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Meeting Abstracts

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4th Symposium of Immunology: Tumor Immunobiology

Botucatu Medical School
Universidade Estadual Paulista Júlio Mesquita Filho - UNESP

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Botucatu, SP, BRAZIL
May 20-22, 2011

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11:35 – 12:15 Luiz Rodolfo Travassos – Unifesp/SP: Development of anti-tumor peptides
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Editorial

Ramon Kaneno[1] and Denise Fecchio[2]

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

May 20, 2011

Dear Participant,

Welcome to the 4th Symposium of Immunology: Tumor Immunobiology at the Botucatu Medical School (FMB), São Paulo State University, Botucatu, SP, Brazil. It is a great pleasure to have you as a guest at our University for this 3-day meeting.

This Symposium was organized by the Department of Microbiology and Immunology of the Biosciences Institute (IBB) in association with the Department of Pathology (School of Medicine) of São Paulo State University and intends to give the opportunity for researchers and students to discuss some of the main trends and challenges in Tumor Immunology.

During this 4th edition, we will have the participation of recognized Brazilian researchers, all of whom have grants from the National Council of Research and Development (CNPq) or State Research Foundations, as well as international speakers, from the US, Argentina and Chile. We thank them so much for responding to our invitation and the rich contribution they will give to the meeting.

We have programmed 2 days for speeches and a poster section, as well as one day for short courses. All the abstracts accepted for the poster section will be published in a regular issue of the Annual Reviews of Biomedical Sciences (ARBS), an online open access journal. The three best posters will be selected for oral presentation and winners will be awarded by The Brazilian Society of Immunology with free registration for the next Brazilian Immunology Congress in Iguassu Falls, PR, Brazil.

We thank The São Paulo State University (*Pró-Reitoria de Pós-Graduação* – PROEX), the Graduate Programs in Pathology (FMB), and Basic and Applied Biology (IBB), *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP) and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for supporting this Symposium. We also thank the members of the Organizing Committee, who have worked hard during the last months, and *Fundação do Instituto de Biociências* (FUNDIBIO), *Serviço Técnico de Informática* (STI) and *Núcleo de Educação a Distância e Tecnologias da Informação em Saúde* (NEAD.TIS) for their technical support, as well as all the enterprises that sponsored this event.

Thank you for attending to the 4th Symposium of Immunology and enjoy the meeting,

Conference Abstracts

Tumour Immunology and ABC transporters

Vivian M Rumjanek

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Rumjanek VM. Tumour Immunology and ABC transporters. Annu Rev Biomed Sci 2011;13:A8.*ABC transporters, such as ABCB1, ABCC1 and ABCG2 have been described in tumour cells under the context of multidrug resistance to chemotherapy. However, these molecules are also expressed in normal cells of the immune system were they might play a physiological role unrelated to the extrusion of xenobiotics. Furthermore, the expression and function of these transporters in immune cells may vary during cell differentiation and activation, and their importance during an inflammatory and an immune response is under study. The relationship between tumour growth and the immune response against it, is a complex phenomenon. Products from tumour cells are also capable of regulating immune cells diferentiation and function and this adds yet another perspective to the local balance of tumour growth versus immune reactivity. The present work discusses the expression and role of ABC transporters on lymphocytes (alfa-beta and gamma-delta), natural killer cells, macrophages and dendritic cells, as well as the impact produced by the use, during tumour therapy, of substances capable of inhibiting such a transport.

Financial support: CNPq, FAPERJ and FINEP
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Immunotherapy of Gastrointestinal Carcinomas with IL-12 and Cyclophosphamide: the Importance of Overcoming Immune Suppression

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Mazzolini GD. Immunotherapy of Gastrointestinal Carcinomas with IL-12 and Cyclophosphamide: the Importance of Overcoming Immune Suppression. Annu Rev Biomed Sci 2011;13:A9.*Immunotherapy-based strategies for gastrointestinal carcinomas (GIC) have to face strong mechanisms of immune escape induced by tumours. Sub-therapeutic doses of an adenovirus expressing IL-12 genes (AdIL-12) mediated a potent antitumour effect against subcutaneous (s.c.) colorectal carcinomas (CRC) in mice pre-treated with low doses of cyclophosphamide (Cy). In our study, we used this combination to treat disseminated CRC and pancreatic cancer (PC) in mice and to assess its impact on the immunosuppressive microenvironment. M&M: Liver metastatic CRC and s.c. PC models were used. Cy (50 mg/ Kg) in combination with AdIL-12 (10^9 TCID50) were administered sequentially. Immunological studies were carried out in samples of peripheral blood, spleen as well as in tumour. CD4+CD25+ and CD4+CD25- T lymphocytes used for the experiments were isolated by magnetic separation. We found that Cy+AdIL-12 were able to eradicate liver metastatic CRC (47%) and PC tumour nodules (40%) and to significantly prolong animal survival. Furthermore, non-responder mice failed to decrease Tregs in tumour, spleen and peripheral blood. Reconstitution of Tregs into tumour-bearing mice treated with combined therapy abolished the antitumoural effect. In addition, Cy+AdIL-12 also modified Tregs functionality by inhibiting the *in vitro* secretion of IL-10 and TGF-beta and their ability to inhibit dendritic cells (DCs) activation by LPS. Combined treatment decreased the number of myeloid-derived suppressor cells (MDSCs) in comparison to non-treated mice and, interestingly, administration of Tregs restored splenic MDSCs population. As a result of Tregs and MDSCs depletion, combined therapy potently generated specific IFN-gamma-secreting CD4+ T-cells able to eradicate established CRC tumours after adoptive transfer. The results of this study support the hypothesis that Cy+AdIL-12 might be a valid immunotherapeutic strategy for advanced GIC.

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Low Non-cytotoxic Doses of Anti-neoplastic Agents Modulate DC and Increase the Immunogenicity of Colon Cancer Cells

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Kaneno R. Low Non-cytotoxic Doses of Anti-neoplastic Agents Modulate DC and Increase the Immunogenicity of Colon Cancer Cells. Annu Rev Biomed Sci 2011;13:A10.*Colon is one of the most infected tissues but the maintenance of parasite:host equilibrium is guaranteed by the high number of immunocompetent cells in this microenvironment as well as the competition of pathogens with components of the microbial flora. A delicate equilibrium between defensive immune reaction and regulation mechanisms avoiding strong unspecific reaction seems to be essential for protect colon against carcinogenesis, whereas the individual ability for inducing a specific antitumor immunoresponse can be essential to overcome the natural regulatory microenvironment of this organ. Our purpose in the present speech is to show that low non-cytotoxic concentration (NTC) rather than minimal effective (cytostatic) concentrations (MEC) of selected antineoplastic agents is able to both modulate DC maturation/stimulation and modify the immunogenicity of tumor cells, enhancing the generation of specific anti-colon cancer cells. We first observed that DCs treated in vitro with very low concentration of selected antineoplastic chemotherapeutic agents showed increased expression of CD83, CD80 and CD40 molecules, as well as their ability to stimulate the proliferation of allogeneic T lymphocytes. Therefore, low NTC of chemotherapeutic agents can directly enhance DC maturation and function. Next we investigated how NTC of paclitaxel (PAC) and doxorubicin (DOX) can affect the immunogenicity of tumor cells and their interaction with the immune system. HCT-116 colon cancer cells treated with these drugs, showed alterations on gene transcription as screened by DNA microarray. Increased expression of calmodulin and proteasome 26 was induced by PAC. Since the product of these genes are involved in the cytosolic route of antigen processing mechanism, we next evaluated whether such alterations were able to change the synthesis of antigen processing machinery (APM) components of three different colon cancer cell lines. We observed that the treatments increased the intracellular expression of APC components such as calmodulin, LMP2, LMP7, TAP1 and tapasin in HCT -116 and HCT-WT cell lines. In vitro treatment of HCT-116 cells with PAC increased the immunogenicity of these cells and DC pulsed with HCT/PAC cell lysate showed higher ability to induce the generation of specific antitumor CTL. Finally, we observed that pretreatment of tumor cells with NTC became them more sensitive to CTL activity. Transfection of normal DC with RNA of HT-29 cells pretreatment with NTC of PAC or 5-fluorouracil (5-FU), but not with MEC slightly increased the ability of DC to stimulated the generation of specific cytotoxic T cells. DC loading with tumor lysates pre-treated with NTC of 5-FU, was also more efficient than cytostatic one, for inducing the stimulation of allogeneic T cells (MLR). Taken together, our data corroborate our initial observations that low NTC of anti-neoplastic agents are able to enhance the in vitro generation of specific anti-colon cancer cells, opening the possibility for the use of such NTC for improving the generation of DC vaccines.

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Anti-Tumor Peptides Derived from Complementarity Determining Regions (CDRs) of Immunoglobulins

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Travassos LR. Anti-Tumor Peptides Derived from Complementarity Determining Regions (CDRs) of Immunoglobulins. Annu Rev Biomed Sci 2011;13:A11-2.*Peptides have been associated with anti-microbial and anti-tumor activities and have been investigated as a potential basis for drug development. The sources of bioactive peptides are numerous including a variety of organisms, microbes, proteins and enzymatic products. Natural and synthetic peptides mediate biological and immunobiological responses or may exert antibiotic and cytotoxic activities. Peptides of various sizes can induce necrosis, apoptosis, cell function-blocking activities and inhibition of angiogenesis. To become an effective anti-tumor agent, a peptide should target cancer rather than normal cells, have a good penetration and favorable pharmacokinetics. Procedures to protect the peptide from degradation and renal clearance are therefore important concerns in this kind of study. Linear peptide fragments from antibodies were found to display cytotoxic activities in a series of microorganisms, an a killer peptide of only 10 amino acids is a prototype in these studies conducted at the University of Parma, Italy. The killer peptide had no effect, however, on mammalian cells. In collaboration with the Parma group, we focused on the direct cytotoxic effect in tumor cells of synthetic peptides with sequences identical to CDRs from monoclonal antibodies. These peptides, were therefore derived from hypervariable regions, and with the exception of V_H CDR3 could occur in antibodies of different specificities. In a pivotal work we showed antifungal, antiviral and antitumor activities of CDRs from 3 different monoclonal antibodies. The anti-melanoma (B16F10) effects of V_H CDR 2 (H2) and V_L CDR 1 (L1) from mAbs C7 and HuA directed to a *Candida albicans* adherence factor and human blood group A, respectively, were demonstrated. The V_H CDR3 (H3) of immunoglobulins has unique properties. Tested as a synthetic peptide it can very often act as a microantibody: it competes with the original antibody for antigen binding and may have similar biological activities usually with different affinity. Linear and cyclic extended H3 peptides from anti-melanoma mAbs (A4 and A4M) competed with the original mAbs for binding to B16F10 melanoma cells. Mab A4 H3 peptide induced DNA degradation and inhibited tumor cell growth similarly to the original antibody. Other mAb H3 showed immunomodulatory property acting on macrophages. The CDR peptides that were cytotoxic to Candida albicans, HIV-1 and murine melanoma (C7H2 and HuAL1) were anti-metastatic using the endovenous B16F10 syngeneic model in mice. We now show that underivatized C7H2 peptide induces apoptosis in several human tumor cell lines at similar concentrations, suggestive of a common mechanism of action. Binding of annexin V, chromatic condensation, DNA degradation (TUNEL), lamin disintegration and caspase 3 and 8 production were observed in various susceptible tumor cells. Abundant anion superoxide production and extensive cytoplasmic alterations involving organelles were also seen. Since C7H2 is not cytotoxic in normal mice and to non-tumorigenic cell lines, it is a potential candidate for drug development. The C7H2 peptide binds to alpha-actin at the surface of cancer cells (Arruda et al., in preparation). Alpha-actin, bound to biotinylated-C7H2-streptavidin, was identified by mass spectrometry and

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C7H2 colocalized with phalloidin-rhodamine to the actin filamentous network in permeabilized cells. Furthermore, C7H2 induced polymerization of G-actin presumably leading to F-actin stabilization. Apparently, the major disturbance in actin dynamics caused by C7H2 may be coupled to mitochondrial alterations and cell apoptosis. Other CDR-derived cytotoxic peptides were identified (e.g. HuAL1) but the molecular targets were not the same as for C7H2.

