

Cuban Research in Current International Journals

The following selection—alphabetical by title—reflects Cuban publishing in international medical and population health journals over the last quarter on an array of topics. Links to these journal articles may be found at www.medicc.org/mediccreview.

Childhood atopic diseases and early life circumstances: an ecological study in Cuba. van der Werff SD, Polman K, Ponce MC, Twisk JW, Junco Díaz R, Gorbea MB, et al. *PLoS One*. 2012;7(6):e39892. Epub 2012 Jun 29.

Background Children are especially vulnerable during periods of resource shortage such as economic embargoes. They are likely to suffer most from poor nutrition, infectious diseases, and other ensuing short-term threats. Moreover, early life circumstances can have important consequences for long-term health. We examined the relationship between early childhood exposure to the Cuban economic situation in the nineties and the occurrence of atopic diseases later in childhood. **Methodology/Principal Findings** A cross-sectional study of 1321 primary schoolchildren aged 4–14 was conducted in two Cuban municipalities. Asthma, allergic rhinoconjunctivitis and atopic dermatitis were diagnosed using the International Study of Asthma and Allergies in Childhood questionnaire. Children were divided into three groups of exposure to the economic situation in the nineties according to birth date: (1) unexposed; (2) exposed during infancy; (3) exposed during infancy and early childhood. Associations were assessed using multiple logistic regression models. Exposure during infancy had a significant inverse association with the occurrence of asthma (OR 0.56, 95%CI 0.33–0.94) and allergic rhinoconjunctivitis (OR 0.46, 95%CI 0.25–0.85). The associations were stronger after longer exposure, i.e. during infancy and early childhood, for asthma (OR 0.40, 95% CI 0.17–0.95) and allergic rhinoconjunctivitis (OR 0.29, 95%CI 0.11–0.77). No significant associations were found for atopic dermatitis. **Conclusions/Significance** Exposure to the economic situation in the nineties during infancy and early childhood was inversely associated with asthma and allergic rhinoconjunctivitis occurrence later in childhood. We hypothesize that factors related to this period, such as infectious diseases and undernutrition, may have an attenuating effect on atopic disease development. The exact cause and underlying mechanisms need to be further elucidated.

Complete definition of immunological correlates of protection and clearance of hepatitis C virus infection: a relevant pending task for vaccine development. Alvarez-Lajonchere L, Dueñas-Carrera S. *Int Rev Immunol*. 2012 Jun;31(3):223–42.

Hepatitis C virus (HCV) infects approximately 3% of global population. This pathogen is one of the main causes of chronic viral hepatitis, cirrhosis, and liver cancer, as well as the principal

reason for liver transplant in Western countries. Therapy against HCV infection is effective in only half of treated patients. There is no vaccine available against HCV. Some vaccine candidates have reached the clinical trials but several factors, including the incomplete definition of immunological correlates of protection and treatment-related clearance have slowed down vaccine development. Precisely, the present review discusses the state of the art in the establishment of parameters related with immunity against HCV. Validity and limitations of the information accumulated from chimpanzees and other animal models, analysis of studies in humans infected with HCV, and relevance of aspects like type, strength, duration, and specificity of immune response related to successful outcome are evaluated in detail. Moreover, the immune responses induced in some clinical trials with vaccine candidates resemble the theoretical immunological correlates, raising questions about the validity of those correlates. When all facts are taken together, complete definition of immunological correlates for protection or treatment-related clearance is an urgent priority. A limited or wrong criterion with respect to this relevant matter might cause incorrect vaccine design and selection of immunization strategies or erroneous clinical evaluation.

Diagnosis of vascular cognitive impairment and its main categories. Rodríguez García PL, Rodríguez García D. *Neurología*. 2012 Jun 26. [Epub ahead of print]

