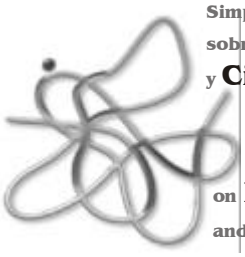

Simposio Internacional
sobre **Interferón**
y **Citoquinas**

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

La Habana, Cuba.
Diciembre 2-5, 2001

Havana, Cuba.
December 2-5, 2001

Centrará su atención en la actualización de los últimos resultados clínicos, nuevas indicaciones y/o alternativas terapéuticas con el uso de interferón y otras citoquinas en entidades oncológicas.

The Simposium will focus on the latest clinical trials, new indications and/or alternative therapeutics using the IFNs and other cytokines in oncology, as well as on angiogenesis mechanisms.

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Interferón alfa-recombinante post tratamiento de inducción con radio/quimioterapia en pacientes con linfomas no Hodgkin de bajo e intermedio grado de malignidad

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Se realizó un estudio prospectivo no aleatorizado (diciembre/1985 y diciembre/1988), con 28 pacientes portadores de un Linfoma no Hodgkin en cualquier estadio de la enfermedad (Ann Arbor), clasificados de bajo e intermedio grado de malignidad (Working Formulation) excluidos los difusos de células grandes, con respuesta completa (RC) al tratamiento con Poliquimio/radioterapia (Grupo A). Se empleó CHOP (modificado) o COP y la radioterapia sobre campos afectos. Al evaluarse la RC recibieron Interferón alfa-recombinante (6 MU intramuscular, tres veces a la semana, 12 meses, o hasta progresión o toxicidad limitante.

El intervalo libre de enfermedad (ILE) y supervivencia (SV) del grupo A fue comparada con las de 74 pacientes (grupo B) con iguales características y criterios de tratamiento, sin interferón después de la RC. En el grupo A predominó el sexo femenino (20 Vs 8), los pacientes entre 30 y 59 años (19 de 28), y los estadios III y IV (19 y 5 pacientes). Según la histología: 3 foliculares de células pequeñas (LFCP) compatibles con LLC, 6 foliculares de células pequeñas (LFCP), 10 difusos de células pequeñas (LDCP), 2 foliculares de células grandes y pequeñas (LFCGP), 5 difusos de células grandes y pequeñas (LDCGP) y 2 foliculares de células grandes (LFCG).

En el grupo B no hubo diferencias con relación al sexo, y predominaron tanto los estadios III y IV (41 de 74) como los pacientes mayores de 60 años (48%). Predominaron los subtipos de intermedio grado de malignidad: LDCPH en 28 pacientes y el LFCGP en 13 pacientes, y el LDCGP en 11 pacientes. Las adenopatias periféricas fueron el síntoma más frecuente en ambos grupos. El ILE del grupo A fue 60%, 55% y 37%, y para el grupo B de 57%, 53% y 53% a los 5, 10 y 15 años respectivamente, sin encontrar diferencias significativas entre ambos grupos (p=0.65), aunque discretamente superior para el grupo A durante los primeros 5 años. La SV del grupo A fue 92%, 74% y 55%, y para el B de 70%, 52% y 48% a los 5, 10 y 15 años respectivamente, con diferencias significativas a favor del grupo interferón (p=0.02), con mayor diferencia en los primeros 10 años y menor a los 15 años del tratamiento. Estos resultados apoyan el tratamiento con Interferón en los pacientes que obtienen una RC con el tratamiento convencional, con el criterio de prolongar el ILE y la SV.

$CD4^+$ T-cells Augment Skin Carcinogenic Activity of the Polyaromatic Hydrocarbon DMBA and Elaborate Both IL-4 and IFN-γ While $CD8^+$ T-cells Produce IFN-γ and Little or No IL-4

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The objective of this study was to characterize the T-cells that are activated following topical application of polyaromatic hydrocarbons (PAH), to determine whether they participate in PAH-induced skin tumorigenesis and to investigate their cytokine profiles. Application of the carcinogenic PAH, 7,12-dimethylbenz(a)anthracene (DMBA) to the skin of C3H/HeN mice resulted in the development of contact hypersensitivity to that agent.