Human Papillomavirus (HPV) Associated Tumor Microenvironment and Modulation of Host's Immune Responses

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Lepique AP. Human Papillomavirus (HPV) Associated Tumor Microenvironment and Modulation of Host's Immune Responses. Annu Rev Biomed Sci 2011;13:A13.*Cervical cancer is one of the leading causes of women's death in developing countries. Persistent infection with high risk HPV types is the main cause for cervical carcinoma development. HPV belong to a family of epitheliotropic DNA viruses and are very prevalent among young women, approximately 30% of the sexually active population. Most women naturally eliminate the infection; however, some display persistent infections that may result in viral genome integration, cell immortalization and eventually transformation. HPV display several immune evasion mechanisms that have been described by several research groups. There is a general consensus that viral antigens are poorly presented and that low grade lesions do not generate enough inflammation to activate adaptive responses. Indeed, while women that spontaneously eliminate infections display CD4 Th1 anti-virus responses, lymphocytes from women with cancer are not activated by virus antigens. This phenomenon seems to be determined by HPV specific regulatory T cells. Our laboratory has been investigating the mechanisms by which HPV associated tumor cells trigger tolerance towards virus antigens in the host. We have shown that keratinocytes expressing the E6 and E7 HPV proteins, both necessary for maintenance of the cell transformed phenotype, recruit monocytes through CCL2 signaling and induce IL-10 expression in the later cells. The same is observed in tumors growing in mice, where the inflammatory infiltrate also expresses IL-10, and is more abundant in tumors from HPV positive cell lines than in HPV negative tumors. We have recently shown that IL-10 is important for generation of a specific regulatory response to viral antigens and that IL-10 depletion unleashes cellular anti-HPV responses reducing tumor growth. Although our data present evidence that the tumor inflammatory infiltrate may have a role in immune evasion, we also gathered evidence that the tumor has systemic effects altering, for example, the environment on secondary lymphoid organs. Our data contribute to the understanding of the modulation of immune responses by HPV transformed cells, which may in the future, allow us to design more efficient anti-HPV immunotherapies.

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Genomic and Transcriptomic Integration Analysis in Penile Carcinomas According HPV Status

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Rogatto SR. Genomic and Transcriptomic Integration Analysis in Penile Carcinomas According HPV Status. Annu Rev Biomed Sci 2011;13:A14.*Penile cancer is a rare malignancy that occurs after the sixty decade of life. Molecular genetic data in penile cancer are extremely limited. In this study, it was evaluated copy number variations and gene expression alterations by large-scale analysis in penile carcinomas according to HPV genotype. All the cases were genotyped using the Linear Array HPV Genotyping Test (Roche). Genomic copy number variations and gene expression were assessed by 4x44K platforms (Agilent). aCGH data from 31 cases were extracted with Feature Extraction 10.1.1.1.1 software and analyzed by Nexus 5.0 software (Biodiscovery), statistical algorithm FASST segmentation and sensitivity threshold of 1.00E-5. Gene expression data were obtained in 29 samples and analyzed using TMeV 4.5 software (http://www.tm4.org) with t test and P<0,05. In 29 cases evaluated by two methodologies, integrative analysis was done including all the genes with copy number alterations and differentially expressed using Pearson's correlation. In addition, pathways and network analyses were determined using the Ingenuity Pathways Analysis (IPA) software (Ingenuity Systems). The aCGH data showed 450 copy number alterations (in average, 14.52±10.17 CNV/individual). Unsupervised hierarchical clustering revealed three major groups based on the genomic alteration profile and distinct clinicopathological features. The most common copy number gains were detected in 3q, 8q, 9p, 9q and 22q. Chromosomal losses were found in 3p, 4q, 8p, 10p, 10q, 19p, 21p, and Y. These specific alterations were correlated with worst prognosis. Losses at 3p and gains at 3q were correlated with shorter overall survival. Gains and losses were accessed and compared between two groups based on the HPV infection. It was found a higher number of genomic imbalances in HPV- tumors. In addition, gains on 19q13.32 were detected exclusively in HPV+ cases. More than 3.000 differentially expressed genes were found by expression oligoarrays analysis. Two cell cycle control pathways were involved after IPA analysis: checkpoint G2/M regulation and cell cycle and cyclin regulation. The network analysis presented several over and downexpressed central genes, including those related to cell cycle (*CDK1, CCNB1, CCNA2, CDKN2A, E2F1, PCNA, MAD2L1*), immune modulation (*STAT1*) and matrix metalloproteinases (*MMP9*). Two hundred seventy nine genes had differentially transcript expression in the comparison between HPV+ and HPV- tumors. Nine out of 10 HPV+ cases were correctly clustered. Recurrent alterations in pathways and networks related to immune response and inflammation, including the interferon signaling pathway, IL-22 pathway, IL-15 pathway and the central network genes *PI3, STAT1, IRF1* and *TNFSF13B* were verified. The integrative genomic and transcriptomic analysis revealed 163 genes significantly correlated (gains/overexpression and losses/ downexpression). The network analysis revealed several central genes, as *cMYC, TNFSF10, S100A11* and *FLI1*. In cases HPV+, 139 genes were significantly correlated (gain/overexpression and loss/ downexpression). Both, transcript expression and integrative analysis in HPV+ tumors revealed a involvement of large number of immune modulation and inflammation genes. These data suggest a role of HPV in deregulating specific immune genes that result in inflammation and cancer. To the best of our knowledge, this is the first study in penile carcinoma using integrative analysis. Overall, it was demonstrated nonrandom genetic alterations that may drive specific genes and their transcripts and contribute with penile carcinoma pathobiology.

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Monocyte-Derived Dendritic Cells in Human Cancer: their Status in Patients and Possible Therapeutic Applications

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Barbuto JAM. Monocyte-Derived Dendritic Cells in Human Cancer: Their Status in Patients and Possible Therapeutic Applications. Annu Rev Biomed Sci 2011;13:A15.*Monocyte-derived dendritic cells have become a powerful tool for immunomodulation. Since the description of the possibility of their *in vitro* generation, the investigation in the field has grown steadily and many clinical protocols have been developed, mainly for cancer treatment. These approaches are supported by the fact that dendritic cells are clearly affected in cancer patients, presenting deficits of activation/maturation within the tumor microenvironment, thus theoretically failing in the presentation of tumor antigens to the immune system. In this context, the generation of mature dendritic cells *in vivo* and their priming with tumor antigens presents itself as a potentially effective strategy of immunotherapy against cancer. However, in spite of their rationale, dendritic-cell based vaccines for cancer, though achieving positive results in some circumstances have been less successful than one could expect. It would possible to argue that this is due to the actual immune system inability to control tumors, however, it should be pointed out also that the *in vitro* generated dendritic cells constitute a very heterogeneous population that still needs to be better defined and selected in order to achieve the expected clinical results. In the case of cancer patients, it is noteworthy that monocyte-derived dendritic cells present a series of phenotypic and functional alterations, which lead to a clear bias toward the induction of regulatory T cells. Therefore, either these deficits are corrected or different sources of dendritic cells should be considered for cancer patients´ vaccination. One strategy that has been tested by our group is the use of allogeneic dendritic cells from healthy unrelated donors fused to tumor cells obtained from the patients. Again, though obtaining some positive results, this approach also lagged behind the ideally expected. In conclusion, hence, dendritic cells-based vaccines for the treatment of cancer still remain a potentially effective approach that needs, however, further investigation and refinement before achieving a definitive position in the clinical management of patients.

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Immunotherapy of Malignant Neoplasias with Interferon and Dendritic Cells Vaccine

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Murta EFC. Immunotherapy of Malignant Neoplasias with Interferon and Dendritic Cells Vaccine. Annu Rev Biomed Sci 2011;13:A16.*Conservative treatment with interferons (IFNs) has the advantage of preserving reproductive capacity in patients with grade II or III cervical intraepithelial neoplasia (CIN). The objective of this work was to study patients with high-grade CIN treated with intralesional IFN α-2b and to analyze the expression of Th1, Th2 and Treg cytokines in cervical stroma, vaginal secretion and serum (peripheral blood). We observed that patients with a satisfactory response (60%) to treatment with IFN α-2b expressed more Th1 (IFN-g, TNF-α, IL-2) cytokines, with a significant reduction in the viral load of high -risk human papillomavirus (p = 0.0313). All patients with therapeutic failure were smokers and had higher expression of cytokines Th2 (IL-4) or Treg (TGF-β2 and TGF-β3). In vaginal secretion, interleukin 6 and TNF-α concentrations were raised at the sixth application for the patient group who failed to respond to therapy compared to the responsive group (p= 0.0357). The concentration of IL-12 in the serum in the twelfth day of application it is elevated in the patients' group that had therapeutic answer compared to the ones what had therapeutic failure. Patients that had a good response had lowers concentration of inflammatory cytokines than did non-responders, and showing increase of the Th1 profile. Innate and acquired immunity form an integrated system of host defense, where the several cell types act synergistically and are responsible for antitumor immunity. However, the real association and action of these two defense lines are still not fully elucidated. Therefore, the purpose of this study was to evaluate the influence of immunotherapy with dendritic cells (DCs) on cell populations involved in innate and acquired immunity. We evaluated four patients with advanced cancer undergoing immunotherapy with DCs and performed cellular analysis before beginning therapy and for 11 consecutive vaccinations. Autologous DCs were obtained by differentiation of peripheral blood mononuclear cells. After differentiation in culture, DCs were electroporated with tumor antigen obtained through patient's biopsy. The vaccine was administered subcutaneously with an average interval of 15 days. Peripheral blood samples were collected for analysis of the immune cell populations and performed by flow cytometry using antibodies (BD Biosciences) for the following markers: macrophages (a-CD14 PE), NK cells (a-CD56 PE), cytotoxic T lymphocytes (a-CD8 PE), total T cells (α-CD3 PE), helper T cells (α-CD4 PE) and B cells (α-CD19 PE). As results, there was noticeable raise of cytotoxic T lymphocytes, there was significant enhance in pre-therapy to the 2nd post-therapy (p=0.0562). Thus, the increase in these cell populations indicates that immunotherapy with DCs influences the innate and acquired immunity, and further studies on the mechanisms involved in this environment, especially what concerns to the expression of cytokines, might further elucidate the effects on the immune response after therapy with dendritic cells.

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Immunological and Clinical Outcomes of a New DC-Based Vaccine

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Salazar-Onfray F. Immunological and Clinical Outcomes of a New DC-Based Vaccine. Annu Rev Biomed Sci 2011;13:A17.*We developed an original method for production of therapeutic dendritic-like cells named Tumor Antigen Presenting Cells (TAPCells®) using an allogeneic melanoma-derived cell lysate (TRIMEL®) as activation factor and antigen provider. TAPCells-based immunotherapy induced T cell-mediated immune responses and improved long-term survival of stage IV patients in studies involving more than 100 individuals (*López et al. 2009, J Clin Oncol*; *Aguilera et al. 2011, Clin Cancer Res*). Importantly, 61% of tested patients (58 out of 94) showed a Delayed Type Hypersensitivity (DTH) reaction against TRIMEL indicating the development of anti-tumor immunological memory that correlates with prolonged patient survival. The *in vitro* analysis of TRIMEL showed that it contains damage associated molecular patterns such as HMBG-1 protein, induced by heat shock, capable to improve, through TLRs, the DC maturation and antigen cross-presentation. Biopsies of DTH tissues revealed the presence of $CD45RO^+$ $CD4^+$ and $CD8^+$ T cells capable to release pro-inflammatory cytokines upon *in vitro* stimulation. DTH response against TRIMEL was associated with prolonged survival of the stage IV responder melanoma patients (DTH +; 35 months) compared to the non-responders (DTH -; 11 months). Moreover, 70% of vaccinated stage III melanoma patients (n=22) showed long-term disease stability without progression signals. Furthermore, we observed that DC-vaccination resulted in a three-fold augment of Th1 cell population releasing IFN-γ and a two-fold increase of Th17 lymphocyte population capable to produce IL-17 in the PBL of DTH+ patients respect to DTH- ones. A direct correlation between increased Th1 and Th17 production in the blood of DTH+ patients was observed suggesting that those profiles may favor an anti-melanoma response. Additionally, we confirmed the presence of the Th1/Th17 response in the DTH- associated T lymphocytes by immunofluorescence and *in vitro* activation assays. Taken together, our results indicate that TAPCells immunization resulted in two different pattern of response associated to the immunological and clinical outcome. Our study may contribute to the better understanding of clinical immunological responses produced by DC-vaccines and to the development of improved DC-based vaccines.

