

Forèiæ D, Mažuran R. Modulation of $[Ca^{2+}]_i$ in freshly isolated mouse lymphocytes with in vivo priming. *Immunol Lett* 1999;67:23-30. Institute of Immunology, Zagreb, Croatia
Studied at the level of the individual cell, the pattern of $[Ca^{2+}]_i$ mobilization of in vivo sensitized mouse lymphocytes by T-dependent antigen (KLH), challenged in vitro by ConA, PHA or anti-CD3e mAb in different periods after immunization, was as follows. In the entire DLN lymphocyte population and in tested T cell subsets from immunized mice, baseline $[Ca^{2+}]_i$ was significantly increased and cells were able to respond additionally to stimuli. In KLH-primed DLN lymphocytes, calcium mobilization in response to membrane receptor-dependent stimuli (anti-CD3e, PHA, and ConA) was increased. Enhancement of Ca^{2+} mobilization is parallel with changed immunophenotype. These findings suggested that: a) $[Ca^{2+}]_i$ mobilization could correlate with lymphocyte behavior during immunization and that mobilization clearly depended on kinetics of immune reaction; b) the higher level of activity among sensitized lymphocytes was due to the increased number of specific B-cells (Iak+) and gdTCR+ cells; c) the quantitative measurement of $[Ca^{2+}]_i$ could be an important biochemical parameter to study cellular reaction to a specific antigen.

Grèeviaæ D, Batiniaæ D, Ascensao JL, Marušiæ M. Pretreatment of transplant bone marrow cells with hydrocortisone and cyclosporine A alleviates graft-versus-host reaction in a murine allogeneic host-donor combination. *Bone Marrow Transpl* 1999;23:1145-52. Department of Physiology, Zagreb University School of Medicine, Zagreb, Croatia
The aim of the study was to alleviate graft-versus-host reaction (GVHR) by pre-treatment of the bone marrow (BM) transplant with hydrocortisone (HC) and cyclosporin A (CsA) in C57BL/6J (donor) @ CBA/J (recipient) mouse combination. BM cells were exposed to HC and CsA for 1 h at 37°C and then injected into lethally irradiated (9.5 Gy) mice at a dose of 2×10^6 BM cells/mouse. Hematopoietic recovery was assessed on day 12, and survival was followed for 100 days. Combinations of 1000 mg/mL HC and 100 mg/mL CsA, and 100 mg/mL HC and 10 mg/mL CsA significantly reduced MLR and additively mitigated GVHR in vivo, achieving 40% and 26% survival rates, respectively. However, HC and CsA altered neither the peripheral blood cell counts nor in vitro and in vivo BM cell clonogenic potential. Additional studies have shown that HC and CsA blocked con A-driven differentiation of CD8+ and CD4+CD8+ lymph node cells (LNC) and progression of LNC to S+(G2+M) cell cycle phases, and inhibited IL-1, IL-2 and TGF- β while enhancing GM-CSF gene expression in BM cells. These data indicate that the pre-treatment of the BM transplant with HC and CsA results in inactivation of GVHR effector cells and mitigation of GVHR while sparing BM repopulating capacity.

Lampret BR, Kidric J, Kralj B, *Vitale Lj, Pokorny M, Renko M. Lapstatin, a new aminopeptidase inhibitor produced by *Streptomyces rimosus*, inhibits autogenous aminopeptidases. *Arch Microbiol* 1999;171:397-404. *Rudjer Boškoviæ Institute, Zagreb, Croatia
Lapstatin, a low-molecular-weight aminopeptidase inhibitor, was purified to homogeneity from *Streptomyces rimosus* culture filtrates. The purification procedure included extraction with methanol, followed by chromatography on Dowex 50WX4, AG50WX4, and HPLC RP C18 columns. By amino acid analysis, mass spectrometry, and NMR spectroscopy, the structure of lapstatin was shown to be 3-amino-2-hydroxy-4-methylpentanoylvaline. Lapstatin inhibited the extracellular leucine aminopeptidases from *Streptomyces rimosus*, *Streptomyces griseus*, and *Aeromonas proteolytica* with an IC50 in the range of 0.3-2.4 mM. IC50 values for other enzymes tested were at least tenfold higher. Leucine aminopeptidase from *Streptomyces griseus* was inhibited in a competitive manner, with an inhibition constant of 5×10^{-7} M. Lapstatin is the first low-molecular-weight compound isolated from streptomycetes shown to inhibit an autogenous aminopeptidase.

