

RESÚMENES DE LAS PRESENTACIONES DEL IV ENCUENTRO DE INVESTIGACIÓN DE LA ASOCIACIÓN COLOMBIANA DE INMUNOLOGÍA (ACOI), REALIZADO EN NEIVA, COLOMBIA, EL 15 Y 16 DE MARZO DE 2018

PRESENTACIONES ORALES

A low degranulation ability of activated CD8⁺ T cells is associated with the persistent systemic immune activation in HIV-infected patients, despite viral suppression induced by the anti-retroviral therapy

Federico Perdomo-Celis¹, Natalia A. Taborda^{1,2}, María T. Rugeles¹.

¹ Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia, UdeA, Medellín, Colombia.

² Grupo de Investigaciones Biomédicas Uniremington, Programa de Medicina, Facultad de Ciencias de la Salud, Corporación Universitaria Remington, Medellín, Colombia.

Immune activation is the hallmark of the human immunodeficiency virus type 1 (HIV-1) infection, identified by the expression of cellular markers such as HLA-DR and CD38, and plasma molecules such as soluble CD14 (sCD14), that remain increased despite the viral control induced by the highly active anti-retroviral therapy (HAART). This hyper-activation induces dysfunction in immune cells, including CD8⁺ T cells, that might contribute to the development of therapy failure. Particularly, a low degranulation ability and production of cytotoxic molecules by CD8⁺ T cells has been observed in untreated HIV-infected patients, but the reconstitution of the response of these cells, in the setting of HAART-induced viral suppression and its relation with the immune activation state is unclear. In this study, we aimed to evaluate the effect of HAART on the degranulation ability and production of cytotoxic molecules and interferon (IFN)- γ by CD8⁺ T cells from HIV-1-infected patients and the association with the activation state. By flow cytometry, we characterized the functionality of activated CD8⁺ T cells (HLA-DR⁺ and/or CD38⁺) by measuring the degranulation ability (expression of CD107a), *de novo* production of cytotoxic molecules (CD107a plus granzyme B and/or perforin) and the expression of IFN- γ . In addition, plasma levels of sCD14 were evaluated by ELISA. Peripheral blood mononuclear cells stimulated *ex vivo* with PMA-ionomycin were analyzed in 30 HIV-1-infected patients receiving HAART (viral load <50 HIV RNA copies/mL), and compared with cells from 10 seronegative healthy

individuals. After stimulation, the expression of CD107a and production of IFN- γ and *de novo* granzyme B and perforin by total CD8⁺ T cells was similar between HIV-1-infected and seronegative individuals. Although in basal conditions, total CD8⁺ T cells exhibited similar levels of activation in both groups, after polyclonal stimulation, HIV-1-infected patients had a higher frequency of HLA-DR⁺ CD38⁺ CD8⁺ T cells, characterized by a low expression of CD107a and *de novo* granzyme B and perforin. In addition, sCD14 levels were increased in HIV-infected patients and negatively correlated with the frequency of HLA-DR⁺ CD38⁺ CD107⁺ CD8⁺ T cells. Our findings suggest that the degranulation ability and *de novo* production of cytotoxic molecules by activated CD8⁺ T cells is impaired in HIV-1-infected patients despite HAART-induced viral suppression, indicating partial immune reconstitution, and these alterations are associated with a persistent systemic immune activation.

Sequential dengue and Zika virus infection induce a higher neutralizing activity and stronger T-cell responses against Zika virus

Félix Delgado^{1,3}, Karina Torres¹, Jaime Castellanos¹, Consuelo Romero-Sánchez², Etienne Simon-Lorière^{3,4}, Anavaj Sakuntabhai^{3,4}, Claude Roth^{3,4}

¹ Grupo de Virología, Universidad El Bosque, Bogotá, D. C., Colombia.

² Unidad de Investigación Básica Oral, Universidad El Bosque, Bogotá, D. C., Colombia.

³ Functional Genetics of Infectious Diseases Unit, Institut Pasteur, Paris, France.

⁴ CNRS URA 3012, F-75724 Paris, France.

The high level of dengue seroprevalence in areas where Zika virus (ZIKV) is circulating have raised concerns on the risk of increased Zika disease severity for patients with a history of previous dengue virus (DENV) infection. Recent studies have shown that anti-DENV pre-existing antibodies may enhance ZIKV infection and increase disease severity. However, little has been shown about the ability of these antibodies to neutralize ZIKV in the same context and regarding the T cell epitopes that are specific or shared between both of these viruses. Given these facts, to determine the role of DENV pre-immunity in ZIKV infection, the aim of this study was to analyze the T and B cell responses against ZIKV. Using PBMC from blood donors with previous history of DENV/ZIKV or ZIKV infection, we have identified ZIKV epitopes by screening T-cell responses against overlapping peptides spanning the ZIKV proteome by IFN-gamma enzyme-linked immunospot analysis. Furthermore, plasma samples were analyzed to quantify the neutralizing and enhancing activities of antibodies against DENV and ZIKV infections using a flow cytometry-based assay. Our results show that the ZIKV non-structural proteins NS1, NS3 and NS5 contain most of the immunodominant

peptides that induce a strong T-cell response. Interestingly, in donors with a history of DENV infection, specific peptides were also identified as DENV CD8+ T-cell epitopes and the strongest T-cell responses observed in these donors correspond to sequences with a high level of amino acid identity with the four serotypes of DENV. These results strongly support the activation of cross-reactive T-cells in this context. Additionally, plasma samples from ZIKV-infected donors exhibited neutralizing activity only against ZIKV, and one donor showed enhancing activity for DENV4 infection. The highest neutralizing activity against ZIKV infection was observed in samples from donors with previous DENV infection, strongly suggesting the induction of cross-reacting antibodies induced upon sequential DENV and ZIKV infection. These data have crucial implications for future ZIKV and DENV vaccines and provide new opportunities to study the role of subsets of DENV- or ZIKV-specific T cells in the induction of broadly neutralizing antibodies in the context of sequential flavivirus infections, which could modulate disease severity.

Reduction in the frequency of IL10-producing B lymphocytes in patients with atherosclerosis

Héctor Rincón-Arévalo¹, Julio Quintero¹, Mauricio Rojas^{1,2}, Fernando Fortich³, Gloria Vásquez^{1,4}, Lina Yassin⁵, Diana Castaño¹

¹ Grupo de Inmunología Celular e Inmunogenética, Instituto de Investigaciones Médicas, Facultad de Medicina, Universidad de Antioquia UdeA, calle 70 No. 52-21, Medellín, Colombia.

² Unidad de Citometría, Facultad de Medicina, sede de Investigación Universitaria, Universidad de Antioquia.

³ Sección de Cardiología. Hospital Universitario San Vicente Fundación. Medellín, Colombia.

⁴ Grupo de Reumatología, Universidad de Antioquia, Medellín, Colombia.

⁵ Grupo de Investigaciones Biomédicas. Corporación Universitaria Remington. Medellín, Colombia.

E-mail contacto: diana.castano@udea.edu.co, yascatorce@yahoo.com.

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Introduction: Atherosclerosis is a systemic inflammatory process characterized by the accumulation of lipids and infiltration of immune system cells in the intima. B cells have been attributed both a protective and proatherogenic role, which apparently depends on the subpopulation involved and the previous antigenic contact. During the last years, the existence of B cells with the ability to regulate the secretion of TNF- α

and IFN- γ by T cells through the production of IL-10 in the context of different inflammatory diseases has been described.

Objective: To compare the frequency, phenotype and regulatory function of some circulating B cell subsets, between patients with atherosclerosis and low cardiovascular risk controls.

Methodology: Circulating mononuclear cells were obtained from patients with atherosclerosis and controls. B cells were characterized into immature, transitional, mature, memory and plasmablast subsets and were cultured using two methodologies. *Ex vivo*: 5 h with Lipopolysaccharide (LPS) or CpG in presence of phorbol myristate acetate (PMA), Ionomycin and Brefeldin A (PIB). *In vitro*: 48 h with anti-CD40 and restimulation in the last 5 h with LPS or CpG in presence of PIB. Cells were labeled with monoclonal antibodies and acquired in the flow cytometer. In each subpopulation, the positivity for IL-10 was evaluated. In some cultures, each B cell subset was selectively removed from mononuclear cells and stimulated for 48 h with anti-CD3 ϵ . In the last 5 h, restimulation with PIB was performed. The frequency of CD4 + TNF- α + and IFN- γ + T cells was evaluated, in presence or absence of an IL-10R blockage.

Results: There were no differences in the distribution of B cell subsets between the study groups. In patients, increased frequency of CD40+ transitional, mature and memory cells, and CD80+ transitional and mature subsets were found. Reduction in the total frequency of IL-10+ B cells was found in patients with atherosclerosis compared to healthy controls, in both the *ex vivo* culture and *in vitro* stimulated with LPS or CpG. That reduction was observed in the immature, transitional, mature and memory subsets with both types of culture stimulated with LPS. The stimulation with CpG induced reduction in all *ex vivo* evaluated subsets, while in *in vitro* culture this stimulation induced reduction of immature, mature and memory B subsets. It was observed that *ex vivo* and *in vitro*, the CD4+ T cells from patients showed higher frequency of TNF- α + cells in cultures under B cell subsets depletion compared with controls. No differential effect was observed with the presence of the IL-10R blockage.

