Immunology

Chair

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HuR prevents c-fos mRNA degradation by proteasome-associated endoribonuclease in vitro

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Introduction

HuR is an ubiquitously expressed protein known to selectively bind ARE-containing mRNAs and protects them from degradation. HuR is supposed to play a role in cancerogenesis since its expression is elevated in many cancer types and it stabilizes a lot of mRNAs encoding proteins involved in oncogenesis. Previously, it was shown that proteasome in addition to its main function – protein degradation – may act as a selective RNase. Since HuR and proteasome have common targets – c-myc and c-fos proto-oncogene mRNAs, the goal was to estimate HuR protective activity against proteasome-associated endoribonuclease.

Material & methods

HuR-GST fusion protein has been cloned, expressed and purified by affinity chromatography. Fragments of c-myc and c-fos were cloned and mRNAs have been transcribed in vitro. Proteasomes have been isolated from K562 and Im-9 cells. mRNAs were treated by proteasomes in presence and absence of HuR. The estimation of mRNA cleavage was held by gel-electrophoresis.

Results

GST-HuR has specifically bound ARE-containing fragments of c-myc and c-fos mRNAs. Proteasomes extracted from Im-9 and K562 cells cleaved target mRNAs in absence of HuR. It was shown that HuR prevents degradation of c-fos mRNA by proteasomal endoribonuclease, whereas c-myc mRNA was cleaved in the same conditions. GST protein didn't bind with target mRNAs and didn't affect proteasome cleavage activity.

Conclusion

HuR protects c-fos mRNA from proteasome endoribonuclease cleavage in vitro, but can't prevent c-myc mRNA degradation. HuR and proteasome compete with each other for the manifestation of their opposite activities. Thus, a new mechanism of regulation of proto-oncogenes expression was observed.

Alterations of multipotent mesenchymal stromal cells characteristics after co-cultivation with lymphocytes

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Introduction

Multipotent mesenchymal stromal cells (MSCs) are used for prevention and treatment of graft versus host disease after allogeneic hematopoietic stem cells transplantation due to their immunomodulatory properties. The MSCs fate in the organism of the patient is unknown. The aim of this study was to analyze the changes in MSCs properties when co-cultured with allogeneic lymphocytes in vitro to simulate their interactions in vivo.

Material & methods

The bone marrow from 13 donors (7 male and 6 female aged 22 to 62 years, median 27 years) was used. MSCs were co-cultured with allogeneic lymphocytes in a ratio of about 1:10 for 4 days and their basic properties were analyzed over time. Lymphocytes were activated by adding to the culture medium 5 mg/ml of PHA (PHA-lymphocytes). Determination of IDO1 gene expression level was performed by reverse transcription polymerase chain reaction in real time (modification of the Taq-Man) and of HLA-DR expression on MSCs by flow cytometry.

Results

Co-cultivation of MSCs with lymphocytes led to significant progressive increase of HLA-DR median fluorescence intensity (MFI) on MSCs 1.4 times in a day and then 1.7; 1.7 and 2 times ($p = 0.03$) respectively. When co-culturing MSCs with activated lymphocytes level HLA-DR expression on MSCs increased significantly greater than when co-cultured with non-activated lymphocytes of 2.8 times in a day and then 9.1; 12.2 and 12.3 times, respectively ($p < 0.05$ in all cases). Relative expression level (REL) of IDO1 increased dramatically when co-cultured with lymphocytes (400 times) but to the 4th day of culturing gradually decreases to 4 times. When co-culturing MSCs with activated lymphocytes IDO1 REL raised 3000-4000 times and reduction did not occurred during further cultivation.

Conclusion

MSCs when co-cultured with lymphocytes lose their immune privileged properties. Thus their immunomodulating properties (REL of IDO1) increased by dozens of times. These data suggest that MSCs could be quickly eliminated from the patient's organism, but efficiently inhibit the lymphocytes activity during the first few days.