Markotiaæ A, Dašiæ G, Gagro A, Sabioncello A, Rabatiaæ S, Kuzman I, et al. Role of peripheral-blood mononuclear cell (PBMC) phenotype changes in the pathogenesis of hemorrhagic fever with renal syndrome (HFRS). *Clin Exp Immunol* 1999;115:329-34. Institute of Immunology, Zagreb, Croatia
Blood samples from 22 HFRS-positive, six seronegative patients and 15 healthy controls were examined in 1995, during the largest HFRS epidemic in Croatia. Results of immunofluorescence analysis showed an increased percentage of cytotoxic T cells (CD3+CD8+) in seropositive patients compared with seronegatives and healthy controls. The majority of seropositive HFRS patients expressed activation and memory antigens on T and B lymphocytes. The percentage of CD23+ and CD21+ B lymphocytes was lower in seropositive patients. HFRS patients had elevated levels of sCD23 and five had elevated total IgE. The increased expression of both early and late T cell

activation antigens (CD25, CD71 and HLA-DR), memory cells and sCD23 positively correlated with biochemical parameters (AST, ALT, urea, α 2-globulin) during the acute phase of HFRS. The phenotypic changes observed, especially activation markers and memory cells, could be useful parameters in the evaluation of HFRS course, and prognostic factors of HFRS severity.

Martinoviæ I, Baraè L, Furaè I, Janièjjeviæ B, Kubat M, Perièiæ M, et al. Str polymorphisms in the population of the island of Hvar. *Human Biol* 1999;71:341-52. Institute for Anthropological Research, Zagreb, Croatia

The aim of this study is to analyze short tandem repeat (STR) variation using data on 9 loci (D3S1358, VWA, FGA, THO1, TPOX, CSF1PO, D5S818, D13S317, D7S820) from the subpopulations of 6 villages on the island of Hvar, Croatia. The STR data help us to analyze the genetic structure of Hvar. The analysis of STR data in this study indicated genetic homogeneity among the village subpopulations on Hvar and the lack of the so-called east-west dichotomy, which had been indicated by some previous multidisciplinary anthropological studies. The observed value of GST (0.030) is most probably a consequence of high STR mutation rates, which produce a high level of within-group (village) diversity relative to total diversity of the population. The validity of STR markers in assessing genetic structure of small populations and relationships among closely related and reproductively isolated groups remains to be further evaluated.

Marušiæ A, Kataviæ V, Grèeviæ D, Lukiaè IK. Genetic variability of new bone induction in mice. *Bone* 1999;25:25-32.

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The authors studied differences in ectopic osteoinduction in eight mouse inbred strains and an outbred strain. Antigen-extracted autolyzed rat bone gelatin was implanted under hind limb muscle fascia of 12-week-old males, and new bone formation was morphologically assessed on serial sections. Four weeks after implantation, less than half of the implants from CBA/J, A/J, BALB/cJ, and C3Hf/Bu mice showed induction of only cartilage. New cartilage was observed in all, and bone and bone marrow in 80% of the implants from AKR/J, C57BL/6J, DBA/2J, and RFM/Rij mice. Volume of the newly formed tissue ranged from 1.3% of the old matrix in A/J strain to 74.6% in DBA/2J strain. Outbred CD1 mice showed only weak cartilage induction. The "good" responders differed among themselves in the volume and type of newly induced tissue: DBA/2J, RFM/Rij, and AKR/J mice had a similar ratio of new bone and cartilage and abundant bone marrow, whereas the predominant newly induced tissue in C57Bl/6J mice was cartilage. The pattern of the expression of BMP-2, -4, and -7, alkaline phosphatase, osteocalcin, IFN-g and GM-CSF measured by RT-PCR did not correlate with the type and the quantity of the newly induced tissue. The results show that adult mice of inbred strains differ not only in the peak bone mass and morphology, but also ability to form new bone after an osteoinductive stimulus.

