Abstracts from the Joint 17th ISG/34th SOMED Meeting in Yokohama, 19–22 May 2011

Guest Editors: Professor Shigeru Kamiya, MD, PhD and Professor Shunichi Kato, MD, PhD
## Contents

### Symposium Presentations

- Microbial ecology between *Helicobacter pylori* and microbiota in gastric mucosa  
  *Shigeru Kamiya* 
  1

- Hematopoietic stem cell transplantation (HSCT)  
  *Shunichi Kato* 
  1

- The current situation and progress in haploidentical hematopoietic stem cell transplantation  
  *Xiaojun Huang* 
  2

- Emerging roles of noninherited maternal alloantigens (NIMAs) and inherited paternal alloantigens (IPAs) in HLA-mismatched hematopoietic cell transplantation  
  *Tatsuo Ichinohe* 
  3

- Properties of sialic acid-binding adhesin of *Streptococcus gordonii*, an oral bacterium as a member of dental plaque organisms and etiological agent for infective endocarditis  
  *Kiyoshi Konishi and Yukihiro Takahashi* 
  3

- Intestinal bacterial microbiota: lessons learned  
  *Marika Mikelsaar* 
  4

- Molecular ecology of butyrate-producing bacteria from the human gut  
  *Petra Louis* 
  5

- Study of human hematopoietic stem cell aging using immune-deficient mice  
  *Kiyoshi Ando* 
  6

- Human-induced pluripotent stem cell-derived blood cells toward clinical application  
  *Naoya Takayama* 
  6

- CMV reactivation and immune reconstitution after CBT  
  *Satoshi Takahashi* 
  7

- The immunobiology of hematopoietic stem cell transplantation  
  *Anders Fasth* 
  7

- Microbial metabolic functions in Crohn’s disease patients  
  *Elisabeth Norin* 
  8

- Application of metabolomics approaches to study energy metabolism and reveals the hepatic glycogen accumulation in germ-free mice  
  *Hsiao-Li Chuang, Yen-Te Huang, Chia-Chung Hou, and Chi-Chang Huang* 
  10

- Microbial ecology and shelf life of ready-to-eat pomegranate arils packaged under modified atmosphere  
  *Athanasios Alexopoulos, Elisabeth Chatzivassiliou, Staeros Plessas, Maria Alexakoudi, Irene Theodoridou, Maria Fournomiti, Ioanna Mantzourani, Chryssa Voidarou, Elizabeth Stavropoulou and Eugenia Bezirtzoglou* 
  11
| Title                                                                 | Authors                                                                 | Page |
|----------------------------------------------------------------------|-------------------------------------------------------------------------|------|
| Germfree animal studies lead to the revelation of new functions of vitamin K | Michio Komai and Hitoshi Shirakawa                                       | 11   |
| Blood stream infections in allogeneic hematopoietic stem cell transplant recipients: reemergence of Gram-negative rods and increasing antibiotic resistance | M. Mikulska, V. Del Bono, A.M. Raiola, B. Bruno, F. Gualandi D. Occhini, C. di Grazia, A. Bacigalupo, Francesco Frassoni and C. Viscoli | 12   |
| Fungal infection in HSCT                                              | Shinichiro Mori                                                         | 13   |
| Infectious complications following reduced-intensity cord blood transplantation | Shuichi Taniguchi                                                       | 13   |
| Contribution of intestinal flora to homeostasis and ibd pathogenesis  | Toshifumi Hibi, Tadakazu Hisamatsu and Takanori Kanai                    | 14   |
| Mucosal decisions for immunity to co-habitation of microflora         | Hiroshi Kiyono                                                          | 15   |
| Control of immune responses by commensal bacteria during acute gastrointestinal infections | Liliane M. dos Santos, Timothy Hand, Nicolas Bouladoux and Yasmine Belkaid | 15   |
| Physiological role of indigenous Lactobacilli/\textit{Helicobacter pylori} in the stomach | Yasuhiro Koga                                                           | 16   |
| Development of novel methods for the search of antibiofilm agents     | Reiko Kariyama and Hiromi Kumon                                         | 17   |
| Two-component signal transduction systems and biofilm formation of \textit{Staphylococcus epidermidis} | Di Qu, Yang Wu, Shiqing Han, Xu Shen and Hualiang Jiang                | 18   |
| Biofilm formation of \textit{Helicobacter pylori}                     | Hideo Yonezawa and Shigeru Kamiya                                       | 18   |
| Immune response of the gnotobiotic mouse model induced mycoplasmal pneumonia and evaluation of antimicrobials using the model | Haruhiko Taguchi, Satoshi Kurata, Ken Arae, Tsuyoshi Onogawa and Shigeru Kamiya | 19   |
| Epidemiology, diagnosis, medication and vaccination of avian mycoplasmosis in Taiwan | Michael M.Y. Lin                                                        | 20   |
| Diagnosis of \textit{Mycoplasma pneumoniae} and other respiratory tract infections using gene amplification method ‘loop-mediated isothermal amplification (LAMP)’ | Yoshinori Ota, Manabu Yoshino, Toshimitsu Annaka, Tsugunori Notomi and Hidetoshi Kanda | 20   |
| Autologous hematopoietic stem cell preservation in nuclear plant workers | Shuichi Taniguchi                                                       | 21   |
| Enterococci as probiotics or autoprobiotics in treatment of the gastrointestinal diseases | Alexander Stucorov, Vladimir Simanenkov, Elena Ermolenko, Viktoria Kolodjieva, Anna Teapieva, Natalia Zaharova and Olga Solovieva | 22   |
Host/bacteria interaction and influence of beneficial bacteria on mucosal immune responses against opportunistic/enteric pathogens: possible cellular-molecular mechanisms and practical approaches

