**Annotated Bibilography**

Johnson, S., Knight, R., Marmer, D. J., & Steele, R. W. (1981). Immune deficiency in fetal alcohol syndrome. *Pediatric Research, 15*(6), 908-911. doi:10.1203/00006450-198106000-00005

In this observational study, Johnson et al. evaluated the clinical histories and immune functions of 13 children with fetal alcohol syndrome in order to determine immune deficiencies that may increase susceptibility to infections. Although the total white blood cell counts of the children with fetal alcohol syndrome were similar to those of control children, the children with fetal alcohol syndrome were observed to have abnormal lymphocytes and a greater incidence of both life-threatening and minor infectious diseases. The authors speculate that possible abnormalities of phagocytic mechanisms may be responsible for many of the observed clinical infections. Overall, the authors suggest that increased susceptibility to infection among children with fetal alcohol syndrome may be due to defects of the immune system caused by alcohol exposure in utero.

Nelson, S., Bagby, G. J., Bainton, B. G., & Summer, W. R. (1989). The effects of acute and chronic alcoholism on tumor necrosis factor and the inflammatory response. *The Journal of Infectious Diseases, 160*(3), 422-429. doi:10.1093/infdis/160.3.422

Nelson et al. investigated the effects of acute and chronic alcoholism on lipopolysaccharide-induced activity of the cytokine tumour necrosis factor, which is involved in inflammatory responses. Using rats to model acute and chronic alcoholism, lipopolysaccharide was injected directly into the trachea. Then, bronchoalveolar lavage fluid was analyzed to quantitate tumour necrosis factor levels in the lungs. Similar levels of tumour necrosis factor was observed in chronic alcoholic rats compared to control rats, but acute intraperitoneal injection of alcohol significantly ethanol decreased tumour necrosis factor levels found in the bronchoalveolar lavage fluid of both chronic alcoholic rats and control rats. However, within alveolar macrophages recovered by lavage, tumour necrosis factor levels were significantly lower in chronic alcoholic mice compared to control mice, while acute alcohol intoxication did not result in significant differences in tumour necrosis factor levels. Overall, the findings illustrate that inflammatory cytokine levels in the lungs decrease in response to acute alcohol intoxication but not chronic alcoholism, while inflammatory cytokine levels within alveolar macrophages decrease in response to chronic alcoholism, but not acute alcohol intoxication. Although this investigation examined the effect of chronic alcohol in vivo, this study did not address whether chronic alcoholism or acute alcohol intoxication by pregnant mothers produce the same effects in offspring.

Yirmiya, R., Pilati, M. L., Chiappelli, F., & Taylor, A. N. (1993). Fetal alcohol exposure attenuates lipopolysaccharide-induced fever in rats. *Alcoholism: Clinical and Experimental Research, 17*(4), 906-910. doi:10.1111/j.1530-0277.1993.tb00862.x

Using a murine model, Yirmiya et al. investigated the effect of alcohol exposure in utero on LPS-induced fever to show that fetal alcohol exposure results in lower resistance to infectious agents. Rats that experienced fetal alcohol exposure were observed to require higher doses of LPS in order to induce hyperthermia. Furthermore, the fevers of rats that were exposed to alcohol in utero were of lower temperatures and declined faster compared to control animals. These findings provide support that prenatal alcohol exposure causes impairment of immune responses.

Yirmiya, R., Tio, D. L., & Taylor, A. N. (1996). Effects of fetal alcohol exposure on fever, sickness behavior, and Pituitary–Adrenal activation induced by interleukin-1β in young adult rats. *Brain Behavior and Immunity, 10*(3), 205-220. doi:10.1006/brbi.1996.0019

In this second paper by Yirmiya et al., the effects of IL-1β administration into rats prenatally exposed to alcohol were examined in order to further elucidate the mechanisms that result in abnormalities of the immune system in response to fetal alcohol exposure. IL-1β was found to induce fevers that were of lower temperature in fetal alcohol-exposed rats compared to control rats. Through this follow-up study, Yirmiya et al. provides further evidence that prenatal alcohol exposure results in impairments of mechanisms that mediate the effects of cytokines normally involved in host defence.

