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IMMUNOCYTOCHEMICAL CHARACTERIZATION OF IMMUNE CELLS IN LESIONS OF ORAL LICHEN PLANUS AND ORAL MUCOSA OF HEALTHY INDIVIDUALS.

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Lichen Planus is a relatively common skin disease of unknown etiology, that may affects the oral mucosa. The histologic analysis shows a marked cellular inflammatory infiltrate toward the basement membrane. The aim of this study was to determine the presence of CD4+ T helper cells, CD8+ T cytotoxic cells and CD1a epithelial dendritic cells in biopsies from patients with oral Lichen Planus (OLP). Oral mucosa from healthy individuals (OMHI) was used as control. The immunocytochemical analysis wes carried out in patients with OLP (n=18) and OMHI (n=10) using an avidin-biotin-immunoperoxidase technique, to detect the above mentioned leukocyte immunophenotypes. Cells were quantified under a microscope with a calibrated monitor to determine the number of cells per mm2 and the information was expressed as mean ± standard error of the mean. Leukocyte cell densities were higher in OLP than in OMHI. The results showed augmented numbers in OLP, CD8+ T cells (1551 ± 202,83 cells/mm^2), CD4+ T cells (1456,72± 195,5) , epithelial dendritic CD1a+ cells (162.94± 27,3). The results suggest that Lichen Planus is a disease of inflammatory character where T lymphocytes are responsible for the cytotoxic processes.

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Oxygen Stress in *Fusobacterium nucleatum* Grown in Continuous Culture. P.I. DIAZ*, P.S. ZILM and A.H. ROGERS (Microbiology Laboratory, Dental School, University of Adelaide, South Australia).

Although *Fusobacterium nucleatum* (*F.n*), is considered an obligate anaerobe, it has been isolated from both supragingival and subgingival plaques and has also been associated with gingivitis and periodontal disease. Recent studies have indicated its importance in the establishment of other oral anaerobes in biofilm communities, possibly by protecting them from oxidative damage. The aim of this study was to determine whether *F.n* has the ability to survive and grow in the presence of oxygen. It was grown in continuous culture under anaerobic conditions and, following steady state, the oxygen concentration in the culture was gradually increased. Cell extracts from different growth conditions were analysed for the presence of NADH oxidase, superoxide dismutase (SOD), catalase and peroxidase activities. No significant differences were seen in the optical density of the culture when the atmosphere was changed gradually from nitrogen to a mixture of air/O_2 (60:40). Although the optical density decreased at a mixture of air/O_2 (40:60), due to wash out, the percentage of oxygen dissolved in the culture remained at zero. High levels of NADH oxidase were detected under all conditions, with a decreased activity when the cells were clearly oxygen-stressed. Low levels of SOD were detected in anaerobically grown cells, but increased slightly as the dissolved oxygen concentration was raised. No peroxidase or catalase activities were detected. These results showed that *F.n* is able to metabolize oxygen by producing the enzymes SOD and NADH oxidase which may be responsible for protection against reactive oxygen species.

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Candida albicans Adhesins Recognising Salivary Basic Proline-Rich Proteins. H-W. JENG*, A.R. HOLMES, G.R. TOMPKINS and R.D. CANNON (School of Dentistry, University of Otago, Dunedin, New Zealand).

Salivary basic proline-rich proteins (bPRPs) act as receptors for the adherence of *Candida albicans* and this adhesion interaction may be important for oral colonization and the development of mucosal *Candida* infections. The aim of this project was to identify the *C. albicans* adhesin(s) for bPRPs by isolating a binding-inhibitory component from a *C. albicans* cell wall extract. Cell wall material was released by mild treatment with the glucanase Zymolase and was shown to competitively inhibit (>50%) the adherence of whole *C. albicans* yeast cells to both saliva-coated hydroxyapatite beads and also to electrophorectically separated bPRPs immobilised on nitrocellulose membranes. Heat treatment of the crude extract inactivated the inhibitory activity, indicating a proteinaceous nature. The extract was partially purified by size exclusion chromatography and by either Concanavalin A (ConA) sepharose or lentil lectin (LcH) sepharose affinity chromatography. Two polypeptides were identified as major components of eluted fractions that possessed inhibitory activity: a 97.4 kDa band eluted from the ConA column and a 32 kDa band from the LcH column. Both fractions showed higher specific activity of inhibition than the crude extract. The 97.4 kDa polypeptide was transferred to PVDF membrane and subjected to N-terminal sequence analysis. There were no sequences in protein databases identical to the N-terminal of the 97.4 kDa polypeptide. This study has identified a novel mannoprotein from the *C. albicans* cell wall that is an adhesin for salivary proteins. This study was supported by a University of Otago Research Grant. H-W.J. gratefully acknowledges the award of a Fanny Evans Scholarship.

