

Characterization of the glycoconjugates of boar testis and epididymis

A. Calvo¹, L.M. Pastor^{2*}, S. Bonef, E. Pinart³ and M. Ventura²

¹Department of Histology, School of Medicine, University of Navarra, Pamplona, Spain; ²Department of Cellular Biology, Section of Histology and General Embryology, Medical School, University of Murcia, Espinardo, 3100 Murcia, Spain; and ³Reproductive Biology Unit, Department of Biology, Faculty of Experimental Sciences and Health, University of Girona, 17071 Girona, Spain

Abstract of:

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Lectin histochemistry was used to perform *in situ* characterization of the glycoconjugates present in boar testis and epididymis. Thirteen horseradish peroxidase- or digoxigenin-labelled lectins were used in samples obtained from healthy fertile boars. The acrosomes of the spermatids were stained intensely by lectins with affinity for galactose and *N*-acetyl-galactosamine residues, these being soybean, peanut and *Ricinus communis* agglutinins. Sertoli cells were stained selectively by *Maackia amurensis* agglutinin. The lamina propria of seminiferous tubules showed the most intense staining with fucose-binding lectins. The Golgi area and the apical part of the principal cells of the epididymis were stained intensely with many lectins and their distribution was similar in the three zones of the epididymis. On the basis of lectin affinity, both testis and epididymis appear to have *N*- and *O*-linked glycoconjugates. Spermatozoa from different epididymal regions showed different expression of terminal galactose and *N*-acetyl-galactosamine. Sialic acid (specifically α 2,3 neuraminic-5 acid) was probably incorporated into spermatozoa along the extratesticular ducts. These findings indicate that the development and maturation of boar spermatozoa are accompanied by changes in glycoconjugates. As some lectins stain cellular or extracellular compartments specifically, these lectins could be useful markers in histopathological evaluation of diseases of boar testis and epididymis.

Deep-brain stimulation of the subthalamic nucleus or the pars interna of the globus pallidus in parkinson's disease. The deep-brain stimulation for parkinson's disease study group*

*M. C Rodríguez-Oroz, M. Rodríguez, J Guridi, K. Mewes, V. Chockman, J. Vitek, M.R. DeLong & J. Obeso

Clinica Universitaria, Pamplona

Abstract of:

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Background: Increased neuronal activity in the subthalamic nucleus and the pars interna of the globus pallidus is thought to account for motor dysfunction in patients with Parkinson's disease. Although creating lesions in these structures improves motor function in monkeys with induced parkinsonism and patients with Parkinson's disease, such lesions are associated with neurologic deficits, particularly when they are created bilaterally. Deep-brain stimulation simulates the effects of a lesion without destroying brain tissue.

Methods: We performed a prospective, double-blind, crossover study in patients with advanced Parkinson's disease, in whom electrodes were implanted in the subthalamic nucleus or pars interna of the globus pallidus and who then underwent bilateral high-frequency deep-brain stimulation. We compared scores on the motor portion of the Unified Parkinson's Disease Rating Scale when the stimulation was randomly assigned to be turned on or off. We performed unblinded evaluations of motor function preoperatively and one, three, and six months postoperatively.

Results: Electrodes were implanted bilaterally in 96 patients in the subthalamic-nucleus group and 38 patients in the globus-pallidus group. Three months after the procedures were performed, double-blind, crossover evaluations demonstrated that stimulation of the subthalamic nucleus was associated with a median improvement in the motor score (as compared with no stimulation) of 49 percent, and stimulation of the pars interna of the globus pallidus with a median improvement of 37 percent ($P < 0.001$ for both comparisons). Between the preoperative and six-month visits, the percentage of time during the day that patients had good mobility without involuntary movements increased from 27 percent to 74 percent ($P < 0.001$) with subthalamic stimulation and from 28 percent to 64 percent ($P < 0.001$) with pallidal stimulation. Adverse events included intracranial hemorrhage in seven patients and infection necessitating removal of the leads in two.

Conclusions: Bilateral stimulation of the subthalamic nucleus or pars interna of the globus pallidus is associated with significant improvement in motor function in patients with Parkinson's disease whose condition cannot be further improved with medical therapy.

