SESSION IV—INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

LOOKING AT AUTISM AS AN INFECTIOUS DISEASE

Finegold, S.M.*
West Los Angeles VA Medical Center and UCLA School of Medicine, Los Angeles, CA USA

There is evidence to indicate that intestinal bacteria, specifically clostridia, may play a role in certain cases of autism (late onset disease with prominent gastrointestinal features). Looking at this type of autism as an infectious disease, one can envision a scenario that could explain (1) the relapse of the disease following therapy with oral vancomycin, (2) the unexplained increasing incidence of autism, and (3) the unexplained increase in multiple cases in the same family. This scenario would revolve around the clostridial spores.

Relapse following clinical improvement in response to an antimicrobial agent would be related to persistence of spores (which are resistant to antimicrobials) during therapy and then germination of the spores after discontinuation of the antimicrobial to form new vegetative cells of the clostridia. This is the situation that we face in relapsing *Clostridium difficile*-related colitis.

The increased incidence of autism again parallels the situation with *C. difficile*-induced colitis. Just as contamination of the hospital environment in the case of *C. difficile* spores accounts for spread of the infection, contamination of the home environment with spores of whichever clostridia may be involved may account for the increased incidence of autism. This presupposes a susceptible host which, in the case of autism, may mean immunoincompetence related to genetic factors or to environmental factors (even just receipt of an antimicrobial which may select out responsible clostridia by suppressing other elements of the bowel flora which may be protective). It is well known that the usual hospital cleansing procedures and germicides typically are not adequate to remove *C. difficile* spores and that spores can persist for months. This would certainly be a likely problem in the home setting as well. In addition, young autistic children's lack of hygiene would lead to significant contamination of the home environment.

Exposure of susceptible siblings in the home would account for increased incidence of multiple cases of autism in the family setting, in the manner discussed in the above paragraph.

The above hypotheses suggest the need for additional research to the role of spores in the environment.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

BACTERIAL VAGINOSIS AND PRETERM BIRTH: NEW INSIGHTS INTO HOST-MICROBE INTERACTIONS

Simhan, H.*
University of Pittsburgh, Pittsburgh, PA USA

The stark disparity in preterm birth between black and white women in the US continues to be a critical and worsening public health crisis, not explained by sociodemographic factors alone. Infection is the etiology of many spontaneous preterm births, especially the earliest births that have the greatest morbidity and mortality. Black women are more likely to have alterations in the lower genital tract inflammatory environment that predispose them to early preterm birth and intrauterine inflammation. Black women are also more likely than white women to have gene polymorphisms that are related to infection-mediated preterm birth. Therefore, in order to understand the racial disparity of preterm birth, it is critical to clarify the influence of environmental factors and gene-environment interactions on lower genital tract inflammation.

Bacterial vaginosis (BV) is one of the most prevalent vaginal disorders, affecting 30% of reproductive age women. BV is a syndrome characterized by a relative lack of lactobacillus and an increase prevalence of anaerobic bacteria, *G. vaginalis, Mobiluncus spp*., and *M. hominis*. There is a strong and consistent association of bacterial vaginosis during pregnancy with preterm birth. The risk factor with the greatest magnitude of association with BV is black race. The 3-fold increased risk of BV among black women is not completely explained by an increased frequency of douching, coital frequency, lower socioeconomic status, and the use of vaginal products.

The risk of preterm birth attributable to BV and vaginal inflammation is greater among African-American women than their Caucasian counterparts. The question of why BV is associated with preterm birth in some women but not in others remains unanswered. The mechanism by which BV may effect the initiation of preterm labor is also unclear. Antibiotic treatment trials of BV among women in the general obstetric population have not demonstrated a reduction in the frequency of spontaneous preterm birth. There is a critical need to identify subgroups of pregnant women who are at highest risk for adverse sequelae of BV.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

INDIGENOUS ANAEROBIC OXALATE DEGRADING BACTERIA AND THE PREVENTION OF CALCIUM OXALATE KIDNEY STONE DISEASE

Abratt, V.R.;* Reid, S.J.; Kullin, B.; Lewanika, T.R.; Magwira, C.
Department of Molecular and Cell Biology, University of Cape Town, Cape Town, South Africa

