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The Francisella tularensis-containing phagosome (FCP) matures to a late-endosome-like phagosome prior to bacterial escape into the cytosol of macrophages, where bacterial proliferation occurs. The authors data show that within the first 15 min after infection of primary human monocyte-derived macrophages (hMDMs), approximately 90% of the FCPs acquire the proton vacuolar ATPase (vATPase) pump and the lysomotropic dye LysoTracker, which concentrates in acidic compartments, similar to phagosomes harboring the Listeria monocytogenes control. The acquired proton vATPase pump and lysomotropic dye are gradually lost by 30 to 60 min postinfection, which coincides with bacterial escape into the cytosols of hMDMs. Colocalization of phagosomes harboring the iglD mutant with the vATPase pump and the LysoTracker dye was also transient, and the loss of colocalization was faster than that observed for the wild-type strain, which is consistent with the faster escape of the iglD mutant into the macrophage cytosol. In contrast, colocalization of both makers with phagosomes harboring the iglC mutant was persistent, which is consistent with fusion to the lysosomes and failure of the iglC mutant to escape into the macrophage cytosol. The authors have utilized a fluorescence microscopy-based phagosome integrity assay for differential labeling of vacuolar versus cytosolic bacteria, using antibacterial antibodies loaded into the cytosols of live hMDMs. Colocalization of phagosomes harboring the iglD mutant with the vATPase pump and the LysoTracker dye was also transient, and the loss of colocalization was faster than that observed for the wild-type strain, which is consistent with the faster escape of the iglD mutant into the macrophage cytosol. In contrast, colocalization of both markers with phagosomes harboring the iglC mutant was persistent, which is consistent with fusion to the lysosomes and failure of the iglC mutant to escape into the macrophage cytosol. The authors have utilized a fluorescence microscopy-based phagosome integrity assay for differential labeling of vacuolar versus cytosolic bacteria, using antibacterial antibodies loaded into the cytosols of live hMDMs. Colocalization of phagosomes harboring the iglD mutant with the vATPase pump and the LysoTracker dye was also transient, and the loss of colocalization was faster than that observed for the wild-type strain, which is consistent with the faster escape of the iglD mutant into the macrophage cytosol. In contrast, colocalization of both markers with phagosomes harboring the iglC mutant was persistent, which is consistent with fusion to the lysosomes and failure of the iglC mutant to escape into the macrophage cytosol.


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The aim of this study was to further elucidate the mechanisms involved in adaptive changes of GABA(A) receptors following prolonged exposure to flumazenil, the antagonist of benzodiazepine binding sites on GABA(A) receptors. The effects of prolonged flumazenil treatment were studied on recombinant alpha1beta2gamma2S GABA(A) receptors stably expressed in human embryonic kidney (HEK 293) cells. Using radioligand binding experiments we found enhancement in the maximum number of [3H]muscimol labeled binding sites in different preparations of HEK 293 cells. The parallel increase of [3H]flunitrazepam binding sites in the membranes was reduced in the presence of actinomycin D and cycloheximide, inhibitors of RNA and protein synthesis, respectively. Chronic flumazenil also raised the steady-state level of mRNA encoding alpha1b2gamma2S receptor subunit. The results suggest that the up-regulation of GABA(A) receptors, observed after prolonged flumazenil treatment is at least partly due to increased de novo synthesis of receptor proteins at both transcriptional and translational level.


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Ubiquitin (Ub) is a small protein modifier that regulates many biological processes, including gene transcription, cell-cycle...