Objective A review of current criteria for the diagnosis of categories related with vascular cognitive impairment, in particular the nomenclature, diagnostic criteria, and differential clinical-radiological findings. **Development** The criteria for the diagnosis of vascular cognitive impairment have evolved, but available criteria were designed basically for differentiating between vascular dementia and dementia due to Alzheimer disease, and for research purposes. Nevertheless, in clinical practice precise elements are required for: 1) Clinical diagnosis of dementia and mild cognitive impairment; 2) Clinical and neuroimaging criteria for identification of the various cerebrovascular lesions associated with cognitive dysfunction, and 3) A formulation of the aetiological-pathogenic relationship between cognitive impairment and cerebrovascular lesions. For this reason, a review was carried out on the diagnostic elements of vascular cognitive impairment categories, classification, and their most relevant characteristics. It highlights the characteristic for the diagnosis of multi-infarction dementia, strategic single infarct dementia, small vessel disease with dementia,

mixed dementia, and vascular mild cognitive impairment. **Conclusions** Standardisation is required, by a multidisciplinary expert team, as regards nomenclature and criteria for the diagnosis of the full spectrum associated with vascular cognitive impairment and especially for vascular dementia and its categories.

Diamel Therapy in Polycystic Ovary Syndrome Reduces Hyperinsulinaemia, Insulin Resistance, and Hyperandrogenaemia. Hernández-Yero A, Santana Pérez F, Ovies Carballo G, Cabrera-Rode E. *Int J Endocrinol*. 2012;2012:382719. Epub 2012 Jun 21.

For to determine the effect of Diamel on the insulin resistance, insulin sensitivity, and sexual hormones results in women with polycystic ovary syndrome (PCOS). A study was carried out on 37 patients with this disorder. A triple-blind clinical trial was designed in which the Diamel food supplement was compared with a placebo. The women with reproductive ages were randomly distributed in two groups, with 18 and 19 women respectively, and they took Diamel or placebo and were followed up during 6 months with clinical and biochemical evaluation. A significant decrease in the HOMA-IR from the initial value at six months was observed in the group with Diamel. The insulin sensitivity improved considerably in this group. The rate of menstrual recovery was higher in the group with Diamel, and two patients from this group obtained pregnancy. The hormone levels shows a significant decrease in testosterone at 3 months in the group with Diamel compared with the control group. The LH also decreases in the same group when comparing the start with 6 months. We concluded that the Diamel decreases insulin resistance and improves sensitivity to this hormone in women with PCOS, with improvement in the levels of LH and testosterone.

Frequency of antibodies to *Toxocara* in Cuban schoolchildren. Sariego I, Kanobana K, Junco R, Vereecken K, Núñez FA, Polman K, et al. *Trop Med Int Health*. 2012 Jun;17(6):711–4.

Objective The aim of the study was to determine the frequency of antibodies to *Toxocara* in Cuban schoolchildren. **Methods** The frequency of antibodies to *Toxocara canis* was assessed with a commercial enzyme-linked immunosorbent assays kit in school-aged children from two municipalities of Cuba. Univariate analysis and a multivariable logistic regression analysis adjusted for age, sex, municipality and co-infection with helminth and/or protozoa were conducted. **Results** The percentage of chil-

dren with antibodies to *Toxocara* was 38.8% (392/1011; 95% CI = 36.8–42.8). Antibody positivity was significantly associated with gender and co-infections with intestinal parasites, but not with age or municipality. **Conclusion** Cuban children are highly exposed to the *Toxocara* parasite, corresponding well with reported environmental contamination with *Toxocara* parasite eggs and *T. canis* prevalences in dogs in Cuba. Relevant policy makers and the Cuban population need to be better informed about this preventable infection.

Gated-SPECT myocardial perfusion imaging and coronary calcium score for evaluation of patients with acute chest pain and a normal or nondiagnostic electrocardiogram. Peix A, Batista E, Cabrera LO, Rodríguez L, Padrón K, Sainz B, et al. Coron Artery Dis. 2012 Aug 3. [Epub ahead of print]

Objective To assess the ability of rest myocardial perfusion imaging (MPI) to rule out an acute coronary syndrome (ACS) in emergency department patients, as well as to investigate whether there exists a concordance between MPI and coronary calcium. **Materials and Methods** Fifty-five patients with chest pain and a normal or nondiagnostic ECG were included. Clinical follow-up was carried out within 1 year. **Results** Sixteen patients (29%) showed an abnormal rest MPI, and in 11 (20%) the MPI was equivocal. There was a weak concordance between MPI and coronary arteries calcium score (CACS) (κ : 0.25). Coronary angiogram driven by a positive MPI was performed in 12 patients (23%), resulting in percutaneous coronary intervention in nine cases (75%). A positive MPI (abnormal or equivocal results) was associated with the occurrence of events in the follow-up ($\chi^2=19.961$, $P<0.0001$). For a patient presenting to the emergency department with acute chest pain and a normal or nondiagnostic ECG, with a positive MPI, the relative risk of having events during the first year was 7.5 (95% confidence interval: 2.8-19.2), $P<0.05$, but with a positive CACS this was 1.77 (95% confidence interval: 0.69-4.56), $P=NS$. At 1 year 68.6% of patients were free of events. **Conclusion** Patients presenting with acute chest pain and a low-to-intermediate likelihood of coronary artery disease with a normal rest MPI have a very low probability of cardiac events during the first year. Coronary calcium score was not helpful in risk-stratifying these patients.