Oxalate-degrading bacteria present in the human colon have been shown to play an important role in host oxalate homeostasis, thereby reducing urinary oxalate concentration and consequently, calcium oxalate kidney stone formation. To establish whether these bacteria may contribute to the low incidence of stone formation (less than 1%) in black people in South Africa, the colonization of gastrointestinal oxalate-degrading bacteria was studied in two groups of male subjects (n = 45), having either a high or low risk of kidney stone formation. DGGE analysis of the faecal microbiota showed that significantly different populations of *Lactobacillus* spp were present in these two groups (Mann-Whitney U test; $p = 0.03$), and that the overall oxalate degrading capacity of culturable *Lactobacillus* spp from the low risk group was higher than that of the high risk group. Oxalate enrichment of faecal samples from the low risk group was used to isolate and identify several possible oxalate degrading *Lactobacillus* species for potential use as probiotics in controlling gut oxalate levels. The presence of a cluster of genes potentially involved in oxalate degradation was confirmed in several *Lactobacillus* candidate species by PCR amplification and DNA sequencing. One of these species, *L. gasseri,* was tested in a three-stage continuous culture system (CCS), inoculated with faecal bacteria, to model environmental conditions in the human proximal and distal colons. A freeze-dried *L. gasseri* probiotic preparation was successfully established in the colon simulator under steady-state growth conditions and promoted oxalate degradation in the first stage of the CCS. The results of the study suggest that *Lactobacillus* strains may play a role in protecting individuals from developing calcium oxalate kidney stone disease and may have potential therapeutic use in managing this disease.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

ENTEROTOXIGENIC *BACTEROIDES FRAGILIS* (ETBF): INSTIGATOR OF COLITIS AND COLONIC ADENOMAS IN MICE

Sears, C.L.;* Rhee, K-J.; Rabidazeh, S.; Housseau, F.; Pardoll, D.M.; Huso, D.; Wu, X.; Wu, S.
Johns Hopkins University School of Medicine, Baltimore, MD USA

ETBF are associated with inflammatory diarrheal illnesses in humans and livestock. Some individuals (~4-30%) appear to be colonized asymptomatically with ETBF. The key virulence factor of ETBF is the *B. fragilis* toxin (BFT), a 20 kDa metalloprotease toxin. BFT binds to a receptor and stimulates cleavage of E-cadherin, increased intestinal permeability, secretion of interleukin-8 and epithelial proliferation. We hypothesized that ETBF colonization is detrimental to the host, instigating colonic inflammation. To test this hypothesis, we evaluated the sequelae of ETBF colonization in C57Bl/6 and multiple intestinal neoplasia (Min) mice. Min mice are adenomatous polyposis coli (APC) heterozygotes, a nearly universal mutation in human colon cancer. Our results indicate that ETBF and nontoxigenic *B. fragilis* (NTBF) colonize C57Bl/6 mice chronically (> 10^9 CFU/gm stool). However, only ETBF colonization of C57Bl/6 mice triggers E-cadherin cleavage and symptomatic colonic inflammation by 18 hours that clinically resolves within one week. Although ETBF-colonized C57Bl/6 mice are healthy after one week, colon pathology reveals persistent colitis up to 16 months later. Recombinant BFT expression by NTBF or *B. thetaiotaomicron* is sufficient to induce colitis. Intracellular cytokine staining at one week reveals a dominant Th17 CD4 cell response in ETBF, but not NTBF, mice. Chronic colonization of Min mice with ETBF also results in a predominant Th17 CD4 cell response as well as acceleration of colonic adenoma formation that is localized to the distal colon similar to human disease. At 4 weeks, 1.5 + 0.5, 3 + 0.3 and 13 + 2 colonic adenomas are visible in sham-inoculated, NTBF- and ETBF-colonized Min mice, respectively ($P<0.007$). Induction of adenomas in ETBF-colonized mice is augmented by other commensal flora. Pathology reveals increased acute inflammation associated with adenomas in ETBF mice. These data indicate that ETBF is a proinflammatory, oncogenic colonic commensal and suggest the hypothesis that colonic ETBF colonization in humans may modulate the commensal flora and induce chronic, deleterious inflammation.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

PREVOTELLA SP IN PUSTULAR ACNE LESIONS: PREVALENCE AND ANTIMICROBIAL RESISTANCE

Bahar, H.;* Ozdemir, S.; Cilingir, H.; Altinkum, S.; Mamal Torun, M.
Istanbul University, Cerrahpasa Medical Faculty, Department of Microbiology & Clinical Microbiology, Istanbul, Turkey

Background: Pustular acne lesions are red at the base and characterized by an inflamed, pus-filled inner region. *Propionibacterium acnes* is the most commonly encountered microorganism in acne lesions, but secondary infection with other microorganisms is not rare. The aim of this study was to present the prevalence of *Prevotella* species in pustular acne lesions and to determine their beta–lactamase activity and their resistance to tetracycline, erythromycin, and clindamycin known as antimicrobial agents mostly used in acne treatment.