In vivo administration of anti-CD8 antibodies prior to sensitization abrogated the response, whereas *in vivo* depletion with anti-CD4 antibodies augmented DMBA contact hypersensitivity. Treatment of $CD4^+$ knockout mice on a C3H background with DMBA was also found to increase DMBA contact hypersensitivity. When ELISAs were performed on T-cells from DMBA treated mice, $CD8^+$ T-cells were found to produce interferon-γ and little or no IL-4, whereas $CD4^+$ T-cells were found to elaborate both IL-4 and interferon-γ. To determine if $CD4^+$ T-cells participated in PAH skin tumorigenesis, $CD4^+$ knockout and wild type mice were subjected to a DMBA skin tumorigenesis protocol. $CD4^+$ knockout mice developed 80% fewer tumors than wild type mice.

The percent of mice with tumors and the number of tumors/tumor bearing mice were also decreased in $CD4^+$ knockout mice. Immunopreventive strategies which suppress the activation of $CD4^+$ T-cells may be successful in reducing PAH skin cancer development.

Intralesional Injection of Recombinant Interferon Alpha 2b in Basal Cell Carcinoma

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The purpose of this work is to show the efficacy of recombinant IFN-α2b in the treatment of BCC comparing two different doses. A double blind two-phase controlled trial comparing treatment with two doses of recombinant IFN-α2b (HEBERON ALFA R, Heber Biotec, Havana), was performed.

Eighty consecutive patients with histopathologically-proven BCC, who did not receive specific treatment for 6 months and lesions between 0.5 and 4 cm large were enrolled and randomly assigned to Group I or Group II. After informed consents were obtained, lesions were injected with recombinant IFN-α2b 1.5 x 10^6 IU (group I) or 5 x 10^6 IU (group II).

IFN-α2b injections were given intralesionally three times per week for 3 weeks. Their evaluation was done two months after treatment. The main evaluation criterion was clinical, defined as complete (CR) (total disappearance of lesion), partial (PR) (> 50% lesion reduction), stable disease (SR) (< 50% lesion reduction) or progression (P) (increase of the lesion). Seventy-five patients received the treatment but seventy-eight lesions were evaluated by intention-to-treat.

The overall response (R = CR+PR) were 92.5% and 84.2% for groups II, and I respectively. None of the patients developed anti-IFN neutralizing antibodies. Side effects included vomits, nausea, perilesional erithema, asthenia, and pruritus but were reversible with the use of paracetamol or dipirone. At one-year follow-up no relapses were observed.

We conclude that treatment of BCC with intralesional recombinant IFN-α2b induced tumor regression, represents an effective and is an encouraging alternative to surgery and other treatments. Both doses of recombinant IFN-α2b were similarly effective.

Recombinant Human Interleukin-2 Inhibits Proliferation of HPV-associated Tumor Cells and Halts Tumor Growth In Vivo

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Previous studies have shown inhibition of cervical cancer cells growth by treatment with high concentrations of IL-2.

In the present study we evaluated the *in vitro* and *in vivo* effects of recombinant human IL-2 (rhIL-2) on HPV-associated tumor cells (3T3-16). Treatment of 3T3-16 cells with rhIL-2 for 72 hours inhibited cell growth in a dose dependent manner and this effect was evidenced at nanomolar concentrations ($P < 0.05$).

These tumor cells expressed mRNA for the beta and gamma subunits of the IL-2 receptor, which are required for signal transduction. In experiments to explore the effect of IL-2 on the growth of the HPV-associated tumor, mice received rhIL-2 through different routes: (i) intraperitoneal; (ii) subcutaneous, at the tumor inoculation site; or (iii) subcutaneous, distant from the tumor inoculation site. An effective antitumor response was only observed in those animals that received IL-2 at the tumor site ($P < 0.01$).