High proportion of mannosidosis and fucosidosis among lysosomal storage diseases in Cuba. Menéndez-Sainz C, González-Quevedo A, González-García S, Peña-Sánchez M, Giugliani R. Genet Mol Res. 2012 Aug 13;11(3):2352–9.

Although lysosomal storage disorders (LSDs) are considered individually rare, as a group they present a non-negligible frequency. Few studies have been made of populational occurrence of LSDs; they have been conducted predomi-

nantly on Caucasian populations. We studied the occurrence of LSDs in Cuba. Data from individuals who had been referred to the Institute of Neurology and Neurosurgery in Havana from hospitals all over the country between January 1990 and December 2005 were analyzed. This institute was the only laboratory to provide enzyme-based diagnostic testing for 19 LSDs in Cuba during this period. Occurrence rates were calculated by dividing the number of post-natal diagnoses by the number of births during the study period. The combined occurrence of LSDs in Cuba was 5.6 per 100,000, lower than that reported in other studies conducted on Caucasian populations. The most frequent individual LSDs were: mucopolysaccharidosis type I (1.01 per 100,000) and, surprisingly, alpha-mannosidosis (0.72 per 100,000) and fucosidosis (0.62 per 100,000). These findings may be related to specific genetic characteristics and admixture of the Cuban population. This is the first comprehensive study of the occurrence of LSDs in Cuba. We conclude that the epidemiology of these diseases can vary regionally, and we stress the need for similar surveys in other Latin American countries.

Interfacial behavior and structural properties of a clinical lung surfactant from porcine source. Blanco O, Cruz A, Ospina OL, López-Rodríguez E, Vázquez L, Pérez-Gil J. Biochim Biophys Acta. 2012 Jul 4. [Epub ahead of print]

Surfacen® is a clinical surfactant preparation of porcine origin, partly depleted of cholesterol, which is widely used in Cuba to treat pre-term babies at risk or already suffering neonatal respiratory distress. In the present study we have characterized the interfacial behavior of Surfacen in several in vitro functional models, including spreading and compression-expansion cycling isotherms in surface balances and in a captive bubble surfactometer, in comparison with the functional properties of whole native surfactant purified from porcine lungs and its reconstituted organic extract, the material from which Surfacen is derived. Surfacen exhibited similar properties to native porcine surfactant or its organic extract to efficiently form stable surface active films at the air-liquid interface, able to consistently reach surface tensions below 5mN/m upon repetitive compression-expansion cycling. Surfacen films, however, showed a substantially larger and stable compression-driven segregation of condensed lipid phases than exhibited by films formed by native surfactant or its organic extract. In spite of structural differences observed at microscopic level, Surfacen membranes showed a similar thermotropic behavior to membranes from native surfactant or its organic extract, characterized by calorimetry or fluorescence spectroscopy of samples doped with the Laurdan probe. On the other hand, analysis by atomic force microscopy of films formed by Surfacen or by the organic extract of native porcine surfactant revealed a similar network of interconnected condensed nanostructures, suggesting that the organization of the films at the submicroscopic level is

the essential feature to support the proper stability and mechanical properties permitting the interfacial surfactant films to facilitate the work of breathing.

Kaposi's Sarcoma and Human Herpesvirus 8 in Cuba: Evidence of subtype B expansion. Kourí V, Martínez PA, Capó V, Blanco O, Rodríguez ME, Jiménez N, et al. Virology. 2012 Oct 25;432(2):361–9.