The subthalamic nucleus in Parkinson's disease: somatotopic organization and physiological characteristics

Maria C. Rodríguez-Oroz,¹ Manuel Rodríguez,³ Jorge Guridi,^{2*} Klaus Mewes,⁴ Vijay Chockman,⁴ Jerrold Vitek,⁴ Mahlon R. DeLong⁴ and Jose A. Obeso¹

¹Movement Disorders and Basal Ganglia Group, Department of Neurology and Neurosurgery, Neuroscience Center, Clinica Universitaria and Medical School, University of Navarra, Pamplona, ²Service of Neurosurgery, Clinica Quirón, San Sebastian, ³Experimental Neurology and Neurobiology Laboratory, Department of Physiology, University of La Laguna Medical School,

Tenertfe, Spain and ⁴Department of Neurology, Emory University Hospital, Atlanta, USA

Abstract of:
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Single-cell recording of the subthalamic nucleus (STN) was undertaken in 14 patients with Parkinson's disease submitted to surgery. Three hundred and fifty neurones were recorded and assessed for their response to passive and active movements. Thirty-two per cent were activated by passive and active movement of the limbs, oromandibular region and abdominal wall. All neurones with sensorimotor responses were in the dorsolateral region of the STN. Arm-related neurones were lateral (≥ 14 mm plane) to leg-related neurones, which were found more medially (≤ 12 mm). Representation of the oromandibular musculature was in the middle of the sensorimotor region (~ 13 mm plane) and ventral to the arm and leg. Two hundred neurones were adequately isolated for 'off-line' analysis. The mean frequency of discharge was 33 ± 17 Hz (13-117 Hz). Three types of neuronal discharges were distinguished: irregular (60.5%), tonic (24%) and oscillatory (15.5%). They were statistically differentiated on the basis of their mean firing frequency and the coefficient of variation of the interspike interval. Neurones responding to movement were of the irregular or tonic type, and were found in the dorsolateral region of the STN. Neurones with oscillatory and low frequency activity did not respond to movement and were in the ventral one-third of the nucleus. Thirty-eight tremor-related neurones were recorded. The majority (84%) of these were sensitive to movement and were located in the dorsolateral region of the STN. Cross power analysis ($n = 16$) between the rhythmic neuronal activity and tremor in the limbs showed a peak frequency of 5 Hz (4-8 Hz). Neuronal activity of the substantia nigra pars reticulata was recorded 0.5-3 mm below the STN. Eighty neurones were recorded 'on-line' and 27 were isolated for 'off-line' analysis. A tonic pattern of discharge characterized by a mean firing rate of 71 ± 28 Hz (35-122 Hz) with a mean coefficient of variation of the interspike interval of 0.85 ± 0.29 ms was found. In only three neurones (11%) was there a response to sensorimotor stimulation. The findings of this study indicate that the somatotopic arrangement and electrophysiological features of the STN in Parkinson's disease patients are similar to those found in monkeys.

Immunocytochemical and ultrastructural characterization of endocrine cells in the larval stomach of the frog *Rana temporaria* tadpoles: a comparison with adult specimens

A.C. Villaro, J. Rovira, M.E. Bodegas, M.A. Burrell, D. García-Ros, P. Sesma

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Tissue & Cell, 2001 33 (5) 462-477

According to immunostaining and ultrastructural patterns, *Rana temporaria* tadpole stomach displays a well-differentiated endocrine population comprising, at least, six cellular types:

ECL, EC [serotonin], D [somatostatin] -all three of them abundant-, P [bombesin] -less numerous-, CCK-8 [cholecystokinifl/gastrin] and A [glucagon/glicentin] - both very scarce. Larval endocrine cells are mainly located in the surface epithelium and show open or closed morphologies. Cellular diversity is similar in tadpoles and frogs, with the exception of immunoreactivity for gastrin-17, found in adults in numerous cells. Larval cells display mature ultrastructural traits, although with smaller secretory granules. The different distribution of endocrine cells, which in adults are preferentially located in the glands, probably refers to different functional requirements. However, the rich vascular plexus present in larval mucosa may be an efficient transport medium of surface hormones to gastric targets. The enhancement in adults of endocrine population and correlative increase in hormonal secretion indicates a more active functional role, probably related to the shift from herbivorous to carnivorous habits. In summary, the tadpole gastric endocrine population, although not as numerous as that of adult frogs, displays histological traits that indicate a relevant (immunoreactive and ultrastructural properties, cellular diversity) and specific (surface location, relative abundance of open-type cells) role of local regulatory factors in amphibian larval gastric function.