These results indicate the potential adequacy of therapeutic strategies based on local administration of rhIL-2 for cervical carcinoma, not only based on the ability of this cytokine to stimulate cellular-mediated immunity but also on its direct effects on HPV-associated tumor cells.

Expresión de citocinas en linfocitos asociados a tumor y en ganglios regionales de pacientes con cáncer de mama

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Los linfocitos tumor-asociados y de ganglios regionales tienen su función suprimida por factores del tumor y de la interacción tumor-sistema inmune del huésped. Estudiamos el patrón de citocinas en el microambiente de tumor y ganglios de pacientes con cáncer de mama y la expresión de moléculas accesorias y proteínas de activación linfocitaria.

Se estudiaron en 19 pacientes con diagnóstico confirmado de cáncer de mama, algunos marcadores de superficie por citometría de flujo en linfocitos periféricos. Se realizó la extracción de RNA y proteínas de los linfocitos, con Trizol. Se determinó la expresión del RNAm de citocinas TH1 y TH2 a través de RT-PCR, utilizando iniciadores específicos.

Los marcadores de superficie se comportaron dentro de niveles normales, excepto 3 pacientes donde se invirtió la relación CD4:CD8. Más del 50% expresó IL-10 en sus linfocitos tumor-asociados pero no hubo expresión de IFN-γ en ningún caso. La pérdida de expresión de IFN-γ pudiera resultar en un empeoramiento de la respuesta inmune antitumoral unido al efecto inhibitorio de la inmunidad celular que ejerce la IL-10. Los linfocitos de ganglios regionales de las 19 pacientes resultaron negativos a IL-10 y solo una mostró expresión de IFN-γ.

Se evidenció un predominio TH2 en los linfocitos periféricos en 9 de las pacientes y su estimulación con IL-2r promovió un cambio a citocinas TH1.

Nuestros resultados sugieren que las citocinas supresoras pueden ser responsables de la afectación de la inmunovigilancia y los mecanismos inmunológicos celulares, lo cual puede favorecer la progresión tumoral.

Regression of Infancy Hemangioma with Intralesional Recombinant Interferon Alpha 2b Treatment

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Interferon (IFN) alpha is known for its antitumor and antiangiogenic effects. There are antecedents of its use for the treatment of infancy hemangiomas, which are the most frequent tumors of the first ages.

We have previously reported the successful use of subcutaneous IFN alpha in children with hemangioma (Garmendia *et al*. J Interferon and Cytokine Res 2001;21:31-8). In this work, the intralesional way of administration was assayed. Thirty-two children (24 girls; age 1 month – 10 years) with hemangioma were treated with interferon alpha-2b (HEBERON ALFA R, Heber Biotec, Havana) 3 x 10^6 IU/m^2 intralesionally, twice per week for eight months. Inclusion criteria were tumor size > 2 cm, life threatening or life limiting hemangioma and parents' informed consent. Treatment was well tolerated. The main side effect was fever (55%).

There was no effect on psychomotor or physical development. Four patients abandoned. The evaluation of the 26 patients who completed 8 months of treatment is that 21 (71%) had tumor regression and 5 (16%) had stable disease. No progression was seen. Regression was more frequent (85.7%) in patients between 1 and 5 years old, children's without family pathological antecedents and whit tumor at born.

This result shows that the intralesional route is as effective and relatively safer than the systemic administration of a similar dose.