Nadiiya Volodymyriena Boyko

23

Partially Purified Bacteriocin and Molecular Characterization Using 16S rRNA From Endemic Tropical Fruit Fermentation of Yellow Marquise (Passiflora edulis var. flavicarpa) In Indonesia

Samaryati Syukur, Habibi Hidayat and Endang Purwati

24

The University Of Chicago Gnotobiotic Research Animal Facility (GRAF): a new member in the gnotobiotic community

Betty R. Theriault, Christina Olivares, Alan Vest and George P. Langan

25

Somatic cell nuclear transfer for genetic modification and preservation of gnotobiotic miniature pigs

Hoon Taek Lee

27

Posters

Establishment of gnotobiotic miniature swine for xenotransplantation research program in Konkuk University, Seoul, Korea

Jeong Ho Hwang, Sung Han Jung, Sang Eun Kim, Yoon B. Kim and Hoon Taek Lee

28

Lactobacillus monoassociated mice might be downregulated by the serum antibody levels induced by dietary antigen compared with Bacteroides monoassociated mice

Yuji Hamamoto, Akira Hosono, Masato Tsuda, Daiki Kamoi, Satoshi Hachimura, Yoshika Momose, Kikuji Itoh, Kazuhiro Hirayama, Kyoko Takahashi and Shuichi Kaminogawa

28

High incidence of radiation-induced cavernous hemangioma in long-term survivors who underwent hematopoietic stem cell transplantation with radiation therapy during childhood or adolescence

Takashi Koike, Noriharu Yanagimachi, Hiroyuki Ishiguro, Hiromasa Yabe, Miharu Yabe, Tsuyoshi Morimoto, Takashi Shimizu, Hiromitsu Takakura and Shunichi Kato

29

Problems in chimerism analysis using buccal mucosa after hematopoietic stem cell transplantation

Osamu Hyodo, Humiko Tsuchida, Tatsuya Sugimoto, Kaoru Sato, Hiromasa Yabe, Fumiaki Yoshiba and Shunichi Kato

29

Characteristic of Candida from fungoid-bacterial associations at inflammatory disorders of the upper respiratory tract

Anatoliy P. Godovalov, Liliya P. Bykova, Anatoliy I. Morgunenko and Sergey Gris

30

The role of sugars in the minor correction of immune status of beings

Olga Sergeevna Korneeva and I.V. Cheremuskina

31

Characteristics of interaction between tick-born encephalitis virus and human immune system

Liliya P. Bykova, Rafael Z. Kaziaev, Anatoliy P. Godovalov and Sergey Gris

31

Changes in phenotypic and functional properties of mononuclear leukocytes as a result of bacterial vaccine application under induced immunosuppression

Olga V. Lebedinskaya, Elena A. Lebedinskaya, Nelly K. Akhmatova, Anatoliy P. Godovalov, Sergey Gris

32

Anti-inflammatory properties of Bifidobacterium spp. Strains isolated from healthy infant feces

Ekaterina V. Khokhlova, Boris A. Efimov, Lyudmila I. Kafarskaik, Svetalana I. Pavlova and Andrei N. Shkoporov

34
Physiological changes of liver induced by fluoroquinolones and hepatoprotective effect of CAP18/LL-37 synthetic peptide in mice
Shiaki Takagi, Yoko Sugawara, Katsuya Inada, Hiroshi Isogai and Emiko Isogai

Role of IL-10 and IL-17 in the Mycoplasma pneumoniae experimental pneumonia model
Satoshi Kurata, Haruhiko Taguchi, Takako Osaki, Tomoko Hanawa, Hideo Yonezawa and Shigeru Kamiya

Wild deer have antibodies recognizing tick defensin with tertiary structure
Emiko Isogai, Nayuta Isogai, Kazuki Sato, Hiroshi Yoneyama, Tomoichi Fukuda, Yoshideke Deguchi, Kazuei Matsubara, Hideo Murata, Takamitsu Tuboi, Makoto Haritani, Toru Miyamoto and Hiroshi Isogai

Gut indigenous microbiota and epigenetics
Boris A. Shenderov

Impact of dihydrodaidzein-producing Clostridium-like intestinal bacterium, strain TM-40 on in vitro metabolism of daidzein by equol-producing bacterium and fecal microbiota from human
Motoi Tamura, Sachiko Hori, Yukie Kurasu and Hiroyuki Nakagawa

Microflora of pregnant women and methods of its correction at disbiosysis
Taras M. Lyaskovsky

Comparative analysis of gastric bacterial microbiota in Mongolian gerbil after long-term infection with Helicobacter pylori
Takako Osaki, Cynthia Zaman, Takahiro Matsuki, Takashi Asahara, Hideo Yonezawa, Tomoko Hanawa, Satoshi Kurata, Fuhito Hojo, Haruhiko Taguchi and Shigeru Kamiya

Gut colonization shapes the host’s metabolism
Sandrine P. Claus, Léa Maitre, Steve Robinette, Sunil Kochhar and Jeremy K. Nicholson

Features of intestinal microbial biocenoses in dysbiotic individuals
Elena F. Zaigorodnyaya and Lyudmila A. Stashkevich