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The Role and Control of Regulatory Dendritic Cells in Cancer

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Shurin GV. The Role and Control of Regulatory Dendritic Cells in Cancer. Annu Rev Biomed Sci 2011;13:A18.*The role of myeloid cells, such as myeloid-derived suppressor cells (MDSC), macrophages, granulocytes and dendritic cells (DC) in tumor progression and spreading is relatively well described and characterized. However, very limited and highly controversial data are available for understanding the appearance, function and significance of so-called regulatory DC (regDC) in the tumor milieu. The immunosuppressive function of regDC is already established in autoimmune models, but tumor-associated regDC are not characterized. We have recently established the animal tumor model, where tumor progression is associated with emergence of regDC without accumulation of MDSC and regulatory T cells at the tumor site and lymphoid tissues. This allows assessing the role of regDC in tumor progression without interference with immunosuppressive function of MDSC and Treg cells in vivo. We have also determined that conventional DC (cDC) may be converted into regDC in vitro, which provided a tool to study immunobiology of regDC both in vitro and in vivo. Using these experimental approaches, we have demonstrated that regDC significantly blocked proliferation of pre-activated T cells in vitro and accelerate tumor growth and inhibit development of the antitumor immunity in vivo. While evaluation of the mechanisms of immunosuppressive properties of regDC is in progress in our laboratory, we have revealed that microtubule-targeting agent taxol can prevent formation of regDC in the tumor milieu, by probably affecting activity of the small Rho GTPase proteins in DC, if is used in ultra low doses. Interestingly, both depletion of total DC and prevention of regDC accumulation in tumor-bearing mice markedly up-regulated the antitumor activity of DC vaccines, suggesting that immunosuppressive regDC might play an important protumorigenic role in the tumor models where MDSC and Treg cells are not functionally involved. In summary, our data demonstrate that accumulation of myeloid regulatory cells is cancer-specific and different myeloid regulators might play different roles in tumor progression and inhibition of the antitumor immunity. Our data also provide new targets and new approaches for controlling cancer-associated immunosuppression and tolerance.

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Ligand-Directed Therapy and Molecular Imaging Based on *In Vivo* Phage Display Technology

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Pasqualini R, Arap W. Ligand-Directed Therapy and Molecular Imaging Based on In Vivo Phage Display Technology. Annu Rev Biomed Sci 2011;13:A19.*Our group has originally developed broad vascular targeting technology platforms to uncover and exploit ligand-receptor interactions in the context of human disease, with emphasis in the use of combinatorial selection of peptide libraries in patient. Essentially, over the past decade, we have been probing the molecular diversity (for example, of the vascular and lymphatic endothelium or of the humoral immune system) to find unique cell surface addresses--endothelial and otherwise--for delivery to selective cell types or cell populations, vasculature of tissues and/or organ systems. There are many potential, as yet unrecognized, protein-protein interactions that may lead to applications such as targeted vascular-mediated tissue repair or acute hemorrhagic control of non-surgical bleeding. Such set of ligand-receptor interactions can encompass applications in different organ-specific vascular beds in health and diseased conditions. The aggregate of the data generated thus far indicate that a new ligand-directed pharmacology and its ramifications is now unequivocally at hand. Development of a Program including--but not limited--to vascular and lymphatic targeting, molecular-genetic imaging, and other medical applications or toolkits has been funded by DARPA. Prioritized goals in this project focus on the optimization of wound stasis and treatment of traumatic or bleeding injuries by delivery of targeted polymer-based procoagulants to the vascular endothelium. Rapid elimination of resistant infections is also feasible by incorporation of a newly developed peptidomimetic-based antibiotic. Applications such as treatment of traumatic or bleeding injuries by delivery of payloads to the vascular endothelium and field targeted imaging are feasible. To evaluate the application of these technologies to the care of the critically injured patient we employed swine models developed at the US Army Institute of Surgical Research in San Antonio, TX. We hypothesized that reliable markers of tissue injury can be identified and will enable the design and validation of targeted entities to be leveraged towards wound stasis. Conditions evaluated focused on liver injury (grade V), and compound fracture of a femur. The animal models selected are those that mimic significant trauma in humans. We have performed an *in vivo* combinatorial screening of phage libraries to select for peptides that can preferentially localize to hemorrhaging wounds and implemented a bioinformatics infrastructure (the "Phage System") to mine extensive data sets generated. A candidate selection algorithm has been subjected to bioinformatics analysis and rigorous statistical tests to identify individual candidate phage and for the production of sublibraries for validation.

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Targeting Immune Regulators in the Tumor Microenvironment

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Shurin MR. Targeting Immune Regulators in the Tumor Microenvironment. Annu Rev Biomed Sci 2011;13:A20.*The tumor microenvironment consists of a variable combination of tumor cells, stromal fibroblasts, endothelial cells and infiltrating leukocytes, including macrophages, T lymphocytes, B lymphocytes, NK cells, granulocytes, and dendritic cells. Tumor progression is often associated with suppression or malfunction of the immune system, including appearance of regulatory T cells, myeloid-derived suppressor cells (MDSCs), M2 or regulatory macrophages, and regulatory or toleragenic dendritic cell (DC) subsets, as well as dysbalance in the intratumoral cytokine network and protumorigenic polarization of Th1/Th2/Th3/Th17/Treg subsets. For instance, functional inhibition of conventional immunogenic DCs and emergence of tolerogenic or immunosuppressive DCs in the tumor environment play an important role in tumor escape from immune recognition and failure of many common immunotherapeutic approaches. We have recently demonstrated that certain antineoplastic chemotherapeutic agents could directly up-regulate development, maturation, and functional activation of DCs in vitro and in vivo if used in ultra low noncytotoxic concentrations. These data suggest that several well characterized chemotherapeutic drugs are able to stimulate immune cells in noncytotoxic/ noncytostatic concentrations without inducing cell death or inhibiting cell cycle. This unexpected new phenomenon was termed *chemomodulation* to distinguish it from conventional or moderately low-dose chemotherapies, which are based on direct cellular toxicity. Our new data revealed that ultra low-dose nontoxic chemomodulation not only directly activates DCs, but also decreases tumor-induced immunosuppression of DCs in the tumor microenvironment. This effect was mediated by two independent pathways: (i) increased resistance of DCs to tumor-derived factors and (ii) blockage of tumor cells to express immunosuppressive molecules after pre-treatment with ultra low noncytotoxic concentrations of chemotherapeutic agents. Most importantly, chemomodulation was able to convert tumor-induced regulatory DCs into immunostimulatory DC subsets, suggesting that both resident DCs as well as vaccine, i.e., administered, DCs cells can be targeted by ultra low-dose nontoxic chemomodulation in the tumor milieu. Furthermore, ultra low-dose nontoxic chemomodulation down-regulated formation and activity of MDSCs and regulatory T cells in the tumor microenvironment. Interestingly, chemomodulation of MDSCs supported their differentiation into conventional DCs. Finally, antineoplastic chemotherapeutic agents in low nontoxic concentrations increased expression of antigen-processing machinery components in tumor cells, increased tumor cell recognition by tumor-specific cytotoxic T lymphocytes, and, thus, increased immunogenicity of tumor cells. Together, these new data suggest that the modulation of the tumor microenvironment by ultra low-dose noncytotoxic chemomodulation that affects different targets in the tumor milieu may serve as a new powerful neoadjuvant for different immunotherapeutic modalities in cancer. In fact, application of low-dose chemomodulation prior to DC vaccines in the animal tumor models resulted in significant inhibition of primary and metastatic tumor growth in vivo. Thus, chemomodulation of the tumor environment with nontoxic doses of several common chemotherapeutic agents might target different cell populations, decrease tumor-induced immunosuppression, and improve the efficacy of modern immunotherapeutic approaches for cancer.

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AWARDED WORK

The Development of Dendritic Cells is Affected in Different Stages by Leukemic Cell Products

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Motta JM, Rumjanek VM. The Development of Dendritic Cells is Affected in Different Stages by Leukemic Cell Products. Annu Rev Biomed Sci 2011;13:A21. Dendritic cells (DCs) are professional antigen-presenting cells that specialize in activating T lymphocytes. DCs play an important role in controlling tumors, since they are able to recognize and initiate an antitumoral immune response. However, tumor cells have developed mechanisms to inhibit immune responses, thereby favoring tumor progression. Some products secreted by tumor cells present immunosuppressive characteristics and can affect different types of immune cells, including DCs. There are many studies involving products released by solid tumors and how they modulate DCs, but there is a paucity of information about products released by leukemic cells. This study aimed to analyze the development and function of DCs in different stages and the influence of leukemic cell products on this process. For this, the tumor cell line used was K562, derived from chronic myeloid leukemia. Monocytes obtained from healthy volunteers were cultured in the presence of IL-4 and GM-CSF, stimuli for DC differentiation, and in the presence of K562 supernatant. After 5 days, CD14, CD1a expression and dextran phagocytosis were evaluated in these cells by flow cytometry. In another experiment assessing the effect of tumor products on DC activation, immature DCs were cultured with TNF-α for an additional 2 days. After this period, CD83 expression by DCs was measured by flow cytometry. Moreover, activated DCs were co-cultured with lymphocytes obtained from a second donor for 24 hours and lymphocyte expression of CD69 was evaluated. During a control differentiation, monocytes down-regulate CD14 expression and start to express CD1a. In this stage, immature DCs present high phagocytosis capacity. In the presence of tumor supernatant, CD14 expression remained high and CD1a expression was low. However, the addition of tumor supernatant did not interfere with dextran phagocytosis by monocytes stimulated to differentiate into DCs. If activated, DCs considerably increase CD83 expression and acquire a high ability to activate lymphocytes. It was observed that monocytes differentiated in the presence of tumor supernatant and then stimulated to activation expressed less CD83. Moreover, tumor products appear to inhibit the appearance of CD69 expression on lymphocytes co-cultured with DCs differentiated with tumor supernatants and then activated. Finally, these results suggest that soluble products released by leukemic cells affect DC differentiation and activation, suggesting that, in this condition, these cells become unable to complete their development.

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The Use of Nanovesicles from Mature Dendritic Cells as Adjuvants to Induce Antitumoral Response

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Romagnoli GG, Toniolo PA, Migliori IK, Bergami-Santos PC, Barbuto JAM. The Use of Nanovesicles from Mature Dendritic Cells as Adjuvants to Induce Antitumoral Response. Annu Rev Biomed Sci 2011;13:A22. Exosomes (Exo) result from the fusion of multivesicular bodies with the plasma membrane and are involved in the intercellular communication in the body. Exo originated from dendritic cells (DCs) are able to induce direct and indirect lymphocyte responses and can lead to maturation/activation of immature DCs. Since these vesicles contain many of the molecules involved in antigen presentation, the present work was designed to evaluate their potential for transferring these molecules to tumor cells thus converting them into immunogenic cells. Mature DCs (mDCs) were differentiated from healthy donors' blood monocytes in culture for seven days in the presence of GM-CSF, IL-4, and, for the last 2 days, TNF-α. Culture supernatant of mDCs was cleared from cells and submitted to ultracentrifugation to isolate nanovesicles, which were characterized by flow cytometry for the expression of typical DC and Exo markers. After phenotyping, Exo at different concentrations were added to cultures of the human breast adenocarcinoma cell line SK-BR-3. After varying time intervals, the tumor cells' expression of the Exo-carried molecules was evaluated by flow cytometry. All different Exo preparations carried HLA-ABC, CD86, CD11c, CD81 and CD18. HLA-DR and CD54 were present in some preparations, but not in others. Exo-treated (60-130 ug/10^6 cells) SK-BR-3 tumor cells expressed the class I and class II HLA molecules CD18, CD80, CD86 and CD83, molecules carried by the Exo and absent in non-treated SK-BR-3 cells. The highest detection level was observed 6-8 hours after treatment with Exo, when up to 43% of cells reacted with class II specific antibodies (with an increase in Median Fluorescence Intensity – MFI – ranging from 150 to 300%). MFI for CD86 increased up to 125% among treated cells and CD18 showed an increase of up to 170% in MFI. CD80 and CD83 were also detected in treated cells, but not in all experiments. Furthermore, CD9, a molecule already expressed by a fraction of tumor cells, and also carried by Exo, was increased in Exo-treated tumor cells, with an average MFI increase of 135%. These preliminary results suggest the potential of DC-derived Exo to affect tumor cell surface molecule expression, thus possibly transforming non-immunogenic cells into immunogenic tumor cells, by virtue of HLA and costimulatory molecule expression.