Materials and Methods: Between April 2004 – November 2007, pus taken from pustular acne lesions of 410 patients were studied for the presence of anaerobic bacteria. 282 of these acne lesions were from the face, 67 were from the back, 33 from the chest, 19 from the nape, and 9 were from the upper arms. Pus specimens were inoculated on the basal medium prepared with brucella agar supplemented with hemin, vitamin K1, 5% sheep blood, and additionally inoculated on phenylethyl alcohol anaerobic agar, on kanamycin vancomycin anaerobic agar prepared with this basal medium. Media were incubated 5 days on anaerobic conditions obtained with Anaero-Gen (Oxoid & Mitsubishi Gas Company) in anaerobic jars (Oxoid). Anaerobic bacteria were identified by API 32 ID (BioMerieux). Susceptibility testing for tetracycline, erythromycin, and clindamycin was performed with E-test (AB Biodisk) and nitrocefin disks were used to determine the β-lactamase production of each strain.

Results: *P. acnes* was isolated from 391 (95.3 %) pus specimens of 410 acne lesions and *Prevotella spp* was isolated from 62 (15%) of these lesions together with *P. acnes.* 41 (66.1 %) of them were *P. melaninogenica* and 23 (37%) were *P. intermedia.* 48 (12.2%) srains of *P. acnes* was found resistant to erythromycin, 21 (5.3%) resistant to tetracycline and 9 (2.3%) resistant to clindamycin. Beta lactamase production was found in 11 (17.7%) strains (8 strains of *P. melaninogenica* and 3 strains of *P. intermedia*). 6 (9.6%) strains were found resistant to erythromycin, 4 (6.4%) strains were found resistant to tetracycline.Clindamycin resistance was not found in these *Prevotella species*.

Conclusion: The role of *Prevotella sp* in pustular acne lesions need to be explained with more studies. *P. acnes* could act as a reservoir for resistance genes.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

MIXED ANAEROBIC INFECTION PRESENTING AS PERISPINAL MASS

Ghafghaichi, L.; Santos, S.; Banaei, N.; Baron, E.J.*
Stanford University Medical Center, Stanford, CA USA

Association between *Actinomyces* species and *Haemophilus* (*Actinobacillus*) *actinomycetemcomitans* has been recognized for many years, but many younger microbiologists and physicians have not seen such cases. Therefore, we thought this classical presentation was instructive. The 56 year old patient, a previously heavy smoker, presented with progressive lower extremity weakness after a month of increasing midthoracic back pain. His CT showed a T6 compression fracture and perispinal mass invasive to the epidural space which was originally thought to be lung cancer with metastases. At surgery, purulent discharge and an inflammatory process were observed, leading to a subjective diagnosis of infectious osteomyelitis and abscess. Specimens were sent for pathology and culture (anaerobic, routine, AFB, fungal). Histopathologic examination showed "sulfur granules" containing branching gram-positive filamentous rods. Cultures first yielded *Haemophilus actinomycetemcomitans* and *Fusobacterium nucleatum*. An *Actinomyces* species was eventually isolated in pure culture after multiple attempts. Due to inconsistent results with spot tests and Rapid ANA II (Remel), the *Actinomyces* was sequenced for final identification. The patient was initially given metronidazole, cefipime, and vancomycin. He was discharged on I.V. ertapenem (1 gm) for 6 weeks and with rehabilitation was recovering very slowly. On more extensive history-taking by the infectious disease specialist, the patient admitted having two wisdom teeth removed 3-4 months prior to this illness.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

POPULATION DYNAMICS OF ANAEROBIC BACTERIA IN THE SPUTUM OF PATIENTS WITH CYSTIC FIBROSIS DURING AN ACUTE PULMONARY EXACERBATION

Field, T.R.;* Sibley C.D.; Parkins, M.D.; Rabin, H.R.; Surette, M.G.
University of Calgary, Calgary, Alberta, Canada

Background: Cystic Fibrosis (CF) is the most common lethal inherited disease of the Caucasian population with chronic, progressive pulmonary infection, the leading cause of morbidity and mortality. Anaerobic bacteria have been demonstrated to be present in the sputum of patients with CF during both the stable chronic phase of infection and during acute pulmonary exacerbations. These previous studies have been cross-sectional and employed either culture or molecular profiling techniques to examine the bacteriology of the sputum samples.