Evaluation of sterility tests for cord blood banking in the Tokai University Cord Blood Bank
Tatsuya Sugimoto, Hitomi Sasaki, Rie Ando, Sachio Sakama, Chie Nakashioya, Fumiko Tsujiya, Kaoru Sato and Shunichi Kato

The study of the bacterial contamination in the spray water of electronic toilets and in the gluteal and inguinal regions due to splashing following spray water
Hideki Katano, Kumi Yokoyama, Yasushi Takei, Hideaki Matsuki, Mami Tsukiji and Seiki Tazume

Molecular epidemiological analysis of methicillin-resistant Staphylococci in a neonatal intensive care unit
Yasushi Takei, Kumi Yokoyama, Hideki Katano, Mami Tsukiji, Takayuki Ezaki and Seiki Tazume

Isolation of toxigenic and nontoxigenic Corynebacterium diphtheriae strains upon preventive examination of hospital patients
Irina Nicolaenia Alekseeva, Svetlana Evgenievna Ivanova, Natalia Vladimirovna Kastyanenko, Elena Vladimirovna Loshchuk, Natalia Igorevna Dorogovtseva and Gennady Frankovich Rakitskiy

Inhibition of biofilm formation and virulence factor expression via control of quorum sensing in pathogenic bacteria
Tsukasa Ikeda and Tomohiro Morohoshi
Deletion of alarmon synthetase altered physiology and biofilm formation of *Bordetella pertussis*
Kentaro Sugisaki, Tomoko Hanawa, Hideo Yonezawa, Takako Osaki, Satoshi Kurata, H. Kawakami and Shigeru Kamiya

Inhibition of *Staphylococcus aureus* biofilm formation and nasal colonization by a commensal bacterium *Staphylococcus epidermidis*
Tadayuki Iwase, Yoshio Uehara, Hitomi Shinnji, Akiko Tajima, Hiromi Seo, Sinya Sugimoto, Toshihiko Agata, Koji Takada and Yoshimitsu Mizunoe

Probiotics and innate immunity: regulation of anticancer activity
Vyacheslav Abramov, Valentin Khlebnikov, Irina Chikileva, Vadim Sakulin, Igor Kosarev, Raisa Vasilenko, Mikhail Kiselevsky and Vyacheslav Melnikov

Possible health promoting benefits of heat-killed *Lactobacillus gasseri* TMC0356, a new selected immune regulatory probiotics strain
Kenji Miyazawa, Fang He, Kazutoyo Yoda, Manabu Kawase, Akira Kubota and Masaru Hiramatsu

Effects of probiotics on gut microbiota composition in familiar Mediterranean fever and Crohn's disease patients
Anahit M. Manelyan, Marine A. Balayan, Elya S. Pepoyan, Susanna S. Mirzabekyan, Lena M. Malkhasyan, Varndan V. Tsaturyan and Astghik Z. Pepoyan

Development of probiotic preparations and functional food products on the base of lactic acid bacteria
Nadiia K. Kovalenko

Beneficial effects of fermented milk containing *Lactobacillus GG* on DSS-induced colitis in mice
Kazutoyo Yoda, Fang He, Kenji Miyazawa, Manabu Kawase, Akira Kubota, Masaru Hiramatsu and Fang Yan

Influence of probiotic Enterococci on the gastrointestinal tract of rats before and after the treatment of antibiotic-associated dysbiosis
Alexander Suroman, Ludmila Gromova, Yuri Borschev, Alena Karaseva and Andrei Gruzdkov

Development of the Caucasian lactic acid bacterial culture collection for the potential industrial use
Nina Chanishvili

Probiotic ‘Lactovit-K’ fights against coccidiosis of poultry and honey bee diseases
Nina N. Gavrilova, Irina A. Ratnikova and Vyacheslav G. Melnikov

Biological active compounds from probiotic bacteria
Galina Novik, Elena Kiseleva, Vyacheslav Melnikov, Andzrej Gamian, Yuriy Knirel and Estera Szwajcer Dey

The role of probiotics in rehabilitation of sportsmen after postmatch exertion
Gagik Hoveyan, Marine Badalyan, Ani Hoveyan, Elya Pepoyan, Varndan Tsaturyan and Astghik Pepoyan

Breakdown of *Bifidobacterium* flora by species in the process of intestinal biocenosis development in newborns and in dysbiotic individuals
Elena Fedorovna Zavgorodnyaya

Efficacy of the probiotic strain *Clostridium butyricum* MIYAIRI 588 on poultry and piglet zootechnical performance and prevention of necrotic enteritis
Motomichi Takahashi, Miroslava Piskoriková, Kaoruko Yuge, Kentaro Oka, Koji Uno, Elinor McCartney and Shigeru Kamiya
Reactivation of latent HIV-1 by a wide variety of butyric acid producing bacteria

_Bacteria Kenichi Imai, Kiyoshi Yamada, Muneaki Tamura and Kuniyasu Ochiai_ 52

Extracellular HIV-1 TAT (ExTat) regulates bacterial superoxide dismutase (SOD) allowing upregulation of reactive oxygen species (ROS) in _Porphyromonas gingivalis_ and _Fusobacterium nucleatum_

_Marni Cueno, Muneaki Tamura, Kenichi Imai, Megumi Hamadate and Kuniyasu Ochiai_ 53