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Candida albicans colonization on thermocycled maxillofacial polymeric materials. H. NIKAWA[a], C. JIN, T. HAMADA, S. MAKIHIRA and G. POLYZOIS [1]. (Hiroshima Univ., Hiroshima, Japan. [1] Univ. of Athene, Athene, Greece).

Although resilient materials are valuable in fabricating of oro-facial protheses, they are known to be easily colonizd by *Candida albicans*, leading to infections such as, denture stomatitis, gastrointestinal and pulmonary candidosis. Hence we examined the colonization patterns of a single isolate of *C. albicans* on saliva- or serum-coated and protein free (uncoated), thermocycled (4°C-70°C for 1min, respectively; 0, 1000 and 10000 times) 15 different commercially available maxillofacial materials using a previously described adenosine triphosphate (ATP) analysis (J Oral Rahabil 24: 594-604, 1997). In control specimens which were not thermocycled and protein-free, the fungal colonization varied depending upon the type of the commercial products used. Thus, the lowest colonization was observed with addition-silicone materials and visible light cured, soft acrylic liners (except for one product), whereas single paste or single gel, visible light curing liners exhibited the highest colonization capacity; cold cured acrylic liners exhibited an intermediate position. In contrast, yeast colonization on all the materials could be significantly enhanced by thermal cycling (ANOVA; $p<0.01$) or by a layer of protein (ANOVA; saliva, $p<0.01$; serum, $p<0.01$). When the relationship between the fungal colonization and the surface hydrophobicity of the materials were analysed, fungal colonization on 1000- and 10000-thermocycled materials correlated well with the contact angles of the materials (Student t-test, $p<0.01$), being consistent with the thermodynamic theory. These results, taken together, suggest that the aging of the materials and the biological fluids of the host promote yeast colonization on maxillofacial materials.

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Amphipatic Peptides with Anti-*Candida albicans* Activity Neutralize HIV-1. J.G.M. BOLSCHER*, A.L.A. RUISSEN, E.J. HELMERHORST, J. GROENINK, K. NAZMI, W. VAN 'T HOF, F.A. VAN ENGELENBURG[1], H. SCHUITEMAKER[1], E.C.I. VEERMAN, A.V. NIEUW AMERONGEN (ACTA and [1]CLB, Amsterdam, The Netherlands)

Mucosal candidiasis, one of the most common opportunistic fungal infections in HIV-1 infected patients, is an early sign of clinical overt AIDS, and an important cause of morbidity. Histatin in human saliva is a natural inhibitor of *Candida albicans*. We have designed amphipatic peptides based on the functional domain of histatin-5 (KRKFHEKHHSHRGY) with strongly increased fungicidal activity. These membrane-disturbing peptides were analyzed for their effect on enveloped viruses, including HIV-1. Peptides with increased amphipathicity in either linear or lateral direction were synthesized. Antifungal activities of these peptides were compared with antiviral activities. A two-fold serial dilution of the peptides was incubated with yeast suspension of 10^7 CFU/ml or with 500 $TCID_{50}$ /ml HIV-1. The IC_{50} towards *Candida* spp was calculated after plating the incubation mixture. Neutralizing activity towards HIV-1 was determined by analyzing the cytopathic effect on MT-2 cells after 7, 14 and 21 days. Cytotoxic and hemolytic effects were determined using up to 800 µg/ml of the peptides. In comparison with the natural histatin-5, all peptides showed striking increase in fungicidal effect (IC_{50} of 2.5 µM for histatin-5, versus 0.4 - 1.6 µM for the modified peptides). Histatin-5 and three synthetic peptides showed no neutralization of HIV-1, as tested up to 800 µg/ml. Two peptides completely neutralized HIV-1 at a concentration of 200 µg/ml. One peptide neutralized HIV-1 at the lowest concentration tested (25 µg/ml). No correlation between the antifungal and antiviral activities was found since peptides displaying identical anti-HIV-1 activity exhibited different antifungal activities (IC_{50} of 0.4 and 1.6 µM, respectively). Thus we have found HIV-1 neutralizing peptides that combines antiviral capacity with antifungal activity. (supported by STW grant VTH 3950 and The Netherlands AIDS-foundation grant 434-95.006)

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Virulance Factors in case of Fluconazole Resistance in *Candida albicans* G.NAGY**,K. FEKETE-FORGÁCS[b], M.MADLÉNA[a],B. LENKEY[b] (a: Univ. of Medicine, Sch. of Dentistry, b: Kossuth L. Univ., Debrecen, Hungary)