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The aim of this study was to assess ethical issues in everyday clinical practice among physicians and nurses of the University Hospital Rijeka, Rijeka, Croatia. The authors surveyed the entire population of internal medicine, oncology and intensive care specialists and associated nurses employed at the (n=532). An anonymous questionnaire was used to explore the type and frequency of ethical dilemmas, rank of their difficulty, access to and use of ethics support services, training in ethics and confidence about knowledge in ethics. Physicians (n=113, 55% female) ranged in age from 27 to 61 years, and nurses (n=251, 95% female), from 20 to 52. The most frequent ethical dilemmas concerned uncertain or impaired decision-making capacity (66% of physicians, 47% of nurses, p=0.008), limitation of treatment at the end of life (60% of physicians, 31% of nurses, p<0.001) and disagreements among family members (47% of physicians, 31% of nurses, p=0.025). The most difficult dilemmas concerned euthanasia and physician-assisted suicide (49% of physicians, 52% of nurses) and limitation of treatment at the end of life (14% of physicians, 18% of nurses). Only a minority reported ever using any kind of ethics support services (12% of physicians, 3% of nurses, p=0.001) or being very confident about knowledge in ethics (5% of physicians, 6% of nurses). In conclusion, similar ethical difficulties are present in the clinical practice of both physicians and nurses, with important differences in access and use of ethics support services. A need for systematic ethics educational activities was identified. Inclusion of individual ethics consultants in Croatian healthcare ethics support services is strongly advised.


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Melanoma inhibitory activity (MIA) protein was identified in significant quantities in primary and metastatic malignant melanomas, where it has an important role in promoting tumor development and progression. The authors’ hypothesis was that MIA serum level will be elevated in patients with metastases or local spreading of the disease before any symptom of such progression is clinically apparent. The authors compared MIA serum levels in two groups of patients with primary melanoma: those with positive as opposed to those with negative sentinel lymph nodes. In addition, MIA serum levels were studied in two control groups: patients with dysplastic nevi and patients with basal cell carcinoma. A blood sample was obtained from each patient included in the study and MIA levels were assessed using standard enzyme-linked immunosorbent assay method. Patients with histologically positive sentinel lymph nodes, meaning that tumor cells were found in the lymph nodes, had much higher mean MIA values than any other patient group considered in this study. With mean value of 14.53 ng/mL, it was almost twice as high as mean MIA value in patients with histologically negative sentinel lymph nodes (7.32 ng/ml) and more than twice as high than any of the two control groups (p<0.001). However, neither the classification by Clarke nor the classification by Breslow could be used to distinguish patients with positive sentinel lymph nodes from those with negative sentinel lymph nodes. In authors’ opinion, MIA serum level is the ideal test for screening the tumor spread to sentinel lymph nodes.

his study aimed to determine the relationship between serum levels of interleukin (IL)-6, IL-8 and IL-10, measured by enzyme-immunoassay, and the clinical characteristics and outcomes in 46 untreated patients with diffuse large B-cell lymphoma (DLBCL). Serum IL-6, IL-8 and IL-10 levels were higher in DLBCL patients than in control subjects. Elevated levels of IL-6, IL-8 and IL-10 correlated with more adverse disease features. Consequently, patients with elevated IL-6, IL-8 and IL-10 levels prior to treatment had a lower response to therapy. Furthermore, those with elevated IL-6 and IL-10 levels had poor median, 3-year and 5-year survival, while elevated serum IL-8 level did not correlate with overall survival. Worse survival was also confirmed in patients with combined elevated pretreatment serum levels of IL-6, IL-8 and IL-10 (none, one, two or three elevated). Multivariate analysis identified elevated values of IL-6 and IL-10 and response to therapy as significant predictors for overall survival. Serum levels of IL-6, IL-8 and IL-10 before treatment of patients with newly diagnosed DLBCL may give some insight into the possible prognosis and thus facilitate the decisions regarding therapeutic approaches for individual patients.


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Dendritic cells (DC)/natural killer (NK) cells interactions in the decidua of early human pregnancies were analyzed in vitro. Phenotype, cytokine expression and/or cytolytic mediators’ expression were measured by flow cytometry in NK and DC from the freshly isolated decidual mononuclear cells or after their purification and co-culture in vitro. Pro-
Poor periodontal conditions in post-war areas could be significant risk factors for periodontal disease and attachment loss tend to increase with age and physical activity and decrease with education level, higher frequency of tooth brushing tendency requirements, forced expiratory volume in 1 second, and serum IgE levels, were also decreased. Clinical variables, such as symptom score, mediators of cytokine and cytolytic mediator expression. These results suggest that decidual CD1a+ cells regulate and shape NK cell function more profoundly than CD83+ cells in decidua.