Evaluation of survival and infectivity of bacteriophage isolates from environmental samples in function of temperature and pH

_Nima Bahador_ 53

Coinfection of mammalian and tick cells with pathogenic and nonpathogenic spotted fever group rickettsiae

_Tsuneo Uchiyama and Hiromi Fujita_ 54

_Leptotrichia_ genus bacteria biotopes in the human body

_Natalia Viktorovna Strebnikova, Alexandra Anatoliieva Antonova and Elena Borisovna Polozova_ 54

Nasopharyngeal microbial biocenosis in children in the Amur River area of Russia

_Galina Nikolaevna Kholodok and Vladimir Kirillovich Kozlov_ 55

Implication of the role of stringent response in the expression of adenylate cyclase toxin in _Bordetella pertussis_

_Tomoko Hanawa, Kentaro Sugisaki, Hideo Yonezawa, Takako Osaki, Satoshi Kurata, Cynthia Zaman and Shigeru Kamiya_ 56

Examination of growth conditions and nitrogen fixation capability of legumes selected from various sources in Northern Greece

_Stavros Kazakos, Stavros Plessas, Athanasios Alexopoulos and Eugenia Bezirtzoglou_ 56

Biochemical studies on the virulence factors of fungi associated with she-camel milk

_Abd El-Aziz Mosaad, Ahmed El-Kirdasy, Mostafa Al-Sherif, Said Fathalla, Abd El-Rahman M El-Bagory and Gamal Abd El. Gaber_ 57

_In vivo_ antibacterial activity of Phx-3 against _Helicobacter pylori_

_Fuuhto Hojo, Takako Osaki, Tomoko Hanawa, Akio Tomoda and Shigeru Kamiya_ 58

Detection of _Campylobacter_ is possible using their physical characters

_Majid Baserisalehi_ 58

Bacillus probiotics: biological and clinical effects

_Larisa A. Safronova_ 58

Monoclonal antibodies against the accumulation-associated protein influence EPS biosynthesis and enhance bacterial accumulation of _Staphylococcus epidermidis_

_Yang Wu, Jian Hu, Tao Xu, Huayong Liu, Youcong Wu and Di Qu_ 59

Eubiotics in medicine

_Nina N. Gavriloa and Irina A. Ratnikova_ 60

Early and quantitative assay to detect HHV-6 viremia and evaluation of cellular response specific against HHV-6 after hematopoietic stem cell transplantation

_Shunichi Kato, Hiromasa Yabe, Chie Nakashioya, Fumiko Tsuchida, Tatsuya Sugimoto, Kimiyasu Shiraki and Masayuki Saijo_ 60
Microbial ecology between Helicobacter pylori and microbiota in gastric mucosa

Shigeru Kamiya*
Department of Infectious Diseases, Kyorin University School of Medicine, Mitaka, Tokyo, Japan

Mongolian gerbils are frequently used to study Helicobacter pylori-induced gastritis and its consequences. The presence of some gastric microbiota with a suppressive effect on H. pylori suggests inhibitory gastric bacteria against H. pylori infection. The aim of the present study was to analyze the microbiota in the stomach of Mongolian gerbils with H. pylori infection.

In the first infection experiment, according to the frequency of detection of H. pylori urea in fecal samples, the infected gerbils were divided into three groups (frequently detected, moderately detected and infrequently detected). Eubacterium limosum and Lactobacillus spp. were isolated from the frequently detected group and infrequently detected group, respectively. In the second infection experiment, the gastric mucosa samples of H. pylori negative and positive gerbils were orally inoculated to five (group 1) and six (group 2) gerbils, respectively, and these gerbils were challenged with H. pylori infection. Colonization rate (40%) of H. pylori in group 1 was lower than that (67%) in group 2 gerbils. Culture filtrate of gastric mucosa samples of group 1 gerbils inhibited the in vitro growth of H. pylori.

Three lactobacilli species of Lactobacillus reuteri, Lactobacillus johnsonii and Lactobacillus murinus were isolated by anerobic culture from the gerbils in groups 1 and 2 and identified by genomic sequencing method. Although these lactobacilli showed no inhibitory effect on adhesion of H. pylori to gastric cells, it was demonstrated that L. murinus exhibited an inhibitory effect on the in vitro growth of H. pylori.

Microbial ecology between H. pylori and gastric microbiota in Mongolian gerbil was analyzed by two infection experiments. The results from the experiments, the presence of gastric bacteria with inhibitory effect on H. pylori, were detected. It was suggested that L. murinus isolated from gastric mucosa with inhibitory effect on H. pylori might be a novel probiotics candidate against H. pylori infection.

Recent research data obtained by molecular analysis of gastric microbiota will be also presented in the lecture.

Hematopoietic stem cell transplantation (HSCT)

Shunichi Kato*
Department of Cell Transplantation, Tokai University School of Medicine, Isehara, Japan

Bone marrow transplantation (BMT) was started in 1970s in Japan, and Seattle-type regimen was introduced by several BMT centers in early 1980s.

Peripheral blood stem cell transplantation (PBSCT) and cord blood transplantation (CBT) were added into new HSCT in 1990s.

Unrelated HSCT became available through the Japan Marrow Donor Program (JMDP) established in 1991 and the Japan Cord Blood Bank Network (JCBBN) established in 1999.

The numbers of allogeneic HSCT have been increased year by year and reached 4500 per year in 2008, while the numbers of autologous HSCT have been almost stable in the last 10 years, approximately 1500 per year (Fig. 1).