Maintenance Biotherapy with Interleukin-2 (IL-2) and Granulocyte-macrophage Colony Stimulating Factor (GM-CSF) Following Concurrent Biochemotherapy for Patients with Metastatic Melanoma

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Concurrent biochemotherapy (C-BCT) for metastatic melanoma results in overall response rates of 50-60%, complete remission in 20% of patients, and a median duration of survival of approximately 12 months. However, initial trials of C-BCT demonstrated significant toxicity as well. In efforts to improve efficacy and decrease toxicity, we modified C-BCT with decrescendo dosing of interleukin-2 (IL-2) and post-treatment granulocyte colony-stimulating factor (G-CSF). We have recently developed a maintenance biotherapy regimen with IL-2 and granulocyte-macrophage colony stimulating factor (GM-CSF) for patients responding to C-BCT to improve time to progression (TTP) and overall survival (OS).

The trial of modified C-BCT included 45 patients with poor prognosis metastatic melanoma who were treated in a community hospital oncology unit affiliated with the John Wayne Cancer Institute (Santa Monica, CA) between July 1995 and September 1997. The 5-day inpatient regimen consisted of dacarbazine, vinblastine, cisplatin, decrescendo IL-2, interferon alfa-2b and tamoxifen and was repeated every 21 days. G-CSF was administered beginning on day 6 for duration of 7 to 10 days. The overall response rate was 57% (95% confidence interval, 42-72%), the complete response rate was 23%, and partial response rate was 34%. Complete remissions were achieved in an additional 11% of patients by surgical resection of residual disease after biochemotherapy. The median time to progression was 6.3 months and the median duration of survival was 11.4 months. A follow-up at 36 months showed 32% of patients were alive (range, 10-36 months) and 14% were alive and free of disease. Decrescendo IL-2 dosing and administration of G-CSF appeared to reduce toxicity, hospital stay and readmission rates. No patient required intensive care unit monitoring and there were no treatment-related deaths. The data from this study demonstrated that a modified regimen of C-BCT could reduce the toxicity of C-BCT without any apparent decrease in overall or complete response rate. No improvement in response rate was observed with the addition of tamoxifen.

Despite the high activity of C-BCT in metastatic melanoma, only 10-20% of patients will achieve durable remission and long-term survival. Furthermore, for the majority of C-BCT patients (approximately 60%) who achieve only partial remission (PR) or stable disease (SD) to treatment, the prognosis remains poor (median survival, 6 months; nearly 100% disease progression at 12 months), in spite of their response to C-BCT.

In efforts to prolong TTP and OS in this poor prognosis group of PR and SD patients, we developed a program of maintenance biotherapy (MBT) to be instituted at the completion of C-BCT. The regimen consisted of twelve 28-day (d) outpatient cycles of low-dose IL-2 (1 MIU/m2 by subcutaneous (SQ) injection, Monday-Friday) and GM-CSF (125 mg/m2 SQ, d 1-14). Patients received intermittent pulses of high-dose decrescendo IL-2 over two days in the hospital for a total dose of 54 MIU/m2, administered as follows: 18 MIU/m2 by continuous infusion (CI) over the first 6 hours, 18 MIU/m2 CI over the next 12 hours, and 18 MIU/m2 CI over the final 24 hours on d 1-3 of cycles 2, 3, 5, 6, 8, 10, and 12.

Between May 1998 and November 2000, 186 cycles of MBT were administered to 33 post-C-BCT patients (19 PR, 14 SD). As of June 2001, a total of 9 patients (27%) remained alive with follow-up ranging between 12 and 37 months, beginning day one of C-BCT. Kaplan-Meier estimates of progression and survival showed a significantly longer median TTP (8.1mos. vs. 5.9mos; p=0.0008, by log-rank) and longer median OS (18.5 vs. 9.3 mos; p=0.0027, by log-rank) in study patients compared to 34 similar patients who achieved PR or SD to C-BCT but did not receive MBT. Five patients (15%) successfully achieved CR from all clinically evident metastatic disease; 4 additional patients (12%) maintained SD for a period of at least 6 months from the start of study therapy.