Methylation and expression of TLR2 and TLR4 and their role in asthma

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Introduction

Asthma is known to be one of the most prevalent chronic noncommunicable diseases. And epigenitics of this pathology forms a field which has expanded greatly so far. It's clear now that two main processes take place in asthma development. They are immune component, which leads to the allergy, and epigenetic mechanisms, which consist of DNA methylation and regulation of gene expression by miRNA (mainly). We aimed to concentrate on DNA methylation and expression studings in order to find some new marks of asthma. We supposed this could help to predict and prevent the disease. Thus there were investigated some genes of innate immunity (TLR2, TLR4) and their potential role in bronchial asthma.

Material & methods

We examined 38 patients with bronchial asthma from the age of 3 to 12 years old and 10 healthy children of the same age. Determinants of miRNA expression in scrapings from the mucous membranes of the respiratory tract and peripheral leukocytes were carried out by PCR-RT. Mann - Whitney U-test was used to estimate the criteria of trustworthiness.

Results

In scrapes from patients with moderate asthma a significant increase was found in the gene expression of TLR2 and TLR4, 3 times and 10 times respectively that in the control group. In children with severe asthma we also noticed an increase in the gene expression of TLR2 4.8 times more than the same rate in the healthy group ($p \leq 0.05$). Indicators of TLR4 gene expression in these patients have a tendency to increase, but this rise is not statistically significant.

Conclusion

TLR2 and TLR4 are one of the most important markers of innate immunity. They might be used in early case detection and in further epigenetic discovery of bronchial asthma. Although there were carried many researches only a few have investigated the whole epigenome. It's highly important to know as much epigenetic signatures as possible, as this will help to develop refined predictive algorithms for the purpose of asthma prevention and treatment.

A novel modality of Bryophyllum pinnatum leaves ethanol extract inhibit maturation and promote apoptosis of B cells in pristane-induced SLE BALB/c mice model: in silico and in vitro study approach

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Introduction

Investigating the potential effect of Bryophyllum pinnatum leaves ethanol extract to deplete B cells by inhibiting maturation and increasing apoptosis of B cells in pristane-induce systemic lupus erythematosus (SLE) BALB/c mice model.

Material & methods

In silico study was conducted to understand affinity of Bryophillin A, Bryophillin B, Bryotoxin A, Bryotoxin B, Kaempferol, p-coumaric acid in Bryophyllum pinnatum against BAFF and its receptors: BAFF-R, BCMA, and BCMA. In vitro study was done by culturing splenocytes from pristane-induced SLE BALB/c mice's spleen. B cells were activated by BAFF yielding CD19+>80%. B cells were cultured by adding BAFF with and without Bryophyllum pinnatum leaves ethanol extract (0, 0.02, 0.1, or 0.5 µg/ml) for 72 hours. Percentages of maturation (CD19+/CD38+) and apoptosis (Annexin V+/PI+) of B cells were determined using flow cytometry. Further analysis to determine the expression of protein controller of maturation and apoptosis of B cells, NF-κB p65, was performed using immunocytochemistry.

Results

Docking results suggest that all of Bryophyllum pinnatum compounds interact with BAFF-R, TACI, BCMA and BAFF through hydrogen bonds and hydrophobic interactions. Flow cytometry assay showed significant decrease in percentages of maturation of B cells in all doses ($p \leq 0.006$) and significant increase in percentages of apoptotic B cells in dose 0.5 ug/ml ($p=0.002$). Immunocytochemistry results showed significant decreased expression of NF-κB p65 in all doses ($p \leq 0.013$). Between percentage of maturation, apoptosis, and expression of NF-κB p65 of B cells have a significant correlation each other ($p \leq 0.001$).

Conclusion

In silico and in vitro study in pristane-induce SLE BALB/c mice model revealed that Bryophyllum pinnatum is a potential natural product which could be used as B cell targeted therapy for SLE.

