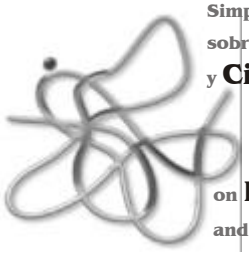

Simposio Internacional sobre **Interferón** y **Citoquinas**

International Symposium on **Interferon** and **Cytokines**

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ENFERMEDADES INFECCIOSAS

Los trabajos comprendidos en este simposio evidencian la participación de las citoquinas en el mecanismo de patogénesis de diferentes enfermedades infecciosas, su influencia en el curso natural de las mismas y la eficacia de su empleo como agentes terapéuticos.

In this session, there are presentations in order to evidence the participation of cytokines in the pathogenesis of several infectious diseases, also to demonstrate their influences in the natural courses of evolution and their efficacy as therapeutic agents.

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Safety and Efficacy of Heberon Alfa R for the Treatment of Chronic Hepatitis C Infected Subjects with Transfusion Dependent Thalassemia in Iran

Mirmomen SH, Ghofrani H, Daryani NE, Fourotan Pishbijary H, Farahvash MJ, Sharifian RA, Azmodeh F

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Up to 30% of Iranian adult multi-transfused thalassemic patients are infected with hepatitis C virus (HCV) that can intensify the progression of liver disease, caused by iron overload, in this group of patients. Our aim was to assess the biochemical and virological response of Heberon Alfa R and its safety in thalassemic patients with chronic HCV infection.

This trial was a single center, open label, single treatment prospective study of Heberon Alfa R(3 MU, every other day) for a period 12 months. 29 subjects, 13 to 56 years old (mean 25.1 ± 10.4 SD years), whose serum HCV-RNA was positive and mean ALT remained greater than 1.5 times upper limit of normal in the past 6 months before the study were enrolled. A percutaneous liver biopsy performed before treatment and all patients underwent monthly assessment of any adverse events and monitoring of serum ALT. Qualitative serum HCV-RNA was obtained in months 3, 6 and at the end of therapy.

Liver biopsy showed mild fibrosis in 33.3%, moderate fibrosis in 56.7% and cirrhosis in 10% of patients. Siderosis was severe in 14 patients (46.7%). 2 nonsplenectomized patients discontinued Heberon because of mild cytopenia, which resolved in less than one week after interruption of therapy. One patient discontinued therapy due to occurrence of hypothyroidism in months 11 of therapy.

Another patient withdrew from the study because of illegal concurrent medication usage at after 10 months of therapy. The following were some of the important adverse events, observed during the study period: Flu syndrome in 29(100%), chills or fever>39° in 14(48%), local pain in 14(48%), transient gastrointestinal symptoms in 13(44%), weakness in 5(17%), local induration in 3(10%) and edema in 2(7%) of patients. By the end of 12 months of therapy: 15 patients out of 27 (55.6%) had a normal ALT and negative HCV-RNA (complete response), 2 patients (7.5%) had negative PCR but abnormal ALT and 2 other patients had normal ALT with positive PCR. 10 of responders followed for a mean duration of 12 months (range 8 to 21 months) and 6 of them (60%) relapsed (abnormal ALT with positive PCR). Viral clearance was a delayed event in our patients (29% by the end of months 3 and 63% by months 7) but ALT normalization occurred in 94% of responders by the end of months 3.

Our experience indicate that the cure of HCV-related liver disease in thalassemic patients is not an unrealistic aim and may be reached with the safe and inexpensive Interferon preparation (Heberon Alfa R) in a sizable portion of cases. In opposite to non-thalassemic patients which most of the viral responses happens in the first 3 months of therapy in this group of thalassemic patients we find that maximum virologic response happened between 3 to 6 months of therapy. Although interferon-α is an effective drug for initial response in thalassemic patients infected with HCV its efficacy, with above dose and duration, for maintaining long term remission is under question.

Expresión diferencial de TGF-b en animales inmunizados con proteínas recombinantes de Entamoeba Histolytica

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Inmunidad parcial o completa hacia el absceso hepático amibiano (AHA) ha sido inducida por infección experimental o por vacunación de animales con proteínas de *E. histolytica.*

Con el fin de estudiar la respuesta inmune celular hacia la lectina amibiana de 220 kDa, se obtuvieron proteínas de fusión derivadas de ésta (GST-L1b y M11).