MBT was well tolerated with expected inpatient toxicities and outpatient toxicities limited mostly to grade 1 and 2 constitutional symptoms and dermatologic (injection site) reactions. Significant toxicities included one episode of severe respiratory distress requiring ICU monitoring, 2 cases of severe urticarial reactions requiring temporary suspension of study drug administration, 2 catheter-related infections, and one DVT. Also of note were 2 cases of hypothyroidism necessitating replacement therapy and 5 cases (15%) of vitiligo. No patient required IL-2-pulse dose reductions. In this pilot study, MBT following C-BCT appears to significantly prolong TTP and OS compared to C-BCT alone, and does so with low treatment-related toxicity. These preliminary results will be subject to further investigation in a larger multicenter trial now underway.

Inducción de la expresión de moléculas MHC-I con IFN-γ en células de cáncer cervical

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Se sabe que IFN-γ tiene un efecto inductor de la expresión de moléculas del complejo principal de histocompatibilidad clase I (MHC-I) y de moléculas asociadas con el procesamiento de antígenos (MAPA) tales como: las subunidades catalíticas-β de los proteasomas (LMP2, LMP7, LMP10); las reguladoras del proteasoma (PA28α y PA28β) y los transporadores de péptidos (TAP1 y TAP2). En este trabajo se analizó la inducción de la expresión de moléculas MHC-I con IFN-γ en ocho líneas celulares de cáncer cérvico-uterino (CALO, INBL, HELA y VIPA, positivas para HPV-18; CASKI Y SIHA positivas para HPV-16: y C33A y VIBO, negativas para HPV) y su relación con defectos en la expresión de MAPA.

La expresión de MHC-I se realizó mediante citometría de flujo utilizando el anticuerpo monoclonal W6/32 que reconoce la porción constante de estas moléculas, mientras que la expresión de MAPA a través de RT-PCR utilizando los oligonucleótidos apropiados.

El cultivo de las líneas celulares en presencia de 100U/ml de IFN-γ indujo un incremento entre un 30-90% en la expresión de moléculas MHC-I en las líneas positivas para HPV-16 y 18 quienes expresaron de manera normal a la MAPA. En tanto que en las líneas negativas para HPV, la carencia de MAPA influyó de manera notable en la inducción de la expresión de moléculas MHC-I: mientras en la línea VIBO, deficiente en LMP10, la inducción con IFN-γ fue de más del 300%, en la línea C33A, deficiente en LMP7 y LMP10, la inducción fue de aproxi- madamente 10%.

Estos datos nos muestran la influencia diferencial que tiene la carencia de subunidades importantes del proteasoma en la inducción de moléculas MHC-I con IFN-γ en células de cáncer cervical, remarcando la importancia que tiene LMP7 en este fenómeno.

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Cytokine Genemedicine™ Products for Cancer Immunotherapy

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Immunotherapy with recombinant cytokines such as Interleukin-2 and Interferon-a represents an effective modality of treatment for some cancers but is limited by severe systemic toxicities. The unique properties of gene-based cytokine immunotherapeutics offer the potential for local and systemic anti-tumor effect without the toxicity and dosing problems associated with recombinant proteins.

Plasmid-based therapeutics represent an attractive new class of biopharmaceutical products that are being used in an attempt to induce the human body to produce proteins in a natural and controllable manner, thus potentially enabling an improved quality of medical treatment.

This presentation reports recent positive preclinical and clinical results with several cytokine GeneMedicine™ products as cancer immunotherapeutics. Data demonstrated that intratumoral administration of a cationic lipid-based IL-2 GeneMedicine™ product, with or without chemotherapy, results in systemic anti-tumor activity in several animal cancer models and protection against specific tumor re-challenge. A randomized, controlled Phase II clinical trial comparing chemotherapy with or without IL-2 GeneMedicine™ product in advanced head and neck cancer patients showed a trend toward delaying disease progression for the combination, with no incremental severe toxicities. Intratumoral administration of a combination of PINC™ polymer-based IL-12 and IFN-α GeneMedicine™ products to syngeneic murine tumors resulted in a synergistic anti-tumor response and protection against re-challenge. Over the past few years, advanced gene delivery systems have been designed to enable passive targeting to disseminated tumors and metastases by systemic administration.