Chiappelli, F., Kung, M. A., Tio, D. L., Tritt, S. H., Yirmiya, R., & Taylor, A. N. (1997). Fetal alcohol exposure augments the blunting of tumor necrosis factor production in vitro resulting from in vivo priming with lipopolysaccharide in young adult male but not female rats. *Alcoholism: Clinical and Experimental Research, 21*(8), 1542-1546. doi:10.1111/j.1530-0277.1997.tb04487.x

Chiappelli et al. investigated the effects of fetal alcohol exposure on cytokine production mediated by blood macrophages in response to pathogen-associated molecular patterns such is lipopolysaccharide. The study found that prenatally exposed male rats demonstrated significantly decreased production of the cytokine TNF-α compared to control males. However, female rats did not show any significant difference in TNF-α production when comparing experimental and control groups. In contrast to previous investigations by other researchers, the findings by Chiappelli et al. suggest that there are sexually dimorphic effects of fetal alcohol exposure on immune function.

Goral, J., Karavitis, J., & Kovacs, E. J. (2008). Exposure-dependent effects of ethanol on the innate immune system. *Alcohol, 42*(4), 237-247. doi:10.1016/j.alcohol.2008.02.003

In this review, Goral et al. draw attention to growing evidence that suggests that alcohol is a significant immunomodulatory factor. In particular, alcohol has been found to have dose-dependent effects that alter inflammatory responses through affecting intracellular signalling events that activate transcription factors involved in regulating pro-inflammatory cytokine production. Acute alcohol exposure has been shown to be associated with a decrease in production of inflammatory mediators. In contrast, chronic alcohol exposure has been linked to increased circulating levels of pro-inflammatory cytokines in the blood. However, it is important to note that a study by Nelson et al., mentioned previously, illustrated exceptions to the generalizations suggested by Goral et al.

Kvigne, V. L., Leonardson, G. R., Borzelleca, J., Neff-Smith, M., & Welty, T. K. (2009). Hospitalizations of children who have fetal alcohol syndrome or incomplete fetal alcohol syndrome. South Dakota Medicine : *The Journal of the South Dakota State Medical Association, 62*(3), 97, 99, 101.

In this retrospective observational study, Kvigne et al. investigate the hospitalization rates and medical diagnoses of children with fetal alcohol syndrome compared to children who did not have fetal alcohol syndrome. In the first year of life, children with fetal alcohol syndrome were observed to have had more hospitalizations as well as longer lengths of hospitalization compared to control children, with pneumonia being the most common reason for hospitalization among children with fetal alcohol syndrome. When combining the findings of previous studies, which suggest that alcohol exposure in utero may result in defects in the immune system, with the findings by Kvigne et al., which show that pneumonia is the most common cause of hospitalization among children with fetal alcohol syndrome, there appears to be an association between prenatal alcohol exposure and increased lung infections.

Lawrenz, M. B., Fodah, R. A., Gutierrez, M. G., & Warawa, J. (2014). Intubation-mediated intratracheal (IMIT) instillation: A noninvasive, lung-specific delivery system. *Journal of Visualized Experiments : JoVE*, (93), e52261. doi:10.3791/52261

Lawrenz et al. outline a protocol for intubation-mediated intratracheal instillation and rationalize why it is a favourable method for infecting the lungs of mouse models. Compared to humans, mice have significantly larger upper respiratory tracts, normalized against total lung capacity, which may significantly influence disease progression in the lower respiratory tract. Therefore, by allowing the means to infect the respiratory tract directly without involvement by the upper respiratory tract, intubation-mediated intratracheal instillation allows researchers to better extrapolate the results of studies involving lower respiratory tract infections from mouse models to humans.

Han, H., & Ziegler, S. (2013). Bronchoalveolar lavage and lung tissue digestion. *Bio-Protocol, 3*(16) doi:10.21769/BioProtoc.859

Han and Ziegler describe a protocol for bronchoalveolar lavage with mice, which can be performed to extract cells from the lungs for subsequent examination. The general procedure involves passing a bronchoscope through the mouth and into the lungs, adding fluid to the lungs, and recovering the fluid which now contains components from the respiratory tract.

Abcam. Flow cytometry intracellular staining protocol. (n.d.). Retrieved from http://www.abcam.com/protocols/flow-cytometry-intracellular-staining-protocol

A protocol for intracellular staining of secreted proteins and subsequent analysis by a flow cytometer is described by the biotech company, Abcam. The general procedure involves blocking protein secretion by treating cells with Brefeldin A, permeabilizing cells to allow entry of antibodies, and using fluorescent antibodies against proteins of interest which can then be detected by flow cytometry. As cytokines are proteins that are secreted by immune cells, intracellular staining and flow cytometry allows cytokine production levels to be quantified.