The numbers of unrelated HSCT have been increasing steadily. The cumulative transplant numbers exceed 12,500 in UCBT and 7000 in UCBT. In 2011, UCBT was 1200 and UCBT was 1000 (Fig. 3). UPBSCT was first introduced in 2011, and only a few transplants were performed until now.

Japan is isolated from the rest of the world, and the population is genetically homogeneous. There has been no major blood mixture in the last 1000 years. Therefore, the chance to find an HLA-matched BM or CB donor is much higher than in other ethnic groups.
The current situation and progress in haploidentical hematopoietic stem cell transplantation

Xiaojun Huang¹²*

¹Peking University Institute of Hematology, Beijing, China; ²Department of Hematology, Peking University People’s Hospital, Beijing, China

Extensive ex vivo T-cell depleted (TCD) or unmanipulated haploidentical transplantation provides benefits of rapid and near universal donor availability for patients without a HLA-identical sibling donor or those who urgently need transplant. However, CD34 selected haplotype mismatched transplantation was limited by delayed immune reconstitution (IR), although this protocol has now been an acceptable approach. Recently, Peking University researchers developed a novel approach to HLA-mismatched/haploidentical blood and marrow transplantation without in vitro TCD (GIAC protocol). Our clinical data showed that G-BM combined with PBSC from haploidentical family donors, without in vitro TCD, might be a good source of stem cells for allo-HSCT. Applying this transplant setting can achieve comparable outcomes with HLA-identical sibling transplantation and even better graft-versus-leukemia effect. To improve the outcomes of patients, we modified the donor lymphocyte infusion (DLI) protocol by using G-CSF-mobilized PB progenitor cells (GPBPCs) instead of traditional steady-donor lymphocytes in therapeutic infusion and further demonstrated the feasibility of applying this strategy against leukemia recurrence from therapeutic DLI to prophylaxis DLI for patients with advanced hematological malignancies undergoing haploidentical transplants. Moreover, much progress has also been made in controlling graft-versus-host disease (GVHD) through manipulating the cell contents.
or function of graft using various kinds of stimulating factors and improving the recovery of IR via novel approach.

Emerging roles of noninherited maternal alloantigens (NIMAs) and inherited paternal alloantigens (IPAs) in HLA-mismatched hematopoietic cell transplantation

Tatsuo Ichinohe*

Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga University, Saga, Japan

HLA compatibility between the donor and recipient has long been recognized as an essential prerequisite for successful allogeneic hematopoietic cell transplantation (HCT). However, increasing needs for allogeneic HCT have now expanded the availability of alternative stem cell sources such as unrelated cord blood units and HLA-haploidentical-related family members.

During pregnancy, fetal immune system needs to suppress harmful responses against noninherited maternal alloantigens (NIMAs), and vice versa, maternal immune system must tolerate inherited paternal alloantigens (IPAs) of the fetus, suggesting the presence of natural mechanisms to generate a form of bidirectional immune tolerance between the mother and her HLA-haploidentical fetus. Nevertheless, a substantial proportion of mothers are believed to subsequently become sensitized against IPAs and umbilical cord blood of their offspring is shown to paradoxically contain cytotoxic T-cells against NIMAs. Therefore, we hypothesized that better understanding of the fetomaternal immunology may shed new light on alternative strategies for HLA-mismatched allogeneic HCT.

In 1950s, (1) first provided experimental evidence that the introduction of maternal antigens into the fetus during pregnancy gives rise to a form of immunologic hyporesponsiveness to maternal alloantigens later in life. To elucidate the mechanism by which immune tolerance against maternal alloantigens is maintained in adults, we developed a murine model of NIMA-mismatched HCT by use of the F1 x P backcross breeding model. Intriguingly, CD4⁺ T-cells from NIMA-exposed offspring compared with those from NIMA-nonexposed controls showed reduced proliferative responses and IFN-γ-production in response to NIMA-expressing allogeneic antigen presenting cells. Furthermore, allogeneic HCT from a NIMA-exposed mouse to MHC-incompatible but NIMA-expressing recipients was associated with compromised severity of graft-versus-host disease and superior survival in a NIMA-specific manner. Notably, such tolerogenic effect was abolished when the hematopoietic cell graft from a NIMA-exposed donor was depleted of CD4⁺ CD25⁺ T-cells, suggesting that NIMA-specific tolerance is maintained by a subset of T-cells harboring regulatory properties.

We next examined the effect of maternal exposure to IPAs when mothers are used as donors for HCT by use of a similar murine model. CD4⁺ T-cells isolated from IPA-exposed mice compared with those from non-IPA-exposed mice showed comparable proliferative responses to IPA-expressing antigen presenting cells, although maternal cells are generally believed to be sensitized against IPAs. Transplants from IPA-exposed mice to MHC-incompatible IPA-expressing recipients also showed similar but not inferior survival rates compared with transplants from non-IPA-exposed controls.

In line with these observations, recent clinical evidence has indicated that the use of NIMA-mismatched or maternal stem cell sources may improve outcomes of allogeneic HCT in selected series of patients. To confirm the presence of such beneficial ‘fetomaternal effects’, a prospective study is warranted to compare the outcomes of transplants using hematopoietic stem cell grafts mismatched for NIMAs/IPAs and those using grafts not mismatched for NIMAs/IPAs.