A cationic lipid-based system has been shown in preclinical models to preferentially distribute to pulmonary tumor endothelium thus providing regional and sustained expression of cytokines in lung tumors. Such an approach has resulted in a significant reduction of lung tumor nodules in mice after intravenous administration of an IL-2 GeneMedicine™ product.

These tantalizing preclinical data associated with a good safety profile in rabbits and non-human primates toxicology studies has led to the initiation of a Phase I clinical trial in patients with lung cancer.

The Role of Vascular Endothelial Growth Factor (VEGF) in Tumor Angiogenesis Induced by EGFR-type Proto-oncogenic Tyrosine Kinases

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The epidermal growth factor receptor (EGFR) family of tyrosine kinases comprises at least four trans-membrane receptors, which are involved in cell growth, differentiation and survival. Two members of the family, EGFR itself, and its most related homolog HER-2/Neu, have been directly implicated in tumorigenesis. These receptors are often over-expressed and/or overactive in a variety of cancers of epithelial origin, including breast, ovarian, lung, cervix, and tumors of the head and neck, where they act as prototypical oncogenes.

The role of EGFR-type receptors in such malignancies have been suggested by numerous preclinical experiments, but the most convincing evidence comes from various clinical trials, which have demonstrated significant anti-tumor activity of different type of biological agents directed against these receptors. Classic examples of these agents are the monoclonal antibodies Herceptin™ and Cetuximab, a specific type of inhibitors of receptor tyrosine kinases, directed against the extracellular domain of HER-2/Neu and EGFR, respectively.

Despite the now extensive use of these drugs in pre-clinical and clinical studies, little is known about their definitive mechanisms of action *in vivo*.

One of the aims of our laboratory is to understand how these drugs act based on the biological functions of EGFR-type kinases *in vivo*, i.e. in a tumor growing in an organism. We are taking this approach based on evidence obtained by us and others, that EGFR-type kinases, when activated are able to induce tumor angiogenesis, i.e., the sprouting growth of blood vessels necessary for the expansion of the tumor mass.

The most common way by which these kinases stimulate angiogenesis is by up-regulating the expression of the potent pro-angiogenic molecule, vascular endothelial growth factor/vascular permeability factor (VEGF/VPF). We present some evidence that the connection between EGFR-type kinases and angiogenesis can explain part of the mechanism of action of EGFR-blocking antibodies.

We have found using the EGFR-overexpressing cell line A431 that different anti-EGFR antibodies cause an anti-angiogenic-like tumor response *in vivo*, which appears to be mediated, at least in part, by the VEGF down-regulation caused by these agents.

Furthermore, we have generated some variants of A431 cells resistant to EGFR-blocking antibodies *in vivo*, most of which overexpress VEGF. Enforced VEGF overexpression induced by gene transfection of A431 cells made them significantly resistant to EGFR-blocking antibodies *in vivo*, which points to VEGF overexpression as one of the effector mechanisms of resistance to these type of drugs.

The results suggest that tumor angiogenesis — mediated in this case by VEGF— should be considered as part of the targeting strategy when treating EGFR-dependent tumors. However, it must be taken into consideration that VEGF is not the only molecule driving angiogenesis by EGFR-type oncogenes. We have generated an adult mouse cell line (MDF528) lacking the VEGF gene to ask the question whether or not VEGF is required for tumor angiogenesis and *in vivo* growth of cells transformed with EGFR-type kinases. VEGF knockout MDF-528 cells transformed with HER-2/Neu oncogene exhibit rapid growth *in vivo*. The high vessel density and endothelial cell proliferation of MDF528/HER-2 tumors suggests that they are indeed angiogenic.