Aim: The aim of this study was to examine population dynamics of anaerobic bacteria in the sputum of CF patients, longitudinally during the course of an acute pulmonary exacerbation using both culture and molecular profiling techniques.

Methods: Sputum samples were collected from patients upon admission to hospital prior to commencement of antibiotic therapy and every one to two days until discharge. Anaerobic bacteria were cultured using a variety of media, under strict anaerobic conditions, from sputum samples obtained upon admission, and a second sputum sample obtained during the course of the exacerbation. DNA was isolated from all of the sputum samples collected over the course of an exacerbation for selected patients and the bacterial communities within the sputum samples were examined using T-RFLP profiling.

Results: Consistent with previous results, anaerobic bacteria were found to be present in 100% of the sputum samples of CF patients admitted to hospital with an acute pulmonary exacerbation. In these admission samples the anaerobic bacteria were present in large numbers (within one log of the CFU of the numerically dominant aerobic pathogen detected) and declined in number as the exacerbation resolved. 100 independent isolates have been cultured from 6 patients (30 sputum samples) and are currently being identified by 16S sequencing and to date 3 have been identified as *Prevotella*. Analysis of the bacterial communities via T-RFLP profiling correlated with the quantitative microbiology. This combination of molecular and culture based techniques provide a powerful approach to elucidate the contribution of anaerobes to polymicrobial infection in CF.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

VAGINAL ACIDIFICATION AND CHARACTERIZATION OF THE LACTOBACILLI IN CHLAMYDIAL CERVICITIS

Romanik, M.; Aptekorz, M.; Ekiel, A.; Martirosian, G.*
Department of Medical Microbiology Medcial University of Silesia, Katowice, Poland

Lactobacillus spp. play a key role in preventing an overgrowth of a variety of anaerobic bacteria associated with bacterial vaginosis (BV) and sexually transmitted infections including *Chlamydia trachomatis* (*C. trachomatis*). Acid and hydrogen peroxide (H_2O_2) production by vaginal flora is one of the most protective mechanisms of the upper genital tract and obstetric and gynecological complications. Limited information exists about vaginal pH and *Lactobacillus* communities in women with chlamydial cervicitis.

The group of 122 sexually active (18-40 years old), nonpregnant women with cervicitis was studied. Following the vaginal pH determination (using colorimetric pH test card), two vaginal swabs were taken for preparation of Gram staining slides and culturing on MRS agar and one endocervical swab for *C. trachomatis* antigen detection (using Chlamydia direct IF assay bioMerieux). The diagnosis of BV was based on Nugent criteria. Identification of lactobacilli was based on Gram staining, catalase activity, biochemical tests and two steps PCR; the H_2O_2 production by *Lactobacillus* spp. was also determined.

By using Nugent criteria in 11.5% studied women, BV and intermediate microflora were confirmed. The vaginal pH value ≥ 4.5 was detected in 22.1% of 122 women with cervicitis, including 30.9% (17/55) women with chlamydial cervicitis and 14.9% (10/67) women with nonchlamydial/nongonococcal cervicitis. The significant difference (p=0.034, RR=0.81, OR=0.39) between vaginal pH value from women with chlamydial cervicitis (mean pH =4.5, ranged 3.8 - 6.8) and nonchlamydial/nongonococcal cervicitis (mean pH =4.2, ranged 3.8 - 6.2) was observed. The H_2O_2 producing lactobacilli were detected very often in both group of women with chlamydial and nonchlamydial cervicitis (77.7% and 69.6% of isolated strains, respectively). The H_2O_2 producing *Lactobacilli* belonged to genus: *L. crispatus* (26.9%), *L. gasseri* (19%), *L. salivarius* (7.6%), *L. jensenii* (3.8%) and *L. reuteri* (7.6%).