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The aim of this study was to assess the periodontal conditions of people in post-war area villages in relation to oral hygiene, habits and war stress. The study was conducted on a sample of 282 subjects (mean age, 41.5±17.8 yr.) in seven Vukovar villages using the questionnaire and clinical periodontal examination. Five years after the war in Croatia the population was still very poor, low educated, and had a low level of periodontal health and oral hygiene habits. The level of periodontal disease and attachment loss tended to increase with age and physical activity and decrease with education level, higher frequency of tooth brushing and toothbrush replacement, dental visits and utilisation of auxiliary devices. Subjects exposed to any kind of war stress had a significantly worse periodontal status and more extended sextants than those who were not exposed to such stress experience (p<0.05). Better periodontal conditions were found in individuals that were refugees, than those who were in war, wounded or lost a dear person (p<0.05).

Individuals who were in war rarely brushed their teeth, visited dentist and changed toothbrushes, but more frequently drank alcohol and smoked, in comparison to other groups, especially those who have not been exposed to war stress. In conclusion, specific socio-economic and psychological conditions in post-war areas could be significant risk factors for poor periodontal conditions.

Aiming to develop more reliable methods for determination of fetal gender from maternal plasma the authors compared three different systems of polymerase chain reaction (PCR) detection of Y-chromosome DNA. Cell-free DNA was isolated from 96 samples of maternal plasma and (1) amplified using AmpFLSTR-Identifiler (15 autosomal STR loci and amelogenin) or AmpFLSTR-Yfiler (16 Y-chromosome STR loci) kits and subsequently analyzed on ABI-PRISM 310 Genetic Analyzer, or (2) analyzed using Quantifiler-Y DNA-Quantification kit. Gender of fetuses was confirmed by cytogenetic analysis or phenotypically at birth. AmpFLSTR-Identifiler and Quantifiler-Y Human-Quantification kits were rather reliable in determining fetal gender (92.5 and 98.1%, respectively), but false negatives were still present in both systems. AmpFLSTR-Yfiler was found to be fully reliable as it amplified Y-chromosome in all cases of male fetuses, and was thus 100% correct in determining fetal gender. In addition, it enabled comparison of polymorphic Y-chromosome loci between father and a child, thus further supporting specificity of obtained results.


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To evaluate the effect of specific immunotherapy (SIT) on the induction of regulatory T cells (Treg) in house dust mite-allergic children and on the expression of specific Treg cell markers (cytotoxic T-lymphocyte-associated protein 4, [CTLA-4], IL-10, and TGF-BETA1). In this uncontrolled open-label study, the percentage of peripheral blood CD4+ Treg cells and the expression of molecules associated with their functions (CTLA-4, TGF-BETA1, and IL-10) were analyzed using flow cytometry in 16 children allergic to house dust mites before and at 3 and 12 months of subcutaneous SIT. Clinical variables, such as symptom score, medication requirements, forced expiratory volume in 1 second, peak expiratory flow rate, and serum IgE levels, were also de-
terminated. Ten healthy children were included as controls. All the clinical variables improved during immunotherapy. The percentage of CD4+CD25+CD69-CD45RO+ Treg cells remained unchanged. The percentage of CTLA-4+ expressing Treg cells transiently increased after 3 months of immunotherapy, whereas the percentage of FOXP3+ Treg cells did not change after 1 year of immunotherapy. Levels of IL-10+ cells transiently decreased after 3 months of immunotherapy. Four children who required inhaled fluticasone propionate administration for significant symptom worsening had no statistically significant increase in TGF-BETA1-secreting T cells at 12 months of SIT, in contrast to 12 children without inhaled corticosteroid treatment. In conclusion, the increase in TGF-BETA1-positive T cells only in children without significant symptom worsening requiring inhaled corticosteroid treatment limits the usefulness of TGF-BETA1 in monitoring response to allergen immunotherapy.