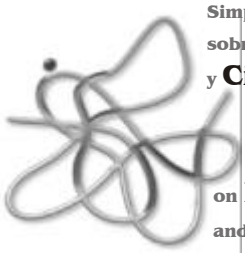

Simposio Internacional sobre Interferón y Citoquinas

International Symposium on Interferon and Cytokines

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Este simposio agrupa trabajos que muestra la estrecha correlación de las citoquinas con la fisiopatogenia de enfermedades de origen autoinmune, alérgico e idiopático. Se muestran resultados de estudios de aplicación terapéutica del interferon gamma y se describen productos capaces de modular la expresión de citoquinas en diferentes modelos experimentales.

The presentations in this session show the correlation between cytokines and physiopathology of idiopathic, allergic and autoimmune diseases. Therapeutic trials with application of interferon gamma are shown and products able to modulate the cytokines expression in different experimental models are described

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In Vitro *and* In Vivo *IFN Gamma Regulation of Chemokine Receptors in Peripheral Blood Leukocytes from Atopic Asthmatics*

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IFN γ exerts major pro-inflammatory, regulatory and anti-inflammatory actions on immune defense responses. Eosinophil, neutrophil, monocyte, and lymphocyte infiltrations are critical events in asthma. Chemokines stimulate the migration of the susceptible subset of inflammatory cells. The chemokine receptors CCR-3 are mainly expressed on eosinophils, basophils and Th2 cells while CCR-5 are expressed on Th1 cells. More recently it has been demonstrated that the IFN γ down-regulates the expression of some chemokine receptors. The effect of this cytokine *in vitro* on some parameters in asthmatic patients was studied. PBL from patients and controls were isolated by Ficoll-Hypaque gradients. IgE determinations were done using an ELISA for total IgE. The cells were incubated in the absence or the presence of 500 or 1000 IU/mL of rhu IFN γ for different times and washed and lysed for RT-PCR analysis. The RT-PCR was performed using a Perkin-Elmer kit. The amplified bands were runned in 2% agarose gels and quantified. The basal levels of CCR-3 in asthmatic patients tend to be higher than in controls. IFN γ, through the modification of the expression of chemokine receptors in Th1 and Th2 cells from atopic asthmatics could exert a beneficial effect on these patients, regulating the migration of some inflammatory cells involved in the pathogenesis of the disease.

Adverse Reactions Associated with Heberon Alpha R

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Heberon alpha R (interferon alpha-2b, recombinant) is a useful treatment for many diseases such as viral hepatitis, Papillomatosis, condylomata acuminata, Peyronié's disease, renal cell carcinoma, multiple myeloma, AIDS-related Kaposi's sarcoma, non-Hodgkin's lymphoma, bladder cancer, mycosis fungoides, infancy hemangioma, chronic myelogenous leukemia and melanoma.

The purpose of this study was to describe the frequency of adverse reaction associated with Heberon alfa R therapy. The adverse events reported in seven clinical trials (N = 507) with Heberon alfa: papillomatosis in infancy and adults, hemangioma, chronic hepatitis B, multiple sclerosis, superficial bladder cancer, Peyronié's disease, comparative bioequivalency and pharmacokinetics of interferon, basocelular carcinoma and mycosis fungoides, were analyzed. The very common adverse events (> 10%) were "flu-like" symptoms, particularly fever, chills, headache, myalgia, arthralgia and asthenia. The common adverse events (1-10%) included weight loss, nausea, vomiting, general malaise, tachicardia, hypotension, pruritus, increased transaminases, bone pain, alopecia, decrease of libido, back pain, pain or inflammation at injection site, dizziness, diarrhea, insomnia, mouth dryness, myelosuppression, allergic reactions, cistitis and metallic taste. Other adverse events were less frequent (< 1%). Discontinuation of treatment because of adverse reactions was reported in 2.7% of the cases. In summary, Heberon alfa R is a relatively safe drug causing similar adverse reactions to those reported for other interferon alpha-2 preparations.

Citocinas como inmunoreguladores del proceso reproductivo

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El proceso reproductivo comprende diferentes etapas, entre ellas las más destacadas son: Gametogénesis, Fecundación, Implantación y Desarrollo. En cada una de estas etapas se ha demostrado que existen factores inmunoreguladores que intervienen para mantener un equilibrio funcional ya sea a nivel de tracto reproductor masculino y femenino así como durante la implantación y gestación. Entre estos factores se encuentran las citocinas, que de acuerdo a evidencias *in vivo* e *in vitro*, establecerían una red de interacción entre ellas que estimulando o inhibiendo la respuesta inmune cumplen el rol inmunoregulador de los eventos que caracterizan a este proceso (Comhaire et al.,1999).