Grupos de hámsteres fueron inmunizados con GST-L1b y M11 y retados con *E. histolytica.* Los esplenocitos de los hámsteres inmunizados e inmunizados y retados mostraron una respuesta proliferativa hacia las proteínas GST-L1b y M11. Para caracterizar la respuesta inducida por estas proteínas, a partir de los esplenocitos se determinaron las citocinas IL-10, TNF-a y TGF-b por RT-PCR. M11 induce IL-10 y TGF-b en esplenocitos de hámsteres inmunizados con M11 y L1b y retados, sin embargo TNF-a sólo se induce en los animales inmunizados con L1b y retados.

L1b induce IL-10, TNF-a y TGF-b en esplenocitos de animales inmunizados con M11 y retados, pero ninguna de ellas esta presente en esplenocitos de animales inmunizados con L1b y retados. En estas células no se detectó TGF-b sugiriendo que L1b es capaz de inducir respuestas de tipo Th1. En ensayos de protección se observó que L1b induce protección parcial hacia el desarrollo del AHA (85.06 ± 5.25 %) comparado con los animales no inmunizados e infectados.

Síntesis de IFN-beta y Protección a la infección por adenovirus tipo 6 en células HEp-2 en presencia de un ARNt de origen fúngico

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La utilización de inductores de la síntesis de Interferón endógeno (IFN) para el tratamiento de afecciones virales, bacterianas o tumorales viene desde la década de los 50. La ausencia de resultados concretos y reproducibles hace que aún se busque a la molécula que cumpla esta función adecuadamente. Actualmente se trabaja con moléculas modificadas a partir de las originales buscando aún al inductor de IFN ideal. En este trabajo se mostrarán los resultados obtenidos al utilizar a un ácido ribonucléico de transferencia modificado (ARNt) como agente inductor de la síntesis de IFN; éstos demostraron que la molécula empleada fue capaz de promover la síntesis de IFN-beta así como de proteger a células HEp-2 de la infección por el adenovirus tipo 6.

Se utilizaron células HEp-2, las cuales fueron incubadas con distintas concentraciones de un ARNt (las concentraciones utilizadas estuvieron entre 0.001 a 1.0 mg/mL) por 24 horas, en este momento se infectaron con el adenovirus tipo 6 y posteriormente se valoró el grado de protección por diferentes métodos. Otros cultivos celulares fueron incubados bajo las mismas condiciones pero utilizando al inductor sintético polyI:polyC o bien IFN-alfa recombinante. Cultivos celulares adicionales fueron utilizados para valorar viabilidad celular, así como su actividad celular. El ARN se obtuvo por medio de la técnica de TRIZOL y utilizando primers específicos para IFN-beta se comprobó la síntesis de novo por medio de la técnica de RT-PCR.

En un grupo de 7 voluntarios clínicamente sanos a quienes se aplicó 100 mg del ARNt se determinó la concentración de IFN total en plasma por medio de la técnica de inhibición del efecto citopático empleando al virus de la estomatitis vesicular.

Las células incubadas con el ARNt mostraron un mayor grado de protección que las células incubadas con el inductor sintético, en un grado similar a las células incubadas con el IFN-alfa recombinante; asimismo la viabilidad no fue afectada pero sí la actividad celular. Los resultados obtenidos por medio de la técnica de RT-PCR demostraron la síntesis de IFN-beta de novo. Las determinaciones de IFN en el plasma de los voluntarios demostraron que la concentración de IFN se elevó de manera significativa después de aplicado el ARNt.

Immunogenicity of Hepatitis B Vaccine in Multi-transfused Thalassemic Patients with and Without Hepatitis C Infection

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Hepatitis C virus (HCV) infection is highly prevalent in thalassemic patients, this may decrease serum antibody response to hepatitis B virus (HBV) vaccine. There is also some alteration of the immune system in multi-transfused thalassemic patients, as a consequence of iron overload. We investigated whether HCV infection may reduce the effectiveness of HBV vaccine in multi-transfused thalassemic patients.