Reference

1. Billingaham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. Nature 1953; 172: 603-6.

* Tatsuo Ichinohe

E-mail: nohe@cc.saga-u.ac.jp

Properties of sialic acid-binding adhesin of *Streptococcus gordonii*, an oral bacterium as a member of dental plaque organisms and etiological agent for infective endocarditis

Kiyoshi Konishi* and Yukihiro Takahashi

Department of Microbiology, Nippon Dental University School of Life Dentistry, Tokyo, Japan

Citation: Microbial Ecology in Health & Disease 2012, 23: 17462 - http://dx.doi.org/10.3402/mehd.v23i0.17462 (page number not for citation purpose)
It is currently well known that the poor oral health contribute to many systemic diseases, such as infective endocarditis explained as an odontogenic (dental) focal infection. Viridans streptococci, Staphylococcus aureus, enterococci, Candida albicans, and others colonize damaged heart valves and frequently identified bacteria acting as etiological agents of infective endocarditis. One of these etiological agents, Streptococcus gordonii, is a member of the biofilm community that comprise a numerically prominent group of oral bacteria, which occur primarily on the human tooth surface, commonly referred to as dental plaque. For the mechanism of infective endocarditis, attachment of blood-borne bacteria, which intrude from oral bacterial flora or dental plaque to platelets of target site, is a postulated central event after platelets and fibrin bound to endothelial cells at the site of injury cardiac valves. Some papers indicated erythrocytes also contribute somewhat to the infective endocarditis.

S. gordonii adhere to saliva-coated hydroxyapatite, an experimental model of the tooth surface, and attach to host cells such as erythrocytes and platelets. A common mechanism in these interactions is to recognize surface-associated host sialoglycoconjugates. Recently, such interactions have been found to involve the binding of streptococcal adhesins identified as large serine-rich glycoproteins to membrane-sialoglycoproteins of host cells. We previously reported that the S. gordonii DL1 hsa gene encoded a large serine-rich repeat protein (Hsa) composed of 2178 amino acid residues. Hsa binds α2-3-linked sialic acid termini of O-glycosylated musin-type glycoproteins and consists of an N-terminal nonrepetitive region (NR1, containing signal sequence), a serine-rich repeat region (SR1), a second nonrepetitive region (NR2), a second serine-rich repeat region (SR2) and a C-terminal cell wall anchoring domain. We also reported that an insertional mutation in hsa gene resulted in a significant reduction of the infection rate of the organism and inflammatory reaction in the rat aortic valve with experimental endocarditis, suggesting that the Hsa contributes to the infectivity of the organism for heart valves. We have identified that the receptors of erythrocyte for Hsa is glycopherin A and band 3, using expressed recombinant NR2, a putative binding domain of Hsa, fused with GST in Escherichia coli BL21. We have also identified GPlba and GPlib as platelet receptors for S. gordonii DL1 Hsa. More recently, we have reported that monocyte stimulated with S. gordonii DL1 rapidly undergo monocyte-to-dendritic cell differentiation through interaction with the Hsa and suggested that this response may be attribute to the initial step in infective endocarditis by oral streptococci.

*Kiyoshi Konishi
E-mail: konikiyo@tky.ndu.ac.jp

Intestinal bacterial microbiota: lessons learned

Marika Mikelsaar*

Department of Microbiology, University of Tartu, Estonia, Ravila, Tartu, Estonia

In the 1990s, our understanding of microbial diversity in intestinal microbiota expanded to include new taxonomic and functional characteristics. Previously, by cultivation on selective and nonselective media, anaerobes such as Bacteroides, eubacteria, bifidobacteria, peptostreptococci and fusobacteria were thought to predominate over the coliforms, Lactobacillus/Enterococcus and staphylococci (1). However, newly developed molecular technologies have revealed nearly 100-fold greater total numbers of bacteria in the large intestines, with an abundance of 3–12% for some incultrable bacteria, such as Atopobium/Eubacterium, Clostridium cocoides and the Clostridium leptum groups (2). Postgenomic approaches have enabled study of the diversity and functionality of this complex ecosystem using new methods, such as metabolomics, proteomics, transcriptomics and genomics (3). Several environmental factors, such as diet and medical/pharmaceutical interventions, were shown to influence microbiota composition, which is intimately associated with GI function in health and disease. The overall aim has mainly been to identify the intervention(s) responsible for these effects on bacteria on an individual and temporal scale.

Another possibility is to elucidate the impact of some established components of this microbiota on human health indices. Based on the diverse functions of GI microbiota, incrementally obtained knowledge on the expression of microflora-associated characteristics (MAC) (4) in macroorganisms has been further elaborated by studies on microbial-host crosstalk (5). Currently, this crosstalk involves the recognition of self and nonself by toll-like receptors on host dendritic cells and macrophages (6), which shape the maturation of GI cells and the development of innate and obtained immunity. Still, the most important recent discoveries are the elucidation of the mechanisms by which intestinal microbiota impact host metabolism. Several experimental and clinical studies have revealed that intestinal microbiota, which live in mutual, beneficial symbiosis with the host organism, are important regulators of energy uptake and storage (7, 8), suggesting a link between microbiota and metabolic diseases, such as type 2 diabetes and metabolic syndrome.