Conclusion: These results suggest that atherosclerotic patients present altered regulatory capacity of some circulating B cells, which could contribute to the chronic inflammatory process present in atherosclerotic plaques.

Role of microparticles from patients with systemic lupus erythematosus in the induction of inflammatory mediators in monocytes

Karen Álvarez¹, Blanca Lucía Ortiz¹, Adriana Vanegas-García³, Carlos H. Muñoz-Vahos³, Mauricio Rojas^{1,2}, Gloria Vásquez¹

¹ Grupo de Inmunología Celular e Inmunogenética, Facultad de Medicina, Universidad de Antioquia, calle 70A No. 52-21, Medellín-Colombia.

² Unidad de Citometría de Flujo, sede de Investigación Universitaria, Universidad de Antioquia, calle 70A No. 52-21, Medellín-Colombia.

³ Grupo de Reumatología de la Universidad de Antioquia.

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Background: Microparticles (MPs) are small vesicles mainly produced during cell activation and apoptosis; they contain several components of parent cells and possess a wide spectrum of biological activities on intercellular communication. Higher levels of MPs have been reported in SLE and distinct groups of proteins, especially immunoglobulins, complement components and damage-associated molecular patterns (DAMPs) are increased in MPs from SLE patients. Several studies suggest that the recognition of MPs by immune cells could contribute to the chronic inflammatory process seen in SLE. We propose that MPs can be internalized by different populations of peripheral blood mononuclear cells (PBMC). It is known that monocytes contribute to the clearance and depuration of dead cell-derived materials, and have an important role in the development of SLE. We propose that MPs from SLE patients can be internalized by monocytes and can induce the production of inflammatory mediators.

Methods: MPs were isolated from platelet-poor plasma from SLE patients and healthy controls. We evaluated by flow cytometry the union/internalization by PBMC of MP labelled with carboxyfluorescein succinimidyl ester. Monocytes were stimulated with MPs from different source. Cytokines (IL-1 β , TNF- α , IL-8, IL-6 and IL-10) and eicosanoids (prostaglandin E2 (PGE2) and Leukotriene B4 (LTB4)) production were determined by flow cytometry and ELISA, respectively.

Results: MPs were mainly internalized by monocytes rather than T and B lymphocytes. Monocytes and B lymphocytes of SLE patients presented decreased MP union/internalization compared to healthy controls. Monocytes treated with patients-derived MP exhibited high IL-6, IL-1 β , LTB4 and PGE2 levels compared with monocytes treated with control-derived microparticles.

Conclusion: Circulating MPs from SLE patients could be internalized by monocytes inducing the production of inflammatory mediators, which could contribute to the chronic inflammatory process seen in SLE.

Association between carbamylated antibodies to specific epitopes of the human fibrinogen β -chain in early Rheumatoid Arthritis

Chila-Moreno L.³, Rodríguez L.S.⁴, De Ávila J.³, Bautista-Molano W.^{2,3}, Bello-Gualtero J.M.^{1,2}, Romero-Sánchez C.^{1,2,3}

¹ Hospital Militar Central, Bogotá-Colombia.

² Universidad Militar Nueva Granada, Bogotá-Colombia.

³ Universidad El Bosque, Bogotá-Colombia.

⁴ Pontificia Universidad Javeriana.

Background: Post-translational modifications such as carbamylation in rheumatoid arthritis (RA) is involved in the generation of auto-antigens, carbamylated proteins change their lysines by homocitrulines and activate T cells inducing the formation of autoantibodies. Recently anti carbamylated antibodies are associated with the severity of the disease, however, the precise target antigen of anti-CarP antibodies has not been elucidated. The objective of this study was to determine the frequency of specific antibodies directed against a homocitrulinated peptides of the fibrinogen beta chain and associate them with rheumatic indices in patients with early RA (ARt).

Methods: A cross-sectional study involving 51 RA Patients with the diagnosis of early rheumatoid arthritis (eRA) were studied at the Hospital Militar Central in Bogota-Colombia. Three peptides of the β chain of human fibrinogen were selected, and the epitope prediction was made by Bitered, BepiPred 1.0 for B cell epitopes, and for the selection of T epitopes, the ProPred and MHCpred programs, the epitopes that having a BCPreds cutoff score >0.8 were selected, to finds regions of local similarity between sequences we used BLAST Uniprot. We synthesized a native peptide (anti-Fib1) 37-52 [NEEGFFSA RHRPLDKK] as a control and two modified peptides (anti-Ca-Fib2) [NEEGFFSAHomoCitHRPLDKK] with a homocitrulin at position 45 and (anti-Ca-Fib3) with a homocitrulline at position 52. Regions of similarity between sequences were analyzed by PSI-BLAST Uniprot- Disease activity was assessed by scales DAS 28. The comparison was made by McNemar and Wilcoxon test and the association was made by chi-square, fisher test and U Mann Whitney. this study was support by the Hospital Militar Central -2015-047..

Results: Fifty-one patients diagnosed with eRA were evaluated, had an mean age of 48.55 ± 10.93 years, the highest proportion were women in 80.4%. The presence of comorbidities was in 54.9% of patients. A higher frequency of anti-FCS-Carp positive was found in the group of eRA compared to the control group (CTRL) (47.1% vs. 17.8%, respectively, OR 7.6 95% CI: 2.3-39.88 p = 0.0001). The frequency of the

antibodies against the carbamylated peptides was as follows: the anti-Ca-Fib2 positives were also found in a higher percentage 47.1% eRA vs 13.7% in CTRL (OR 6.6 95% CI: 1.97-35.0 $p = 0.0005$) while no differences were found in the frequency of anti-Ca-Fib3 between eRA and CTRL. (31.4% vs 21.6% respectively OR 1.71 95% CI: 0.62-5.13, $p = 0.359$), additionally, anti-Fib1 $\geq 1/50$ antibody titers were found in 68.6% of ART vs. 15.7% in CTRL (OR 6.4 95% CI: 2.47- 21.04 $p = 0.001$ showing the autoimmune burden in this disease. The anti-Ca-Fib2 antibodies were associated with positive RF ($p = 0.022$) and with ACPA > 60 U ($p = 0.032$), The anti-Ca-Fib3 was associated with the activity index of the disease DAS28-VSG ($p = 0.046$), Statistically significant differences were found between the presence of anti-Ca-Fib3 and anti-Ca-Fib2 antibodies ($p = 0.001$), no associations were found with the anti-peptide antibodies with serological markers, however the 23,52% had RF+ APCA+ anti-Ca-Fib2+ and 1,72% had RF+ APCA+ anti-Ca-Fib3+..

Conclusions: humoral response in ARt may be restricted to specific regions of the fibrinogen β - chain that contain homocitrullines and may be considered in the future as a biomarker of this disease in a subgroup of patients. In addition, the response of the different peptides was not similar, point toward that the carbamylation site may have an influence on the recognition and generation of these antibodies.

Sulfasalazine promote the production of IL-17 by CD8⁺ T cells from human immunodeficiency virus-1 (HIV-1)-infected patients on highly active antiretroviral therapy (HAART), *in vitro*

Manuel G. Feria¹, Federico Perdomo-Celis¹, Natalia A. Taborda^{1,2}, María T. Rugeles¹

¹ Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia, UdeA, Medellín, Colombia.

² Grupo de Investigaciones Biomédicas Uniremington, Programa de Medicina, Facultad de Ciencias de la Salud, Corporación Universitaria Remington, Medellín, Colombia.