The opportunistic pathogenic yeast *Candida albicans* is an obligate associate of human beings. It is frequently encountered as a harmless commensal of the digestive system. Multiple characteristics of *C. albicans* have been proposed as virulence traits. The aim of this study was to investigate the virulence factor of a fluconazole-sensitive (MIC $_{fluconazole}$ 5 µg/ml) clinical isolate (FS) and a fluconazole resistant (MIC $_{fluconazole}$ >80µg/ml)laboratory mutant *Candida albicans* strain (FR). In order to model the situation that may develop in human organism under fluconazole therapy, the mutant strain was developed from the sensitive one using the method of permanent shaking of fluconazole-sensitive cells at increasing fluconazole concentrations. We studied putative virulence factors including germination, adherence ability to either buccal epithelial cell or acrylate surface, the aspartic proteinase and the extracellular phospholipase activity of the two strains as well as their growth. The fluconazole-resistant strain proved to be superior in all the virulence factors tested than the original strain : 1./ the resistant strain considerably surpassed the FS strain in germinating capacity. 2./ the FR strain showed ~3.2 times higher adhesion to buccal epithelial cells compared with FS strain 3./ the aspartic proteinase activity of the FR strain was more than ten times higher, the phospholipase activity of this strain was two times higher than these values of the sensitive strain. These results suggest that the development of fluconazole resistance can accompany with serious morphological and physiological changes; several putative virulence traits, moreover the *in vivo* virulence can increase simultaneously.

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The effect of chlorhexidine gluconate on the germ tube formation of *Candida albicans* and its relatedness to post-antifungal effect. A.N.B.ELLEPOLA* & L.P.SAMARANAYAKE (University of Hong Kong).

Adherence of *Candida albicans* has been implicated as the first step in the pathogenesis of oral candidosis, and germ tube formation a contributory attribute. Recently, these organisms have also been implicated in persistent apical periodontitis. Chlorhexidine gluconate is by far the commonest antiseptic mouth wash prescribed in dentistry. As the intraoral concentrations of this antiseptic fluctuate considerably due to the dynamics of the oral cavity, the main objective of this study was to investigate the effect of brief exposure to three different sub-therapeutic concentrations of chlorhexidine gluconate (0.005%, 0.0025% and 0.00125%) on the germ tube formation of *C. albicans*. These findings were then correlated with the chlorhexidine induced post antifungal effect (PAFE) values. Ten oral isolates of *C. albicans* were exposed to three different concentrations of chlorhexidine gluconate for 30 minutes, the antiseptic removed, and the germ tube formation of these isolates quantified following subsequent incubation in a germ tube inducing medium. The PAFE was evaluated by turbidometric measurement of growth. When compared with the controls, exposure to 0.005%, 0.0025% and 0.00125% chlorhexidine gluconate suppressed the ability to form germ tubes by 81.23% ($p < 0.01$), 42.74% ($p < 0.01$) and 9.13% ($p > 0.05$), respectively, while eliciting a mean PAFE of 9.91 hours, 1.65 hours and 0.67 hours respectively. On regression analysis a significant positive correlation was observed between these two parameters ($p < 0.0001$: $r = 0.7325$). These findings, imply that short exposure to sub-therapeutic levels of chlorhexidine gluconate may modulate candidal germ tube formation as well as its growth, thereby suppressing its pathogenicity

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Genotypic comparison of *Candida albicans* and *C. dubliniensis* oral isolates. D.W. WILLIAMS*, W.A. COULTER[1], M.J. WILSON, A.J.C. POTTS, M.A.O. LEWIS (Dental School, UWCM, Cardiff, and [1]School of Clinical Dentistry, The Queen's University Belfast, N. Ireland, UK).

Previously, differentiation between *Candida albicans* and the recently described *C. dubliniensis* (Sullivan *et al.*, 1995 *Microbiol*; 141:1507-21) has been difficult due to the high level of phenotypic similarity. The aim of this study was to identify *C. albicans* (n=5) and *C. dubliniensis* (n=7) using electrophoretic karyotyping (EK) and PCR. DNA extraction for EK involved the treatment of candida in agarose plugs with lyticase (1 mg/ml) buffer (72 h; 37°C), followed by proteinase K (1 mg/ml) treatment (72 h; 45°C). The plugs were washed (×3) in 50mM EDTA and placed in the wells of a 0·9% agarose gel (in 1× Tris Borate EDTA (TBE) buffer). Pulsed-field gel electrophoresis was performed in 1× TBE buffer for 24 h at 4 V/cm (300s switch interval) followed by 48 h at 2·7 V/cm (1000s switch interval). DNA banding patterns were analysed with a GelDoc 1000 system (BioRad) and associated software. PCR on candidal DNA targeted the intergenic spacer regions of the ribosomal DNA operon with primers ITS1 and ITS4 (Williams *et al.*, 1995 *J Clin Micro;* 33:2476-79). Resulting products were sequenced and compared using CLUSTALW software. EK revealed differences between *C. dubliniensis* and *C. albicans*, with a lower molecular weight band (~800 Kb) evident in the former. Differentiation of the species was also achieved by PCR sequencing with over 20 consistent base differences evident in the PCR products. Subsequent restriction digestion of PCR products using *Msp*AII was also found to differentiate the species. These results demonstrate the value of EK and PCR in the discrimination of *C. dubliniensis* and *C. albicans*. Of the two approaches, PCR is a non-subjective approach and less laborious than EK.