*Saboliæ I, Herak-Kramberger CM, Breton S, Brown D. Na/K-ATPase in intercalated cells along the rat nephron revealed by antigen retrieval. *J Am Soc Nephrol* 1999;10:913-22.

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An antigen retrieval technique was used to reexamine the presence of Na/K-ATPase in intercalated cells (IC) along the rat nephron using monoclonal antibodies against the Na/K-ATPase alpha-subunit. Subtypes of IC along the nephron were identified by their distinctive staining with polyclonal and monoclonal antibodies to the 31-kD vacuolar H⁺-ATPase subunit, whereas principal cells (PC) were labeled with a polyclonal antibody to the water channel aquaporin-4 (AQP-4). In PC, the Na/K-ATPase and AQP-4 staining colocalized basolaterally. In contrast to previous reports, IC of all types showed basolateral labeling with the anti-Na/K-ATPase antibody. The staining was quantified by fluorescence image analysis. It was weak to moderate in IC of cortical and outer medullary collecting ducts and most intense in IC of the initial inner medullary collecting duct. IC in the initial inner medulla showed a staining intensity that was equivalent or stronger to that in adjacent principal cells. Models of ion transport at the cellular and epithelial level in rat kidney, therefore, must take into account the potential role of a basolateral Na/K-ATPase in intercalated cell function.

Stanèiæ MF, Miæoviæ V, Potoènjak M. The anatomy of the Berrettini branch: implications for carpal tunnel release. *J Neurosurg* 1999;91:1027-30. Division of Neurosurgery, Pula General Hospital, Croatia

OBJECT: Dissections were performed in 100 fresh cadaver palms to determine the frequency with

which superficial palmar communication between the median and ulnar nerves occurs and to what extent it might incur iatrogenic injury during endoscopic carpal tunnel release. **METHODS:** Superficial palmar communication between the median and ulnar nerves was present in 81% of the dissected hands. Superficial palmar communication, also known as the Berrettini branch, has been classified into four distinct types by Ferrari and Gilbert. Twelve hands were classified as Group 1 (communication in an oblique course from the ulnar to the median nerve originating >4 mm above the distal margin of the transverse carpal ligament [TCL]), 16 hands were classified as Group 2 (communication parallel to the distal margin of the TCL), and 53 hands were classified as Group 3 (communication in an oblique course from the ulnar nerve to the third common digital nerve, originating below the distal margin of the TCL). No hand fit the Group 4 classification (atypical communication). **CONCLUSIONS:** The Berrettini branch can be considered a normal anatomical finding. In 28% of the hands in this study, the branch was proximal to the edge of the distal ligament and, therefore, prone to iatrogenic injury in both one-portal and two-portal endoscopic surgery.

Stanèiæ MF, Potoènjak M, Miæoviaiæ V, *Krpmotiaiæ A, Mackinnon SE. Evaluation of functional nerve recovery shows that allogeneic nerve graft treated with ICAM-1 and LFA-1 mAbs can be good alternative to syngeneic graft. *Acta Neurochir (Wien)* 1999;141:875-9. *Neurosurgical Laboratory, Rijeka University School of Medicine, Croatia