Immune activation, main immunopathogenic mechanism of HIV-1 infection, is caused by several factors, including intestinal microbial translocation. This phenomenon, at least in part, is due to the decrease and/or dysfunction of interleukin (IL)-17-producing T cells, a cytokine that promotes the integrity of the intestinal barrier. Among IL-17-producing cells, it has been described a population of CD8⁺ T cells (Tc17 cells); however, its relation with microbial translocation and immune activation during HIV-1 infection is unknown. Until now, we had demonstrated that during HIV-1 infection there is a lower frequency of circulating Tc17 cells that are characterized by an activation state and by their ability to produce low levels of IL-17, despite of the viral

suppression induced by HAART. Therefore, the search of therapies that modulate this process and reverse the dysfunctionality of Tc17 cells, constitute an important field of research. In this sense, there is the possibility that sulfasalazine (SSZ), an anti-inflammatory component used empirically, but successfully in HIV-1-infected patients, can restore the function of Tc17 cells, contributing to decrease microbial translocation. Thus, here we aimed to evaluate the effect of SSZ on the immune activation and the production of IL-17 by Tc17 cells from HIV-1-infected patients under viral suppression by HAART. In peripheral blood mononuclear cells (PBMC), from HIV-1-infected patients on HAART and healthy donors, treated with LPS in the presence or absence of 1mM SSZ and stimulated with PMA and ionomycin, the immune activation (expression of HLA DR and CD38) and the production of IL-17 in Tc17 cells were determined by flow cytometry. Additionally, in the supernatants of cell cultures, the IL-1 β levels were quantified by ELISA. The immunomodulatory effect of SSZ was evidenced by the decrease in the IL-1 β levels in supernatants of cell cultures. Additionally, it was observed that SSZ induced the death of HLA-DR⁺ CD38⁺ CD8⁺ T cells and an increase of the frequency of total Tc17 cells in HIV-1 infected patients. Similarly, the frequency of IL-17-producing HLA-DR⁻ CD38⁺, HLA-DR⁺ CD38⁺ and HLA-DR⁺ CD38⁻ CD8⁺ T cells increased after SSZ treatment. Remarkably, after the treatment with SSZ, the frequency of activated HLA-DR⁺ CD38⁺ Tc17 cells from HIV-1-infected patients reached similar levels to those obtained in healthy individuals. Finally, in the presence of SSZ, the frequency of HLA-DR⁺ CD38⁺ Tc17 cells was positively correlated with the frequency of non-viable HLA-DR⁺ CD38⁺ CD8⁺ T cells and negatively correlated with the frequency of total HLA-DR⁺ CD38⁺ CD8⁺ T cells and IL-1 β levels. Thus, we suggest that SSZ inhibits the release of IL-1 β , limiting the activation of CD8⁺ T cells, favoring the increase of the frequency of Tc17 cells.

Humoral immune response against synthetic peptides derived from LACK and KMP-11 proteins from *Leishmania* spp.

Sánchez Lizeth Paola, Flórez Magda Melissa, Delgado Gabriela

Grupo de Investigación en Inmunotoxicología, Departamento de Farmacia, Universidad Nacional de Colombia.

Introduction: Leishmaniasis is a parasitic disease caused by the intracellular protozoan *Leishmania* spp., and it affects about 1.2 million people in the world, including Colombia, where about 10,000 cases occur each year, being the most prevalent clinical form cutaneous leishmaniasis in 95-98% of cases. As a control strategy for this disease it is necessary to identify epitopes of the parasite, that can be useful as well as candidates for more sensitive and specific diagnosis than conventional ones, by using synthetic

peptides. Therefore, our objective was to evaluate the production of antibodies against synthetic peptides derived from LACK and KMP-11 proteins of *Leishmania spp.* in patients with a clinical history of leishmaniasis.

Materials and Methods: The presence of total IgG as well as the subclasses of IgG was evaluated in sera against 5 synthetic peptides derived from LACK protein and 5 from KMP-11 protein of *Leishmania spp.* The human volunteers were classified in different clinical groups according to their history of leishmaniasis as follows: *i*) individuals with active cutaneous leishmaniasis; *ii*) Individuals positive leishmanin skin test without clinical signs; *iii*) leishmanin skin test negative individuals; *iv*) individuals with completed treatment and *v*) individuals with Chagas disease. The antibodies were detected by ELISA technique.

Results: The evaluation of reactivity of the sera against the soluble antigen of *Leishmania* (SLA), showed antibody titers in 66.6% of active individuals, in the other groups no detectable antibody titres were found. 50% of reactivity in active leishmaniasis volunteers were observed against each LACK and KMP-11 recombinant proteins. On the other hand, from the 10 synthetic compounds evaluated derived from LACK and KMP-11, LACK_{xx-xx}, KMP-11₆₁₋₈₀ and LACK₁₂₁₋₁₄₀ showed the highest reactivity in sera from individuals with active cutaneous leishmaniasis, (77.7%, 55.5% and 44.4% respectively). Additionally, the cross-reaction with Chagas disease ranged between 0-20%. About the detection of IgG subclasses, it was observed that for SLA, the 4 subclasses of IgG were found, with a slight increase of IgG3. Against KMP-11₆₁₋₈₀ was observed predominance of IgG1 and IgG4, for LACK_{xx-xx} was observed predominance of IgG3 and slightly IgG1 and finally, LACK₁₂₁₋₁₄₀ did not show a characteristic subclass pattern.

Conclusions: We conclude that, the identification of peptide sequences was achieved, as natural epitopes in the infection by *Leishmania spp.*, representing, immunodominant linear epitopes of the proteins LACK and KMP-11, in human B cells, since they induce a marked production of antibodies after natural exposure to the parasite. The experimental approach used here may be useful for the characterization of immunogenic epitopes for the study of diagnostic candidates for leishmaniasis.

Immunoproteomic identification of the G3PDH and P α 3S proteins as potential candidates for a molecularly-defined vaccine against cutaneous leishmaniasis

Christian Piedrahíta-Ochoa, Lina Orrego, Paula Correa, Janny Villa, José R. Ramírez-Pineda

Grupo Inmunomodulación (GIM), Universidad de Antioquia.

Cutaneous leishmaniasis (CL) is a highly prevalent parasitic disease in tropical countries like Colombia. Current control strategies against CL are suboptimal and the most promising preventive strategy that can be envisioned is the development of an effective vaccine. By using a murine model of LC by *Leishmania (Viannia) panamensis* (Lp) we observed the development of progressive cutaneous ulcers during the first 5-6 weeks, with a subsequent plateau and stabilization by the weeks 7-10 post-infection. Lesion stabilization was associated with maximal levels of IFN- γ production in spleen cells. Moreover, mice cured the lesions by week 15-20 and were immune to reinfection. Based on these observations, we hypothesized that a humoral immunoproteomic approach that detects IgG2a antibodies would allow the identification of IFN- γ -inducing antigens and vaccine candidates. BALB/c mice were infected and the auto-resolutive and immunity-inducing character of the model was confirmed. By using ELISA and western blot, increasing anti-Lp IgG2a antibody reactivity was observed, with maximal levels at the 9th week post-infection. With serum pools from the same animals we identified 22 antigens with a preferential IgG2a immunoreactivity by using 2D/GE/MS and western blot. Two antigens, the Glyceraldehyde 3 Phosphate Dehydrogenase (G3PDH) and the Proteasome 3 α Subunit (P α 3S) were selected to be cloned and produced as recombinant proteins. Polyclonal sera were also risen against recombinants and both were shown to recognize proteins with the expected molecular weight and localization in parasite lysates and intact promastigotes by immunoblot and immunofluorescence microscopy, respectively. G3PDH and P α 3S-reactive IgG2a antibodies were evidenced in serum samples from infected mice by ELISA and immunoblot, validating the pattern previously observed with native parasite protein in 2D gels. Of more importance, spleen and/or lymph node cells from 10-week-infected mice were stimulated to produce IFN- γ in response to recombinants or to pools of overlapping synthetic peptides derived from the protein sequences. Finally, serum samples from cured/reinfected mice recognized rG3PDH and rP α 3S with a biased IgG2a over IgG1 reactivity. Altogether, our results indicate that using an humoral immunoproteomic approach that detects IgG2a antibodies is possible to identify antigens able to induce IFN- γ (Th1 response) in infected mice, which, in turn, can be proposed as potential vaccine candidates for a molecularly-defined vaccine. The prophylactic efficacy of the G3PDH and P α 3S proteins is currently being investigated.

PRESENTACIONES EN FORMA DE PÓSTER

Prediction of binding of Peptidyl arginine deiminase citrullinated peptides of *Porphyromonas gingivalis* with hypervariable regions of Immunoglobuline G. A Rheumatoid arthritis and periodontal disease model: In-silico Docking approach

Mónica Acevedo^{1,2}, Paula Hernández^{1,5}, Adriana Amaya^{1,2}, Diana M. Castillo P.², J.E. Castellanos^{1,4}, J. Bello-Gualtero³, W. Bautista-Molano^{2,3}, Consuelo Romero-Sánchez^{1,2,3}

¹ Master in Biological Science Program, Universidad El Bosque, Bogotá, Colombia.

² Unit of Basic Oral Investigation (UIBO), School of Dentistry, Universidad El Bosque, Bogotá, Colombia.

³ Rheumatology and Immunology Department, Hospital Militar Central. Clinical Immunology Group, School of Medicine, Universidad Militar Nueva Granada, Bogotá, Colombia.

⁴ Virology Group, Universidad El Bosque, Bogotá, Colombia.

⁵ Parasitology Lab., Universidad El Bosque, Bogotá, Colombia.

Introduction: Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease characterized by the presence of IgG against citrullinated peptides which are the most specific clinical markers used in the diagnosis of the disease. The periodontal disease commonly caused by *Porphyromonas gingivalis* is associated with RA. These bacteria express Peptidylarginine Deiminase (PPAD); this enzyme is capable of exchanging arginine for citrulline in some proteins. Similar situation occurs in human PAD. Such modifications can alter the tertiary structure and expose epitopes that can be recognized by IgG antibodies.

Objective: To evaluate the geometric complementarity and estimates the interface area of the complex and atomic contact energy (ACE) between PPAD peptides with the hypervariable regions of IgG(HVG) in the in-silico docking model.