Peptidylglycine α -amidating monooxygenase- and proadrenomedullin-derived peptide-associated neuroendocrine differentiation are induced by androgen deprivation in the neoplastic prostate

Nuria Jiménez^{1,*} Johan Jongsma², Alfonso Calvo¹, Theodorus H. Van Der Kwast², Anthony M. Treston³, Frank Cuttitta³, Fritz H. Schröder², Luis M. Montuenga¹ and Gert J. Van Steenbrugge²

¹Department of Histology and Pathology, University of Navarra, Pamplona, Spain. ²Department of Experimental Urology and Pathology, Josephine Nefkens Institute, Rotterdam, The Netherlands. ³National Cancer Institute, National Institutes of Health, Rockville, MD, USA

Abstract of:
Int. J. Cancer: 94, 28-34 (2001)

Most PCs show NE differentiation. Several studies have tried to correlate NE expression with disease status, but the reported findings have been contradictory. Prostatic NE cells synthesize peptides with a wide spectrum of potential functions. Some of these active peptides, such as PAMP, are amidated. PAM is the only carboxy-terminal peptide-amidating enzyme identified. We studied expression of PAMP and PAM in

normal prostate and prostatic tumors (clinical specimens and human xenograft models) with or without prior androgen-deprivation therapy and found a wide distribution of both molecules in NE subpopulations of all kinds. Although the correlation of either marker to tumor grade, clinical progression or disease prognosis did not reach statistical significance, PAMP- or PAM-immunoreactive cells were induced after androgen-blockade therapy. In the PC-3 10 and PC-295 androgen-dependent models, PAMP or PAM NE differentiation was induced after castration in different ways, being higher in PC-3 10, which might explain its long-term survival after androgen deprivation. We show induction of expression of 2 new NE markers in clinical specimens and xenografted PC after endocrine therapy.

Inmunohistochemical Mapping of Endothelin in the Developing and Adult Mouse Lung

Laura Guebbe and Ana C. Villaro

Department of Histology and Pathology, University of Navarra, Pamplona, Spain

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Endothelins (ET) are a family of regulatory peptides displaying, among other abilities, potent constrictor actions. We studied the perinatal time course expression and distribution of ET in the mouse airway epithelium. In fetal mouse, ET-immunoreactivity (IR) appeared earlier (gestational Day 18) in the epithelium of upper (bronchi and large bronchioles) than in lower airways, being scarce and mainly located in the apical cytoplasm. As the lung developed, ET-IR became gradually stronger and extended throughout the cell in both bronchi and bronchioles. ET-IR was found in most airway epithelial cells. Clara cells were positive for ET, whereas ciliated and endocrine cells were not. In adult lungs, part of the myocytes and parenchymal cells also showed ET-IR. In both developing and adult mouse lungs, the cell distribution of ET-IR in the epithelium is compatible with apical and/ or basal secretion. The presence of ET in mouse airway epithelium during the perinatal period may indicate a role for ET as a growth factor in lung development and its involvement in control of lung ventilation at birth.

The Enterococcal Surface Protein, Esp, Is Involved in *Enterococcus faecalis* Biofilm Formation

Alejandro Toledo-Arana¹, Jaione Valle¹, Cristina Solano¹, Maria Jesus Arrizubieta¹,

Carme Cucarella², Marta Lamata³, Beatriz Amorena¹, José Leiva³, José Rafael Penadés² and Iñigo Lasa^{1*}

¹Instituto de Agrobiotecnología y Recursos Naturales and Departamento de Producción Agraria, Universidad Pública de Navarra-Consejo Superior de Investigaciones Científicas, Campus de Arrosadía, 31006 Pamplona. ²Unit of Biochemistry, Department of Basic Biomedical Sciences, Cardenal Herrera-CEU University, 46113 Moncada, and ³Department of Microbiology, University Clinics, 31008 Pamplona, Spain

Abstract of:

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The enterococcal surface protein, Esp, is a high-molecular-weight surface protein of unknown function whose frequency is significantly increased among infection-derived *Enterococcus faecalis* isolates. In this work, a global structural similarity was found between Bap, a biofilm-associated protein of *Staphylococcus aureus*, and Esp. Analysis of the relationship between the presence of the Esp-encoding gene (*esp*) and the biofilm formation capacity in *E. faecalis* demonstrated that the presence of the *esp* gene is highly associated ($P < 0.0001$) with the capacity of *E. faecalis* to form a biofilm on a polystyrene surface, since 93.5% of the *E. faecalis esp*-positive isolates were capable of forming a biofilm. Moreover, none of the *E. faecalis esp*-deficient isolates were biofilm producers. Depending on the *E. faecalis* isolate, insertional mutagenesis of *esp* caused either a complete loss of the biofilm formation phenotype or no apparent phenotypic defect. Complementation studies revealed that Esp expression in an *E. faecalis esp*-deficient strain promoted primary attachment and biofilm formation on polystyrene and polyvinyl chloride plastic from urine collection bags. Together, these results demonstrate that (i) biofilm formation capacity is widespread among clinical *E. faecalis* isolates, (ii) the biofilm formation capacity is restricted to the *E. faecalis* strains harboring *esp*, and (iii) Esp promotes primary attachment and biofilm formation of *E. faecalis* on abiotic surfaces.

Virulent strains of *Salmonella enteritidis* disrupt the epithelial barrier of Caco-2 and HEp-2 cells

Cristina Solano, Begoña Sesma, Miguel Álvarez, Elena Urdaneta, David García-Ros, Alfonso Calvo, Carlos Gamazo

Abstract of:

Arch Microbiol (2000)175:46-51

To confirm the existence in nature of *Salmonella enteritidis* strains of different degrees of virulence and to elucidate the mechanisms underlying the effects of such strains on the

epithelial barrier function, the consequences of infection of Caco-2 cells and HEp-2 cells with 15 *S. enteritidis* strains in a chicken infection model were examined. The more virulent strains of *S. enteritidis*, which are biofilm producers in adherence test medium, were able to disrupt HEp-2 and Caco-2 monolayers, as shown by transmonolayer electrical resistance and lactate dehydrogenase activity. In contrast, the low-virulence strains of *S. enteritidis*, which do not produce biofilms in adherence test medium, had no effect on the same cells. An avirulent rough mutant of *Salmonella minnesota* exhibited a pattern of behaviour similar to that of the low virulence strains of *S. enteritidis*, whilst a clinical *Salmonella typhi* strain caused rapid injury to the monolayers. The effect of supernatants of *Salmonella* cultures in adherence test medium on the integrity of Caco-2 cell monolayers indicated that the high-virulence *S. enteritidis* strains, but not the low-virulence strains, release a soluble factor when incubated under optimum biofilm-forming conditions, which enables the disruption of the integrity of Caco-2 monolayers.

A *Brucella ovis* antigenic complex bearing poly- ϵ -caprolactone microparticles confer protection against experimental brucellosis in mice

M. Munillo^a, M.J. Grilló^b, J. Reñé^a, C.M. Marín^b, M. Barberán^b, M.M. Goñi^a, J.M. Blasco^b, J.M. Irache^a, C. Garmazo^c

^aDepartment of Technological Pharmacy, University of Navarra, 31008. Pamplona. Spain. ^bAnimal Health Unit, SIA -DGA, 50080, Zaragoza, Spain. ^cDepartment of Microbiology, University of Navarra, 31008, Pamplona, Spain

Abstract of:

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A hot saline antigenic extract (HS) from *Brucella ovis* was encapsulated in poly- ϵ -caprolactone microparticles (PEC), and tested as a vaccine against *B. ovis* and *B. abortus* infections in mice. Subcutaneous but not oral administration in BALB/c mice of the HS-PEC induced high amounts of IFN- γ and IL-2 but low quantities of IL-4 suggesting a combined Th1/Th2 cellular immune response. The vaccine administered either subcutaneously or orally protected mice against *B. ovis* infection. Such protection was similar to that provided by the reference living attenuated *B. melitensis* Rev. 1 vaccine. By contrast, only the subcutaneous vaccination with HS-PEC was as effective as Rev. 1 in conferring protection against *B. abortus* infection. The use of free HS or empty PEC microparticles did not produce any protective effect.

Key words: Microparticles. Brucellosis. Poly- ϵ -caprolactone.