The objective of the study is to establish recovery results of tibial nerve defects reconstructed using allogeneic and xenogeneic graft, in host immunosuppressed with Intercellular Adhesion Molecule-1 (ICAM-1) and Lymphocyte Function Antigen-1 (LFA-1) monoclonal antibodies (mAbs). A pilot study was conducted in fifteen Fischer rats by forming a 1 cm right tibial nerve gap, then reconstructing it with 1.2 cm long grafts, namely, Wistar allogeneic, Black mouse xenogeneic, and syngeneic (n = 5/group). The main study included forty-eight rats allocated to the following groups (n=12/group): 1) Allograft without treatment as control group. 2) Allograft with intraperitoneal ICAM-1 and LFA-1 mAbs treatment. 3) Allograft preserved in Belzers' solution including ICAM-1 mAbs plus standard intraperitoneal treatment. 4) Syngraft as benchmark. At 3, 6 and 9 weeks postengraftment walking track analysis was performed and expressed as Tibial Functional Index (TFI). Motor and compound nerve action potential across the graft conduction velocities were measured at week 10. Xenograft did not show any functional recovery and was therefore excluded from main study. However, pilot and main study results showed recovery results in both treated allogeneic groups and were comparable to benchmark syngraft. Therefore, allogeneic nerve graft could be an alternative in peripheral nerve reconstruction and spinal cord grafting.

Schönwald S, Kuzman I, Oreškoviæ K, Burek V, Škerk V, Car V, et al. Azithromycin single 1.5 g dose in the treatment of patients with atypical pneumonia syndrome – a randomized study. *Infection* 1999;27:198-202.

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An open comparative study was undertaken in order to assess the efficacy and safety of a single dose of azithromycin in the treatment of community-acquired atypical pneumonia. A total of 100 adult patients with atypical pneumonia syndrome were randomized to receive 1.5 g of azithromycin as a single dose, or 500 mg once daily for 3 days. The presence of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Coxiella burnetii*, and *Legionella pneumophila* infection was diagnosed by serological tests. Control clinical examinations were performed 72 h, 10-12 days and 4 weeks after treatment initiation. Among 96 evaluable patients (48 in each group) *M. pneumoniae* infection was confirmed in 24, *C. pneumoniae* in nine, *C. psittaci* in five, *C. burnetii* in six, and *L. pneumophila* in five. Forty-seven patients (97.9%) in each group were cured. Side effects were observed in two patients in the single-dose group, and one patient in the 3-day group. In conclusion, a single 1.5 g dose of azithromycin may be an alternative to the standard 3-day azithromycin regimen in the treatment of outpatients with atypical pneumonia syndrome.

*Supek S, Aine CJ, Ranken D, Best E, Flynn ER, Wood CC. Single vs. paired visual-stimulation – superposition of early neuromagnetic responses and retinotopy in extrastriate cortex in humans. *Brain Res* 1999;830:43-55.

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Neuromagnetic techniques were used in conjunction with magnetic resonance imaging (MRI) techniques to: 1) localize and characterize cortical sources evoked by visual stimuli presented at different locations in the lower right visual field; 2) examine the superposition of cortical responses by comparing the summation of responses to the presentation of single stimuli with responses to paired stimuli; and 3) examine the spatial resolution of magnetoencephalographic (MEG) techniques by

comparing the identified source locations evoked by the presentation of single vs. paired stimuli. Using multi-dipole, non-linear minimization analyses, three sources were localized for each stimulus condition during the initial 80-170 ms poststimulus interval for all subjects. In addition to an occipital source, two extrastriate sources were identified: occipital-parietal and occipital-temporal. Each source evidenced a systematic shift in location associated with changes in stimulus placement parallel to the vertical meridian. To our knowledge, this is the first demonstration of retinotopic organization of extrastriate areas, using non-invasive neuromagnetic techniques. The paired presentation of stimuli reflected superposition of the responses evoked by single stimuli but only for early activity up to 150 ms poststimulus. Undersummation was evident after 150 ms. All sources identified for single stimuli were also identified in the paired-stimulus responses; but at the expense of larger errors for some of the estimated parameters.