Objective To evaluate the temporal distribution (1991-2009) and associated variation of KSHV subtypes in Cuba. **Method** Phylogenetic characterization based on the KSHV K1 gene was performed using 90 KSHV positive samples. **Results** Molecular characterization confirmed the prevalence of a wide range of KSHV subtypes (A: n=48 [A5=12]; C: n=15; B: n=22; and E: n=5). In the current study, we observed a significant increase in HHV-8 subtype B after 2004 ($p=0.0063$). This Subtype B in Cuba was associated with: heterosexual behaviour (OR: 3.63, CI: 1.2-10.98; $p=0.03$), with the antecedent of acquiring HIV/KSHV in Africa ($p=0.0003$), with nodular stage of KS lesions (OR 4.2, CI: 1.1 to 15.7; $p=0.04$). **Conclusion** Our study is the first to report KSHV Subtype B expansion in Cuba, that might be reflective of a change in human behavioural pattern.

Observations of fallout from the Fukushima reactor accident in Cienfuegos, Cuba. Alonso-Hernandez CM, Guillén-Arrebarena A, Cartas-Aguila H, Morera-Gómez Y, Díaz-Asencio M. Bull Environ Contam Toxicol. 2012 May;88(5):752–4.

Following the recent accident at the Fukushima Daiichi nuclear power plant in Japan, radioactive contamination was observed near the reactor site. As a contribution towards the understanding of the worldwide impact of the accident, we collected fallout samples in Cienfuegos, Cuba, and examined them for the presence of above normal amounts of radioactivity. Gamma ray spectra measured from these samples showed clear evidence of fission products ^{131}I and ^{137}Cs . However, the fallout levels measured for these isotopes ($135 \pm 4.78 \text{ mBq m}^{-2} \text{ day}^{-1}$ for ^{131}I and $10.7 \pm 0.38 \text{ mBq m}^{-2} \text{ day}^{-1}$ for ^{137}Cs) were very low and posed no health risk to the public. The doses received as consequence to the Fukushima fallout by the Cienfuegos population's (0.002 mSv per year) don't overcome the limit of dose (1 mSv per year) fixed for the public in Cuba.

QSPR in Oral Bioavailability: Specificity or Integrality? Cabrera-Perez MA, Pham-The H, Bermejo M, Alvarez IG, Alvarez MG, Garrigues TM. Mini Rev Med Chem. 2012 Jun 1;12(6):534–50.

During the last decade the technological advances in drug discovery changed the absorption, distribution, metabolism, excretion and toxicity

(ADMET) profiles of New Chemical Entities (NCEs). Among ADMET processes, absorption plays an important role in the research and development of more effective orally administered drugs. Although significant progress has been made in *in vitro*, *in situ* and *in vivo* experimental determinations of absorption, the development of *in silico* methodologies has emerged as a cheaper and fast alternative to predict them. Even though several *in silico* models have been described in the literature to predict oral bioavailability and related properties, the prediction accuracy and their potential use is still limited. The low precision and high variability of data, the lack of a complete experimental and theoretical validation of *in silico* approach, and above all, the multifactorial nature of the oral absorption term, make the development of predictive *in silico* models a thorny task. The present review discusses several important advances regarding the QSPR approaches used in the development of predictive oral bioavailability models. The importance of fixing the problem associated with data resource, as well as improving the reliability of *in silico* results is highlighted. Optimization of individual properties along the absorption process must be integrated in a multi-objective scenario for studying oral bioavailability behavior in the early drug discovery and development.

Type 1 diabetes associated and tissue transglutaminase autoantibodies in patients without type 1 diabetes and coeliac disease with confirmed viral infections. Sarmiento L, Galvan JA, Cabrera-Rode E, Aira L, Correa C, Sariego S, et al. *J Med Virol.* 2012 Jul;84(7):1049–53.