Evaluation of three methods to measure anti-*Brucella* IgM antibodies and interference of IgA in the interpretation of mercaptan-based tests

T. Marrodan*, R. Nenova-Poliakova* ||, M Rubio*, J. Ariza†, E. Clavijo‡, H.L. Smits§ and R. Diaz*

*Departamento de Microbiología, Servicio de Microbiología Clínica, Universidad de Navarra, Pamplona Spain. †Servicio de Enfermedades Infecciosas, Hospital de Bellvitge, Barcelona, Spain. ‡Servicio de Microbiología, Hospital Virgen de la Victoria, Málaga, Spain and §Department of Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands

Abstract of:

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The results of a dipstick assay for the detection of immunoglobulin NI (IgM) to *Brucella* smooth lipopolysaccharide (S-LPS) correlated with those of an enzyme-linked immunosorbent assay (ELISA) for IgM and of the serum agglutination test (SAT) performed with and without dithiothreitol. Two sera which were dithiothreitol-sensitive and were dipstick negative were shown to contain specific IgA. The dipstick assay is recommended as a simple method for detecting specific IgM antibodies in acute-phase brucellosis patients.

Promotion of platelet aggregation by sera from brucellosis patients with antiphosphatidylcholine antibodies

M. Ángeles Casao, Ramón Díaz, Antonio Orduña* and Carlos Gamazo

Departamento Microbiología. Universidad de Navarra, Clínica Universitaria de Navarra. 31080, Pamplona and*Departamento Microbiología, Hospital Universitario, Facultad de Medicina, Universidad de Valladolid, Spain

Abstract of:

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Results obtained in this study suggest that in human brucellosis there is an antibody response against platelet-activating factor (PAF) and phosphatidylcholine (PC). The specificity of the antiphospholipid response was determined by inhibition assays. The PAF molecule was able to inhibit the anti-PC activity of the brucellosis-control serum. This inhibition capacity of PAF was similar to that of the phosphorylcholine (PYC) group. These results suggest that the inhibition activity could be attributed to the PYC group present in both PAF and PC molecules. Consequently, these findings support an immunodominant role of PYC in the antiphospholipid response

of brucellosis. Furthermore, sera from patients infected with *Brucella* organisms were able to cause platelet aggregation, as were brucella phospholipids, suggesting a possible role of the antiphospholipid antibodies and phospholipids in the inflammatory response in brucellosis.

Random Amplified Polymorphic DNA Typing Applied to the Study of Cross-Contamination by *Listeria monocytogenes* in Processed Food Products

V. Aguado, A.I. Vitas, and I. García-Jalón*

Department of Microbiology, University of Navarra, C/ Irunlarrea 1, 31008 Pamplona, Spain

Abstract of:

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The presence of *Listeria spp.* was investigated in 369 samples of cooked meat products and 52 of smoked salmon. Incidences of 17.6% for cooked meat and 38.5% for smoked salmon samples were found. All *Listeria monocytogenes* isolates (34 from meat products and 16 from smoked salmon) were typed serologically and by random amplified polymorphic DNA (RAPD) typing using primers HLWL74 (5'-ACGTATCTGC-3'), HLWL85 (5'-ACAACTGCTC3'), and OMP-01 (5-GTTGGTGGCT-3'). Strains from cooked meat products were characterized and compared in relation to their origin. The detection of identical strains in products of different type and brand packed on the same date suggested cross-contamination probably during the slicing process. All *L. monocytogenes* isolates from smoked salmon were indistinguishable by serotyping and RAPD, suggesting that this strain was highly disseminated and adapted to the treatment used for the preservation of this food. RAPD subtypes were analyzed using GelCompar version 4.1 software and the unweighted pair method using arithmetic averages, and six groups with at least 78% similarity were established. Serotyping and RAPD results were in concordance, although RAPD showed a higher discriminatory power with *L. monocytogenes* isolates from meat products. RAPD is an easy method that could be useful to detect cross-contamination occurring during postprocessing manipulations.