Bacterial suspension activates B cell differentiation and IL-10 production via Toll-Like Receptor-2 recognition

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Introduction

Bacterial suspensions (BS) are used to improve immune response in patients with respiratory infections. BS are constituted by a mixture of bacterial antigens derived from different bacterial species; species included in these lysates are 14 different bacteria: S. pneumoniae, K. pneumoniae, B. catarrahalis, S. aureus, H. influenza, S. alpha, and beta, S. faecalis, S. epidermidis, B. pertussis, Proteus sp., Pseudomonas sp., E. coli, and C. pseudodiphteriae (BS-14) It has been suggested that BS activate innate response through TLR, however if BS-14 are able to induce B cell activation is not known and this was the aim of our study.

Material & methods

Peripheral mononuclear blood cells (PBMC) were obtained from healthy donors and cultured with/without BS-14 (IPI ASAC Pharma®) at different concentrations, after stimulation with BS-14, supernatant were collected and IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A (BD Biosciences®), were measured with cytometric bead arrays. PBMC were harvested at 24, 48 and 72 h, labelled against anti- CD19, CD38, CD5, CD1d, CD69, IL-10 or TLR2 (BD Biosciences®), and analysed by flow cytometry.

Results

We observed dose-dependent increased activation of CD19+. After 72 h we observed a diminished frequency of B cells precursors and expansion of transitional B cells, these cells were TLR2+ cells. A significant increased concentration of IL-10 and IL-6 was observed at 24h and 48 h after BS-14 stimulation.

Conclusion

BS generates activation of CD19+, TLR2+ and IL10 cell phenotype, contributing to inmune regulation. BS could be used therapeutically not only to impove immune response but also to modulate immune response in infectious diseases and allergies contributing for a better lifestyle. BS induces expansion of transitional B cells and IL-10 production through TLR2 recognition.

Preparation and characterization of chitosan and trimethyl-chitosan nanoparticles loaded with influenza virus, with or without alginate coat, for nasal immunization in mice

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Introduction

Influenza virus is a globally important respiratory pathogen which is associated with a high degree of morbidity and mortality annually. Vaccination is still the most effective method of prophylaxis. Nasal influenza vaccination may prove to be a good alternative to parenteral injection because of the enhancement of the mucosal immune response and the ease of vaccine administration. Because of limitations encountered in mucosal vaccinations, potent adjuvants and delivery systems are needed to enhance the immunogenicity and to protect the antigens. This study investigated the Preparation of chitosan and N-trimethyl chitosan (TMC) nanoparticles, loaded with inactivated influenza virus (PR8), with or without alginate coat, and evaluated their adjuvant potential after nasal immunization in BALB/c mice.

Material & methods

PR8:CHT NPs and PR8:TMC NPs were prepared by direct coating of antigen by polymer. Then Both CHT and TMC nanoparticles were directly coated with Alginate and, size and zeta potential of the nanoparticles were studied. Mice were nasally immunized (3 times, 2 weeks interval) with 15 µg of inactivated influenza virus (PR8), coated with chitosan and N-trimethyl chitosan, with or without alginate coat. Ten days after the last immunization, IFN-☒ and IL-4 cytokines and IgG1, IgGtotal, IgG2a and IgA titers were determined by ELISA method.

Results

Among the groups studied, PR8:CHT showed the highest IgG1 titers (<0.001) and PR8:TMC:ALG induced the highest IgG2a titers (<0.001). IgG2a/IgG1 ratio in PR8:TMC:ALG formulation was 1.4.

Conclusion

Regards to the importance of the both cellular and humoral responses for prevention against influenza, PR8:TMC:ALG formulation with IgG2a/IgG1 ratio of 1.4, is a good candidate for nasal immunization against influenza.