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*Presenting author

AWARDED WORK

Induction of Cell Death by p19Arf and IFN-Beta in Tumor Cells Resistant to P53 Gene Therapy

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Medrano RFV, Ribeiro AH, Catani JPP, Strauss BE. Induction of Cell Death by P19arf and Ifn-Beta in Tumor Cells Resistant to P53 Gene Therapy. Annu Rev Biomed Sci 2011;13:A23. One critical step in tumorigenesis is the loss of p53 function, caused either by mutations in the p53 gene or by alterations in its pathway. One common mechanism to inactivate the p53 pathway is the loss of p19Arf and/or overexpression of mdm2. Such alterations may also contribute to the resistance of tumors to p53 gene therapy since the exogenous p53 would be maintained in an inactive form. We propose that the introduction of exogenous p19Arf would be an effective gene therapy strategy in mouse models. For this, recombinant adenoviral vectors (rAd) were constructed containing the transgene of interest, such as p19Arf or eGFP, under the control of a p53-responsive promoter, called PG. First we verified the transcriptional activity of endogenous p53 in both B16 (mouse melanoma) and LLC1 (mouse lung carcinoma) cell lines where the eGFP reporter gene was introduced by the p53-responsive adenoviral vector. When treated with doxorubicin, a chemotherapeutic agent that induces p53 function, an increase in reporter activity was observed by flow cytometry, indicating that endogenous p53 could be stimulated. As expected, the introduction of exogenous p53 (rAdPG-p53) in either B16 or LLC1 cells did not induce death, as revealed by cell cycle analysis, confirming that these cells are resistant to p53 gene therapy. However, when p19Arf was applied in our p53-responsive vector (rAdPG-p19Arf), cell death was induced in both B16 and LLC1 cells. Data from the literature as well as our lab suggest that the p53 pathway, including p19Arf, plays a part in mediating the response to IFNβ, a stimulator of the immune response with several additional anti-neoplastic functions. We propose that combining interferon-beta (IFNβ) with p19Arf may yield an improved treatment strategy. To test this cooperative effect of p19Arf and IFNβ on the p53 pathway, we constructed a recombinant adenovirus for IFNβ (rAdPG-IFNβ)**.** B16 was transduced with rAdPG-p19Arf and rAdPG-IFNβ separately or co-transduced with both vectors. The induction of cell death was greater upon co-transduction as compared to application of a single vector, as analyzed by cell cycle and standard MTT assays, confirming our hypothesis of cooperation between p19Arf and IFNβ. With the p53-responsive adenoviral vectors, we have taken steps to establish a pro-apoptotic and immune-stimulatory interplay between p53, p19Arf and IFNβ. *In vivo* studies are underway to reveal whether this treatment strategy is effective against primary and metastatic foci.

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Poster Session

Assessment of HLA Compatibility among Relatives of Patients Waiting for Bone Marrow Transplantation in the Brazilian States of São Paulo, Rondonia and Mato Grosso

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Cita RF, Nascimento DF, Medeiros L, Araújo MF, Donadi EA. Assessment of HLA Compatibility among Relatives of Patients Waiting for Bone Marrow Transplantation in the Brazilian States of São Paulo, Rondonia and Mato Grosso. Annu Rev Biomed Sci 2011;13:A25.*The process of choosing a donor for hematopoietic stem cell transplantation (HSCT) is customized for each patient, and aims to select a donor who has the highest probability of success as well as few procedure-related complications. Hence, the selected donor will determine possible adjustment of several steps involved in the transplantation process. Moreover, in order to achieve a successful transplantation it is necessary, among other factors, to have compatibility of the molecules encoded by human leukocyte antigen (HLA) genes. Generally, the transplants that have a good prognosis are those performed between HLA -identical siblings. Therefore, the objective of the present study is to assess the compatibility percentage of the bone marrow (BM) donors among relatives of patients that need BM transplantation (BMT) at the Cancer Hospital of Barretos, Sao Paulo state, Brazil. To this end, data were collected in 2009 and 2010 at the Immunogenetic Laboratory database in the Cancer Hospital of Barretos. For the total of 47 cases of patients waiting for BM transplantation, 506 potential donors were screened among their relatives, 27.9% and 50.9% were siblings and cousins (up to fourth degree), respectively. There was compatibility with the patient's family members in 26 (5.2%) cases, of which 3.4% were established among siblings, 1.6% among cousins and 0.1% among mothers. When the donors were evaluated, we obtained a total of 141 siblings typed for donation, of whom 17 (12%) were compatible with the patient that underwent typing while 8 (3.8%) out of 213 cousins were compatible. Our data indicate that, in the brazilian regions studied (Rondonia, Mato Grosso and São Paulo states), the possibility of finding a compatible donor for BMT within the patient's family is promising especially among siblings and cousins.

*Presenting author

Interplay Between Social Stress and Breast Cancer: Women's Experiences

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Amorim MAP, Siqueira KZ. Interplay Between Social Stress and Breast Cancer: Women's Experiences. Annu Rev Biomed Sci 2011;13:A26.* Breast cancer (BC) is the most commonly diagnosed form of cancer among women in Brazil and is the second most frequent internationally. Stress can be conceptualized as a process of adapting the organism to an adverse event. In this process, triggered by stimuli known as stressors, the individual responds with physiological, cognitive and behavioral alterations. This study examined the relationship between stress factors and BC development and provided an important opportunity to explore the impact of everyday stress on the long-term risk of first time incidence of primary BC. The semi-structured interview applied was based on life events, important emotional losses, difficult life situations, and psychological characteristics. The study population investigated was 71 breast cancer patients. Data were collected between January and June 2010. The interviews were conducted individually, face-to-face with the consent of participants, in a place (room of Rede Feminina de Combate ao Câncer de Mama of Blumenau - SC) with adequate conditions of comfort and privacy. The project was approved by the Ethics Committee of the Blumenau Regional University (protocol number 46/2010). Our data show that 76% of women affirmed having experienced stressful events prior to BC diagnosis; 79% of them noticed the influence of these factors in developing cancer. Familial context (79%), death of near relative or partner (34%), financial difficulties (15%), illness of near relative or oneself (11%), work issues (08%), menopausal hormones (04%) and kidnapping (02%) were problems most described. Furthermore, 10 participants reported relapses; four of them affirmed other new stress factors. We concluded that prolonged stress of everyday life results in a persistent activation of stress hormones, which may suppress immune response and may thereby be related to a higher risk of breast cancer or susceptibility to infections.

*Presenting author

Modulation of Macrophage Pro- or Anti-Tumoral Responses by Jacalin

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Polli CD, Scarpino LM, Geraldino TH, Toledo KA, Bisson GS. Modulation of Macrophage Pro- or Anti-Tumoral Responses by Jacalin. Annu Rev Biomed Sci 2011;13:A27. Within the context of tumors, macrophages have been increasingly recognized as central regulators. These cells are able to dramatically affect the course of the disease and depending on their functional orientation, can present both pro- and anti-tumoral activities. The aim of this study was to analyze the modulation of macrophage tumoricidal activity by the lectin jacalin. We show that *in vitro*, jacalin (2.5 to 40µg/ml) induced the production of both pro- and anti-inflammatory mediators by human macrophages. Lower concentrations of this lectin (up to 5µg/ml), when compared to the higher range (from 10 to 40µg/ml), induced the secretion of higher levels of the anti-inflammatory cytokines IL-10 and TGF-β. Similar amounts of the pro-inflammatory cytokines TNF-α and IL-6 were secreted by cells stimulated with jacalin at all of the concentrations tested. For IL-12, high concentrations of the lectin determined the maximal responses. As assessed by MTT assays, when supernatants from macrophages stimulated with higher, but not with lower, concentrations of jacalin were added to cultures of human colon adenocarcinoma cells (HT-29), up to 25% reduction of cell viability was observed. These results indicate that jacalin, through its ability to exert a pro-inflammatory activity, can direct macrophages to an anti-tumor phenotype.

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Effect of Lymphocyte's Heat Stress on the Phenotype and Function of Lymphocyte-Dendritic-Cell Hybrids

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Cruz KSP, Romagnoli GG, Barbuto JAM. Effect of Lymphocyte's Heat Stress on the Phenotype and Function of Lymphocyte-Dendritic-Cell Hybrids. Annu Rev Biomed Sci 2011;13:A28. Dendritic cells are the major antigen-presenting cells and their *in vitro* generation is of huge interest regarding immunotherapy protocols. This cellular type presents great functional heterogeneity; attempts to clarify which factors could influence this characteristic to produce an efficiency gain in their use as immunomodulators have been studied. Among these factors, temperature and the evidence that indicates a key role of fever in immune responses have not been examined thoroughly. Since our group is studying tumoral-dendritic hybrid cells as stimulators of antitumoral response in cancer patients, our aims in this study were to investigate the effects of lymphocyte exposure to fever temperature and to assess whether temperature could effect the hybrid cells generated by the fusion of lymphocytes to allogenic dendritic cells. In this study we have analyzed the effects of lymphocyte exposure to different temperatures (37º C and 40 ºC) and membrane phenotypes (MHC I, MHC II, CD3, CD4, CD8, CD16, CD19 and CD25). Also, we have analyzed the allostimulatory activity of the hybrid cells using proliferative assays to determine the phenotype of responsive cells ($CD4^+$ and $CD8^+$) and the production of cytokines IFN-gamma and IL-10. Our results found no significant differences in cellular recuperation. It was observed that lymphocytes that had undergone thermal stress at 40 ºC presented a phenotypical change in the surface molecules CD4, CD8, CD16 and MHC I, an effect that did not occur in other surface molecules, namely HLA-DR, CD3, CD19 and CD25. A preferential induction of $CD4^+$ lymphocyte proliferation was observed in the groups where dendritic cells and stimulatory lymphocytes were mixed, whereas the groups submitted to the fusion presented the opposite result, a preferential induction of $CD8^+$ lymphocyte proliferation. Our results also showed that there was a significant production IFN-gamma increased in cultures in which cells were mixed only when compared with those cells that were fused. These data do not permit us to make a final conclusion about the effects of temperature in the studied model, but suggest the need for many more studies.

Financial Support: FAPESP

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Characterization of Different Monocyte Sub-Populations Obtained from Human Blood Apheresis Used to Generate Dendritic Cells *In Vitro*: Preliminary Analysis

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Reginato MP, Romagnoli GG, Santos PCB, Barbuto JAM. Characterization of Different Monocyte Sub-Populations Obtained from Human Blood Apheresis Used to Generate Dendritic Cells In Vitro: Preliminary Analysis. Annu Rev Biomed Sci 2011;13:A29. Dendritic cells (DCs) are used for the immunotherapy of cancer with promising, but still not completely satisfactory results. One possible reason for that is the functional deficiency found in patients' monocyte-derived DCs (Mo-DCs). Human blood monocytes have been divided into two distinct subpopulations, one CD14+CD16- and one CD14+CD16+, which differ in their cytokine production pattern but are equally able to differentiate into DCs. However, it is possible that these two subpopulations are differentially affected numerically and functionally by the tumor presence, which could result in functionally altered Mo-DCs. The aim of this study is to characterize, by flow cytometry analysis, the monocyte subpopulations present in apheresis' leukoreduction chambers in order to analyze their differentiation into DCs in the presence of tumor cells. Interestingly, the flow cytometric analysis of mononuclear cells present in the leukoreduction chambers showed three subpopulations instead of the expected two: CD14+CD16- (35.4%), CD14+CD16low (46.5%) and CD14+CD16high (7.4%). It still needs to be determined whether these findings are a consequence of the apheresis procedure or represent a refinement of monocyte sub-classification.

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Infiltrated Immune Cells in the Uterine Cervical Stroma of Patients with Cervical Intraepithelial Neoplasia II - III Treated with Intra-Lesional Interferon

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Machado FA, Michelin MA, Murta EFC. Infiltrated Immune Cells in the Uterine Cervical Stroma of Patients with Cervical Intraepithelial Neoplasia II - III Treated with Intra-Lesional Interferon. Annu Rev Biomed Sci 2011;13:A30.*Infection by human papilloma virus (HPV) induces innate and acquired immune responses in the uterine cervical stroma, which constitute a delicate, balanced and generally unpredictable immunological defense. Advances in our understanding of the immune system and of the definition of antigens on tumor cells have led to many new treatment strategies. As a result, immunotherapy has the potential to be the most specific treatment for tumors, and one that requires elaboration. Recently, immunotherapy with interferon (IFN) has been utilized to treat cervical intraepithelial neoplasia grades II and III (CIN II and CIN III). This study aimed to characterize the immune cells that infiltrated uterine cervical stroma obtained from biopsies collected from patients diagnosed with CIN II or III that were treated with IFN immunotherapy using the technique of immunohistochemistry for T (CD3,CD4,CD8), B lymphocytes (CD20), macrophages (CD68), inducible nitric oxide synthase (iNOS), natural-killer cells (CD16) and perforin (PERFORIN). Our study group consisted of 13 patients with an average age of 33.9 years who were diagnosed with CIN II or III and subjected to treatment with intra-lesional IFN (3,000,000 UI). Two biopsies were collected from each patient, one after the diagnosis of CIN II or III and one after IFN therapy. The slides were examined by immunohistochemistry utilizing common light microscopy with 400x ocular objectives. The statistical analysis was performed with the software 4.0 GraphPad Prism by Fisher's exact test. As to effectiveness, 46.15% of the patients showed a good response to the treatment and 53.85% presented therapy failure. There was no statistically significant variation before and after treatment among the cell types studied. The profile of the studied cells from patients with CIN II or III persisted, regardless of IFN treatment or lesion degree. The treatment did not modify the peritumoral infiltrate. Probably the IFN action mechanism does not influence the finding of peritumoral cells after treatment, regardless of clinical response. The small sample size (n) reflects the difficulty of selecting this type of patient. Therapeutic success of this treatment may occur by direct mechanisms of IFN on the neoplastic cells by inducing regression through apoptosis.