Subjects studied prospectively in three groups: group 1, 125 multi-transfused thalassemic patients with negative serum HCV antibody; group 2, 96 age and sex matched multi-transfused thalassemic patients with positive serum HCV antibody (ELISA II), in at least 2 different occasions; group 3, 100 age and sex healthy subjects. Subjects in all groups had negative serum HBsAg, HBcAb and HBsAb and received three 20 µgr/dose of recombinant HBV vaccine (Heberbiovac HB) in months 0, 1, 6. HBsAb titer was obtained one month after last dose of vaccine and it was considered effective seroconversion if was more than 10 IU/L.

Seroconversion rate was 83.2% in group1 and 80.2% in group 2 (P= 0.34). Seroconversion rate was about 86% in healthy subjects, which didn't significantly differ with HCV positive and negative thalassemics (P> 0.1). There was also no significant change in seroconversion rate between HCV positive subjects with normal or increased serum ALT (88.2% versus 78.5% P=0.29). During vaccination period patients, in all 3 groups, did not show any significant adverse reactions.

Our study shows that three standard dose of HBV vaccine is immunogenic and safe in multi-transfused thalassemic patients with or without HCV infection.

Cytokines and Nitric Oxide in the Immunology of Echinococcus Granulosus *Infection*

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Echinococcosis / Hydatidosis is a zoonotic infection caused by the adult stages (metacestodes) of the cestodes genus Echinococcus. The changeability and gravity of the clinical expression of classic cystic hydatidosis caused by *Echinococcus granulosus* is associated with duration and intensity of the Infection (Touil-Boukoffa *et al*, 1997) and also to the numerous antigens (Rigano *et al*, 2001). In this report, we investigate *in vivo* and *in vitro* Interferon-γ, interleukine-12, Interleukine-4 Interleukine-10 and Nitric oxide (NO) production in hydrated patients with liver, lung cyst and other localization as spleen, kidney and bone. We determine antigen-driven cytokine production. PBMC (peripheral mononuclear cells) cultures are stimulated respectively by Antigen-5, a major antigen and fraction-4, that appears less frequent and more specific.

These antigens are prepared by Chromatographic procedure. Cytokines are evaluated by enzyme-linked immunosorbent and nitrite by Griess assay. We have also used a cytopathogenic assay inhibition test for IFN-γ quantification. The high activities of IFNγ, IL-12, IL-4, IL-10 is observed in the majority of sera from patients, which expressed positive serological reaction against the two.

Antigens. In contrast, healthy controls showed minor levels. Evaluation of cytokines production in PBMC cultures showed distinct Th1/Th2 profiles Th1 cells activation appears to relate to the Antigen-5 and Th2 cell activation to the Fraction-4. In addition, important circulating nitrite levels are noted with interest in subjects with liver (105 µM±7.1), lung hydatidosis (59,1 µM ±7.1) and supernatants of PBMC cultures stimulated with Antigen-5 and/or IFN-γ.

Interestingly, NO synthase -2 is revealed by immunochemical staining method using liver biopsy of six patients with liver hydatidosis. Overall, These finding suggest that lymphocytes of subjects infected with *E. granulosus* contain Th1 and Th2 like subpopulations. The cytokines and nitric oxide with their multiple regulatory functions open new perspectives in the knowledge of the pathogenesis and theimmunosurveillance of this parasitosis.

Enhancement of the Anti-Core Antibody Response Elicited in Mice by HCV DNA-based Vaccine Through the use of IL-15 Gene Adjuvant

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DNA vaccines containing genes for antigenic portions of viruses have been developed as a novel vaccination technology. An important advantage of this vaccination method is that in *vivo*-synthesized viral proteins can enter both major histocompatibility complex (MHC) class I and class II antigen-processing pathways to activate specific immunity. In many animal models for infectious diseases, DNA vaccines induced a broad range of immune responses, including antibody, CD8+ cytotoxic T lymphocytes (CTL) and CD4+ helper T (Th) lymphocyte responses. The magnitude and nature of these immune responses to DNA vaccines can be further manipulated by the use of cytokine genes.

We have investigated the utility of DNA-based immunization as a strategy for the development of an effective vaccine against hepatitis C virus (HCV). The present study was conducted to investigate whether expression of human IL-15 could enhance the immune response elicited by a DNA vaccine expressing the structural proteins of HCV (Core, E1 and E2) in mice. Two eukaryotic expression vectors encoding for human IL-15 were constructed.