Coeliac disease and type 1 diabetes are autoimmune diseases that may share the same initiating environmental factors. In this study, the occurrence of type 1 diabetes associated autoantibodies (GADA and IA-2A) and tissue transglutaminase autoantibodies (TGA) was determined in patients with confirmed viral infections and no signs of type 1 diabetes or coeliac disease. Serum samples from 82 Cuban patients tested positive for PCR and IgG specific to enterovirus (HEV, serotype echovirus 16, 20 samples), Epstein-Barr virus (EBV, 20 samples), cytomegalovirus (CMV, 21 samples), and hepatitis C virus (HCV, 21 samples); and sera from 164 controls negative serologically to EBV, CMV, HCV, and echovirus 16 were enrolled in the study. All subjects were screened for GADA, IA-2A, and TGA. The prevalence of TGA in patients infected with HEV, EBV, CMV, or HCV was 55% (11/20), 25% (5/20), 9.5% (2/21), and 9.5% (2/21), respectively. GADA and IA-2A were found in 15% (3/20) and 25% (5/20) of patients infected with HEV. None of the patients infected by EBV, CMV, and HCV had GADA or IA-2A. All children infected with HEV who were positive for type 1 diabetes-associated autoantibodies were also TGA-positive. None of the sera from unin-

ected subjects were positive for GADA, IA-2A or TGA. In conclusion, TGA can develop during infection with HEV, EBV, CMV, or HCV, while the emergence of islet cell related autoantibodies is restricted to HEV infections. The findings suggest that HEV may be a shared environmental factor for the development of islet and gut-related autoimmunity.

Variation in Inflammatory/Regulatory Cytokines in Secondary, Tertiary, and Quaternary Challenges with Dengue Virus. Sierra B, Pérez AB, Alvarez M, García G, Vogt K, Aguirre E, et al. *Am J Trop Med Hyg.* 2012 Jul 16.

Secondary heterologous dengue infection is a risk factor for severe disease manifestations because of the immune-enhancement phenomenon. Succeeding clinical infections are seldom reported, and the clinical course of tertiary and quaternary dengue infections is not clear. Cuba represents a unique environment to study tertiary/quaternary dengue infections in a population with known clinical and serologic dengue markers and no dengue endemicity. We took advantage of this exceptional epidemiologic condition to study the effect of primary, secondary, tertiary, and quaternary dengue infection exposure on the expression of pro-inflammatory and regulatory cytokines, critical in dengue infection pathogenesis, by using a dengue infection *ex vivo* model. Whereas secondary exposure induced a high cytokine response, we found a significantly lower expression of tumor necrosis factor- α , interferon- γ , interleukin-10, and tumor growth factor- β after tertiary and quaternary infectious challenge. Significant differences in expression of the cytokines were seen between the dengue immune profiles, suggesting that the sequence in which the immune system encounters serotypes may be important in determining the nature of the immune response to subsequent infections.

Very Small Size Proteoliposomes (VSSP) and Montanide combination enhance the humoral immune response in a GnRH based vaccine directed to prostate cancer. Aguilar FF, Barranco JJ, Fuentes EB, Aguilera LC, Sáez YL, Santana MD, et al. *Vaccine.* 2012 Aug 23. [Epub ahead of print]

Very small size proteoliposomes (VSSP) constitute a complex of very small size proteoliposomes that includes proteins, lipids, CpG and gangliosides tumor-associated that provides a potential target for cancer immunotherapy. This compound has been described to stimulate the humoral and cellular response, dendritic cells (DC) activation and differentiation of T-helper cells, specially, in immunocompromised patients with cancer status. This work deals with the stimulating capacity of the VSSP to reach a humoral response when they are used as a component in a peptidic vaccine based on the gonadotrophin releasing hormone (GnRH). This study was carried out in male Copenha-

gen rats, which were immunized with 750 μ g of the GnRH mimetic peptide (GnRHm1-TT) with or without the VSSP. The mixtures were always emulsified with the oil adjuvant Montanide ISA 51. The anti GnRH seroconversion analysis revealed that the group immunized with the peptide GnRHm1-TT/VSSP developed a strong anti GnRH seroconversion. These antibody levels proved to be significant superior to those reached by the use of the GnRHm1-TT peptide solely emulsified in Montanide. Post-mortem analysis on the Testosterone ablation target organs (prostate and testicles) yielded a sudden decrease in their size and weight in respect to the control group. On the other hand, the group submitted to the use of GnRHm1-TT/VSSP, showed a significant difference in the reduction of these target organs in comparison with the group only immunized with GnRHm1-TT adjuvated in Montanide ISA 51. These values turned to be of $p=0.023$ and $p=0.009$ in the prostate and testicles respectively. These findings foreground the VSSP as a useful immunopotentiator to be used as part of a GnRH based vaccine to treat prostate cancer. 