Financial support: FAPEMIG and CNPq.

*Presenting author

Superoxide Dismutase Increases During Interaction Between Amoebae and Leukocytes in the Presence of Melatonin Hormone

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Ferreira MA, Botelho ACF, França JL, França EL, França ACH, Gomes MA. Superoxide Dismutase Increases During Interaction Between Amoebae and Leukocytes in the Presence of Melatonin Hormone. Annu Rev Biomed Sci 2011;13:A31. Amoebiasis is an important parasitic disease that accounts for significant morbidity and mortality in humans. Tissue invasion by trophozoites induces a humoral immune response, which has been described as less effective than the cellular immune response. It remains unclear whether the oxidative stress generated at the inflammatory sites of amoebiasis gives rise to benefits or injuries to the host. The main function of the antioxidant defense of the organism is to inhibit or reduce the damage caused to cells by reactive oxygen species. There are a variety of antioxidant components including superoxide dismutase (SOD). The hormone melatonin has been reported as highly effective in eliminating free radicals, and has shown significant antioxidant action. The present study aimed to verify the superoxide dismutase enzyme during interaction between leukocytes and amoebae in the presence of melatonin. Polymorphonuclear (PMN) and mononuclear (MN) leukocytes were separated by the method of Ficoll-Paque and incubated with trophozoites of *Entamoeba histolytica* (strain HM1-IMSS). The SOD was analyzed by the NBT (Nitro Blue Tetrazolium) reduction method. The SOD concentration increased in the presence of melatonin, suggesting that after leukocyte activation it may also prevent cell damage by increasing SOD levels.

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Melatonin Modulated Superoxide Release During Interaction between Amoebae and Leukocytes

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Abstract

*Ferreira MC, França-Botelho AC, França JL, Fran;ca EL, Honório-França AC, Gomes MA. Annu Rev Biomed Sci 2011;13:A32.*There is strong evidence of the modulating action performed by melatonin in various infections; however, as to its action in the interaction between protozoa and their hosts, the reports are scarce. Amoebiasis is caused by the globally widespread protozoan *Entamoeba histolytica*, although its highest incidence is in places with inadequate basic sanitary conditions. Several aspects of this host-parasite relationship such as parasite virulence and host susceptibility are poorly understood. The infection course begins with inflammatory process that recruits eosinophils, lymphocytes, neutrophils and macrophages. Reactive oxygen species, such as superoxide (O_2^-), are important in the destruction of pathogens by leukocytes. This study aimed to verify the supeoxide release by blood leukocytes in the presence of trophozoites and the melatonin hormone. Polymorphonuclear (PMN) and mononuclear (MN) leukocytes were separated by the Ficoll-Paque method and incubated with trophozoites of *E. histolytica* (HM1-IMSS strain). Superoxide anion was measured with chromogen Ferricitocromo C. The concentration of superoxide anion increased with melatonin, suggesting that this hormone may play a beneficial role in the control of the amoebic lesion, activating leukocytes, and opening up the possibility of using the drug as an adjuvant to antiamoebic therapy.

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LQB-118, a Novel Antineoplastic Agent, Reduces B16F10 Melanoma Growth and Induces Changes in Thymus Cell Subpopulations *In Vivo*

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Abstract

*Eduardo J. S. Salustiano[1], Matheus L. Dumas[1], Chaquip D. Netto [2], Alcides J. M. da Silva[3], Paulo R. R. Costa[3], Vivian M. Rumjanek[1]. LQB-118, a Novel Antineoplastic Agent, Reduces B16F10 Melanoma Growth and Induces Changes in Thymus Cell Subpopulations in vivo. Annu Rev Biomed Sci 2011;13:A33.*The side effects of most antineoplastic agents often contribute to therapeutic failure in the attempt to treat malignant cancers. Therefore, development of novel, safer chemotherapy agents is of great interest. Among natural products with antineoplastic effect, the pterocarpans, isoflavonoids able to induce DNA fragmentation, and the naphthoquinones, known for inducing oxidative stress, have inspired a new hybrid synthetic molecule, LQB-118, which has proven effective against human leukemias and lung cancer *in vitro* by our previous works. Thus, the present study aims to evaluate the effect of LQB-118 on the growth of B16F10 murine melanoma *in vivo*. Safety was also considered, since the observed *in vivo* toxicity highlighted effects on immune system cells. Swiss mice received a single, acute intraperitonial dose of LQB-118 (3.8 mg/kg). After different periods (24h, 72h, 30 days and 90 days) weight alteration and behavior were observed. At the same time, thymus, spleen and bone marrow were excised and cells were analyzed by flow cytometry to detect cell subpopulation alterations. Furthermore, the antineoplastic effect of LQB-118 was evaluated. One hundred thousand (10^5) B16F10 cells were subcutaneously injected into C57BL/6 mice; cells were left to grow for three days and animals were then treated with daily intraperitonial injections of LQB-118 at a chronic dose (0.19 mg/kg/day), for two weeks. Animals were euthanized and tumor mass was excised for evaluation of size and weight. LQB-118 was found to be non-toxic to young and adult mice since intraperitonial administration did not change weight gain, weight of the immune system organs or the absolute number of cells when compared to control group. However, LQB-118 appears to provoke a decrease of T $CD4^+/CD8^+$ cells with concomitant increase of T $CD4^+$ cells in the thymus. Furthermore, *in vivo* experiments showed that LQB-118 exerted an interesting antineoplastic effect *in vivo*, being able to significantly reduce melanoma mass and size after two weeks. Data showed that synthetic LQB-118 presents chemoterapeutical potencial to human patients because of its low toxicity. However, further investigation of LQB-118's effect on T CD4 thymocytes is still needed to understand its impact on the immune system.

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Abrin and Pulchellin Antitumoral Activity in the Absence or Presence of Beta-D-Galactose in Murine Breast Cancer

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Abstract

Matos DC, Ribeiro LCA, Ferreira LS, Polesi MC, Colombo L, Carlos IZ. Abrin and Pulchellin Antitumoral Activity in the Absence or Presence of Beta-D-Galactose in Murine Breast Cancer. Annu Rev Biomed Sci 2011;13:A34. Breast and colon cancers are the most frequent cancer types in women; their respective 2008 estimates in Brazil are 49,400 and 18,680 new cases. Abrin and pulchellin are obtained from seeds of *Abrus precatorius* and *Abrus pulchellus*, respectively. They are type II ribosome-inactivating proteins (RIPs), and consist of two dissimilar, disulfide-linked polypeptide chains. The A-chain presents N-glycosilase enzymatic activity, and the B-chain exerts lectin activity on b-D-galactose, a carbohydrate present in most mammalian cells. To evaluate the antitumoral activity of these proteins, breast cancer was induced in female Balb/c mice and the tumors were measured and weighed after treatment with intratumoral injection of these proteins. Pulchellin (0.75 μg/kg) did not show antitumoral activity, but abrin (0.75 μg/kg) presented low activity ($p<0.05$). The tumors treated with these RIPs in the presence of b-D-galactose or only b-D-galactose presented greater size and weight than tumors treated with the proteins or only the saline solution (PBS, control group). But there is still a doubt as to whether the b-D-galactose reduced the abrin/pulchellin cytotoxicity or if b-D-galactose provided nutrition to the tumors, which will be clarified after histopathologic experiments.

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Phenotypic and Functional Study of "Heterokaryons" Used in Therapeutic Vaccines Against Advanced Cancer

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Abstract

Santos PCB, Ramos RN, Migliori IK, Barbosa BZ, Romagnoli GG, Barbuto JAM. Phenotypic and Functional Study of "Heterokaryons" Used in Therapeutic Vaccines Against Advanced Cancer. Annu Rev Biomed Sci 2011;13:A35. Monocyte-derived dendritic cells (Mo-DCs) can be a very powerful tool for the development of immunotherapeutic strategies against cancer. However, Mo-DCs from cancer patients present a series of phenotypic and functional changes that impair their potential to induce effective anti-tumor responses. To circumvent these deficits, one strategy that we have been testing in various clinical protocols is the generation of Mo-DCs from healthy donors, which are then fused with patients' tumor cells and, after irradiation, injected back into the patients to initiate anti-tumor responses. In the present study we show a partial phenotypic characterization of the heterokaryons, generated by an electric pulse (1000V/cm) into a suspension of Mo-DCs and tumor cells from the SK-BR-3 breast-cancer cell line. Heterokaryons thus generated maintain the expression of both tumor (her-2/neu) and Mo-DC markers (CD11c, HLA-DR) for at least 7 days after fusion. Furthermore, the heterokaryons seem to survive and proliferate better than non-fused cells (both tumor and DCs) in culture. When fused cells were produced with patients' tumor cells and utilized to stimulate patients' lymphocytes (allogeneic in relation to the Mo-DCs), they were able to induce the production of a distinct cytokine pattern, characterized by a higher IFN-gamma and a lower IL-4 production. Interestingly, these fused cells induced a low proliferative response on allogeneic lymphocytes. These data confirm that heterokaryons generated by the electrofusion of tumor and dendritic cells maintain the expression of relevant surface markers of both cell types. Furthermore, they seem to survive and, possibly, present a proliferative advantage over non-fused cells, since they can also induce a distinct lymphocyte response, biased toward a Th1 pattern.

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Influence of Autologous Dendritic Cells Immunotherapy on Cytokine Synthesis in Patients with Invasive Cancer

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Abstract

Aleixo AAR, Abdalla DR, Rodrigues CM, Matias BF, Murta EFC, Michelin MA. Influence of Autologous Dendritic Cells Immunotherapy on Cytokine Synthesis in Patients with Invasive Cancer. Annu Rev Biomed Sci 2011;13:A36. The immune response is of fundamental importance against the development of tumors and the cytokines play a key role in regulating this response. Immunotherapy with dendritic cell aimed at inducing an immune response against tumors in order to eliminate or decrease the progression of the tumor. However, the actual association and activity of these cytokines linked to immunotherapy with dendritic cells (DCs) are not yet fully elucidated. Therefore, this study aimed to evaluate the influence of dendritic cells (DCs) therapy on the synthesis of cytokines involved in immune response. We describe seven patients with invasive cancer who underwent immunotherapy with DCs. The vaccine was produced from peripheral blood samples and tumor biopsy taken from each patient. Mononuclear peripheral blood cells were differentiated into DCs in culture. The tumor antigens obtained from the biopsy were transfected into DCs. Vaccination was administered subcutaneously at 15-day intervals. For analysis of cytokines, peripheral blood cells were tested by flow cytometry, using the equipment FACs Calibur BD using antibodies to mark the following molecules**:** IFN-γ, IL-2, IL-10, IL-12 and TNF-α. Among the results, it was observed that IFN-γ expression tended to increase significantly between pre-therapy vs. third post-therapy ($p = 0.07$), pre-therapy vs. fourth post-treatment ($p = 0.09$) and pre-therapy vs. fifth post-therapy ($p = 0.055$) in monocyte population, while the lymphocyte population presented a non-significant elevation. TNF-α synthesis increased in both cell populations between pre-therapy and the fifth post-therapy, with a strong significant trend in the monocyte population ($p = 0.056$). IL-10 tended to decrease with DC immunotherapy, a reduction present in both populations without a statistical difference. IL-2 expression by lymphocytes increased from pre-therapy to fifth post-therapy whereas IL-12 synthesis by monocytes was found to be unchanged during the therapy. Thus, we conclude that immunotherapy with DCs can increase the expression of cytokines that characterize Th1 profile, a profile of desirable response in fighting tumors.