One of them comprises the coding sequence for human IL-15 including the own signal peptide; in the other one, the IL-15 coding sequence is fused to the erithropoyetin signal peptide. A faster seroconvertion rate and a stronger antibody response against the core protein were observed in mice immunized with the IL-15 encoding plasmids compared with animals vaccinated with the HCV antigens-encoding plasmid alone.These results suggest that co-expression of IL-15 influence the specific immune response elicited after immunization with HCV antigens-ecoding plasmids.

Interferon Alpha 2b as Adjuvant Treatment of Recurrent Respiratory Papillomatosis in Cuba: National Program (1994 – 1999 Report)

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The recurrent nature of Respiratory Papillomatosis is the main life-spoiling feature of this disease. Relapse rates reported reach 80% for the younger ages and 36% for adults. An interferon (IFN) alpha-2b treatment schedule, adjuvant to surgery, was assessed for its contribution to the disease control and the improvement of the patients' quality of life. 169 patients were included (85 children and 84 adults) who were subjected to surgical removal of the lesions followed by intramuscular IFN alpha-2b (Heberon alfa R) schedule, starting with 10^5 IU/Kg of weight in children or 6 x 10^6 IU in adults, 3 times per week. The dose was monthly reduced, as long as no relapses occurred, until reaching a monthly maintenance with 5 x 10^4 IU/Kg of weight in children or 3 x10^6 IU in adults. The treatment lasted 2 years. If there was a relapse, it was removed surgically and the patient returned to the higher dose level. The response was evaluated by comparison of the relapse frequency before and after IFN treatment.

This variable showed a very significant decrease in most of the recurrent patients (69/90, 76.7%; 34/46, 73.9% for children and 35/44, 79.5% for adults). When patients were included in the program after their first papilloma onset, good and partial responses were considered if they had none or only one relapse after treatment, respectively.

These were the majority as well (44/66, 66.7%; 15/33, 45.4% for children and 29/33, 87.8% for adults). As a whole, the treatment benefited 73.1% of the patients (117/160) with respect to relapse frequency. Besides, 118 patients (72.8%; 49, 57.6% for children and 69, 82.1% for adults) concluded the treatment without lesions, while the rest showed a reduction in the number and size of lesions.

IFN was well tolerated. 62 patients (38.3%) did not have adverse events at all. The main side effects were fever (56.8%), chills (22.5%), arthralgias (13.6%), myalgias (13.6%) and headache (9.5%). Only 3 patients withdrew from treatment due to intensity of the adverse events. One patient developed anti-IFN alpha neutralizing antibodies. He became resistant to treatment with recombinant IFN alpha-2b and responded to natural leukocyte IFN alpha.

These results confirm that treatment with IFN alpha as adjuvant to the surgery, represent a favorable and sure therapeutic alternative for patients with Recurrent Respiratory Papillomatosis.

Expresión de citocinas y producción de óxido nítrico inducida por la proteína recombinante Am230 de Amastigotes de Trypanosoma Cruzi

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En humanos y en ratones, la infección por *T. cruzi* va seguida por una severa inmunosupresión mediada por células T y macrófagos. Durante la fase aguda de la infección en ratones se ha demostrado que la inmunosupresión de la linfoproliferación de los esplenocitos es acompañada por la producción de Óxido Nítrico (ON). Las moléculas del parásito que regulan la respuesta inmune del huésped no han sido totalmente identificadas.

Ratones BALB/c fueron inmunizados con la proteína recombinante Am230, estadío específica de Amastigotes de *T. cruzi*. Se observó que Am230 induce la producción de inmunoglobulinas de los isotipos IgG1, IgG2α e IgG2β. Por otra parte, esplenocitos de ratones inmunizados produjeron grandes cantidades de NO. Así mismo macrófagos de peritoneo de animales normales e inmunizados así como también macrófagos de la línea celular J774 fueron capaces de producir NO cuando se estimularon con Am230. En macrófagos se detectó por inmunotransferencia la presencia de la sintasa de óxido nítrico (iNOS). Además, mediante ensayos de RT-PCR se observó que Am230 induce la expresión de citocinas tales como IL-1 IL-6, IL-10, IL-12 y TNF-alpha las cuales participarían en la activación del macrófago, de linfocitos B, respuesta TH2 y producción de NO sugiriendo que Am230 participaría en el proceso inmunoregulatorio durante la fase aguda de la enfermedad de Chagas.