The presence of murine VEGF and the partial response to an anti-VEGF receptor antibody (DC101) of the most aggressive tumors is evidence that VEGF-derived from tumor stroma (host tissue) plays a significant but not necessarily an obligatory role in the *in vivo* growth of these tumors. The dispensability of VEGF in this system is proven by the fact that several tumors grew to significant sizes (larger than1 cm^3) in the total absence of VEGF, including host-derived.

Taken together the results suggest that tumor angiogenesis induced by EGFR-type kinases is a complicated process involving crosstalk between tumor and stromal cells, and that molecular mediators other than VEGF must be taken into consideration.

Induction of IFN-g and Cytokine Producing Cells After Dendritic Cell Based Vaccination in Cancer Patients

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In cancer immunotherapy, the use of dendritic cells (DC) loaded with tumor-associated antigens (TAA) emerged as a promising strategy. In tumor models, cytokines and especially IFN-γ are major players in the development of an effective anti-tumor response. IFN-γ is a pivotal cytokine involved both in the afferent phase (differenciation of Th1 type CD4+ cells, generation of cytotoxic T lymphocytes, CTL) and in the efferent phase (killing of tumor cells) of the anti-tumor response. Moreover, it is able to enhance the expression of HLA molecules at the surface of cancer cells. However, the correlation between CTL induction, IFN-γ secretion and tumor regression is not so clear in human clinical trials. This lead some investigators to propose more recently that Th2 and Tc2 type responses might also be involved through the recruitment of other effector cells such as eosinophils.

At the Erasme Hospital in Brussels, we initiated 3 pilot clinical trials with immunological endpoints using TAA loaded autologous DC. The DC based vaccine was generated from leukapheresis products in closed system by a 7-day culture of circulating monocytes in the presence of GMP IL-4 and clinical grade GM-CSF. The cellular product was loaded with clinical grade HLA class I (A*0101, A*0201, B*4402, B*4403) restricted peptides derived from the TAA MAGE-1 and –3. The targeted tumors included mainly melanomas but also other MAGE positive tumors. These trials showed that this approach was safe and associated with the induction TAA specific IFN-γ responses, which were transient despite the providing a further help through KLH presentation. Subcutaneous (SC) IL-2 administration was associated with long-lasting TAA specific IL-5 production together with hypereosinophilia. Clinical responses were observed in about 1/3 of the patients.

Further improvements will take advantage of the use of a new type of DC (IL-3/IFN-β DC) and of tumor cell-DC hybrids in patients bearing measurable tumor lesions. In those patients with tumor regression, the analysis of a more exhaustive cytokine secretion pattern in conjunction with tetrameric and Vβ staining should help us to understand more precisely the nature of clinically relevant anti-tumor responses.

Interferon Alpha 2b Versus Bacillus Calmette-Guerin as Adjuvant to Surgical or Endoscopic Removal for the Treatment of Superficial Bladder Cancer

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The objective was to evaluate the efficacy and safety of interferon alpha-2b (IFN) versus Bacillus Calmette-Guerin (BCG) as maintenance treatment for superficial bladder carcinoma in terms of relapse rate, relapse free rate, and survival intervals. It was a randomized, open study.

Double blinding was not possible since the treatment schedules were different. Patients had a biopsy-confirmed transicional bladder cancer. Less than 45 days after partial cystectomy or endoscopic removal or the tumor, people received intravesical installations of (i) BCG ("C J Finlay" Company, Havana) 100 mg in 50 mL one per week during 6 weeks, and then once every 3 months up to two years or (ii) IFN (HEBERON ALFA R, Heber Biotec, Havana) 20 x 10^6 IU in 50 mL of saline twice per week up to 3 months, and then monthly up to two years. 43 patients were included in the BCG group and 47 received IFN. Both groups were similar concerning baseline characteristics such as age, time ill, and histological classification of the tumors.