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Increase of IL-2, TNF-α and IFN-γ in the Cell Supernatants Obtained from Mice with DMBA-Induced Breast Tumor Subjected to Physycal Activity

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Abstract

Douglas R Abdalla, André AR Aleixo, Eddie FC Murta, Márcia A Michelin. Increase of IL-2, TNF-α and IFN-γ in the Cell Supernatants Obtained from Mice with DMBA-Induced Breast Tumor Subjected to Physycal Activity. Annu Rev Biomed Sci 2011;13:A37. Epidemiological data report that physical activity is able to provide reductions in morbidity/mortality in patients with cancer, but it remains unclear how to achieve such results. The present study aimed to investigate the influence of aerobic physical activity on the immune response in cancer development. For this we used 35 Balb/c virgin females, weighing between 20 and 30g and aged 8 weeks. Five groups were formed with n = 7: Control (GI) - animals without intervention delay; Sedentary (GII) - animals that received soy oil vehicle (500µl); Trained (GIII) - animals only subjected to physical training, swimming in water 30 ± 4 ° C for 45 minutes, 5 times per week for 8 weeks; Sedentary Tumor (GIV) - sedentary animals that received doses of DMBA (1mg/ml weekly for 6 weeks) and Trained Tumor (GV) - animals that have been trained according to the above protocol, and then subjected to tumor induction like group IV. After the experimental period, spleens and peritoneal macrophages were collected, placed in culture and stimulated with LPS 10 µg/ml. The supernatants were harvested after 24 and 48 hours. The IL-2, TNF-α and IFN-γ from the culture supernatants were measured by ELISA, using BDOpteia kits. Among the results, it was found that the concentration of IFN-γ and TNF-α in macrophages was higher in GIII compared with GI and GII ($p < 0.05$) and between groups GIV and GV ($p<0,05$). Concentrations of IL-2, IFN-γ and TNF-α in spleen cells also showed an increase when subjected to physical activity, evidence derived from comparing GI and GII to GIII and GIV and GV groups, both compared with differences less than 5%, except for IL-2, whose increases did not show significant differences. So the practice of physical activity in the presence or absence of breast tumor, is able to increase the synthesis of IL-2, IFN-γ and TNF-α. This increase suggets an improvement in the immune response by polarizing a Th1 profile, which would be favorable against the tumor.

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Potentiating Effect of Physical Activity in the Synthesis of IFN-γ, IL-12 and TNF-α in Breast Cancer

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Abstract

Abdalla DR, Aleixo AAR, Murta EFC, Michelin MA. Potentiating Effect of Physical Activity in the Synthesis of IFN-γ, IL-12 and TNF-α in Breast Cancer. Annu Rev Biomed Sci 2011;13:A38. Despite growing interest in studies correlating exercise and its beneficial effects in preventing and fighting cancer, existing studies have not been able to elucidate the mechanisms through which these effects occur. Regarding the implementation of physical activity in sick pacients, the mechanisms by which exercise influences the fight against cancer are explained by chance. In order to explain hypotheses, this study aims to investigate the synthesis of cytokines by lymphocytes in the presence of breast tumor and their interaction with physical activity. For this we used 35 8-week-old Balb/c virgin females with body mass between 20 and 30g. Five groups were formed with n = 7: Control (GI) - animals without intervention; Trained (GII) - Animals only subjected to physical training, swimming in water 30 ± 4 ° C for 45 minutes, 5 times per week for 8 weeks; Tumor (GIII) - sedentary animals that received doses of DMBA (1mg/ml weekly for 6 weeks) and Tumor Trained (GIV) - animals subjected to tumor induction like group III and trained according to the above protocol. After the experimental period, spleens and cells were collected and the intracelular cytokines IFN-γ, IL-10, IL-12 and TNF-α were measured by flow cytometry, using the equipment FACs Calibur BD. Our results indicate that the concentrations of IFN-γ increased in the groups that practiced physical activity, with group GIV having the highest increase, with a tendency toward significance. GII and GIV showed the lowest expressions of the cytokine IL-10, also with a tendency toward significance. IL-12 concentrations differed statistically between GIV and the other groups ($p < 0.05$) and finally the synthesis of TNF-α, comparing GI and GII, increased ($p = 0.08$) and, by comparing groups GIII vs. GIV, it also increased ($p = 0.06$). From this analysis, it is possible to infer that regular physical activity can potentiate the synthesis of cytokines IFN-γ, IL-12 and TNF-α, that play important roles in immune response against tumors.

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Immune Effect of Allogeneic Monocyte-Derived Dendritic Cells Lipofected with Survivin mRNA: Implication for Cancer Immunoterapy

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Abstract

Toniolo PA, Demasi MAA, Ramos RN, Migliori IK, Sogayar MC, Barbuto JAM. Immune Effect of Allogeneic Monocyte-Derived Dendritic Cells Lipofected with Survivin Mrna: Implication for Cancer Immunoterapy. Annu Rev Biomed Sci 2011;13:A39. Introduction: The development of dendritic cell (DC)-based cancer immunotherapy has been the target of many studies. For solid tumors, a promising approach based in allogeneic DCs fused to tumor cells has been relatively effective. On the other hand, this approach requires large tumor samples to generate enough DC-tumor cell hybrids. To overcome this problem, tumor mRNA-transfected DCs can be used since mRNA can be amplified *in vitro* and allow unlimited vaccine production. Objective: This work, therefore, aimed to obtain efficient transfection and optimal translation of tumoral mRNA in DCs, using, as a model, the mRNA of survivin, an antigen overexpressed in many types of tumors. Methods: DCs were differentiated from healthy donors' mononuclear peripheral blood. On the 5th day of culture, the survivin mRNA, obtained from *in vitro* transcription reactions, was incubated with lipofectamine 2000 transfection reagent and the liposomal-RNA complex was added to immature DCs. The cells were activated with IL-6, IL-1β, TNF-a and PGE2 seven hours after transfection and were evaluated as to survivin expression, by flow cytometry, up to 72 hours thereafter. Transfected DCs were used as stimulator cells in proliferation assays using allogeneic T cells as responder cells at 1 and 24 hours post DC activation. Results: We obtained transfection in nearly 40% of DCs using this lipofection protocol. The mRNA-transfected DCs started to increase survivin expression 12 hours after activation and peaked at 48 hours. The percentage of cells expressing survivin increased approximately 16%, 28% and 43% at 12, 24 and 48 hours, respectively. Although the increased expression of survivin among transfected DCs was undetectable at 1 hour post activation, in the T cell stimulation assays, we had already observed an increased CD4+ T cell proliferation index – PI – (from 61 to 82) when stimulator cells were DCs transfected with mRNA. At 24 hours post activation, the PI of CD4+ T cells was even higher (from 142 to 543). These data show that mRNA DC transfection can affect immune responses induced by these APCs, apparently favoring CD4+ T cell stimulation. Conclusion: We show the kinetics of survivin mRNA expression after transfection of allogeneic DCs and present data suggesting that these transfected DCs are recognized and stimulate lymphocytes. These findings support the use of tumoral mRNA-transfected DCs for anti-cancer vaccine production and show survivin as a potent antigen to induce CD4+ T cell responses.

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Evaluation of Glucocorticoid Effects on Human Monocyte Subsets

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Abstract

Teixeira MPC; Rumjanek VM. Evaluation of Glucocorticoid Effects on Human Monocyte Subsets. Annu Rev Biomed Sci 2011;13:A40.* Monocytes are mononuclear cells that represent 5 -10% of the leukocytes present in peripheral blood. Circulating monocytes are a heterogeneous population and, based on the expression of CD14 and CD16 molecules, they can be divided into different subpopulations with distinct phenotypic and functional properties. Recently, it has been shown that the small subset of circulating monocytes that express the CD16 molecule ($CD14^+CD16^+$) appears to be expanded in cancer patients. These $CD16^+$ monocytes produce higher levels of tumor necrosis factor (TNF) in response to lipopolysaccharide (LPS) stimulation and express higher levels of HLA-DR, CD40, CD80 and CD86 molecules compared to $CD16^-$ monocytes. Moreover, this subset appears to be more mature because they present features similar to mature tissue macrophages and monocyte-derived macrophages. Since glucocorticoids are part of the treatment of some cancers, the aim of the present work was to study CD14 and CD16 expression by human monocytes after 24 hours of culture in the absence or presence of various concentrations of dexamethasone and hydrocortisone. Furthermore, we sought to better understand the expression dynamics of these two molecules during our culture period. To address these questions, peripheral blood was obtained from healthy volunteers and mononuclear cells were separated by Ficoll-Hypaque centrifugation. CD14 and CD16 expression were assessed, using flow cytometry, on PBMC and after 2 hours of adhesion and 24 hours of culture. Our results show that the adhesion process induced the expression of CD16 on $CD14^+CD16^-$ cells. Regarding the effect of glucocorticoids, we observed that $CD16^+$ monocytes appear to be reduced after 24 hours of culture in the presence of 10^{-5}M and 10^{-7}M of dexamethasone or hydrocortisone. Collectively, these results suggest that CD16 expression is modulated during culture and that glucocorticoids differentially affect monocyte subsets.

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Immune Response in Advanced Breast Cancer and Chemotherapy Treatment

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Abstract

Panis C, Victorino VJ, Herrera ACSA, Rossi T, Campos FC, Colado-Simão AN, Barbosa DS, Pinge-Filho PP, Cecchini R. Immune Response in Advanced Breast Cancer and Chemotherapy Treatment. Annu Rev Biomed Sci 2011;13:A41. Breast cancer involves inflammatory alterations of multiple cellular pathways. Chronic inflammation products in cancer are associated with tumorigenesis due to generation of proinflammatory cytokines. In breast cancer treatment, paclitaxel (PTX) and doxorubicin (DOX) are the chemotherapeutic agents usually employed, although their use is implicated in several side effects. We aimed to evaluate alteration in mediators of immune response during establishment of advanced disease and chemotherapy. Women were divided into the following groups: control (CTR- healthy women, n=30), advanced breast cancer disease (AD-TNM IIIc and IV, n=30), immediately after DOX infusion (DOX-60mg/m^2 intravenously for 1 hour, n=30), and after PTX infusion (PTX-175mg/m^2 intravenously for 1 hour, n=30). Plasma cytokines levels of TNF-a, interleukin 1 (IL-1), interleukin 10 (IL-10) and interleukin 12 (IL-12) and oxidative leukocyte burst were evaluated. Plasma nitrite levels (NO) and C reactive proteins (PCR) were also measured. Statistical analyses were performed using GRAPHPAD PRISM 5.0, Student's unpaired *t* Test, $p<0.05$. Results are given as mean±standard error. Results show that NO levels rose in AD group (CTR= 16.47±0.82mM to AD=21.21±1.78mM) and no alterations were observed during chemotherapy (18.3±1.97mM in DOX, 20.34±1.5mM in PTX). AD group developed a proinflammatory state with higher levels of TNF-a (CTR= 9.47±1.55pg/mL, AD= 23.85±6.22pg/mL) and IL-1b (CTR= 5.72±1.74pg/mL, AD= 13.35±3.74pg/mL). No alterations in IL-12 or IL-10 were observed. Chemotherapy with DOX significantly decreased TNF-α (8.77±2.53pg/mL) and IL-1b (5.2±1.9pg/mL). PTX augmented TNF-a levels (38.27±9.12pg/mL) and did not influence IL-1b (7.14±2.99pg/mL). DOX group presented diminished IL-10 (14.94±1.48pg/mL) and elevated PTX levels (101.2±30.03pg/mL). No alterations in IL-12 levels were observed (CTR= 33.4±0.9, AD= 33.17±1.98, DOX= 31.31±0.94, PTX= 35.04±2.68pg/mL). Reduced leukocyte oxidative burst was observed in AD group (integral area CTR= 9.7e+006±1.208e+006, AD= 4.1e+006±1.5e+006) and during chemotherapy (DOX= 1.413e+006±151322, PTX= 1.544e+006±84862). High PCR levels were found (CTR= 0.66±0.19mg/dL, AD=6.5±1.31mg/dL, DOX=4.8±1.23mg/dL, PTX= 7.12±1.87mg/dL), indicating the activity of inflammation associated with reduction of leukocyte oxidative burst. These dysfunctions of immune response established an immunosuppressive state that may be a tumor-regulated signal to ensure cancer survival by evading immunological response.