Con el propósito de establecer la presencia de las citocinas en fluídos masculinos y femeninos del tracto genital, se determinaron mediante ensayo inmunoenzimático específico (SERO ELISA CytoScreen.Ca.) las concentraciones de IL-6, IL-8, IL-10, TNFα, IFNα en semen de 124 hombres de parejas infértiles para relacionarlas con las características seminales. Así mismo se analizaron las concentraciones de IL-6, IL10, TNFα e IFN α en semen de 7 toros sementales de un Centro de Inseminación Artificial. En cuanto a fluídos femeninos se determinaron las concentraciones de IL-6, TNFα e IFNα en líquido folicular de 59 mujeres que participaron en un Programa de fecundación in vitro. En el Centro de Reproducción Asistida UNIFERTES, que dirige el Dr. Jorge Lerner.

Los resultados obtenidos demuestran que todas las citocinas investigadas se encuentran presentes en semen humano y de bovino así como en líquido folicular humano, aunque en concentraciones variables. Por otra parte la aplicación de la prueba de Spearman reveló que existen correlaciones positivas y negativas, al comparar las concentraciones de una citochina respecto a las otras. Estas correlaciones fueron significativas. Los valores de IL-8 y TNFα se correlacionron con concentración y movilidad espermática en humanos. En el estudio realizado en bovinos se observó que la presencia de concentraciones más elevadas de IL-6 disminuía la movilidad espermática y la morfología normal de los espermatozoides. En cuanto a la presencia de estas citocinas en líquido folicular se demostró su presencia en niveles variables, dependiendo del estado de madurez folicular.

Nuestros hallazgos demuestran que estos compuestos están jugando un rol importante en el tracto genital masculino y femenino y apoyan la hipótesis de su participación como inmunoreguladores en distintos eventos del proceso reproductivo, interactuando con factores inmunoneuroendocrinos, en acciones bidireccionales.

Cytokines Pattern th1/th2 in Animal Model of Induced Arthritis

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The most common chronic inflammatory disease affecting the synovial articulating joints is Rheumatoid Arthritis (RA).

This disease is characterized by the infiltration of immunocompetent cells into the synovial tissue and fluid, proliferation of synovial fibroblasts,and the formation of pannus, tissue which invades and degrades the articular cartilage and subchondral bone.

Recent advances in our understanding of cellular and molecular mechanisms of rheumatoid arthritis have highlighted a critical role for th1 cytokines.

Several experimentally induced rat and mouse models of autoimmune erosive arthritis are currently widely used to evaluate potential etiopathogenetic mechanisms in RA.

Although no model can accurately reflect every aspect of such a heterogeneous disease, each model may represent different pathogenic aspects of relevance and might provide unique insights into the etiopathogenesis of RA and/or related arthropathies. They are also used extensively to evaluate potential new therapeutic agents.

Here we used a model in Lewis rats (RT-1) were arthritis is induced with Micobacterium tuberculosis in complete Freund´s adjuvant. Our purpose is to corroborate, in order to validate this animal model towards assays of novel drugs against RA, that affected rats have a pattern of cytokines th1 similar to the RA in humans. So that a real judgment for evaluating a drug, will be the inversion of cytokine pattern to th2 or tendency to the balance th1/th2.

To validate this animal model upon basis of cytokines profiles will be very useful for understanding the complex relationship between genetic and not genetic factors and will establish basis for generation of novel drugs and new therapies.

Use of IFN Gamma in One Patient with Idiopathic Pulmonary Fibrosis

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Idiopathic Pulmonary Fibrosis (IPF) is characterized by a fibroproliferative response that almost always causes rapid fibrotic destruction of the lungs. The median survival is four to five years after the onset of symptoms. The standard treatment for IPF is oral glucocorticoids but it is often ineffective. We studied one 64 years-old, feminine gender, IPF patient, histologically-verified by peripheral transbronchial biopsy. She had symptoms for 10 months. Complete pulmonary function tests were done at baseline and after 1, 3, 6, and 12 months of treatment and high resolution computed tomography (HRCT) was performed at baseline, 3, 6 and 12 months. The patient was treated for 12 months with IFN gamma 1 000 000 IU three times per week, intramuscularly and daily oral prednisone (60 mg for the first two months, 50 mg for the third month, 40 mg for the fourth month, 30 for the fifth month, 20 mg for the sixth month, 10 mg until the 9th month, and 5 mg until one year.

The patient had grade-2 dyspnea, dry cough, crackles at chest auscultation, a mild restrictive ventilatory defect at pulmonary function test, and a decrease in CO_2 diffusing capacity. HRCT showed basal fibrotic lesions. Before the third month of treatment the patient improved clinically, the dry cough disappeared and dyspnea decreased to grade 1. She reported an improved ability to perform the daily living activities; the ventilation was normal. The gas exchange improved since the first month. At the third month, she had no evidence of fibrosis at HRCT. The treatment with interferon gamma and dose-decreasing prednisone was associated with substantial improvements in this IPF patient's conditions. At present, other 3 patients are under treatment.

