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activation markers in the lymphocyte region of no stimulated PBMC. The early activation marker CD69 showed no differences, CD69% C: 2.9+0.6 (n=15); P: 2.7+0.4 (n=5), but the late activation marker CD25 had a lower expression in P when compared to C. CD25% C: 6.9+1.8 (n=9); P: 3.5+0.8 (n=5), although this difference was not significant. We have detected differences in cell populations other than B cells of SPAD patients: a significant increase in CD14+CD16+ monocytes, for which a crucial role in inflammation and infectious disease in man has been suggested, and a decrease in γδ T cells with respect to normal controls.

43. Different presentation of Autoimmune Hepatitis in IPEX syndrome

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IPEX (Immune, Poliendocrinopathy, Enteropathy, X linked) syndrome is a rare immunodeficiency caused by mutations in Foxp3. Symptoms included autoimmune enteropathy, early onset type 1 diabetes mellitus (TIDM) and dermatitis. We report two IPEX cases diagnosed in our Center with different clinical onsets and outcomes. Case1: Boy born from healthy parents, without family history of immunodeficiency, developed neonatal TIDM. Diarrhea began at 2 years old diagnosed as celiac disease. He was referred to our Unit at 4 years old with fulminant hepatitis (no A B) for hepatic transplantation. In the laboratory findings Coombs positive hemolytic anemia, ASMA autoantibodies, elevated IgG and IgA were observed. IPEX was suspected clinically and was treated with corticosteroids and antithymocyte globulin. He died without response. Punctual mutation (p.F367L) on FoxpP3 confirmed the diagnoses. Case2: Second boy of two siblings born from healthy parents. At 2 month old developed eczema. Chronic diarrhea and failure to thrive began during the second year of age; duodenal biopsy showed eosinophilic infiltration in the lamina propria and villous atrophy. At 4 years old he presented eosinophilia, autoimmune hepatitis (LKM+ autoantibodies) and Coombs positive hemolityc anemia and begun corticosteroids treatment. Insulin dependent diabetes mellitus was diagnosed at 5 years old. He received azatioprime and prednisone as an immunosuppressant during 18 months with good response but after that he developed diarrhea caused by microsporidium, thoracic herpes zoster and two pneumonias. The patient underwent bone marrow transplantation from his HLA matched sibling at 7 years old with reduced intensity conditioning. Actually his on +60 day post transplant. Although hepatic involvement is less common than other autoimmune phenomena on IPEX, our two patients had autoimmune hepatitis with different response to immunosuppressive treatment.

44. Role of Nitric Oxide Synthases in Elastase-Induced Emphysema

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Inducible nitric oxide synthase (iNOS) is overexpressed in the human emphysematous lung. iNOS produces nitric oxide (NO) that in combination with superoxide generates peroxynitrites and induces protein nitration. Although iNOS is a well known pro-inflammatory molecule, its role in this disease is unknown. The aim of this study was to determine whether iNOS contributes to the development of elastase-induced emphysema in mice. iNOS -/- and eNOS -/- mice and mice treated with a pharmacological iNOS inhibitor were intratracheally exposed to elastase. Expression of iNOS, eNOS and inflammatory mediators was evaluated at the protein and mRNA levels. Emphysema was quantified morphometrically. iNOS and eNOS were diffusely upregulated in the lung of elastase-treated mice and a 12-fold increase in the number of 3-nitrotyrosine-expressing cells was observed (80% of alveolar type 2 cells, 20 % macrophages). In elastase-instilled mice, iNOS inactivation reduced protein nitration and increased protein oxidation but had no effect on inflammation, MMP activity and the subsequent development of emphysema. eNOS inactivation had no effect on inflammation and emphysema. In conclusion, in the elastase-injured lung, iNOS mediates protein nitration in alveolar type 2 cells and alleviates oxidative injury. iNOS are not required for the development of elastase-induced inflammation and emphysema.

45. Experimental error control during immune monitoring by flow cytometry of positive reactors to bovine tuberculin skin test

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Flow cytometry (FC) has become the method of choice for studying the immune response to infectious agents. The development of an extensive set of monoclonal antibodies (MAb) to bovine leukocyte differentiation molecules has now made it possible immune response characterization in bovine tuberculosis (Tbc). Before extending these studies it is essential to establish results consistency. Currently indirect labeling (IL) is used in single and multiparameter analysis. This introduces the probability of ex-