Methods: Four peptides of PPAD epitopes recognized by B cells were selected on Bcepred y ABCpred and for T lymphocytes on ProPred and MHCpred: 1. native, 2. with citrullination in Arg1 of the C-terminal end, 3. citrullination in Arg1 of C-terminal and the Arg8 and 4. with aminoacid sequence in random disposition. 3D modelling of peptides was carried out using PEP-FOLD Server and the citrullines were added with ACD/ChemSketch. The modelling of variable domains in HVG(GenBank:CAB44207.1) and light chain(GenBank:BAE98205.1) was obtained with the SwissModel server. The amino acids of the HVG capable of interacting were defined using The ConSurf Server tool. The peptides and variable domains were prepared using Openbabel. Docking used Patchdock. The PyMOL tool was used to rendering the structural complexes.

Results: The native peptide1 has a higher score in the interaction with the light chain, and the heavy chain shows a higher score with the peptide3. Peptide3 has lower ACE for both heavy and light chain therefore interaction is more stable. Aminoacids of contact area of heavy chain that interact with all peptides are Tyr50, Leu98, Trp103, Asn104, Gly106, Phe107, Asp108 and of the light chain are Ser95, Pro96, Gly97, Phe98 and Gly99. The interactions of the light chain and peptide1 were: Arg1 interacts with Gly100, and Arg8 with Glu1 and for peptide3: Cit8 with Thr10 (Cit1 has no contact). Regarding the heavy chain, peptide1 interacts with Arg1 and Trp47 while for peptide3: Cit1 with Lys43, and Cit8 with Pro40. The citrullinated residues of Peptide2 do not interact with the amino acids of HVG but they do with others amino acids in the contact area.

Conclusions: The In-silico model shows that bacterial PPAD peptides with and without citrullination were capable of interacting with human HVG. The citrulline influence the contact area between the hypervariable regions of heavy and light chains. Peptides with modifications of two arginine residues by citrulline, present a higher interaction score and lower ACE, predicting the recognition for IgG. This could be a proposal of RA and periodontal disease pathogenic interaction model.

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Microparticles from systemic lupus erythematosus and rheumatoid arthritis patients as inducers of endothelial damage

Laura Atehortúa Castro^{1,2}, Mauricio Rojas^{1,2}, Gloria Vásquez^{1,3}, Carlos H. Muñoz³, Adriana Vanegas³, Rafael Andrés Posada-Duque⁴, Diana Castaño^{1*}

¹ Grupo de Inmunología Celular e Inmunogenética, Instituto de Investigaciones Médicas, Facultad de Medicina, sede de Investigación Universitaria (SIU), Universidad de Antioquia UdeA, calle 70 No. 52-21, Medellín, Colombia.

² Unidad de Citometría, Facultad de Medicina, SIU, Universidad de Antioquia UdeA, calle 70 No. 52-21, Medellín, Colombia.

³ Sección de Reumatología, Hospital Universitario de San Vicente Fundación, calle 64 No. 51D-154, Medellín, Colombia.

⁴ Grupo de Neurociencias de Antioquia, Instituto de Biología, Facultad de Medicina, SIU, Universidad de Antioquia UdeA, calle 70 No. 52-21, Medellín, Colombia.

* Corresponding author: diana.castano@udea.edu.co.

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are autoimmune diseases with a high endothelial involvement of macro vasculature that manifests with the accelerated development of atherosclerosis and cardiovascular diseases. In addition, they also present an evident endothelial compromise of the micro vasculature, mainly in the organs where the ultrafiltration processes occur. It has been proposed that different components that are common to the immunopathology of RA and SLE, such as auto-antibodies and immune complexes (ICs), should participate in the endothelial damage observed in these patients. Taking this in consideration, we propose that microparticles (MPs), vesicular structures that are increased in the circulation of these patients and form IC (MPs-ICs), could play a role in the induction of endothelial damage. Therefore, our aim was to evaluate the effect of MPs and MPs-ICs from patients with RA and SLE in endothelial cells of micro and macro vasculature.

Primary endothelial cells were cultured for 24 h in the presence or absence of MPs and MPs-ICs isolated from patients with RA and SLE and healthy controls. The production of soluble mediators and the expression of CD54, CD102 and HLA-DR were evaluated by ELISA and flow cytometry, respectively. In addition, the adhesion of classical and non-classical monocytes to the endothelium, the structure and arrangement of the endothelial monolayer (actin filaments and expression of VE-Cadherin), as well as the viability of these cells were studied by epifluorescence microscopy. Finally, the permeability of the endothelial monolayer to dextran was measured by spectrofluorometry.

It was found that MPs and MPs-ICs induced the expression of CD54 and CD102, the production of IL-6 and IL-8 cytokines, and CCL2 and CCL5 chemokines in macro and micro vasculature cells; a higher proportion of classical monocytes was adhered to macro vascular endothelial cells previously treated with MPs and MPs-ICs. Also, these vesicles decreased the expression of VE-cadherin in membrane, induced the depolymerization of actin filaments, and increased the number and size of intercellular spaces. In addition, some endothelial cells died in response to these vesicles, evidenced with fragmentation and condensation of nuclei. These results can explain the increase in endothelial permeability that was observed in response to MPs and MPs-ICs from these patients.

These results show that MPs and MPs-ICs from patients with RA and SLE induce the activation and damage of endothelial cells and participate in the recruitment and adhesion of monocytes to these cells. The increase in the amount of these structures in the circulation of these patients could contribute to endothelial dysfunction, the increase in atherosclerotic plaques, as well as facilitate the recruitment of leukocytes to the organs that are affected in these diseases.

Subpopulations of peripheral blood monocytes in individuals with chronic Chagas disease

Sergio Gómez-Olarte^{1,2}, Natalia Bolaños¹, Mariana Echeverry¹, Juan Vargas¹, Gabriel Durán¹, Adriana Cuéllar³, Concepción Puerta⁴, Alejandro Mariño⁵, John González¹

¹ Grupo de Ciencias Básicas Médicas, Facultad de Medicina, Universidad de los Andes, Bogotá, Colombia.

² Departamento de Ciencias Biológicas, Facultad de Ciencias, Universidad de los Andes, Bogotá, Colombia.

³ Grupo de Inmunobiología y Biología Celular, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia.

⁴ Laboratorio de Parasitología Molecular, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia.

⁵ Clínica de Falla y Trasplante Cardíaco, Hospital Universitario San Ignacio, Bogotá, Colombia.

Introduction: Human monocytes are a heterogeneous cellular population participating in tissue inflammation, repair and patrolling. This cellular population has been classified into three subsets with differential functions based on the expression of CD14 (LPS receptor) and CD16 (low affinity receptor for IgG): classical (CD14++ CD16), intermediate (CD14++ CD16+) and non-classical (CD14+ CD16++). Changes in the percentage of peripheral blood monocytes have been associated with the pathophysiology of viral, bacterial and parasitic infections. It was reported the expansion of CD14+ CD16+ monocytes in asymptomatic children, adults with acute and recent chronic infection by *T. cruzi*. However, variations in the percentage of monocyte subpopulations in chronic Chagas disease have not been evaluated. Therefore, this work is aimed to study if there is any pattern in the alterations of monocytes subpopulations, this could allow the identification of cellular markers for the follow-up of chronically infected individuals.

Materials and methods: The study included Colombian adults with chronic Chagas disease (CCC) who attended the Heart Failure service at the University Hospital San Ignacio (HUSI) in Bogotá between 2015 and 2018, as well as patients with non-Chagas cardiomyopathy (CNC) and healthy individuals without antibodies to *T. cruzi* (CS) paired by sex and age, as control groups. Individuals with history of neoplasms, autoimmune or endocrine diseases and acute infections were excluded. All volunteers were screened by two serological tests for antibodies against *T. cruzi*, also clinical and cardiac functional evaluation were carried out. Peripheral blood mononuclear cell (1×10^6 cells per tube) were obtained, blocked with anti-CD16/32 and human serum

AB, and labeled with anti-CD14 PE, anti-CD16 PE-Cy7 and anti-HLA-DR FITC or the corresponding isotype control. 50,000 events were acquired in a FACSCanto II flow cytometer on the HLA-DR+ population, and the CD14/CD16 subpopulations were gated. The comparison between multiple groups were performed using the Kruskal-Wallis test followed by Dunn's post hoc test in the GraphPad Prism 7 software ($p < 0.05$).

Results: The analysis included 21 samples of patients with Chagas disease, 13 with non-Chagas cardiomyopathy and 11 healthy. The percentage of classical monocytes decreased in patients with chronic Chagas disease ($84.8 \pm 4.5\%$) compared with non-chagasic cardiopaths ($90.2 \pm 4.6\%$, $p=0.0147$) and healthy controls ($89.1 \pm 3.3\%$; $p=0.0225$), while the percentage of the intermediate subpopulation increased in chronic chagasic patients ($10.2 \pm 3.5\%$) compared with non-chagasic cardiopaths ($5.5 \pm 2.3\%$, $p=0.0020$) and healthy individuals ($6.5 \pm 2.0\%$, $p=0.0339$). The analysis of the CD16+ subpopulation (intermediate and non-classical monocytes), named inflammatory, revealed an increase in chronic chagasic patients ($15.2 \pm 4.5\%$) compared with non-chagasic cardiopaths ($9.8 \pm 4.6\%$; $p=0.0147$) and healthy individuals ($10.9 \pm 3.3\%$, $p=0.0225$) (Fig. 1). When we analyzed multiple samples of three patients with chagasic cardiomyopathy, it was observed individual changes in the percentages of monocyte subpopulations according to the time (months or years) of sampling (Fig. 2).

