2809 Pathogenicity of *Abiotrophia* Isolated from Oral Cavity.

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Both *Abiotrophia adiacens* and *Abiotrophia defectiva* are important human pathogens causing infective endocarditis. Our previous study has demonstrated an unexpectedly high colonization frequency (87.1%) of *A. adiacens* in the oral cavity of healthy adults, whereas that of *A. defectiva* (11.8%) was relatively low (Ohara-Nemoto Y. *et al.*, J Clin Microbiol, 1997), in spite of similar isolation frequencies of these two bacteria from the patients. To compare the pathogenic potency, we injected these bacteria (1×10^8 CFU) i.p. into ddy mice pretreated with galactosamine, and counted viable cells in the spleen and blood. After 24h of injection, approximately one hundred-fold more *A. adiacens* (1×10^4 CFU) survived than did *A. defectiva* in the spleen, while both bacteria were not observed in blood 24h after, and nor in the spleen 48h after. These results suggest that the inflammatory response of the host against these bacteria is different. Thus, we firstly investigated extracellular immunoreactive activities produced by *Abiotrophia*, and found that the culture supernatant of *A. adiacens*, not of *A. defectiva*, contained a high molecular mass (approx 1,200 kDa) substance stimulating mouse peritoneal macrophages. The purified substance was dominantly composed with polysaccharide, and induced expression of proinflammatory mediators (i.e., COX-2, iNOS, TNF-α, IL-1 β, IL-6, and IL-8) mRNA in mouse macrophages and also in human peripheral blood mononuclear cells. These results suggest that the bioactive polysaccharide from *A. adiacens* should be a pathogenic agent of the organism. This study was supported by a grant from the Science Research Promotion Fund of the Japan Private Promotion Foundation.

2810 Sulfate-Reducing Bacteria in Periodontal Pockets and Healthy Oral Sites.

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Sulfate-reducing bacteria (SRB) are strictly anaerobic terminal degraders, which depend on an active microflora for appropriate growth conditions in the periodontal pocket. A previous study indicated that SRB occur in pockets of 48 % of periodontal patients. The aim of this study was to determine the distribution of SRB throughout the oral cavity.
This was investigated in patients referred to the periodontal clinic, prior to mechanical therapy. From 20 patients samples were taken from pockets with a depth ≥ 5 mm and from healthy gingival sulci by insertion of a sterile paperpoint. From mucosa samples were scraped with a sterile plastic loop. All samples were immediately transferred to a pre-reduced growth medium containing an $FeSO_4$ indicator, and incubated in an anoxic chamber with an oxygen partial pressure below 5 ppm. Samples positive for sulfate-reducing activity showed strong blackening due to FeS precipitation, and SRB were identified by culture on agarplates and partial 16S rDNA sequence analysis. In 85 % of the patients SRB were present in at least one pocket, and one third of the patients contained SRB in all pockets that were sampled. In 55 % of the patients SRB were not detected in healthy oral sites. In healthy sulci and on the mucosa SRB were found only in low frequency. Detection frequencies of SRB on the tongue and in supragingival plaque were 25 and 20 %, respectively. The observations in these patients indicated a correlation between the presence of sulfate-reducing bacteria and periodontitis.

2811 Strain Replacement in AIDS Related Candidiasis in Zambian Patients.

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Candida albicans is the most frequently isolated species from oral candidiasis in industrialised countries, but our studies have noted a much higher prevalence of non-albicans species in some African countries. This study aimed to ascertain which species of yeast predominate in the oral cavities of a group of rural Zambian AIDS patients and to determine if the presence of *C. albicans* is more frequently associated with clinical diagnoses of oral candidiasis compared with other species.
Yeasts were isolated and colony forming units (cfu/ml) determined from the whole saliva of 107 AIDS patients attending a rural hospital in Zambia. Yeasts were identified using Chromagar, API 32C, PFGE and PCR. Oral and general clinical examinations were performed and CD4+ lymphocyte counts determined. None of the study population were receiving antibiotics or antimycotics.
Salivary culture yielded 108 yeast isolates from 89 (83%) of the 107 patients. Of these, 58% of the patients harboured *C. albicans*, with the remaining isolates constituting a variety of species which are generally considered to be less pathogenic than *C. albicans*. Of the 22 patients presenting with clinical signs of oral candidiasis, 19 (86%) harboured *C. albicans*, while only three patients presented with oral candidiasis due to non-albicans species. Non-albicans species were recovered more frequently (86%) from patients without clinical signs of oral candidiasis compared with candidiasis patients (14%). Patients with high saliva yeast counts (>10,000 cfu/ml) were more likely to harbour *C. albicans* (85%) compared with patients with moderate (1,000 to 10,000 cfu/ml) or low (<1000 cfu/ml) counts. Patients with low counts were most likely to harbour non-albicans species (78%). Oral carriage of *C. albicans* compared with non-albicans species did not appear to be influenced by CD4 counts or by gender.
This study was conducted on a drug-naive AIDS population with a naturally high prevalence of non-albicans species of *Candida* and suggests a selection of *C. albicans* as oral candidiasis develops.