Induction of Humoral Immune Response Using Human Interferon Alfa 2b and Recombinant Anti-hepatitis B Vaccine in Asymptomatic Carrier Patients of Hepatitis B Virus

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The clinical consequences of Hepatitis B Virus (HBV) are extremely variables. Approximately 5-10 % of adults exposed to HBV and the 90 % of perinatally infect children develop a chronic infection.

The nature of chronic infection is also variable. The Asymptomatic Carrier State (ASC) can have relatively normal morphology and function despite the presence of high levels of viral replication. Because the HBV is not cytopathic, the liver injury and the subsequent clearance of virus are mediated by the immune response. An inadequate response leads to chronicity. The use of the recombinant Hepatitis B Virus surface antigen (HBsAg) subunit vaccine has been advocated to overcome the initial inadequate response to the natural infection by widening the immune repertoire against to HBV. We studied the serum HBsAg and anti-envelop antibodies (anti-HBs), detected and quantified by ELISA method, in 111 ASC patients.

They received three standard injections of recombinant anti-Hepatitis B vaccine (20 mg/HBsAg of Heberbivac HB) using 0-1-2 months schedules by intramuscular via together with $5x10^6$ IU of Human Interferon alpha 2b (Herberon Alfa R) in order to potentiate the immune response.

Forty-seven patients reduced HBsAg concentration in more than 50% and it was observed the presence of the anti-HBs response, otherwise these results were not obtained in the patients treated with vaccine alone. In twenty-five patients were detected antigen-antibody complexes.

This study inferred that the combination of vaccine and interferon treatment induce the Anti-HBs production by most patients with chronic hepatitis because usually the antibody response to HVB is undetectable in the serum of these patients. Accordingly, the HBV specific B cell response may be entirely intact in Chronic Hepatitis B.

Intramuscular Administration of Plasmid-based Interferon Alpha Gene Medicine for the Treatment of Hepatitis

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Recombinant interferon alpha, used in combination with ribavarin, is currently the primary treatment of chronic hepatitis C viral infection. Pegylated forms of interferon alpha have recently emerged as a promising new modality of therapy enabling less frequent dosing due to increased protein half-life sustained absorption and slower rate of clearance.

In order to achieve long-term persistence of interferon alpha in blood with further reduction in frequency of injection, we have explored the application of nonviral gene therapy to achieve sustained, stable gene expression after intramuscular administration. In order to provide an added safety margin for an interferon alpha gene medicine, we have also examined the possibility of regulating gene expression using a GeneSwitch™ system that responds to oral antiprogestins, such as mifepristone.

This presentation reports the prolonged, dose-dependent constitutive expression of interferon alpha at therapeutically relevant levels for more than 100 days in mice after a single intramuscular injection of formulated plasmids with electroporation. The biological activity of interferon alpha was demonstrated in mice by measuring the levels of Mx transcripts in white blood cells that were induced proportionally to the levels of expressed interferon alpha.

Using the GeneSwitch Plasmid in combination with the interferon alpha gene medicine, pulsatile expression was achieved in mice in response to administration of mifepristone for several months. These synthetic gene therapy approaches could represent an attractive alternative to the use of recombinant proteins for the treatment of hepatitis by exploiting the patient's muscle to produce interferon alpha in its most natural and efficient form.

LPS-binding Peptides Modulate Cytokine Responses in Sepsis and Improve Survival in Mice

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Sepsis results in activation of numerous mediators, which may damage cells and result in organ injury. Early in sepsis, a rapid increase in circulating levels of inflammatory cytokines is observed and seems to be fundamental for septic shock onset. Additionally, several studies indicate that a shift from Th1 to Th2 responses occurs in late sepsis, contributing to immunosuppression.

Both phenomena have been involved in organ injury, which characterizes severe sepsis and multiple organ dysfunction and influences patient outcome. Therefore, molecules able to modulate cytokine responses in sepsis could be valuable for prophylactic or therapeutic regimes. We studied peptides derived from lipopolysaccharide (LPS)-binding protein (LBP) and Limulus anti-LPS factor (LALF). LBP86-99 and LBPK95A down-regulated TNF-α responses in models of endotoxicosis and live bacteria inoculation. Their inhibitory activity was several times stronger *in vivo* than *in vitro*.