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Myelotoxic Effects of 7,12-Dimethylbenz[a]anthracene on Bone Marrow Cells from Mice Genetically Selected for Inflammatory Reactivity

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Abstract

Katz ISS, Albuquerque LL, Suppa AP, Moreira C, Jensen JR, Starobinas N, Cabrera WHK, Franco M, Borelli P, Ibañez OM, Ribeiro OG. Myelotoxic Effects of 7,12-Dimethylbenz[a] anthracene on Bone Marrow Cells from Mice Genetically Selected for Inflammatory Reactivity. Annu Rev Biomed Sci 2011;13:A42. Polycyclic Aromatic Hydrocarbons (PAHs), such as DMBA, induce a decrease in bone marrow cell (BMC) numbers and hematological alterations resulting in an immunosuppressive state. DMBA metabolism depends on the activation of the aryl hydrocarbon receptor (AhR). Mice genetically selected for high (AIRmax) or low (AIRmin) acute inflammatory response to s.c. injection of Biogel P100 presented a complete segregation of Ahr alleles endowed with low (*Ahr*^{d}) or high (*Ahr*^{b1}) affinity to PAHs, respectively. Accordingly, AIRmax are more resistant than AIRmin to DMBA induced skin and lung carcinogenesis. We investigated the effect of DMBA treatment on BMC of AIR-selected mice and its possible impact on acute inflammatory response. AIRmax and AIRmin mice were treated with a single i.p. dose of 50mg/kg DMBA in olive oil. Flow cytometric analysis was used to determine hematopoietic stem cells (HSC) (Lin^{-}/Sca-1^{+}/c -Kit $^{+}$) and neutrophils (Gr-1^{hi}/CD11b^{hi}). BMC proliferation index was determined in response to GM-CSF stimulus. Acute inflammation response was also evaluated 24 hours after subcutaneous (s.c.) injection of Biogel P100. DMBA treatment resulted in a significant ($p<0.01$) decrease in neutrophil population and increase of HSC in bone marrow in AIRmin mice only. Blast cells from DMBA-treated AIRmin presented a dysplastic nucleus, which is one of the distinguishing features of preleukemia, and myeloid cells showed low proliferation capacity after *in vitro* GM-CSF stimulation. These effects on myeloid BMC reflect an impaired cellular migration to the inflammatory site 24hs after Biogel injection. This investigation demonstrates that AIRmax mice are protected and AIRmin are prone to acute bone marrow cytotoxic and presumable preleukemic effects of DMBA. The complete segregation of alleles at the *Ahr* locus found in AIR mice might contribute to their differential inflammatory responses and to the phenotypes of susceptibility and resistance of BMC to DMBA-induced effects.

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Effect of Cyclopalladated Compounds in Breast Cancer

Marcela B Quilles[1*], Aline Tansini[1*], Camila BA Carli[1], Antonio C Moro[2], Antonio E Mauro[2], Livia CA Ribeiro[1], Danielle CG Maia[1], Flavia A Rezende[1], Eliana A Varanda [1], Iracilda Z Carlos[1]

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Quilles MB, Tansini A, Carli CBA, Moro AC, Mauro AE, Ribeiro LCA, Maia DCG, Rezende FA, Varanda EA, Carlos IZ. Effect of Cyclopalladated Compounds in Breast Cancer. Annu Rev Biomed Sci 2011;13:A43. Cancer, a manifestation originated by the uncontrolled growth of cells, affects million of individuals. Cancer development is strongly associated with a chronic inflammatory process; the production of different cytokines can influence the growth of tumoral cells. Some palladium (II) compounds, general formula: [Pd(dmba)(Cl)tu] and [Pd (dmba)(N3)tu] are known to present anti - tumoral potential. Currently, cis-platinum is the drug most commonly used against cancer. In the present study the anti-tumoral activity of a palladium compound was analyzed. Adherent cells obtained from Ehrlich tumors in their solid form carried by Swiss mice were cultured with [Pd(dmba)(Cl)tu], [Pd(dmba)(N3)tu] and cis-platinum. The cellular viability (IC50) (MTT method), nitric oxide production (NO) (Griess) and production of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-12 (IL-12) (ELISA) were tested. The mutagenecity of these compounds was analyzed via the Ames Test. The IC50 (mM) results were: 137.65±0.22-[Pd(dmba)(Cl)tu], 146.51±0.22-[Pd(dmba)(N3)tu], cis-platinum-113.21±0.28. All tested compounds stimulated production of NO as well as the cytokines TNF-α and IL-12. The [Pd(dmba)(N3)tu] induced higher NO production (27.29±8.01) than cis-platinum (19.33±9.22). The IL-1β production was similar among the substances, with [Pd(dmba)(Cl)tu] (67.2±55.7), [Pd(dmba)(N3)tu] (46.4±80.72) and cis-platinum (39.5±157.1). The same was observed for IL-12 production, with [Pd(dmba)(Cl)tu] (2101±844.1), [Pd(dmba)(N3)tu] (2253±686.6) and cis-platinum (2233±563.9). The compound and its ligands did not present mutations, different from *cis*-platinum, which caused point mutations in the DNA of *S. typhimurium*. The results indicate the Palladium (II) complexes as promising in the development the therapies for cancer treatment.

*Presenting author

Glycine Max (l.) Merrill (Leguminosae) Fermented by Enterococcus Faecium and Lactobacillus Helveticus Modulates Experimental Mammary Tumor Growth

Lívia CA Ribeiro[1*], Lucas S Ferreira[1], Djamile C de Matos[1], Pâmela Alegranci[1], Marcela B Quilles[1], Aline Tansini[1], Marisa C Polesi[1], Elizeu A Rossi[2], Luis C Spolidório[3], Iracilda Z Carlos[1]

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

RibeiroLCA[1], Ferreira LS[1], Matos DC[1], Alegranci P[1], Quilles MB[1], Tansini A[1], Polesi MC[1], Rossi EA[2], Spolidório LC[3], Carlos IZ[1]. Glycine Max (l.) Merrill (leguminosae) Fermented by Enterococcus Faecium and Lactobacillus Helveticus Modulates Experimental Mammary Tumor Growth. Annu Rev Biomed Sci 2011;13:A44.* Cancer is generated by uncontrolled cellular proliferation. Activated macrophages polarize and may stimulate a cellular immune response or a humoral immune response. In theory, an effective immunotherapy can modulate differentiation of involved cells. Immunotherapies include functional food consumption. We intended to observe whether consumption of a soy product fermented by Enterococcus faecium CRL 183 and Lactobacillus helveticus ssp jugurti 416 has the ability to modulate immune system or tumor growth. Balb/c mice received 0.5 mL of saline (SS, control) by gavage, or 0.5 mL of fermented soy product (FSP) daily for 40 days. On day 10, animals were inoculated subcutaneously with LM3 cells ($1.25x10^4$ cells), a murine mammary adenocarcinoma cell line. Animals were sacrificed; tumor volume was observed and an angiogenesis score calculated by Microscopic Angiogenesis Grading System (MAGS) analysis. Macrophages and lymphocytes were cultivated separately with stimuli (LPS and ConA respectivally) for 24hrs. Arginase activity was measured through urea dosage after cell lysis. Supernatant cytokines were measured via capture ELISA kits (BD Biosciences). FSP showed a reduced final tumor volume (FSP: 1.45±0.39 cm3; SS: 3.68±0.76 cm3; $p<0.05$). Angiogenesis inside the tumor did not differ between SS (MAGS Score 13.25±1.88) and FSP (MAGS Score 19.75±4.16) ($p>0.05$). FSP macrophages showed higher arginase-1 activity (FSP: 6.68±0.24 mU; SS: 3.39±0.16 mU; $p<0.001$). Also, splenic lymphocytes presented lower production of IFN-gamma (FSP: 62.61±14.10 pg/mL; SS: 169.40±20.07 pg/mL; $p<0.05$), without affecting IL-10 production (FSP: 416.20±61.03 pg/mL; SS: 337.40±40.88 pg/mL; $p>0.05$). These results indicate that FSP consumption may influence tumor growth by favoring the host, but it appears to stimulate Th2 immune response. Further studies are required to confirm this hypothesis.

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Neutrophil's Functional Impairment in Pediatric Cancer Patients

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Barbosa BZ, Maeques OC, Cordino-Neto A, Carvalho B, Odone-Filho V, Barbuto JAM. Neutrophil's Functional Impairment in Pediatric Cancer Patients. Annu Rev Biomed Sci 2011;13:A45. Neutrophils constitute the main cells of innate response against pathogens and they are the first cells to arrive at the inflammation site. Activation of their microbicidal mechanisms is an essential step in establishing innate immune responses. Some chemotherapy regimens against cancer can cause neutropenia – a life threatening condition. This study aimed to evaluate the functional state of neutrophils from pediatric cancer patients before and after chemotherapy, in order to elucidate whether the function of these cells are also impaired in such cancer patients. Neutrophils from three pediatric cancer patients, with solid tumors, were evaluated by flow cytometry before treatment as to their phagocytosis of *Escherichia coli (E. coli), Staphilococcus aureus (S. aureus)* and *Candida albicans (C. albicans)*. The oxidative burst was evaluated by the intracellular oxidation of non-fluorescent dihydrorhodamine 123 (DHR) to fluorescent rhodamine 123 (DHR) and fluorescent rhodamine 123 after stimulation with *E. coli*, *S. aureus*, *C. Albicans*, phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharide (LPS), also by flow cytometry. We found that neutrophils from these three cancer patients were able to phagocytize *C. albicans, S. aureus* and *E. coli* but less efficiently than controls. Furthermore, these cells produced less reactive oxygen species (ROS) when stimulated with the bacteria and fungi than controls. These preliminary results indicate that the ability of neutrophils from pediatric cancer patients to perform phagocytosis and to produce ROS are impaired even before chemotherapy. The effects of chemotherapy on these cells still need to be evaluated.

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Evaluation of the Cytotoxic Profile of Lectin BJcuL on Gastric Carcinoma Cells (Kato Iii) and Colon Adenocarcinoma (Ht-29)

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

*Damasio D, Nolte S, Esposito SE, Moreno AN. Evaluation of the Cytotoxic Profile of Lectin BJcuL on Gastric Carcinoma Cells (Kato Iii) and Colon Adenocarcinoma (Ht-29). Annu Rev Biomed Sci 2011;13:A46.*Lectins are (glyco) proteins that present potent biological activity by interacting with specific carbohydrates on the cell surface by participating in phenomena of biological importance such as induction of platelet aggregation, hemagglutination, activation of lymphocyte proliferation, involvement in cell growth regulation and the activation of apoptosis. Thus these molecules are of great scientific interest. The antitumoral property of lectins has been demonstrated *in vitro* and *in vivo*, suggesting their role as a therapeutic agent. The lectin extracted from *Bothrops jararacussu* (BJcuL) is specific for D-galactosides. It was described as inhibiting proliferation of human pancreatic carcinoma, glioma and endothelial cells. The goal of this study is to evaluate the *in vitro* effect of BJcuL on the gastric carcinoma (KATO III) and colon carcinoma (HT-29) cell lines. This objective was achieved through the following methods: assay of recognition of surface glycans, assay of cytotoxicity of BJcuL at different concentrations on the carcinoma cell lines (MTT assay), comparison of cell adhesion of Matrigel molecules in the presence and absence of BJcuL and evaluation of lectin interference in cell proliferation and expression of caspase-8. The results show that BJcuL interacts with glycoligand targets on the surface of both carcinoma cell lines, and that this interaction was inhibited in the presence of D-galactose. The interaction between BJcuL and the cellular surface was cytotoxic in a dose-dependent manner and more pronounced for HT-29. A decrease in adhesion to extracellular matrix was also dose-dependent for both cell lines, but when the matrix was previously incubated with the cells, the lectin had no effect on adherence. The analysis of cell-proliferation inhibition, assessed by anti-PCNA, showed that the lectin reduced the proliferation of KATO III but not HT-29. However, caspase-8 expression was intense in HT-29. These results suggest that the cytotoxic effect of BJcuL, assessed by induced cell viability, differs between the two cell lines, since BJcuL inhibited proliferation of KATO III and induced apoptosis in H-T29.

