-19).

In addition, no differences were found in the B cell subsets (follicular and marginal B cells) or in the T cell subsets (active, naive, memory or regulatory T cells) in immune-unchallenged animals (n=6-19; data not shown)



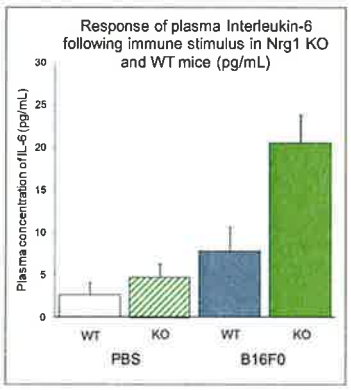
Plasma Cytokine Levels in Nrg1 KO mice compared to WT litter mates

Under basal conditions, with no additional immune stimulus, Nrg1 KO mice showed a tendency towards up-regulation of TNFα, IL-2 and KC (murine IL-8) (NS). Whereas IL-10 and IFNγ demonstrate a trend towards decreased plasma levels (NS). Cytokine levels are expressed in pg/mL+SEM, n=3. IL-1β, IL-4 and IL-12p70 were not detected in our samples.

- A tendency towards increased TNFα, IL-2 and KC (murine IL-8) indicated a potential pro-inflammatory state in Nrg1 KO animals in the absence of an immune stimulus.
- A trend towards decreased IL-10 and IFNγ in the presence of possible increased TNFα suggested dysfunction of cytokine regulation.
- This was consistent with data of schizophrenia patients.

RESULTS Immune Challenge

We therefore challenged Nrg1 and WT mice with a murine melanoma cell line (B16F0) to induce a chronic immune response



Plasma Interleukin-6 levels following B16F0 melanoma challenge in Nrg1 KO compared to WT.

Following a chronic immune stimulus (10 days), the concentration of plasma IL-6 was 20.6 ± 3.2 pg/mL in Nrg1 KO mice compared to 7.8 ± 2.8 pg/mL in wild type litter mates. This represents a 3 fold higher increase in plasma IL-6 levels from control animals in Nrg1 KO mice compared to WT.

B16F0 Melanoma

Image representative of tumour growth in both KO and WT animals with average tumour size 7mm x 4mm measured using calipers following excision of tumour. No difference was found in tumour size or growth rate (data not shown)

DISCUSSION

- IL-6 is produced in the periphery as well as the brain[2]
- IL-6 crosses the blood-brain-barrier from blood to brain via saturable transport mechanisms[3]
- Neurons are responsive to IL-6 signalling - role in neurite outgrowth, differentiation and survival of neurons as well as cognition[4]
- Membrane bound IL-6 receptor has been shown on adult murine and human neurons[4]
- Soluble IL-6 receptor is produced endogenously in brain[5]
- Signal transduction component (gp130) widely distributed in the brain[6]

Relevance to Schizophrenia:

- ✓Patients have consistently demonstrated increased plasma levels of IL-6[5,7]
- ✓Higher plasma levels of IL-6 have been correlated with worse symptomology[7]
- ✓Anti-psychotics reduce plasma IL-6 levels[8]

First genetic neurodevelopmental mouse model of schizophrenia that mimics the neuro-immunology of the illness.

FUTURE DIRECTIONS

- Acute immune stimulation (LPS)
- Cytokine Microdialysis:
  - measure central IL-6 changes
  - following LPS treatment in Nrg1 KO mice
- Trial result:

Measuring IL-6 in the brain using microdialysis

Initial trial of cytokine microdialysis shows an increase in IL-6 in the brain within 2 hours of an LPS injection (0.5mg/kg; time 0). Microdialysis performed using 35kDA culoff probe at 1µl/min flow rate. Amount (pg/mL) is raw data measured using a bead based cytokine array on a Luminex100 flow cytometer. Cytokine recovery rate to be calculated