C. trachomatis infection influences significantly on vaginal pH value, but not on Lactobacilli communities.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

FOUR CASES OF INTRIGUING BACTEREMIA CAUSED BY *FUSOBACTERIUM NUCLEATUM*

Terhes, G.;[1] Urbán, E.;*[1] Piukovics, K.;[2] Hajdú, E.;[1] Nagy, E.[1]
[1]Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Hungary
[2]2nd Department of Internal Medicine, University of Szeged, Szeged, Hungary

Objectives: Our aims were to show four cases of bacteremia caused by *F. nucleatum* and to analyze the microbiological features and the clinical background of these cases and to emphasize the importance of the use of anaerobic blood culture bottles and culture in the case of bloodstream infections.

Methods: During the febrile period, blood was taken from 4 patients, aerobic, and anaerobic blood culture bottles (Becton Dickinson BACTEC™) were incubated until positive signal was generated. Positive samples were processed according to the standard clinical laboratory procedures.

Results: During a 10-month-period, four cases of bacteremia caused by *F. nucleatum* in patients (3 females, 1 male) with hematologic malignancy (the underlying disease was ALL in one case, and AML in three cases) were detected. In the case of three patients, inflammation of the oral mucosa were present, while in one case, no oropharyngeal ulcer was detected, however, this patient received chemotherapy, which may induce the development of oropharyngeal lesions. In all cases, fusobacteria were isolated from anaerobic bottles only. The mean number of hours for the blood cultures to give positive signal was 61.5 hours (2.6 days). Coinfection was not detected in the above cases. In spite of the adequate therapy, two patients died: one of them had septic shock, while the other patient developed pneumonia.

Conclusion: The incidence of bacteremia caused by anaerobic bacteria varies greatly in various publications, and the requirement to use anaerobic bottles to detect causative agents is discussed extensively in the literature. Because of the increase in the number of patients with hematologic and other malignancy, their better survival, the use of wide range of chemotherapeutic agents, and frequent damage to the oral or intestinal mucosa, we would expect increasing incidence of bloodstream infections caused by anaerobic bacteria.

Anaerobe • 2008

The 9th Biennial Congress of the
Anaerobe Society of the Americas

Renaissance Hotel • Long Beach, California USA
June 24-27, 2008

INTERESTING CLINICAL TOPICS IN ANAEROBIC INFECTIONS

DOMINATING ANAEROBIC FLORA DEMONSTRATED IN THE BIOFILM STRUCTURE ON THE INTRA-UTERINE DEVICES

Urbán, E.;*[1] Pál, Z.;[2] Dóczi, I.;[1] Mihalik, E.;[3] Pál, A.;[2] Nagy, E.[1]
[1]Institute of Clinical Microbiology
[2]Department of Obstetrics and Gynaecology
[3]Department of Plant Biology, University of Szeged, Hungary

Objectives: Intrauterine devices (IUDs) are highly effective, long-term methods of contraception; however, IUD use is limited due to concerns about the increased risk of pelvic inflammatory disease (PID) and subsequent complications. The major complication associated with the use of medical implants such as IUDs, intravascular catheters, or tubes is infection. Microorganisms originating from the normal flora can colonize these devices and form biofilms, consisting of layers of host cells and bacteria/fungi embedded within a matrix material. It turned out that these foreign materials are the most probable sites of biofilm formation. The biofilm bacteria are usually resistant to attack by antimicrobial agents and host phagocytes. This is one reason to explain why infections caused by these microorganisms are hard to treat without removal of the devices.

Methods: A retrospective review of clinical and microbiological data of 127 participants was carried out over a 3-year period; IUDs were removed and sent for qualitative and quantitative microbiological examination. A 10-year old IUD was chosen as a representative sample; this was removed because of the symptoms of PID and was investigated via both microbial culture methods and scanning electron microscopy. The primary objective of this study was to examine the bacteria present on removed IUDs of different ages by using aerobic and anaerobic culture methods.

Results: A close association of the distribution of aerobic and anaerobic bacteria on the IUDs with different ages was found. Our data showed a close correlation between the change in the number and type of the microbial flora and the proportion of patients with BV, the longest the IUD was in place. This supports the recommendation for wearing the IUD only for 5 years to ensure safety. The complexity of the biofilm flora and the dominance of the anaerobic bacteria on the IUDs older than 5 years were remarkable, regardless whether the patient had a BV flora in their vagina or had no symptoms or signs of genital infection.

This work was supported by a grant K69044 from the Hungarian National Researches Foundation (OTKA).