In our laboratory, we have developed an approach to characterize the health status of different age groups according to different anthropometrical and clinical characteristics, as well as cellular, biochemical and immunological blood and urine indices (8–11). Using this approach, we have obtained statistical normal reference values for nearly 50 health status indices in different age groups in a geographically distinct Estonian population. Furthermore, we have correlated these data with bacteriologically and molecularly assessed Lactobacillus sp. compositions and describe some species and
strains as risk modulators for increased glucose and triglyceride content in the blood, impaired cholesterol metabolism, high blood pressure, obesity and antimicrobial and immunological competence. The metabolites profile of the defined \textit{Lactobacillus} sp. contains antimicrobial and antioxidative compounds (Mn-superoxide dismutase, glutathione system), as well as nitric mono-oxide and polyamines (12). These findings have aided the prediction and manipulation of the functionality of microbiota, resulting in the ability to transform host ecosystems using pinpointed biotechnological applications of probiotics.

References

1. McFarland LV. Normal floradiversity and functions. MEHD 2000; 12: 193–218.
2. Zoetendahl EG, Vaughan EE, de Vos W. A microbial world within us. Mol Microbiol 2006; 59: 1639–50.
3. Zoetendahl EG, Rajilic M, de Vos WM. High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. Gut 2008; 57: 1605–15.
4. Midtvedt T, Bjørnekrlett A, Carlstedt-Duke B, Gustafsson BE, Hoverstadt T, Lingeas E, et al. The influence of antibiotics upon microflora-associated characteristics in man and mammals. Prog Clin Biol Res 1985; 181: 241–4.
5. Falk PG, Hooper LV, Midtvedt T, Gordon JJ. Creating and maintaining the gastrointestinal ecosystems: what we know and need to know from gnotobiology. Microbiol Mol Biol Rev 1998; 62: 1157–70.
6. Takeda K, Kaino T, Akira S. Toll-like receptors. Annu Rev Immunol 2003; 21: 335–76.
7. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 2004; 101: 15718–23.
8. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis FR, Gordon JJ. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444: 1027–31.
9. Mikelsaar M, Annuk H, Stsepetova J, Mandar R, Sepp E, Björksten B. Intestinal \textit{Lactobacilli} of Estonian and Swedish children. Microb Ecol Health Dis 2002; 14: 75–80.
10. Mikelsaar M, Stsepetova J, Hütt P, Kolk H, Sepp E, Lõivukene K, et al. Intestinal Lactobacillus sp. is associated with some cellular and metabolic characteristics of blood in elderly people. Anaerobe 2010; 16: 240–6.
11. Stsepetova J, Sepp E, Kolk H, Lõivukene K, Songisepp E, Mikelsaar M. Diversity and metabolic impact of intestinal \textit{Lactobacillus} sp. in healthy adults and the elderly. Br J Nutr 2011; 105: 1235–44.
12. Kullisaar T, Songisepp E, Aunapuu M, KilikK, Arend M, Mikelsaar M, Rehema A., M Zilmer M. Complete glutathione system in probiotic \textit{Lactobacillus fermentum} ME-3. Appl Biochem Microbiol 2010;46:481–6.

*Marika Mikelsaar
E-mail: marika.mikelsaar@ut.ee

Molecular ecology of butyrate-producing bacteria from the human gut

Petra Louis*

Rowett Institute of Nutrition and Health, University of Aberdeen, Bucksburn, Aberdeen, United Kingdom

The human large intestine is colonized by a highly diverse microbial community, which is dominated by bacteria belonging to Gram-negative Bacteroidetes and Gram-positive Firmicutes. The gut microbiota receives most of its energy from dietary carbohydrates that cannot be digested by the human host and reach the colon. The quantity and type of dietary carbohydrate ingested is likely to influence the composition and activity of the gut microbiota, with potential consequences for human health. Carbohydrate fermentation by the microbial community as a whole leads to the accumulation of three main short-chain fatty acids: acetate, propionate and butyrate. Other fermentation products such as formate, lactate, succinate and branched-chain fatty acids may accumulate to a lesser degree.

Butyrate has received special attention due to its role as main fuel for the colonic wall and its anticarcinogenic and anti-inflammatory effects. Butyrate producers belong to several different clostridial clusters within Firmicutes. Two major groups of butyrate producers are bacteria related to \textit{Faecalibacterium prausnitzii} within clostridial cluster IV and \textit{Eubacterium rectale} and \textit{Roseburia} spp. within clostridial cluster XIVa. Another cluster XIVa group that is of functional importance for the conversion of lactate to butyrate is lactate-utilizing butyrate producers related to \textit{Eubacterium hallii} and \textit{Anaerostipes} spp. Further species of butyrate producers are phylogenetically interspersed with non butyrate-producing bacteria, which make it difficult to monitor the butyrate-producing capacity of the gut microbiota using molecular approaches based on the 16S rRNA gene. We, therefore, targeted a functional gene involved in butyrate metabolism in the majority of human butyrate producers, butyryl-CoA:acetate CoA-transferase, to investigate the diversity of this functional group and monitor changes in response to prebiotic supplementation in healthy human volunteers (1). Thirty-two different operational taxonomic units (OTUs, <98% sequence identity at DNA level) were detected in fecal samples from 10 volunteers, with each volunteer carrying between 6 and 17 different OTUs. The most prevalent OTUs belonged to the \textit{E. rectale} \textit{Roseburia} sp. group, \textit{E. hallii} and as-yet unnamed strain SS2/1, all within clostridial cluster XIVa. The majority of sequences (88%) belonged to 12 OTUs that were closely related to cultured isolates, while the remaining 12% of sequences belonged to 20 OTUs without cultured
representatives. Thus, OTUs with cultured representatives were mostly abundant, while less-abundant OTUs mostly did not match cultured isolates. This indicates that the lack of cultured isolates for many gut bacteria identified by molecular approaches may be due to their low abundance rather than an inherent unculturability. Supplementation with the prebiotic inulin led to a significant increase in sequences related to *F. prausnitzii*, which confirmed previous microbiota analysis based on the 16S rRNA gene (2). Within the *E. rectale/Roseburia* group, a shift in species composition was noted upon inulin consumption with a significant decrease in *Roseburia inulinivorans* and *Roseburia hominis* and a trend toward higher levels of *E. rectale*. Furthermore, sequences related to strain SS2/1 also showed a trend toward an increase after inulin consumption, which could be confirmed by 16S rRNA gene-based qPCR analysis. These results indicate that the effect of prebiotics on the gut microbiota is more complex than originally thought.