Conclusions: Chronic Chagas patients have an increased percentage of inflammatory monocyte populations (intermediate and CD16+) and a reduction in reparative (classical) monocytes. These variations in the monocytes subpopulations could be correlated with clinical features of the disease.

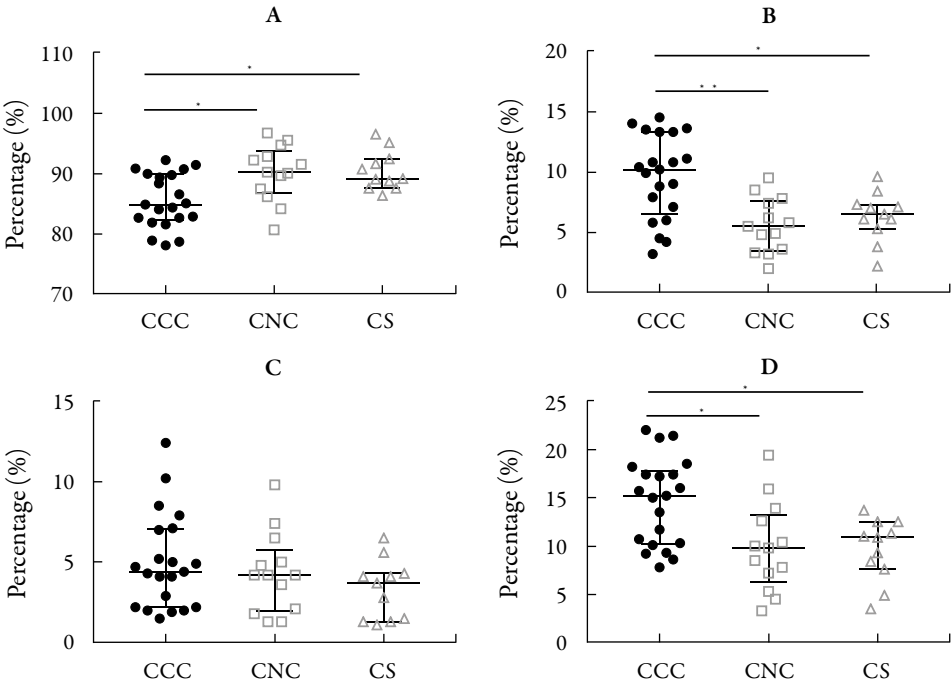


Figure 1. Percentage of subpopulations of classical (A), intermediate (B), non-classical (C) and inflammatory (D) monocytes shown as median and interquartile ranges. * $p<0.05$ and *** $p<0.01$. CCC: chronic chagasic cardiomyopathy; CNC: non-Chagas cardiomyopathy; CS: healthy controls.

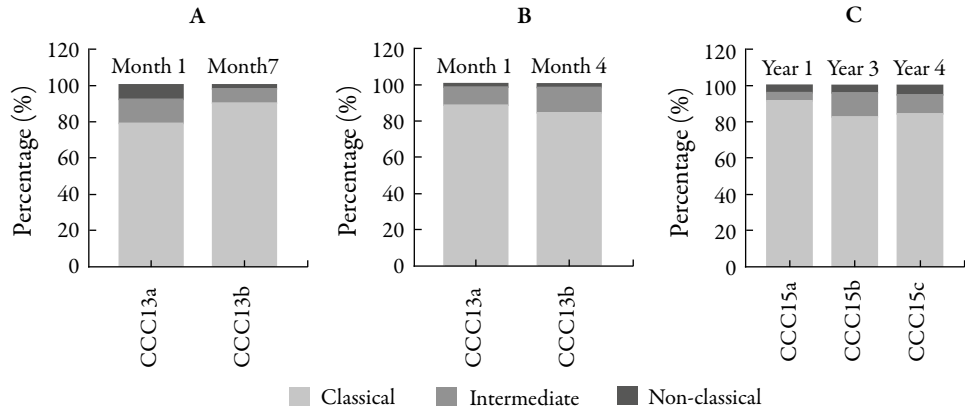


Figure 2. Variation of the percentages of monocyte subpopulations in three patients (A, B and C) with Chagas disease. The analysis were performed with samples taken in a seven months interval (CCC13), four months interval (CCC14), two years between samples a-b and one year between samples b-c (CCC15). CCC: chronic chagasic cardiomyopathy.

Levels of IFN- γ and proinflammatory cytokines in bronchoalveolar lavage of patients with suspected of pulmonary tuberculosis with negative sputum smear

Paula X. Losada¹, Federico Perdomo¹, Marcela Castro^{1,2}, Carol Salcedo^{1,2}, Giovanni Lastra^{1,2}, Carlos E. Narváez¹

¹ Programa de Medicina, Facultad de Salud, Universidad Surcolombiana, Neiva, Colombia.

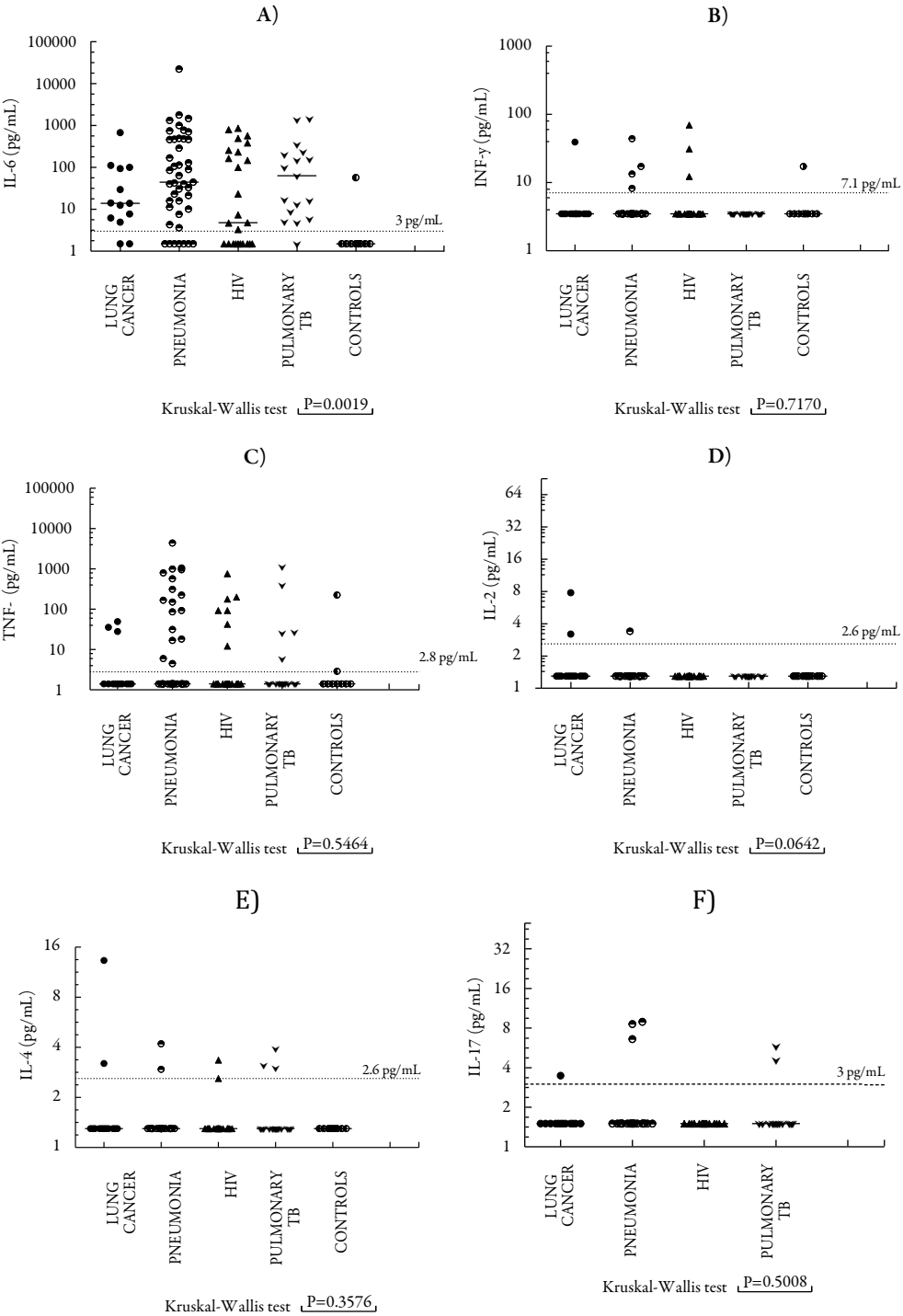
² Hospital Universitario de Neiva, Neiva, Colombia.