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perimental error because of the multiple processing steps needed. The objective of this study was to develop and test a strategy for assuring the reliability of methods for processing peripheral blood mononuclear cells (PBMC) from Tbc positive cattle. PBMC were obtained from 10 Tbc positive cows by density gradient centrifugation (Histopaque1077). Each sample was labeled with four MAb cocktails (antiCD4-IgM/antiCD25-IgG1, antiCD4/antiCD45Ro-IgG, antiCD8-IgM/antiCD25, antiCD8/antiCD45Ro), then they were washed and labeled with antiIgG1-PE/anti-IgM-FITC cocktail. Finally PBMC were fixed, stored and 10000 events were acquired with cytometer FACS-CANTO (Becton-Dickinson). Data were analyzed with FCS Express trial version. A dot plot side light scatter (SSc) vs forward light scatter (FSc) was set to define the mononuclear cell population. Then a dot-plot FSc vs. fluorescence distinguished between unlabeled and labeled cells. Finally a histogram is generated to compare geometric means of fluorescence intensity (GMFs). SAS v 9.2 calculated the paired t test between duplicate GMFs. The variation in labeling between paired samples was very low, p value of paired t test>0.05 in all samples, showing that reliable results can be obtained with minimal differences introduced during sample preparation. When in FC IL is routine, statistical comparison of results from repetitions of the same sample labeled with the same MAb would be strategic as an experimental error control.

46. Molecular Strategies to Detect Gross Gene Lesions in Primary Immunodeficiency (PID) Genes

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The majority of gene disruptions underlying human inherited diseases are microlesions and small deletions (DEL) or insertions (INS), gross lesions are less common, with gross DEL being most frequently found (6%). BTK, CYBB, SH2D1A and Artemis genes showed gross lesions with greatly different incidence (7%, 12.5%, 27.9% and 56% of disease-causing mutations, respectively). In this work, we show molecular strategies to detect and to confirm gross lesions. We studied 2 XLA, 2 CGD-X, 1 XLP and 1 Artemis-SCID patients. The genes were screened by SSCP or RT-PCR, followed by sequencing. When a gross lesion was suspected, additional methodologies were incorporated: PCRs for large gene segments (up to 4 kb), Long Expand PCR for fragments longer than 5 kb and SNP haplotype analysis. In 2 CGD-X patients, that failed to amplify CYBB single exons, PCR for large gene segment showed a 2.3 kb DEL and a 780 bp complex INS-DEL in each other. Screening the BTK gene by using RT-PCR, showed in 2 patients abnormal size fragments, containing each other a DEL and a duplication of exons 2 and 3. These lesions could result from Alu element-induced unequal homologous recombination. A homozygous mutation in Artemis heterozygously carried by her father but not by her mother, suggested DEL inside the maternal allele. By SNP haplotype evaluation we tried to define the size of this DEL, likely arosing from mispairing between 2 highly homologous sequences. In 2 XLP brothers that failed to amplify exon 3 in SH2D1A, Long Expand PCR allowed to counsel their mother, carrier of a 797bp DEL. Once located the deletion breakpoints, specific primers were designed, to perform an accurate genetic counseling for this family. These patients clearly show the importance of having more than one screening strategy for an efficient diagnosis and family genetic counseling. It is also very important to be attentive to the genes having higher frequency of gross lesions to incorporate appropriate study methodologies.

47. Development and validation of an anti-p16 monoclonal antibody for the detection of high risk HPV-associated lesions in cervical biopsies

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Human papillomavirus (HPV) infection is an essential factor for the development of cervical lesions that may lead to cancer. Cervical cancer is an important public health problem in developing countries. However, the diagnosis of HPV is currently based on microscopic observation of the cervix and morphological analysis of lesions in endocervical smears and biopsies, being these methods highly dependent on the criteria and training of the observer. As HPV oncoproteins induce cell cycle deregulation, proteins of the cell cycle may be used as indicators of HPV infection. These proteins may be used in immunochemical techniques to help in the diagnosis of cervical neoplasia. Here, we aimed to develop monoclonal antibodies (mAbs) to proteins that are known to be surrogate markers of high risk HPV-associated neoplasias. p16 is a cyclin dependent kinase inhibitor regulated by retinoblastoma protein (pRb). In high risk HPV-related cervical lesions there is a functional inactivation of pRb by HPV E7 oncoprotein, leading to p16 upregulation. We expressed and purified recombinant p16 protein and produced mAbs using standard technique. From the panel of mAbs obtained, we characterized one anti-p16 mAb that specifically recognized endogenous p16 protein in HPV cancer cell lines, in agreement with reported data, as judged by enzyme-linked immunoassays and Western blots. Importantly, in conventional immunohistochemistry our mAb specifically immunostained paraffin-embedded sections of cervical cancer biopsies, HPV positive, and was not reactive in normal cervical epithelium, in correlation with a commercial anti-p16 mAb immunostaining used as control. Our results suggest that our anti-p16 mAb has high sensitivity for immunohistochemical detection on high risk HPV-related neoplasias, constituting a useful tool to improve diagnostic accuracy at low cost.