TREATMENT OF "INFLUENZA A" INFECTION WITH DIALYZABLE LEUKOCYTE EXTRACT: EVALUATION IN A MURINE MODEL.

Elvia Pérez Soto[1], Carlos Pérez de La Mora[2] Mario García Solís[3], Carlos Cabello Gutiérrez[4], María Eugenia Manjarrez Zavala[4], Consuelo Gómez García[1] and David Guillermo Pérez Ishiwara[1,5]

1. Laboratory of Molecular Biomedicine I, Institutional Program Molecular Biomedicine, ENMyH-IPN,Guillermo Massieu Helguera No. 239, Col. The Ladder, Mexico, C.P.07320, e-mail:elviser_1085@hotmail.com
2. Biological Products for Export S. A.of C. V., México, C. P. 02480
3. Pathology, Hospital Tláhuac; México, C. P. 13200
4. National Institute of Respiratory Diseases (INER); México, C. P. 14080
5. Research Center for Applied Biotechnology (CIBA); Tlaxcala, México, C. P. 90700

Dialyzable Leukocyte Extract (*DLE*), influenza, inflammation

Introduction. Influenza an acute infectious disease, which resolves over a period of 2-5 days, is caused by viruses from the family Orthomixoviridae, mainly of the generous influenza A, which is characterized by a high capacity for mutation, causing pandemics, such as the H1N1 pandemia, where Mexico was severely affected. The severity of infection depend on viral and host factors, such as exacerbated immune response, causing inflammation and even death. There are different strategies to combat the disease, including vaccines for the control and prevention: pharmacological treatment using some antiviral agents such as Tamiflu® (oseltamivir), as well as other drugs that act on the immune response (1). An alternative strategy is the use of some products that modulate the inflammatory response. In 2011, Li and colleagues obtained a *DLE* from pigs vaccinated with the H1N1 virus, observing that there was the activation of T cells in healthy animals (3). No studies have been conducted to show the therapeutic effect of such products in experimental models.

Due the limited number of pharmacological agents for the treatment and/or prevention of influenza and the development of drug resistance, we decided to evaluate the immunomodulatory effect of a Dialyzable Leukocyte Extract (*DLE*) in a murine experimental influenza A infection to determine whether it is capable to modify the development of pulmonary immunopathology.

Methods. H1N1 vacunal pandemic strain 2009 was used to intranasally infect Balb/c mice (6-8 weeks of age). Infections were done using 90 µL suspension of influenza virus with a dose approximately of $1.6x10^6$ p.v.i. corresponding a 8UHE and $3.2x10^6$ p.v.i. referring a 16 UHE per mouse. The profilactic treatment with the *DLE* was done before 7 days of infection and therapeutic treatment was done 1 day post-intection, during 7 days. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (4).
For preparation and isolation of lung tissue for histopatological examination, the mice were sacrificed and lungs were inflated with 10% formalin at constant pressure. Lungs were fixed in 10% formalin and stained with H&E (4).The bronchoalveolar lavage (BAL) was obtained from animals for Th1/Th2 cytokine response determination by immunoassay (ELISA).

Results. Lungs from Infective animals with 8 and 16 UHE respectively, showed interstitial pneumonitis, alveolar collapse, hemorrhage, inflammatory infiltrate and destruction of tissue architecture (Fig.1 B, C). On the other hand, therapeutic and prophylactic treatments with *DLE* showed display a marked reduction of the histophatological damage, from 90% to 80% reduction, respectively. Lung architecture of treated animals resembles the normal lung architecture, preserving the alveoli, the bronchioles and an important inflammation reduction. The biological effects were better using prophylactic treatment in both 8 and 16 UHE infected animals (Fig.1 A,D-G).

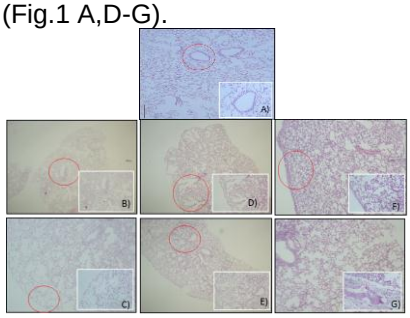

Fig.1 Histopathologic evaluation of *DLE* in influenza A (8 and 16 UHE).

ELISA for IL-10 and IFN-γ using bronchoalveolar lavage showed that the antiviral response decreased in infected group, was strongly increased after *DLE* administration, on the other hand, IL-10, an anti-inflamatory cytokine, increases it expression.

Conclusions. The *DLE* treatment display a benefic effect against influenza infection generating an antiviral and anti-inflammatory response.

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