Pulmonary diseases are a common reason for consultation and one of the most important disease is the pulmonary tuberculosis (TB), which is a cause of relevant morbidity and mortality worldwide. In Colombia, *Mycobacterium tuberculosis* infection is considered a public health problem, as our country reports about 12,000 TB cases annually. The diagnostic methods currently available have limitations that don't allow an efficient diagnosis for the timely and accurate management of the disease. Thus, new diagnostic alternatives are necessities. Some immune factors such as cytokines locally released, have been evaluated as complementary diagnosis method. Here, we propose to evaluate whether level of locally secreted proinflammatory cytokines are related to pulmonary TB or other pulmonary diseases. We evaluated samples of Bronchoalveolar lavage (BAL) collected from 107 patients older than 15 years with initial suspicion of TB with negative serial smear. Diagnostic Fibrobronchoscopy was performed in the Pulmonology unit of the Hospital Universitario de Neiva during the years 2014-2016. They were classified within the groups of pulmonary TB, pneumonia, lung cancer and HIV infection. Each of them was documented on the study and procedure and signed the informed consent. Using a commercially available ELISA kit and flow cytometry, the amount of the IL-17, IL-2, IL-4, IL-6, IL-10, IFN- γ and TNF- α , in BAL were quantified. The clinical and epidemiological characteristics of the patients indicate that the group with HIV infected patients were younger than other groups. The weight loss is more frequently reported in patients with lung cancer as described previously (Table 1). The most of the samples measured were lower than limit detection established for the evaluated cytokines except the IL-6 and the detected concentrations do not allow establishing the specific relationship with any of the pathologies analyzed (Figure 1). Funded: Universidad Surcolombiana.

Table 1. Clinical and epidemiological characteristics of the patients.

| PARAMETERS | PULMONARY TB | NON TB PULMONARY DISEASES | | | CONTROLS | P |
|-----------------------------|-------------------|---------------------------|-----------------|-----------------|------------|---------|
| | | PNEUMONÍA LUNG CANCER | HIV | | | |
| | (A) | (B) | (C) | (D) | (E) | |
| | n= 17 | n=43 | n= 13 | n=25 | n= 9 | |
| AGE[Years] (median, range) | 41(21-67) | 58(17-88) | 58 (21-79) | 35 (23-69) | 53(21-70) | 0,003 a |
| WEIGHT [Kg] (median, range) | 53 (42-75) | 60 (20-90) | 60(50-67) | 59 (40-66) | 62 (41-76) | 0,457 a |
| BMI (median, range) | 19(15-27) | 21 (12-39) | 22,3(15,9-27,2) | 20,8(14,3-24,2) | 22(16-28) | 0,361 a |
| SIGNS Y SYMPTOMS | Sweating (n, %) | 4 (23%) | 5 (38%) | 6 (24%) | 1 (8%) | 0,099 b |
| | Hyporexia (n, %) | 6 (35%) | 5 (38%) | 3(12%) | 2 (15%) | 0.113 b |
| | Fever(n, %) | 12 (70%) | 32 (74%) | 5 (38%) | 16 (64%) | 0.046 b |
| | Cough (n/ %) | 13 (76%) | 37 (86%) | 12 (92%) | 22 (88%) | 0.762 b |
| | Hemoptysis (n, %) | 5 (29%) | 11(25%) | 3 (23%) | 1 (4%) | 0.120 b |
| | Weightloss (n, %) | 6 (35%) | 15(35%) | 10 (77%) | 14 (56%) | 0.021 b |
| | Dispnoea (n, %) | 6 (35%) | 27 (63%) | 8 (61%) | 6 (53%) | 0.081 b |

a Kruskal-Wallis test
b Chi-cuadrado test



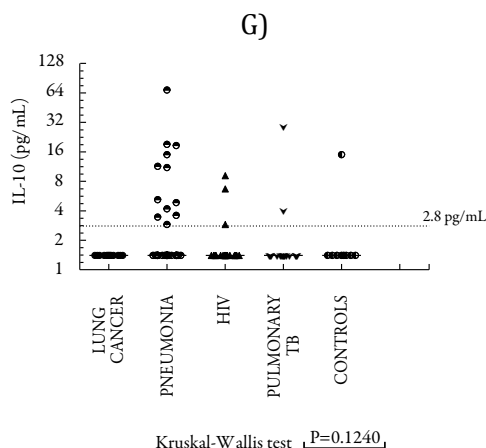


Figure 1. Levels of Proinflammatory cytokines in BAL. Local levels of interleukin (IL)-1, IL-2, IL-4, IL-6, IL-10, IFN- γ and TNF- α , were evaluated by ELISA and flow cytometry in patients with pulmonary TB and other respiratory diseases. Dashed line represented the sensitivity limit of the assay. P value of Kruskal-Wallis test is shown. Each point represents an independent patient. The dotted line represents the sensitivity of the assay.

Genotypic and haplotypic frequencies of Dickkopf-1 gene polymorphisms in patients with early rheumatoid arthritis compared to healthy Colombian controls

Mónica Acevedo-Godoy¹, Alex Darío Cardona-Rincón², Sandra J. Perdomo-Lara¹, Lorena Chila-Moreno¹, Wilson Bautista-Molano^{1,2}, Philippe Chalem Ch.³, Juan Manuel Bello G.², Consuelo Romero-Sánchez^{1,2}

¹ Unit of Basic Oral Investigation (UIBO), Faculty of Dentistry, Universidad el Bosque, Bogotá-Colombia.

² Rheumatology and Immunology Service Hospital Militar Central/Clinical Applied Immunology Group -Faculty of Medicine Universidad Militar Nueva Granada, Bogotá-Colombia.

³ Foundation Institute of Rheumatology Fernando Chalem, Bogotá, Colombia.

Introduction: Rheumatoid arthritis (RA) and periodontal disease (PD) are destructive chronic inflammatory disorders characterized by the dysregulation of the inflammatory response of the host sharing common bone processes. There are few studies on the role of the Wnt pathway in bone loss in patients with RA and diagnosis of PD. DKK1 is a regulator of bone mass and joint remodeling.

Objectives: To evaluate the genotypic and haplotypic frequency of DKK1 polymorphisms related to bone resorption in patients with early RA (eRA) and healthy controls and its association with indexes of periodontal and rheumatic activity.

Methods: 63 patients with eRA and 67 controls were analyzed. X-rays of the hands and feet were evaluated. The DKK1 polymorphisms (rs1896368, rs1896367, rs1528873)

were evaluated by High Resolution Melting and confirmed by sequencing. Autoantibodies, markers of serological and clinical activity and HLADRB1 were measured. Fisher's exact tests and the Wilcoxon rank tests were used. A logistic model was used to analyze the associations between radiological findings, rheumatic activity, laboratory variables and the presence of DKK1 polymorphisms.

Results: 79.4% of the patients were women and 61.7% and 43.3% had positive rheumatoid factor and citrullinated peptide antibodies respectively. 23% had erosions in feet and 17% joint space narrowing. The healthy controls exhibited the same distribution of age and sex. The frequency of the single nucleotide polymorphism (SNP) rs367 was 43,4% and rs368 was 87,5% in eRA and controls. Polymorphism rs368 was associated with overweight ($p=0.041$). The homozygous genotype of polymorphism rs873 in eRA was 86.7% while in controls the heterozygote was 96.9% with a significant difference ($p=0.001$). Both patients with eRA and controls had polymorphic frequencies of 63.3% for 1 or 2 SNPs and 36.7% for 3 SNPs ($p=0.06$). No significant associations were found between the polymorphisms of DKK1 and rheumatic activity, however, the heterozygous genotype of rs367 was associated with greater frequency of erosions ($p=0.026$) and/or joint space narrowing ($p=0.005$) in feet. Both homozygous and heterozygous patients for rs368 had less radiological damage (OR, 0.04, 95% CI, 0.00-0.93, $p=0.05$). Individuals homozygous for rs367 and heterozygous for rs368 were diagnosed more frequently with PD ($p=0.009$ and $p=0.033$ respectively). No associations were found with the HLA DRB01.

Conclusions: There is genetic homogeneity in the SNPs of risk rs367 and rs368 associated with bone mass deterioration between patients with eRA and controls, with frequencies similar to those reported in healthy Colombian population. However, rs367 seems to be associated with a greater radiological compromise and rs368 confers protection against bone damage in Colombian patients with eRA and PD. The presence of haplotypes and genetic variants of DKK can be affected with polymorphisms of other genes involved in the Wnt pathway.

Preclinical development of a molecularly-defined liposomal vaccine against cutaneous leishmaniasis

David Ernesto Bautista-Erazo¹, Natalia García-Valencia¹, Verónica Guzmán-González¹, Gisela M. García-Montoya², José Robinson Ramírez-Pineda^{1,*}

¹ Grupo Inmunomodulación, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia.

² Centro Nacional de Secuenciación Genómica, Sede de Investigación Universitaria, Universidad de Antioquia, Medellín, Colombia.