There were no statistical differences between the products concerning efficacy outcomes. Relapse rate was slightly lower with BCG (BCG: 65 % *vs* IFN> 49 %; p = 0.12) but relapse-free interval was shorter (BCG: 20; IFN: 29 months; p = 0.12) as well as mean survival (BCG: 53; IFN: 67 months; p = 0.66). Only 6 patients have died, 3 in each group of treatment. Both products were well tolerated, but the safety profile seemed better for IFN since cystitis was statistically more frequent in the BCG group (p = 0.016).

These results indicate that intravesical IFN can be considered as an alternative to BCG for the maintenance therapy of superficial bladder carcinoma.

Characterization of Chronic Wounds. Molecular and Immunohistochemical Studies

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Wound healing is a post-inflammatory process that represents the effort of the organism to restore tissue integrity and function following damage. A finely orchestrated cascade of overlapping events makes up the process of tissue repair in ordinary, acute and uncomplicated wounds.

An accurate balance of positive and negative signals governs normal wound repair. Chronic wounds in contrast are recalcitrant to heal and their molecular environment is mostly governed by negative signals that hinder matrix accumulation and turnover, contraction, and re-epithelialization. An example of chronic wound is the diabetic foot ulcer.

This study is focused in: (I) a histopathological characterization skin damages from apparently normal sites in diabetic patients; (II) histopathological characterization of diabetic chronic wounds and of their granulation tissue as compared to that sealing acute defects. Skin and ulcers histosections from diabetic and normal humans were hybridized against a panel of antibodies for growth factors, pro-inflammatory cytokines, stress proteins, cell proliferation, and apoptosis. cDNA was prepared from extracted RNA (skin and granulation tissue) and amplified with specific primers for human RAGE gene.

Our findings suggest that: (i) an aberrant inflammatory infiltrate made up with intermixed neutrophils and macrophages predominate in atonic wounds. (ii) There is a positive pro-inflammatory balance, (III) a qualitative deficit of TGF-beta and of cell proliferation markers. (IV) HSP-27 and 70 appeared increased in diabetic unwounded skin as compared to normal individuals. RAGE is fond over-expressed in all the diabetic samples studied.

Caracterización de las subpoblaciones de linfocitos infiltrantes de tumor y la expresión tisular de ICAM-1 en biopsias de pacientes con cáncer de mama

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Estudios previos han relacionado la presencia de linfocitos como principal característica histológica en múltiples neoplasias malignas incluyendo seminoma, carcinoma gástrico, cáncer de cuello uterino y carcinoma medular de mama. Varios autores han reportado que los linfocitos infiltrantes de tumor (TILs)en cáncer de mama representan predominantemente células T.

Sin embargo, existe controversia con respecto a las subpoblaciones de los mismos,en especial al índice CD4/CD8 en esta patología. Por lo tanto, es propósito de este trabajo determinar la significancia biológica y pronóstica de los TILs y la expresión tisular de ICAM-1 (CD54) en pacientes con cáncer de mama, establecer comparación con patología mamaria benigna y correlacionar los resultados con los factores pronósticos tradicionales.

A tal fin, se determinaron las subpoblaciones de linfocitos infiltrantes de tumor y la expresión tisular de ICAM-1 mediante la técnica de Inmunofluorescencia indirecta, utilizando anticuerpos monoclonales de ratón anti-CD3, -CD4, -CD8 y -CD54 humano en muestras de biopsias de pacientes con patología benigna y cáncer de mama. Los resultados demostraron aumento significativo ($p < 0.0001$) de células CD4 + y CD8+ y de la expresión tisular de ICAM-1 en las lesiones malignas al compararlas con patología benigna de mama. El incremento de las células T CD4+, CD8+ y de la expresión tisular CD54 en cáncer de mama sugiere que la respuesta inmunitaria ejerce un papel importante en la relación tumor-hospedador.

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