2812 A Predominant Colony Phenotype for *Candida albicans* in Denture Stomatitis.

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Candida albicans cells issued from a single progenitor are capable of expressing an assortment of variant colony morphologies which result from differences in the proportion of blastospores, hyphae and pseudohyphae in the colony domes. As switch phenotypes may also affect many of the putative virulence attributes of *Candida*, we tested whether commensal oral *Candida* isolates would display the same repertoire as isolates from denture stomatitis. The study population was a cohort of 235 patients, of which 66% were oral carriers of *Candida albicans* and 101 presented clinical symptoms of denture stomatitis. These were divided in 3 groups according to the severity of clinical symptoms whereas two groups of healthy patients were included: 13 were denture wearers and 26 were patients without prosthesis. *Candida albicans* was enumerated from denture and/or mucosal swabs on Oricult-N dip slides and individual commensal or stomatitis isolates were constituted by pooling all colonies on each dip slide for overnight amplification in Sabouraud broth. The repertoire of morphologies for 100 colonies per isolate was resolved on agar containing the amino-acid rich medium of Lee supplemented in arginine and a limiting concentration of zinc after 9, 21 and 90 days of incubation at 25°C. Five phenotypes were observed but most isolates showed only two colony morphologies. The smooth phenotype was predominant in patients with stomatitis, this morphology increasing up to 90% with the severity of disease. The major phenotype in healthy subjects was a colony with a halo of mycelia spreading into the agar. Present data suggest that environmental constraints for bud-hyphae transition within the denture plaque may differ in health and disease leading to a selection for particular switching systems, some of which possibly associated with increased inflammatory potential.
This study was supported by the Fond de Recherche en Santé du Québec (FRSQ) and the Fond National de Recherche Scientifique Suisse (FNRS).

2813 Intra-oral Colonization of Coliform Bacteria in Irradiated, Dentate, Xerostomic Individuals.

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We investigated the oral colonization of aerobic or facultative anaerobic gram-negative rods and cocci (AGNR & C) in dentate, xerostomic (head and neck irradiated) individuals. They were recruited from a nasopharyngeal carcinoma clinic and were segregated into group A: < 60 yr (n = 25, 48 ± 6 yr, 5 ± 5 yr post-irradiation), and group B: ≥ 60 yr (n = 8, 67 ± 4 yr, 2 ± 2 yr post-irradiation) and were compared with age matched normal individuals, group C: < 60 yr (n = 20) and group D: ≥ 60 yr (n = 60). Selective culture of the oral rinse samples was carried out to isolate, quantify and speciate AGNR & C recovery. All test subjects were put under comprehensive oral and preventive care for 3 months and 12 of group A and 5 of group B subjects were recalled for reassessment of AGNR & C colonization. All identical isolates, pre- and post- hygienic care, were phenotypically (API 20E, Vitek and antibiogram profile) and genotypically (pulsed-field gel electrophoresis, PFGE) evaluated. The AGNR&C isolated included: *Acinetobacter, Neisseria, Chryseomonas, Flavimonas, Pseudomonas, Citrobacter, Enterobacter, Escherichia, Klebsiella, Flavobacterium* and *Weeksella* species. AGNR & C isolation rate were 64/25% and 100/52% for groups A/C and B/D, respectively. *Pseudomonadaceae* were frequently found in group B ($P < 0.05$), where *Pseudomonas aeruginosa* and *Citrobacter freundii* (clu/ml oral rinse) were significantly elevated. The isolation rate of AGNR & C post-hygienic care remained unchanged. On repeat culture, 3/12 and 3/5 subjects in groups A and B, respectively, harbored same AGNR & C. Only 2 pairs of *Klebsiella pneumoniae*, isolated form group B, were found to be identical by PFGE. This may be due to reinfection from the same source or permanent colonization. In conclusion, irradiation induced xerostomia seems to favor frequent, repeated, transient intra-oral colonization of AGNR & C, especially in elderly individuals.
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2814 Denture stomatitis - clinical and microbiological study.

