P081 STUDY OF THE INFLUENCE OF N-3 FATTY ACIDS IN THE SYNTHESIS OF NITRIC OXIDE DURING PARACOCCIDIOIDOMYCOSIS.

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Background: Fatty acid omega-3 has various functions in the body, including by modulating the immune system. Flaxseed and perilla is an excellence vegetable source of alpha-linolenic acid (LNA, 18:3 n-3), approximately 50%. LNA is a precursor of polyunsaturated fatty acids of the n-3 series (EPA and DHA) that have anti-inflammatory characteristics. They regulate enzymes involved in the synthesis of nitric oxide (NO). NO is the principal cytotoxic mediator and an important regulatory species in immune system. Beyond that, NO has been shown to be an important fungicide. Paracoccidioidomycosis (PCM) is the most prevalent systemic mycosis in Latin America, caused by term-dimorphic fungus Paracoccidioides brasiliensis (Pb). The purpose of this study was analyzed the influence of n-3 fatty acid in production of NO during the paracoccidioidomycosis experimental murine.

Methods: Groups of male Swiss mice, infected intraperitoneal with 2×106 yeasts of Paracoccidioides brasiliensis (Pb18), were fed a diet enriched with 3% of flaxseed oil or 10% of perilla meal for eight weeks. Control groups received commercial food. After 1st, 4th and 8th weeks peritoneal lavage was collected. Furthermore, peritoneal macrophages were cultured and challenged in vitro with a Pb fungal suspension. Peritoneal lavage and culture supernatants were collected and the concentration of NO was measured using the standard reaction of Griess.

Results: In the 1st week, omega-3 did not influence the production of NO. In the 4th week there was an increase in NO synthesis ($P < 0.05$) by the groups infected and fed flaxseed or perilla-enriched diet, that continued until 8th week of experiment. These data suggest that omega-3 may affect the microbicidal activity of macrophages by regulate the expression of enzymes that are involved in the production of NO.

Conclusion: Diet with 3% of flaxseed and 10% of perilla stimulated NO synthesis in the 4th week and it remained in the later phase of paracoccidioidomycosis. Therefore, these data suggest that lipids in the diet may affect the microbicidal activity, especially macrophages.

P082 IMPLEMENTATION OF NEXT GENERATION SEQUENCING (NGS) TECHNOLOGY FOR HLA TESTING: KEY LESSONS LEARNED FROM A MULTI-CENTER ALPHA STUDY.

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Background: NGS technologies have significant potential to generate high resolution HLA typing in a high throughput fashion. However, implementation in clinical immunogenetics laboratories involves extensive validation of a multitude of interdependent procedures, instruments, reagents, and software. To address these challenges we participated in March of 2014 in a multicenter Alpha study lead by the Immunogenetics Laboratory of a large academic institute that has developed an NGS-based protocol for HLA typing on the Illumina MiSeq.

Methods: We participated in the Alpha study with 2 main objectives: (1) validate the in-house developed NGS genotyping protocol for the HLA-A, B, C, DRB1 and DQB1 loci; (2) identify key critical steps in the protocol that we had problems with in our own lab prior NGS implementation. This abstract focuses only on key technical lessons learned from the study. The Alpha study results are independently submitted in another abstract.

Results: Participation in the Alpha study identified some critical steps in NGS workflow: (1) the quantitation of genomic DNA needs to be assessed by using methods that directly measure double stranded DNA concentration during primary sample preparation prior to target enrichment, (2) the library preparation reagents need to ensure adequate and uniform coverage throughout the length of the HLA targeted genomic region, (3) use appropriate methods for fragment size selection including magnetic beads and/or specialized electrophoretic instrumentation after library preparation, (4) quantitation and normalization of DNA of each amplicon to a standard DNA amount prior to amplicon pooling, (5) use two independent genotyping algorithms for data analysis to avoid systematic errors of genotype assignments caused by individual analysis software. Specific details about these lessons learned and more technical tips will be provided in this presentation.

Conclusion: In spite of the technical complexity of NGS workflow, identifying key critical steps in the process and identifying troubleshooting strategies are critical in implementing NGS in the clinical laboratories. Inter-laboratory validation studies and seeking the guidance from laboratories who developed NGS expertise is invaluable for expediting the learning curve for the implementation of this technology.

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