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Cytokine Profiles in Serum and Cervical Secretion of Women with Low-Grade Squamous Intraepithelial Lesion, High-Grade Squamous Intraepithelial Lesion and Invasive Squamous Cell Carcinoma

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Marcolino LD, Mösch BM, Silva DF, Polettini J, Marques MEA, Traiman P, Mauad LMQ, Candeias JMG, Silva MG. Cytokine Profiles in Serum and Cervical Secretion of Women with Low-Grade Squamous Intraepithelial Lesion, High-Grade Squamous Intraepithelial Lesion and Invasive Squamous Cell Carcinoma. Annu Rev Biomed Sci 2011;13:A47-8. Infection with oncogenic types of human papillomavirus (HPV) is crucial for development of intraepithelial lesions and invasive squamous cell carcinoma. The immune response that plays an important role in the natural history of uterine cervical HPV infection is involved in viral persistence and progression of lesions. Cytokines are molecules that regulate HPV transcription; the Th1 pattern contributes to the development of cellular immunity against HPV infection and neoplasia, and is related to clearance of infection. The Th2 pattern is associated with persistence of viral infection and the progression of lesions**.** To evaluate the concentrations of IL-2, IL-4, IL-6, IL-10, IL-12, IFN-□ and TNF-□ in cervical secretion and serum of women with low-grade squamous intraepithelial lesion (LSIL) , high-grade intraepithelial lesion (HSIL) and invasive squamous cell carcinoma (EC). The study included 109 women with histological diagnosis of LSIL (n = 16), HSIL (n = 40), EC (n = 13) and 40 women with suspected HPV-induced disease, but no pathological changes in cervical biopsy (control group) attended at the Colposcopy Clinic of Botucatu Medicine School (UNESP) and at the Preventive Gynecology Ambulatory Unit, Hospital Amaral Carvalho, Jau, SP. During speculum examination, cervical secretion was collected with a cytobrush to determine the profile of cytokines. Ten mL of peripheral blood were collected by venipuncture from each woman enrolled in the study and cytokine patterns in serum and cervical secretion were assessed by enzyme immunoassay (ELISA). HPV in the fragments of the biopsies was detected by the technique of polymerase chain reaction (PCR) using specific primers (MY9/11 and GP5+/GP6+) and genotyping was performed by primer-specific PCR and confirmed by the restriction fragment polymorphism technique (RFLP). The socio-demographic and gynecological data were obtained by interviewing patients during study enrollment. Among the patients studied, 78.9% were white, 60.5% reported stable, 23.8% completed high school, 37.6% were smokers, 57.8% had had at least three sexual partners, 8.3% reported a history of previous STD and 36.7% used oral contraceptives. The median age of patients was

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significantly higher in EC compared to other groups. HPV DNA was detected in 90% of samples from the control group, 93.7% in LSIL, 100% in HSIL and 84.6% in EC. The levels of IL-4, IL-6 and IL-10 in cervical secretion were significantly greater in EC patients than controls, LSIL and HSIL. Serum IL-6 levels were significantly increased in HSIL patients. These results corroborate the finding that elevated Th2 cytokines in serum and cervical secretion of women with HSIL and EC is related to the progression of intraepithelial lesions.

ArtinM Lectin Exerts Antitumor Effect on Solid Ehrlich Tumor

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4th Symposium of Immunology: Tumor Immunobiology, May 20-22, 2011, Botucatu, SP, BRAZIL

Abstract

Ricci-Azevedo R, Carvalho F, Roque-Barreira MC. ArtinM Lectin Exerts Antitumor Effect on Solid Ehrlich Tumor. Annu Rev Biomed Sci 2011;13:A49. Several lectins have been described as possessing the capacity to induce cell death or to inhibit tumor growth. The antitumor effects may occur either directly, by interaction with aberrant carbohydrates on tumor cells to trigger apoptosis, or indirectly through a profile of immunomodulation and immunostimulation, which prevents tumor progression. ArtinM, a mannose-binding lectin from *Artocarpus heterophyllus* seeds, is known for its immunomodulatory activity. This lectin primarily increases the serum levels of interleukin 12 by interacting with N-glycans of the toll-like receptor 2 on an antigen-presenting cell surface, and promotes the cellular response against intracellular pathogens. Previous experiments demonstrated ArtinM's potential applicability against tumor cells *in vitro*. Under an *in vivo* Walker tumor model, ArtinM was responsible for increasing the inflammatory cells in the tumor´s microenvironment. The Ehrlich tumor, from breast cancer in mice, is extremely aggressive and develops in both solid and ascitic form. Therefore, it is an important model for studying breast cancer due to its easy manipulation. The present study aimed to evaluate the direct and indirect effect of ArtinM on the Ehrlich tumor. To evaluate the direct effect of the lectin, a binding assay and MTT assay were performed. ArtinM was able to bind to Ehrlich tumor cells but did not induce cell death at 5, 10 or 25µg/ml. To evaluate the indirect effect, the foot pads of Swiss mice were injected with $1x10^5$ tumor cells. Next, the animals were treated with ArtinM (0.5 and 5 µg/mouse/ week) for four weeks. The foot-pad size evolution was monitored and the cytokine profile of the popliteal lymph node was determined. Subcutaneous lectin administration to these animals inhibited tumor progression, especially at 5µg/mouse/week dose. The ArtinM treatment also induced an increase of inflammatory cytokines. However, the Th1 immunity profile was observed only in the group treated with 0.5µg/mouse/week. Taken together, our results indicate that ArtinM reduces tumor progression *in vivo*, probably mediated by the immunostimulatory effect and immunomodulatory potential of the lectin.

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Lectins ArtinM and Jacalin, from *Artocarpus integrifolia* Seeds, Have Distinct Binding and Action on Gastric Carcinoma

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Abstract

Carvalho F, Reis C, Roque-Barreira MC. Lectins ArtinM and Jacalin, from Artocarpus Integrifolia Seeds, Have Distinct Binding and Action on Gastric Carcinoma. Annu Rev Biomed Sci 2011;13:A50. Lectins are carbohydrate-binding proteins capable of recognizing and reverse-binding to glycans without altering their covalent structure. Jacalin exhibits a specific affinity for galactose β1,3 *N*-acetyl-D-galactosamine residues (Galβ1,3GalNAc; O-linked sugar residues). ArtinM exhibits high specificity for the trimannoside Mana1-3[Mana1-6]Man, present in the core of N-glycans. Because cell malignant transformation is often associated with altered expression of cell surface glycans, lectins have been used to identify glycan determinants as markers of clinical interest. In this study we evaluated the interaction of ArtinM and Jacalin with tissue sections of gastric biopsies (n = 20), by histochemistry. There was a pronounced binding of ArtinM to inflammatory cells and to carcinoma cells in 55% of samples. On the other hand, Jacalin was able to bind specifically to the gastric epithelium, mostly in goblet cells and gastric glands, in all samples. The co-localization of Jacalin and MUC2, a mucin aberrantly expressed in gastric carcinoma, opens a new frontier for using lectins as a diagnostic and prognostic tool in gastric carcinoma. Furthermore, we evaluated the interactive, anti-proliferative and anti-invasive effects of both lectins on human gastric carcinoma cell lines (MKN45 and AGS) by fluorescence, MTT assay and Matrigel invasion chamber assay. ArtinM and Jacalin showed binding to gastric carcinoma cell lines at the same intensity, but did not interfere in cell proliferation. ArtinM was able to disturb the invasion capacity of MKN45 cells. An additional advantage of therapy with ArtinM is its immunomodulatory property, responsible for the induction of Th1 immunity, a response that is potentially effective against leukemia progress. The effects of ArtinM on other carcinoma models have been investigated.

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Synthesis of Complement Proteins by Monocyte-Derived Dendritic Cells Developed in The Presence of Tumor Supernatants

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Abstract

Cechim G, Isaac L, Barbuto JAM. Synthesis of Complement Proteins by Monocyte-Derived Dendritic Cells Developed in The Presence of Tumor Supernatants. Annu Rev Biomed Sci 2011;13:A51. Dendritic cells (DC) are the most important antigen-presenting cells (APC) which continuously sample the environment. The outcome of antigen presentation by dendritic cells to T cells depends on their activation status. Several mechanisms of tumor escape/evasion prevent the activation of these cells, thereby interrupting the antigen presentation process, which is crucial for antitumor immune response. Complement proteins seem to be an important element of interaction between DC and tumor cells. Previous studies have shown that C3 participates in the process of activation and maturation of DC cells, a phenomenon that is disrupted in the tumor microenvironment. In this work, we investigated the production of complement proteins – C3, C5 and FB – by monocyte-derived dendritic cells differentiated in the presence of supernatants of two glioma cell lines (U87MG and A172). Monocytes obtained from the blood of healthy donors were differentiated into monocyte-derived dendritic cells by culture, for seven days, in the presence of GM-CSF, IL-4, and, in the last 2 days, LPS. Tumor supernatants were added to the culture at days zero, five and six. The membrane phenotype of these cells was evaluated by flow cytometry and the production of complement proteins measured by Real-Time PCR. In conclusion, the membrane expression of CD14 seems to be negatively influenced by U87MG supernatant, notably when the treatment was added on day zero. It was possible to note the same effect on the expression of the co-stimulatory molecules, CD80 and CD86. In the case of CD83, a negative effect on the membrane expression was noted when the A172 supernatant was added at the sixth day of culture. Contrastingly, when the cells were treated with U87MG supernatant on day five, CD83 expression was increased. The preliminary Real-Time PCR results suggest an increase in FB gene expression provoked by the treatment at all times tested, whereas the C3 gene expression was negatively affected when the supernatant from the A172 tumor cell line was added at days zero and five. These data suggest that tumor secreted factors may affect complement synthesis by dendritic cells, a phenomenon that could modify their function and thus contribute to tumor evasion from the immune system.

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Claudin-10, a Tight-Junction Component, is Upregulates in B16 Murine Melanoma Cells after Interaction With B-Lymphocytes

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Abstract

Pérez EC, Xander P, Novaes RRBE, Mariano M, Lopes DJ. Claudin-10, a Tight-Junction Component, is Upregulates in B16 Murine Melanoma Cells after Interaction With B-Lynphocytes. Annu Rev Biomed Sci 2011;13:A52. B-1 cells are the prevalent lineage of B cells in the peritoneal and pleural cavities of mice and constitute one of the main sources of interleukin-10 (IL-10). Previous studies by our group demonstrated that co-cultivation of B16 melanoma cells with B-1 cells from wild-type C57BL/6 mice (wt), but not from IL-10 knockout C57BL/6 mice (IL-10KO), increases the metastatic potential of melanoma cells. But whether molecules expressed in B-1wt cells can affect the metastatic potential of B16 cells has not yet been fully addressed. Therefore, this work aimed to identify any molecule expressed by B-1 wt, absent in B-1 IL-10KO, interacting with melanoma cells and therefore increasing their metastatic potential. To investigate differential gene expression between B-1 cells from wt and KO mice, total RNA from cultures of these cells was extracted for microarray studies. The expression profiles were generated using the Affymetrix GeneChip Technology-chip GeneChip Mouse Genome 430 2.0 Array. The data were normalized using Robust Multi-chip Average (RMA). The statistical significance of expression chances between B-1 wt and B-1 KO was evaluated via the Significance Analysis of Microarrays (SAM) method. Three independent experiments of microarray analyses demonstrated differential mRNA expression of seven (7) genes between wt and IL-10KO B1 cells. Claudin-10, Lplrap1 and Mid-1 genes involved in cell communication and cell adhesion processes were upregulated in B-1 cells of wt mice. These preliminary results suggest possible participation of these genes since B-1 cells increase the metastatic potential of B16 cells during co-culture of both cells.

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Interferon-Gamma: an Extrahepatic Inducer of Serum Amyloid A Protein in Gliomas

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Abstract

Knebel FH, Albuquerque RC, Campa A. Interferon-Gamma: an Extrahepatic Inducer of Serum Amyloid A Protein in Gliomas. Annu Rev Biomed Sci 2011;13:A53. Interferon-gamma (IFN-g) is an immunoregulatory cytokine produced immediately after inflammation in order to augment the immune response. One manner by which IFN-g promotes this increase is inducing the hepatic production of acute phase proteins including serum amyloid A (SAA). SAA is an apolipoprotein marker of acute inflammatory processes that together with the other acute phase proteins retroactively modulates the inflammatory reaction. Recently, SAA has been recognized as a marker of tumor progression, since its expression and production have been described in many tumors, suggesting an important SAA function in the tumor microenvironment. However, it remains unclear whether the expression and production of SAA protein by the tumor can be induced by IFN-g. These hypotheses were examined in gliomas A172 and T98G, which represent human carcinomas characterized by a highly aggressive biological behavior – by quantitative real-time PCR and ELISA **techniques**. Our investigations showed that SAA is constitutively expressed and produced by both gliomas, with the predominant isoform being SAA1, followed by SAA2 and finally SAA4. Moreover, IFN-g increased mRNA expression of all SAA isoforms – SAA1, SAA2 and SAA4 – and intracellular protein synthesis. These findings suggest that IFN-g may mediate not only systemic but also local inflammatory responses and increase the possible autocrine and paracrine effect of SAA in tumors.

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