* Correspondence: jrobinson.ramirez@udea.edu.co, ramirezpineda@yahoo.com

Cutaneous leishmaniasis (CL) is a neglected tropical disease that represents a serious public health problem in Colombia. Clinical, epidemiological and experimental evidence

suggests that an effective vaccine against CL is, in principle, possible. However, no effective vaccine for human use is currently available. Using a murine model of CL by *Leishmania (Viannia) panamensis* (LVp), the most prevalent species in Colombia, we found that: 1) synthetic CpG oligonucleotides work as a protective adjuvant when combined with total parasite lysate and 2) a liposomal formulation of soluble total antigen was also protective against parasite challenge. Additionally, the protein trypanothione reductase (TR) was identified as a promising vaccine candidate by using a reverse vaccinology strategy, thus suggesting the possibility to develop a molecularly-defined vaccine formulation. Following this line of research, it was proposed, as a general objective, to evaluate the prophylactic efficacy of both the soluble and micro/nanostructured formulation of the recombinant TR (rTR) combined or not with CpG (soluble/liposomal). First, vaccination was performed using different protocols and dosages of the components (rTR as antigen and CpG as an adjuvant) in two independent experiments. One month after the last booster, mice were infected, and the clinical follow-up was performed (Figure 1). It was observed that vaccination with soluble rTR and CpG induces partial protection against the infectious challenge; however, results were not completely reproducible, and a dose-dependent protection was not demonstrated. Hence, it is of great importance to evaluate whether efficacy of this vaccine candidate could be improved by using a particulate delivery system. To achieve this, protein stability tests were first performed to examine the feasibility of the encapsulation of rTR into liposomes, a process that includes the heating of the protein at high temperatures. Moreover, the preparation of the rTR- or CpG-loaded liposomes was standardized (Figure 2). Different factors involved in the preparation of the vesicles were analyzed and several response variables were studied, such as size, zeta potential, and encapsulation efficiency, to optimize those formulations. We found that rTR is feasible to be encapsulated in liposomes because it was not degraded nor form aggregates by thermal action under manufacturing conditions of the vesicles, so it was possible to standardize a platform for the preparation of micrometric cationic liposomes that encapsulate this protein. Furthermore, cationic liposomes of homogenous suprananometric size were prepared that encapsulate CpG with high efficiency. The resulting molecularly-defined liposomal formulations will be used to evaluate whether protective efficacy can be improved by using particulate delivery systems rather than soluble formulations.

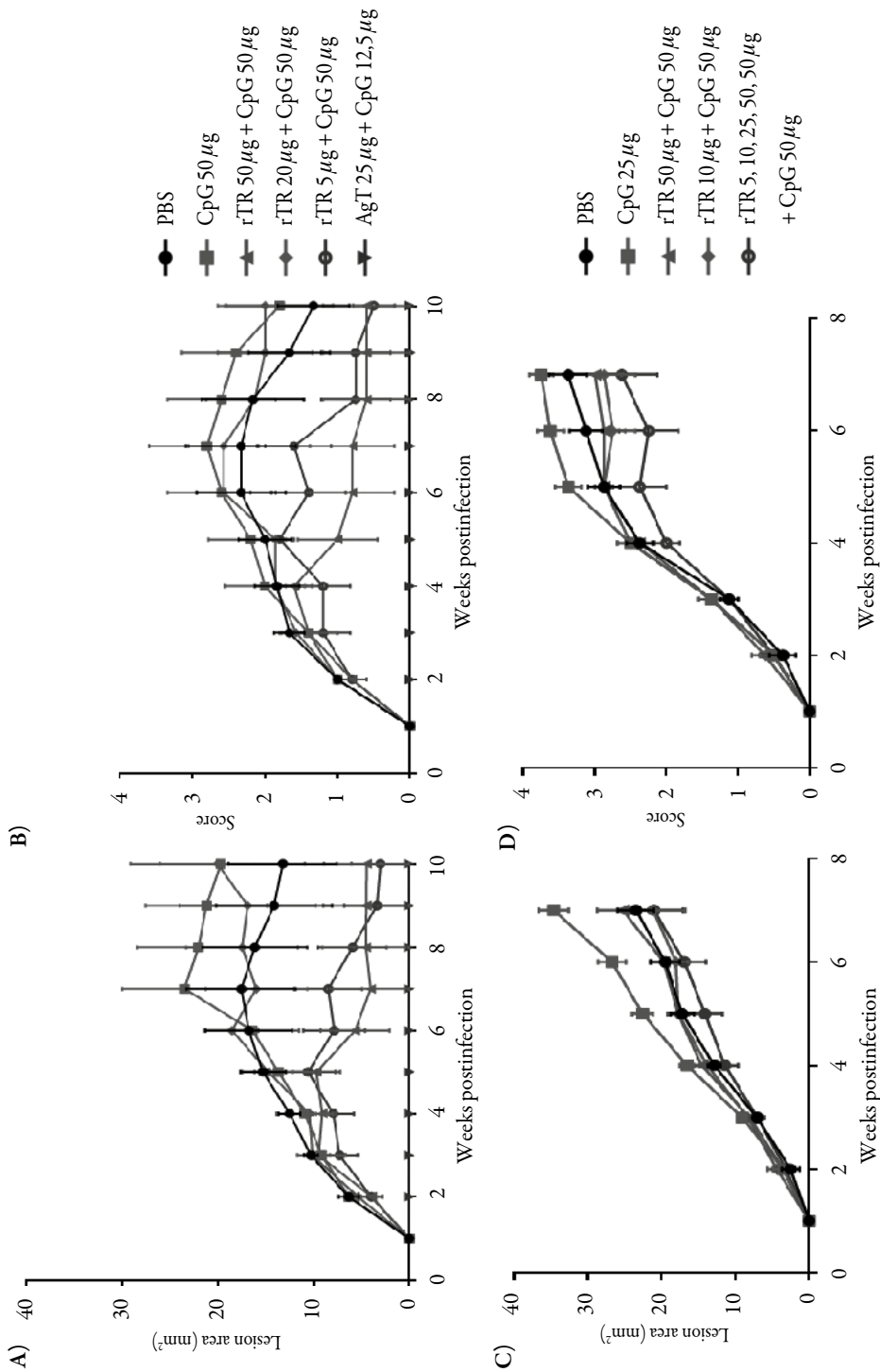


Figure 1. Prophylactic efficacy of soluble rTR. Female BALB/c mice were vaccinated subcutaneously at the base of the tail using different protocols and dosages of the components (rTR as antigen and CpG as adjuvant). One month after the last booster, the animals were infected with 10^5 parasites of *Leishmania (Viannia) panamensis* on the ear. The kinetics of the lesion area (A and C) and the clinical score (B and D) of two independent experiments are plotted. AgT: LVp total antigen.