In vitro evaluation of phase transition temperature of liposomes on human dendritic cells

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Introduction

Dendritic cells (DCs) are population of antigen presenting cells (APCs) which play a critical role in the activation of T cell responses and generate protective immunity against antigens. The intrinsic tendency of liposomes to have interaction with APCs is the main rationale to utilize these nanoparticles as antigen carriers. In this study, the effect of liposome phase transition temperature(Tm) on liposome uptake by human monocyte derived DCs (MoDCs), DCs maturation and immune response activation were investigated.

Material & methods

Two cationic liposomal formulations, DOTAP/cholesterol (1:1 molar-ratio) with low Tm and DSPC/DOTAP/cholesterol (3:1:1) with Tm>50°C, which had shown better results in our previous studies, were prepared using lipid film hydration method. All formulations contained soluble Leishmania antigens (SLA) as a model antigen. Liposomes characterized in terms of their size and surface charge by zetasizer. The amount of SLA was assigned by BCA. The presence of SLA in formulations has been showed by SDS-PAGE. The amount of phospholipid in formulations was determined by Bartlett phosphate assay. Human monocytes were isolated from fresh blood cells. The cells were exposed to GM-CSF and IL-4 till immature DCs to be converted. Liposomes were incubated with immature and mature DCs. Liposome uptake and the expression of DC co-stimulatory markers was evaluated by flow-cytometry using a FACS-Calibur flow cytometer and Cell Quest software.

Results

Cationic liposomes used for current study were 200-400 nm. The entrapment efficacy for SLA was 51.73% in DOTAP liposome and 58.56% in DSPC liposome. The phosphate content for DSPC liposome was 73.28%. We couldn't do phosphate assay for DOTAP formulation because of phosphorous absence in formulation. Liposome uptake by MoDCs was significantly ($p \leq 0.0001$) higher in DSPC formulation than other group and over expression of co-stimulatory (CD40,CD80,CD83) and MHC(HLA-DR) markers in MoDCs was significantly ($p \leq 0.0001$) more than DOTAP liposomes.

Conclusion

Cationic liposomes with high phase transition temperature (e.g. DSPC) have higher uptake and expression of DCs surface markers. So they can be considered as effective adjuvants for delivering antigens to MoDCs and stimulating immune response.

Evaluation of immune response induced by nanoliposomes containing whole leishmania lysate antigen in a mouse model

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Introduction

Cutaneous leishmaniasis is one of the most common tropical infections worldwide and is of major public health importance. Despite the numerous attempts no vaccine is found for effective vaccination against the disease. Parasite fractions or whole killed Leishmania parasites have shown to be more successful for this purpose. In this study, we evaluate the immunization caused by liposomes containing whole Leishmania lysate (WLL) antigen, as a first generation Leishmania vaccine candidate, in mice.

Material & methods

Cationic liposomes formulations containing different WLL concentrations (103, 104, 105, 106 and 107 parasite per 50µ final foodpad injection) were prepared and characterized in terms of their size and surface charge by particle size analyzer. BALB/c mice were immunized, three time with 2 week intervals via subcutaneous injection of formulations, empty liposomes or buffer. To explore the type of immune response generated and the extend of protection, lesion development and parasite burden in foot and spleen, IFN-γ (indicative of Th1 immune response) and IL-4 (indicative of Th2 immune response) production were evaluated. Also titration of IgG and IgG isotypes before and after the challenge was carried out.

Results

Liposomes were 356.6-1455 nm in size (PDI: 0.53 + 0.03) and the zeta potential was -11.2 ± 9.9. It was found that maximum protection was provided by WLL06 (106 parasite per injection) as depicted by the reduction of footpad swelling, reduction in parasite load, increase in IgG2a production and low levels of IL-4 production demonstrating generation of Th1 type of immune response (which leads to protection against the infection).

Conclusion

Our findings highlight the overall superiority of liposomes incorporating WLL, particularly WLL06, over the other groups. Vaccines using whole parasites approach, have been attractive in terms of cost, safety and stability when compared to other types and have better prospects as effective models in developing a new immunization method.