Study of the *in vitro* Sulphidoleukotriene Production In Food-Allergic Patients

Leticia Vila, María L. Sanz, Germán Sánchez, Carina G. Uasuf, Marta Ferrer, Maite Barrio and Isauro Diéguez

Department of Allergy and Clinical Immunology, School of Medicine, University of Navarra, Pamplona, Spain

Abstract of:

J. Invest Allergol Clin Immunol 2001; Vol. 11(4):247-254

Objective and design: To study *in vitro* sulphidoleukotriene (sLT) production by food allergic patients using cellular allergen stimulation test (CAST)-ELISA and to evaluate the reliability of this technique for diagnosing food allergic reactions. **Subjects:** Forty patients with adverse reactions after food intake, 20 healthy controls, and 15 individuals sensitized to inhalant allergens as atopic controls. **Methods:** Skin tests, serum-specific IgE, histamine release test (HRT), CAST-ELISA and food challenges. One-way ANOVA was used to compare tests results between patients and controls and to study mediator release and specific IgE, related to the severity of clinical pictures. Sensitivity and specificity were analyzed by ROC curves. **Results:** Food allergic patients showed higher ($p < 0.05$) Ag-dependent sLT production (836.2 ± 664.1 pg/ml) (mean \pm standard deviation) than both control groups. After stimulus with anti-IgE antibodies, sLT production was higher ($p < 0.05$) by atopic controls (1630.8 ± 696.5 pg/ml) compared to patients and healthy controls. Patients with anaphylactic reactions showed higher Ag-specific and anti-IgE sLT and histamine production than patients with less severe manifestations. Mean serum-specific IgE was significantly lower ($p < 0.05$) in patients presenting oral allergy syndrome compared to patients with more severe clinical pictures. CAST-ELISA was the most sensitive method. Prick by prick test was the most specific. **Conclusions:** CAST-ELISA may provide a useful tool for diagnosing food allergy. Enhanced cell releasability may be linked to the severity of the clinical response to foods.

Keywords: Sulphidoleukotriene. Food allergy. CAST-ELISA. Releasability.

Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics

M.L. Sanz, P. M. Gamboa*, I. Antépara*, C. Uasuf, L. Vila, C. García-Avilés, M. Chazot and A.L. De Week

Department of Allergology and Clinical Immunology, University Clinic of Navarra, School of Medicine, University of Navarra, Pamplona, and *Servicio de Alergología, Basurto Hospital, Bilbao, Basque Country, Spain

Abstract of:

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Background: In this study, we used flow cytometry to determine the percentage of activated basophils that expressed the CD63 marker after *in vitro* stimulation by different betalactam

antibiotics. The diagnostic reliability of the technique was assessed, as well as its correlation with specific IgE.

Methods: Fifty-eight patients with clinical allergy to betalactam antibiotics and presenting positive skin tests to at least one of the allergens (minor determinant mixture (MDM), benzylpenicilloypolylysine (PPL), penicillin, ampicillin, amoxicillin, cephalosporins) were tested. Thirty subjects non-allergic to betalactams were also studied as controls. The flow assay stimulation test (FAST) uses flow cytometry to determine the percentage of basophils that express CD63 as an activation marker after *in vitro* stimulation with allergen. Double labelling with monoclonal antibodies antiCD63-PE and anti-IgE FITC was used.

Results: The allergic patients show a statistically greater number of activated basophils than the control subjects, after the incubation of cells with all the betalactams at various

concentrations. The sensitivity of the technique is 50%, the specificity 93.3%, the likelihood ratio for a positive value 7.46 and the likelihood ratio for a negative value 0.54. In spite of having a greater sensitivity (37.9%) and specificity (86.7%) than CAP, differences between sensitivity and specificities of both techniques (CAP and FAST) do not reach statistical significance.

Conclusion: The basophil activation test is a particularly useful technique in the diagnosis of patients with IgE-mediated allergy to betalactams and allows the identification of 50% of patients. Used in conjunction with CAP, it allows the identification of 65.5% of such patients.

Key words: betalactam allergy. CD63 expression. Flow cytometric basophil activation test. In vitro diagnosis.

Fe de erratas

En la página 55 del número de junio de 2002 de la Revista de Medicina, hay un error en el pie de fotografía de la noticia "Dos graduados de la Universidad de Navarra, entre los diez primeros MIR". La persona que aparece a la derecha de la fotografía no es D. Oscar Alcalde, como indica el pie de foto, sino D. Sebastián Cervantes, Médico Interno Residente, del Dpto. de Neurología de la Clínica Universitaria.