The *in vivo* effect was associated with decrements in organ injury and mice mortality. The LALF31-52 peptide induced *in vitro* the production of IL-2, IL-12, IL-13, IFN-α and IFN-γ by human mononuclear cells, but inhibited the LPS-induced expression of TNF-α, IL-6 and IL-10. *In vivo*, this peptide prevented organ damage and protected mice from death when administered several hours before the septic stimulus. LALF31-52 administration resulted in tissue-selective increments in several cytokines expression. In a model of Gram-positive sepsis, LALF31-52 protective effects were related with increments in IL-2 and IL-10 mRNA levels in the lungs and with decrements in IL-12 and IFN-γ transcripts, as well as with reduced systemic levels of TNF-α and IFN–γ. These studies demonstrated that treatment regimes based on immunomodulating and LPS-neutralizing peptides could abrogate deleterious cytokine responses to septic stimuli and increase host resistance to infection and septic injury.

Surfacen: Inhibe la Liberación de TNF Alfa y la Expresión de ICAM-1 in vitro, *así como el edema pulmonar en un modelo de shock séptico*

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Surfacen, surfactante exógeno natural, compuesto por fosfolípidos y las proteínas hidrófobas SP-B y SP-C, ha sido utilizado con éxito en la terapia del Síndrome de Dificultad Respiratoria del Neonato.

Además de su función tensoactiva, el surfactante pulmonar presenta propiedades de defensa innata en el pulmón, lo cual ha fundamentado aún más su uso en otras enfermedades respiratorias, cuyo denominador común es la inflamación. El objetivo de este trabajo es evaluar las propiedades antinflamatorias de Surfacen en modelos «*in vitro*»e «*in vivo*».

Para esto se evaluó Surfacen en la liberación de TNF alfa en monocitos humanos estimulados con LPS y en la expresión de la molécula de adhesión ICAM-1, así como el efecto de Surfacen en el edema pulmonar en un modelo de shock séptico en ratones Balb/c.

Surfacen fue capaz de inhibir la liberación de TNF alfa en monocitos humanos, así como la expresión de ICAM-1 en células endoteliales de la aorta bovina, por otra parte inhibió el edema pulmonar en una forma dosis-dependiente en el modelo de shock séptico por LPS en ratones.

Yersinia Pseudotuberculosis *and Cytokine Production*

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We investigated the levels of interferon-a (IFN-a), tumor necrosis factor-a (TNF-a), interleukin-1 (IL-1), interleukin-2 (IL-2) and interleukin-6 (IL-6) production in patients with pseudotuberculosis and in experiment in vitro. Totally levels of cytokines production from serum of 21 patients were measured. In experiment 3 different strains of Y. pseudotuberculosis (both 47MDa (pYV) and 82MDa (pVM) plasmid bearing strain, 47 MDa bearing strain and without plasmid derivate) and one strain of Y. enterocolitica were studied. Each strain was incubated for 24 h with whole blood of healthy volunteers at a ratio of 10^6 microbial cells per 1 ml of blood.

Blood without strain was used as control and with lipopolysaccharide of *Y. pseudotuberculosis* (LPS) and superantigen (YPMa) as positives controls. Concentrations of IFN-α, TNF-α, IL-1 and IL-6 were measured by ELISA.

The first week of disease levels of all cytokines production were significantly decreased comparing with their levels in the healthy volunteers. Levels of IL-1 and IL-2 rose on the third week of disease but level of TNF-α remained unchanged and levels of IL-6 continued to decrease. In experiment, LPS and YPMa induced higher levels of IFN-α, TNF–α, IL-1 and IL-6 than all strains of *Y. pseudotuberculosis* and *Y. enterocolitica*. Both pYV and pVM plasmid bearing strain induced lower levels of IFN-α, TNF-α, IL-1 and IL-6 than pYV bearing strain and without plasmid derivate. Levels of IL-2 were unchanged. There were no significant differences between levels of cytokine production induced by pYV bearing strain or without plasmid derivate. YPMa induced higher levels of all cytokines than LPS of Y. pseudotuberculosis.

Y. pseudotuberculosis has significant immunosuppressive properties and diminishes the cytokine production both *in vivo* and *in vitro*. Imunosupression is dependent on plasmid 82 ?da.