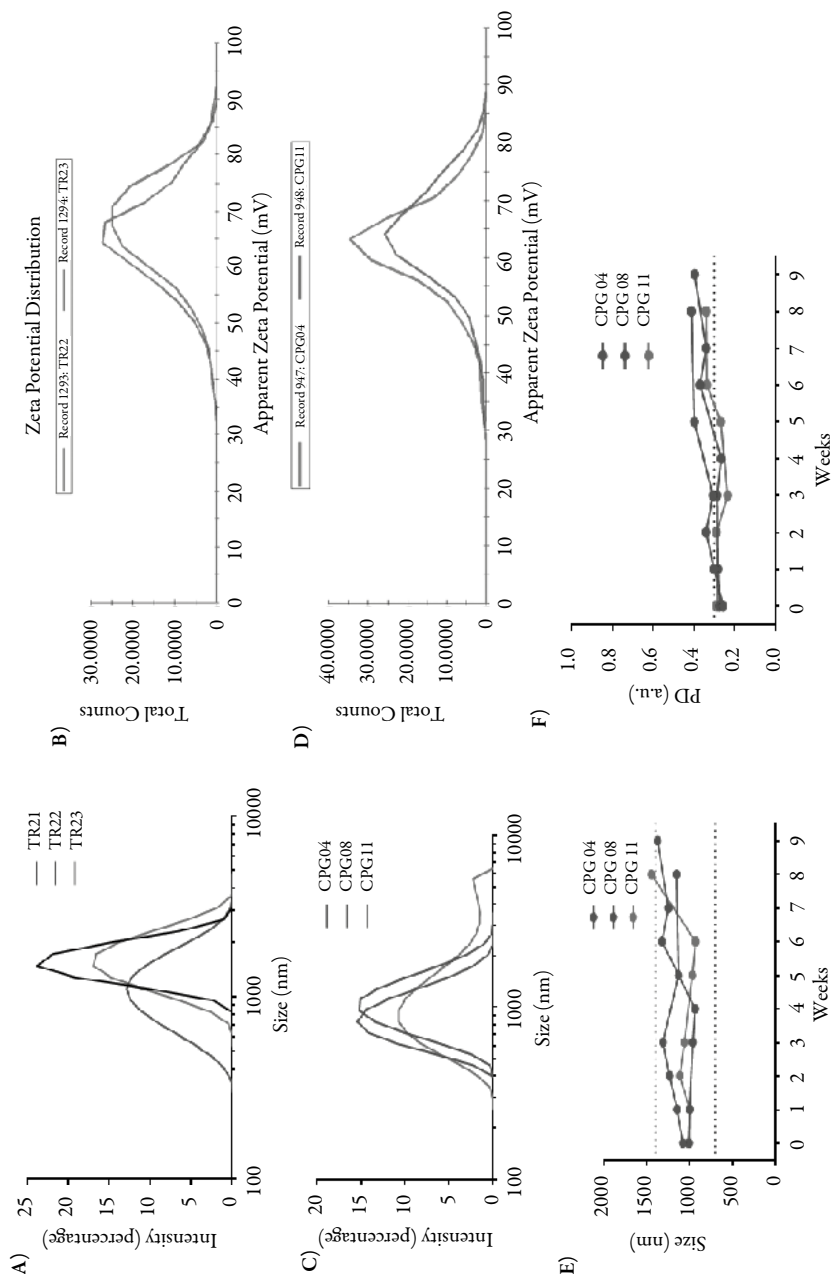


Figure 2. Cationic liposomes containing rTR and CpG. (A) Size distribution of three batches of rTR-loaded liposomes produced under the same conditions. Average PDI: 0.15. (B) Distribution of apparent zeta potential (mV) of two batches of rTR-loaded liposomes which show a similar approximate peak of +65 mV. (C) Size distribution of three batches of CpG-loaded liposomes. (D) Distribution of apparent zeta potential (mV) of two batches of CpG-loaded liposomes which show a similar approximate peak of +65 mV. (E-F) Stability of the CpG-loaded liposomes over time (weeks) in terms of variation of the (E) size (nm) and (F) PDI (a.u.). It is indicated in (E), with dotted horizontal lines, the sizes that are considered adequate: between 700 nm and 1400 nm. In (F), the dotted horizontal line indicates the maximum value of PDI considered acceptable: 0.3. PDI: polydispersity index, a.u.: arbitrary unit.

Standardization of a protocol for RNA isolation from urinary sediment for the search of biomarkers involved in acceptance or rejection of the kidney allograft

Carlos Andrés Carmona, Cristiam Mauricio Álvarez

Grupo de Inmunología Celular e Inmunogenética, Instituto de Investigaciones Médicas, Facultad de Medicina, Universidad de Antioquia.

In spite of the improvement in the management of immunosuppressive treatments as well as in the understanding of the role of the immune system in kidney allograft outcomes, rejection is still the most important limitation for renal graft function and survival.

The growing number of patients requiring a kidney transplant in contrast to the notable decrease in the number of donors, together with the occurrence of rejection events, reveal the importance of addressing research from different perspectives, in order to propose sensitive and non invasive diagnostic, prognostic and follow-up tools.

Searching of biomarkers associated to kidney allograft outcome, has become an important research target in this area. Nevertheless a set of biomolecules related to state, function and outcome of kidney allografts has not been well defined yet.

Based on the above, we propose that differentially expressed transcripts in urine of kidney allograft recipients with different outcomes (either acceptance or rejection), might constitute a useful tool in monitoring the graft as well as in understanding immune processes occurring *in situ* at organ level.

Urine is a non invasive and easy to obtain sample. However, due to its composition is at the same time a hostile environment for RNA. Although researchers in prior studies have implemented commercial kits for RNA isolation from urinary sediment, information regarding to yields and quality and integrity of isolated RNA is not well described. Therefore, a protocol for RNA extraction from urine sediment for usage in next generation sequencing technologies (e.g RNA – Seq) is required.

Among the different protocols that we have implemented in our lab for the isolation of RNA, are included those provided by comercial kits and the Trizol reagent. Comercial kits have not displayed neither good yields (low concentration) nor quality (low 260/280; 260/230 relations) or integrity (no observation of 18S and 28S bands). Conversely, usage of Trizol reagent has displayed both good yields and optimal quality and integrity in terms of nucleic acid concentration, good 260/280; 260/230 relations and presence of 18S and 28S ribosomal RNA bands. The protocol was adapted from *Optimization of total RNA isolation from human urinary sediment*, Monteiro et al., 2016, and is suitable for the isolation of optimal RNA for its implementation in RNA sequencing.

Symptomatic individuals with previous natural dengue infection have a higher frequency of dengue-specific T cells against NS3, NS4b AND NS5 viral proteins

Karina I. Torres^{1*}, Félix G. Delgado^{1,2}, Jaime E. Castellanos¹, María Consuelo Romero², Claude Roth³

¹ Grupo de Virología, Universidad El Bosque, Bogotá, D. C., Colombia.

² Unidad de Investigación Básica Oral, Universidad El Bosque, Bogotá, D. C.

³ Functional Genetics of Infectious Diseases Unit, Pasteur Institute, Paris, France.

Dengue is the most common mosquito-borne human viral diseases that cause a range of clinical outcomes which 70% are asymptomatic. Some authors argue that the cellular immune response could be different between humans who have presented symptoms or not. In addition, it has been showed that T cell responses influences strongly the protection against the infection. The aim of this work was to find differences of T cell response between individuals who had presented evidence of apparent clinic symptoms or not. The frequency of antigen-specific IFN- γ producing T cells against peptides from non-structural viral proteins NS3, NS4b y NS5 of dengue virus (DENV) was quantified in peripheral blood mononuclear cells (PBMCs) from healthy donors. They were assessed by ELISpot in 49 samples using the peptides NS3 (34-44), NS5 (435-443), NS4b (389-397), NS3 (299-316), NS3 (259-267), NS4b (352-359), NS5 (522-531), NS3 (54-63), NS3 (203-211), NS3 (245-254), NS4b (342-350). The sequences of the peptides belong to conserved regions between the four serotypes and restricted to the most common HLA class I alleles in Colombia (A*02, A*24, B*07 y B*35) and are included in a group of epitopes from a DNA vaccine candidate against dengue from the Institut Pasteur (Paris, France). We found that 35 out of 49 donors were identified as seropositive and 8 of them reported characteristic clinical symptoms of the disease. The frequency of dengue-specific T cells against peptides NS5 (522-531) (HLA-B*07 restricted), NS3 (203-211) , NS3 (245-254) and NS4b (342-350) (HLA-B*35 restricted) in this group (median values of 180, 388, 403 y 239 SFC/10⁶ respectively) were significantly higher ($p < 0.005$) than the frequency quantified in individuals without apparent symptoms of disease (median values of 21, 15, 13, 14 SFC/10⁶ PBMCs respectively) . Although it is not clear the correlation between higher T cell response and development of symptoms or severe forms of disease, it is necessary to explore the polyfunctionality of this cellular response in order to get more information about its ability to induce disease protection.

Global DNA methylation in immune cells of patients with systemic lupus erythematosus and its relationship with disease activity and environmental exposure

Research proposal

Carolina Hurtado Montoya¹, Elsa María Vásquez Tres Palacios¹, Rodrigo Antonio Urrego Álvarez², Liliana Yasmín Acevedo Sáenz³, Gloria Vásquez⁴

¹ Escuela de Graduados y Facultad de Medicina, Universidad CES.

² Grupo de Investigación en Ciencias Animales CES (INCA-CES) y Facultad de Medicina Veterinaria y Zootecnia, Universidad CES

³ Grupo de Ciencias Básicas de la Facultad de Medicina y Escuela de Graduados, Universidad CES.

⁴ Grupo de Inmunología Celular e Inmunogenética, GICIG, Universidad de Antioquía, Medellín, Colombia.

Systemic Lupus Erythematosus (SLE) is a disease of multifactorial origin and can affect various systems and organs. Tissue damage is caused by the production of auto-antibodies against different components of the nucleus or cell. The wide spectrum of clinical manifestations explains the heterogeneity in its presentation and causes difficulties in the diagnosis. The classification criteria of the ACR (American College of Rheumatology) or SLICC (Systemic Lupus International Collaborating Clinics) have been adapted, however, they have limited sensitivity and specificity. The diagnostic tests available as ANA (Antinuclear Antibodies) and anti-dsDNA (Antibodies against double-stranded DNA), not only have sensitivity and specificity problems, but are also frequently positive in other autoimmune diseases.

The percentage of global DNA methylation as an epigenetic change has been proposed as a biomarker with specific cut-off points for the early detection of cancer patients. Although changes have been described in the global DNA methylation of different immune cells in patients with SLE, it has not yet been described whether or not there are cut-off points for each cell population, which may maximize the possibility of discriminating between patients with SLE of healthy individuals and between the different states of disease activity.

The technique that is mainly used to measure DNA methylation patterns is bisulfite sequencing, with high costs, risks and technical difficulties. In the present study we propose the measurement of global DNA methylation through flow cytometry, with advantages such as speed, accessibility and lower cost, important benefits in the context of a diagnostic and follow-up test. In addition, cytometry allows to characterize markers of interest cell by cell, which provides simultaneous information of the different cell subtypes.