Vuèkov Š, Nikoljæ H, Kvesjæ A, Bukvjæ N. Our experience in the treatment of the vesicoureteral reflux with Lich-Gregoir antireflux surgical-procedure. *Eur J Pediatr Surg* 1999;9:33-36.
Department of Pediatric Surgery, University Hospital Center, Rijeka, Croatia
During 1980-1990 period the authors applied the operative Lich-Gregoir antireflux method on 166 children and adolescents. The application of the above method yielded a success of 97.8%. There were no intraoperative complications, with early postoperative complications occurring in two patients causing infection of the wound and subsequent ureter stenoses. Recurrent reflux occurred in 4 (2.2%) and the stenoses also in other 4 (2.2%) operated ureters. The follow-up lasted from 4 up to 14 years. The authors added their personal detail to the original Lich-Gregoir antireflux method considering it to be a further improvement in achieving better results. They fixed the ureter to the detrusor with additional stitches in the newly formed hiatus, i.e. at the exit of the ureter out of the new submucous canal. The possibility of arousing postoperative paraostial diverticula is thus avoided. In this way the possibility of postoperative ureter stenoses is reduced. According to authors' experience the antireflux method described above does not give good postoperative results in patients with greatly dilated and aperistaltic ureter, while all other examples show a high percentage of postoperative success.

Vlasak I, Plochl E, Kronberger G, Bergendi E, Rittinger O, Hagemann M, Schmitt K, Blumel P, Glatz J, Fekete G, *Kadrnka-Lovrenjæ M, et al. Screening of patients with Turner syndrome for hidden Y-mosaicism. *Klin Pædiatr* 1999;211:30-4.
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The presence of Y-chromosomal sequences in the cells of patients with Turner-Syndrome (TS) is a risk factor for the development of gonadal tumors. Since demonstration of Y-material usually results in prophylactic gonadectomy, optimal sensitivity and specificity of the diagnosis have to be attempted. The aim of the study was to evaluate the diagnostic potential of cytogenetic investigations as routinely employed in TS. In the most comprehensive study published so far the authors screened 208 TS patients for the presence of Y-chromosomal sequences by polymerase chain reaction (PCR) specific for eight different loci along the Y-chromosome. Six patients (3%) without cytogenetic evidence of Y-chromosome were found to be Y-positive. Among 12 cases with marker chromosomes two more Y-chromosomal fragments were identified. Thus, PCR-screening for Y-specific sequences was shown to be a valuable tool in the clinical management of Turner patients.

Trisler Z, Seme K, *Poljak M, Celanlucu B, Sakoman S. Prevalence of hepatitis-C and hepatitis-G virus infections among intravenous drug users in Slovenia and Croatia. *Scand J Infect Dis* 1999;31:33-5.
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The prevalence of hepatitis C virus (HCV) and hepatitis G virus (HGV) infections was assessed in 115 Slovenian and 102 Croatian intravenous drug users (IVDUs). HCV and HGV infections were detected in 60 (52.2%) and 61 (53.0%) Slovenian IVDUs and in 70 (68.6%) and 39 (38.2%) Croatian IVDUs, respectively. The established prevalence of both HCV and HGV infection in Croatian IVDUs are the lowest found to date among IVDUs. HCV positive Slovenian and Croatian IVDUs were significantly older and reported longer duration of the intravenous drug use in comparison with HCV negative IVDUs. In contrast, no significant differences in both parameters were found among HGV-positive and -negative IVDUs.

Židovec S, Mažuran R. Sendai virus induces various cytokines in human peripheral blood leukocytes – different susceptibility of cytokine molecules to low pH. *Cytokine* 1999;11:140-3.
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Virus infection of cell cultures induces the synthesis of various cytokines which can either inhibit or stimulate virus replication. The Sendai virus induces large quantities of biologically active interferon (IFN- α 3) in human peripheral blood leukocytes (hPBL) in vitro, as well as many other cytokines. The supernatants of Sendai virus-infected hPBL contained biologically active IFN- α 3, significant amounts of immunogenic IFN- γ , monokines (IL-1a, IL-1b, TNF- α), lymphokines (IL-6, TNF- β), growth factor (PDGF-AB) and small concentrations of IL-2 and GM-CSF. The analysis of the influence of the Sendai virus inactivation by lowering pH 2.0 on the cytokine concentrations showed that IL-1a, TNF- α , TNF- β and IFN- γ are susceptible to acid conditions, while IFN- α 3, IL-1b, IL-6 and IL-2 concentrations remained unchanged.