Tratamiento con Interferón Alfa 2b recombinante asociado a amantadina en pacientes con Hepatitis Crónica Tipo C

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Actualmente las tasas de respuesta que se obtienen con los tratamientos disponibles para la hepatitis crónica C(HCC) son desalentadoras, por ese motivo se están desarrollando nuevos tratamientos con distintas drogas o combinaciones de estas. Se ha descrito que la Amantadina puede ser útil en estos pacientes(1). Smith J P.Gastroenterology,1996;110:A 1330.(2)Bonkousky HL.Hepatology 1998;Sep;26(3 suppl 1):143-151.

Evaluar la eficacia y seguridad de un régimen de Interferon (IFN) asociado a Amantadina en un grupo de pacientes con HCC.

Se realizó un estudio aleatorio ,controlado que incluyó 20 pacientes con criterios de HCC que no hubieran recibido tratamiento previo con antivirales e inmunomoduladores A todos se les determino en suero anticuerpos contra el virus C de la hepatitis (Anti-HCV)(UMELISA,Anti-.HCV 3ra G, La Habana,Cuba),la presencia del HCV-RNA mediante PCR(Amplicor HCV,Roche Diagnostic Syst! em), HBsAg y Anti-HIV para descartar infección por estos virus. Se consideró que las cifras de ALT estuvieran 1.5 veces por encima de la cifra normal(hasta 40UI/L), fueron incluidos pacientes entre 18 y 65 años de edad.

Hubo dos grupos de 10 pacientes cada uno(Grupo A y B),Al grupo A se le administro IFN alfa 2b R 3000 000 UI por vía subcutanea 3 veces por semana durante 1 año asociado a Amantadina 200 mg al día por vía oral durante 6 meses y el grupo B a quienes se administró IFN alfa 2b R 3000 000 UI por vía subcutánea tres veces por semana durante un año. Se realizaron evaluaciones clínicas y de laboratorio cada 4 semanas ,ello incluía biometría hemática y pruebas de función hepática. Al final del tratamiento se evaluaron los pacientes considerando como variable principal las cifras de ALT clasificando los pacientes en respondedores y no respondedores. Seis meses después de concluida la terapia, se considero respuesta bioquímica sostenida o no respuesta.

6(60%) de los 10 pacientes tratados con IFN y Amantadina tenían cifras de ALT normales al final del año de tratamiento. En el grupo B 5(50%) de los pacientes eran respondedores al final del tratamiento. En el grupo A se logró una respuesta bioquímica sostenida en 3(30%) pacientes, en el grupo B respondieron de forma sostenida(respuesta bioquímica) 2 (20%) pacientes no observándose diferencia estadísticamente significativa entre ambos grupos de tratamiento. No hubo efectos adversos significativos en el grupo tratado con IFN a diferencia del grupo tratado con IFN y Amantadina donde además de los efectos adversos comunes del IFN hubo 1 (10%) paciente que presentó constipación.

Nuestros resultados muestran que el tratamiento combinado de IFN mas amatadina no aumenta la tasa de respuesta bioquímica en la hepatitis crónica C.

Use of Recombinant Human Interferon Gamma in Patients with Multidrug-resistant Tuberculosis

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Around 1.7 billion people are infected with the *Mycobacterium tuberculosis* at present and 60 million bear the active form of the disease. An increment of patients with multidrug-resistant tuberculosis (MDR-TB) has been detected in the world; therefore the number of deaths has also increased by this cause. Enough evidences exist on the action of interferon gamma (IFN γ) on the macrophages immunoregulatory activity.

The deficiency in production of the cytokine or in its receptor function is associated with TB progression. A pilot, open label trial was carried out. The five MDR-TB patients existing in Cuban received IFN γ (Heberon Gamma R, Heber Biotec, Havana) during 6 months as adjuvant to the indicated chemotherapy,. Treatment efficacy was evaluated by the clinical, bacteriological and radiological evolutions.

Sputum samples for smear and culture were collected at entry and monthly as well as routine clinical assessments and thorax radiography. Sputum acid-fast-bacillus smears and cultures became negative in all patients before three months of treatment. At the end of treatment, the size of cavitary lesions was reduced in all patients; in one case, the lesions disappeared. In all five, bodyweight stabilized or increased, while the leukocyte counts increased in three of them. Interferon g was well tolerated by all patients.

Few adverse events were registered, mostly mild; fever and arthralgia prevailed.

Preliminary data suggest that IFN γ is useful and well tolerated as adjunctive therapy in patients with MDR-TB who are otherwise not responding well to therapy.