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It has generally been assumed that *Candida albicans* and related species play an important role in the pathogenesis of denture stomatitis (Iacopino *et al. JADA*, 123: 46-51, 1992). To evaluate this the frequency and severity of *Candida* infection in 46 patients, acrylic complete denture-wearers suffering from denture stomatitis were assessed. The patients with denture stomatitis were categorised according to the classification of Newton. The prevalence and density of yeasts in the oral mucosa and in the denture were estimated by the imprint culture technique. All yeast isolations were identified by germ tube formation and by API 20C AUX (bioMerieux). The *in vitro* susceptibility of isolated fungal strains was assessed by the use of agar-diffusion method (Diagnostics Pasteur) (Drouhet *et al.* Bull. Soc. Fr. Mycol. Med., 10: 131-134, 1981).
Type I of denture stomatitis occurred most frequently in the patients. 66% of the patients had clinical and microbiological evidence of oral candidal infection. *Candida albicans* were the most frequently isolated yeasts. The mean overall candidal density was significantly higher on the fitting surface of the denture than on the palatal mucosa ($p<0,01$). All yeast isolates were *in vitro* susceptibility tested and 98% of them were found to be sensitive to nystatin.
The results were assessed, using U-Gauss test and Student's t test, where appropriate. The results suggest that denture stomatitis is associated with a proliferation of *Candida* which is primarily within plaque on the denture rather than on the inflamed palatal mucosa. These findings should be taken into account during treatment the patients with *Candida* - associated denture stomatits.

2815 Microbial Analysis of Primary Teeth Dentine expressing Nursing Caries

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Nursing caries constitute a very invalidant pathology of the first age characterized by poor perspectives of therapy certainly correlated with the very few knowledges about this disease. In this way, we examined incisors and molars from 10 children aged 2 1/2 to 5 with nursing bottle syndrome for their microbial flora.
After clinical and radiographic exams, teeth were extrated under local anesthesy and immediatly transferred in RTF (reduced transport fluid). For microbial analysis, apparent dentine at the lesion site was sampled using a sterile excavator. Ten fold serial dilutions of the samples were inoculated on supplemented agar under microaerophilic and anaerobic conditions.
Results were characterized by the non recovery of Bacteroidaceae and especially of pigmented species. Anaerobic flora was detected at low level with *Peptostreptococcus* sp. and *Veillonella* sp. in about 50% of the samples. Among streptococci, no prevalence of *S. mutans* has been observed. *Capnocytophaga* sp. and *Actinomyces* sp. were always present associated with various *Haemophilus*. The most significant result consisted in the constant detection of *Actinobacillus actinomycetemcomitans*. SEM analysis demonstrate that nursing caries consist in an enamel destruction following by dentine attack. Bacteria migration inside the tubuli was correlated with the progressive destruction of the dentine and linked with the progression of the disease. These data indicate that the preliminary most often implicated bacteria in nursing decay (i.e., *P. melaninogenica*) like bacteria implicated in caries (i.e. *S. mutans* and Lactobacilli) are not recovered. On another hand, the presence of *Actinobacillus actinomycetemcomitans* in all the samples may correspond to its implication in the aetiology of this particular dentine destruction process. This study was supported by Pierre Fabre Laboratories.

2816 Pre-school Children with Rampant Caries: Microbial Load and Risk Factors

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Few studies investigated the relationship between risk factors and the development of rampant or nursing caries. The aim of the present study was to describe the microbiological profile of pre-school children with rampant caries and to compare it with that of an age-matched group without or with low caries experience. The impact of several risk factors was investigated. A total of 136 children were allocated to one of the following three groups based on their caries experience: RC=rampant caries (dmft≥6), CF=caries-free (dmft=0) and LC=lower caries experience (0<dmft<6). The RC group comprised 88 children with a mean age of 4.8 (SD: 1.1) years, the CF group 30 children with a mean age of 5.0 (SD: 0.9) years and the LC group 18 children with a mean age of 5.8 (SD: 0.6) years. From each child, besides clinical parameters (e.g. plaque extent, gingival inflammation, carious lesions), socio-economic status and oral health behaviour were scored (the latter by means of a questionnaire and dietary agenda). Moreover samples from the supragingival plaque and the saliva on the tongue were collected in order to evaluate the number of colony forming units of *S. mutans, S. sobrinus* and lactobacilli. RC children scored significantly higher for nearly all risk factors when compared to the CF group. *S.mutans* was isolated in 86 and 87 of tongue-loop and plaque samples of the 88 RC children while this was the case for only 11/30 of tongue-loop and plaque samples from CF children. No significant differences were found in the isolation frequency of Lb between RC (±40%) and CF (±50%) group. *S. sobrinus* was only observed in the RC group (5/88). The variance in dmft-score within the group of children with a low caries experience was again significantly influenced by nearly all risk factors, except the microbial load, bringing the importance of the bacteria per se again in discussion. In the RC group the impact of all risk factors on the further variance in dmft is negligible, except for the sugar consumption and the help during brushing. The other factors seem to have passed their saturation level for what their influence on the dmft is concerned. Therefore the importance of several risk factors in the etiology of caries in young children is highlighted but a certain threshold level above which no further impact should be expected is also suggested.