Recombinant Interferon Gamma in Pediaetric Patients with Juvenile Rheumatoid (Chronic) Arthritis as First or Alternative Remission Inducer Option

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Juvenile chronic arthritis (JCA) is a collagen disease characterized by chronic synovitis and extra-articular symptoms, starting before 16 years old. This work is an open trial, designed with the aim to evaluate the effect of IFN gamma in JCA as first or alternative remission inducer option. Twenty patients (2.5 - 15.7 years old; 11 girls) were included. Five had pauciarticular, 9 poliarticular and 6 systemic JCA.

The main symptoms of the latter were rash (5), hepatosplenomegaly (4), myalgia (3), and pericarditis (2) and adenopathy (2). Three patients had uveitis. Affected joints ranged from 1 to 21. Recombinant IFN gamma (Heberon Gamma R, Heber Biotec, Havana), 50 000 IU/Kg (up to 1 x 10^6 IU) was given intramuscularly: daily, during 4 weeks, then 3 times per week for 3 months, then twice per week to complete 2 years of treatment. Four patients withdrew before 6 months of treatment: 2 moved and were lost from follow-up, and 2 due to adverse reactions. They were considered as failures. The treatment was well tolerated in the rest of the patients. Clinical evaluation included: number of affected joints, presence of systemic symptoms, childhood HAQ, possibility of suspending steroids, adverse reactions to treatment and laboratory parameters. The results showed: complete response (10 patients: 50 %), partial response (3: 15 %) and no response (7: 35 %). Steroids were suspended in 5 patients out of 9 who had them. In none of the patients who did not had received steroids before the IFN schedule, these drugs were necessary. These results suggest that the beneficial effect of IFN gamma treatment in JCA, previously described for very advanced patients, refractory to other procedures, could be extended to patients with less advanced disease and thus prevent functional impairment and deformities.

Characterization of the anti proliferative properties of boifn-w1 obtained from P. Pastoris

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The BoIFN-ω1 obtained from *P. pastoris* has antiluteolitic and antiviral properties in ruminate. The present work pretends the characterization of the anticellular and antiproliferative properties of BoIFN-ω1.

This showed a low capacity to inhibit the colony formation by the MDBK cell line cultured at low cell density. At doses of 500 and 1000 antiviral units/mL, BoIFN-ω1 decreased the number, and size of colonies.

The anticellular capacity of BoIFN-ω1 was very low when you compare to the equivalent doses of OvIFN-τ on MDBK cells. The colony counts showed a significant reduction ($P < 0.01$) on number of visible colonies at 20 units/mL of OvIFN-τ and at 500 units/mL of BoINF-ω1. However, BoINF-ω1 is a potent anticellular agent on swine cell line. Colonies formation by swine cell line Pk-15 cultured at low concentration were inhibit at doses 20 and 100 antiviral units/mL of BoIFN-ω1. Direct colony counts showed that the reduction on number of visible colonies was significant ($P < 0.01$) at 20 and 100 units/mL of BoINF-ω1.

Doses response studies of inhibition of proliferation by BoINF-ω1 indicated that it is efficacious on MDBK cells by accumulative concentration. At 4,5 and 6 days of BoINF-ω1 treatment, only doses of 500 and 1000 antiviral units/mL produced significant reduction in the proliferation. This reduced antiproliferative activity of BoINF-ωl is different to OvINF-τ in the same system. Though, BoINF-ω1 has a potent antiproliferative activity on Pk-15 cell line over a broad range of concentrations.

Actividad antiviral y antiluteolítica del interferón –w 1 bovino recombinante obtenido en la levadura P. pastoris

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El tiempo de vida del cuerpo lúteo es extendido por señales de reconocimiento de la preñez proveniente del trofoblasto. Estas señales están mediadas por una nueva familia de inteferones tipo I denominados τ. Se demostró para los interferones tau y alfa que integran esta familia las propiedades antiluteolíticas de los mismos.

Recientemente se ha corroborado que el IFN-ω1 bovino tiene efecto antiluteolítico en rumiantes. Este trabajo reporta la purificación del IFN-ω1 bovino en la levadura metilotrófica *P. pastoris*.

Se muestra un proceso de purificación con el cual se obtienen hasta 300 mg de proteína pura por litro de fermentación, con una pureza de más del 80% y una actividad antiviral específica in vitro de 108 UI / mg. Además, se demuestra el alargamiento significativo del tiempo de vida del cuerpo lúteo en vacas cicladas.

También se determino la capacidad antiviral *in vivo* de este interferón, mediante la introducción de un estado antiviral en carneros retados con 100 veces la dosis letal 50 (DL 50) del virus Aujeszky.