The butyryl-CoA:acetate CoA-transferase gene sequence targeted here is most closely related to 4-hydroxybutyrate CoA-transferases from several *Clostridium* species. We were unable to detect this gene in the human gut butyrate producer *Eubacterium cylindroides*, which belongs to clostridial cluster XVI. Phosphotransbutyrylase and butyrate kinase, which are used by some gut bacteria to generate butyrate instead of butyryl-CoA:acetate CoA-transferase, could also not be detected. However, we have recently identified another CoA-transferase gene more closely related to propionate CoA-transferases in clostridial cluster XVI isolates from the human and the chicken gut (3). It appears therefore that different types of CoA-transferase genes may have evolved in different bacterial lineages to perform the last step of butyrate generation.

### References

1. Louis P, Young P, Holtrop G, Flint HJ. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. Environ Microbiol 2010; 12: 304-14.
2. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. Br J Nutr 2009; 101: 541–50.
3. Eeckhaut V, Van Immerseel F, Croubels S, De Baere S, Haesebrouck F, Ducatelle R, et al. Butyrate production in phylogenetically diverse *Firmicutes* isolated from the chicken cecum. Microbial Biotechnol 2011; 4: 503-12.

*Petra Louis*
E-mail: p.louis@abdn.ac.uk

---

**Study of human hematopoietic stem cell aging using immune-deficient mice**

Kiyoshi Ando*

Department of Hematology and Oncology, Tokai University School of Medicine, Isehara, Japan

Stem cells of highly regenerative organs including blood are susceptible to endogenous DNA damage caused by both intrinsic and extrinsic stress. Response mechanisms to such stress equipped in hematopoietic stem cells (HSCs) are crucial to sustain hematopoietic homeostasis but remain largely unknown. We demonstrate that replication stress induces intracellular elevation of reactive oxygen species (ROS) that results in accumulated and persistent DNA damage in human HSCs both in vitro and in vivo. This accumulation of DNA damage is demonstrated in HSCs of clinical transplant patients and elderly individuals in addition to a xenotransplantation model. The oxidative DNA damage causes premature senescence among HSCs, leading to loss of stem cell function. Importantly, treatment with an antioxidant can antagonize oxidative DNA damage and consequent HSC dysfunction. Our results reveal that ROS play a causative role for DNA damage, and mechanisms of ROS regulation have a major influence on human HSC aging.

*Kiyoshi Ando*
E-mail: andok@keyaki.cc.u-tokai.ac.jp

---

**Human-induced pluripotent stem cell-derived blood cells toward clinical application**

Naoya Takayama*

Clinical Application Department, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Abstracts

Citation: *Microbial Ecology in Health & Disease* 2012, 23: 17462 - http://dx.doi.org/10.3402/mehd.v23i0.17462
The achievement of ‘regenerative medicine’ needs the global and sophisticated system for translation from the basic science to clinical application. We aim to develop the novel blood transfusion and gene- and cellular-therapy, for example, using human-induced pluripotent stem (iPS) cells. We have so far developed the static culture system whereby human iPS cells can be differentiated into the Sac-like structures that concentrate CD34⁺ hematopoietic progenitors, further generating platelets, erythrocytes or T lymphocytes in vitro. In addition, our research program will focus on the developmental strategies for achievement of safe and stable blood supply for transfusion independently of blood donation using immortalized cells derived from human iPS cells. In this symposium, we would like to introduce recent results on platelet generation from human iPS cells and next step toward clinical application.

*Naoya Takayama
E-mail: naoya.takayama@cira.kyoto-u.ac.jp

CMV reactivation and immune reconstitution after CBT

Satoshi Takahashi*
Institute of Medical Science, University of Tokyo, Tokyo, Japan

Cytomegalovirus (CMV) reactivation is thought as one of most important problems after cord blood transplantation (CBT). On the other hand, we have achieved good clinical results for adults after CBT with histocompatibility antigen [human leukocyte antigen (HLA)]-mismatched single graft. The one of crucial questions in CBT is whether naïvety of lymphocytes could gain antigen-specific cellular immunity during early phase of HLA-mismatched transplant. To answer this, we have analyzed the CMV-specific immune reconstitution process for first 6 months.

Forty adults has received myeloablative regimens including 12 Gy of total body irradiation followed by CBT and a standard cyclosporine and methotrexate combination as GVHD prophylaxis in the Institute of Medical Science, University of Tokyo (IMSUT) and in nine different facilities, which participated for the prospective study using IMSUT regimen. CMV-specific CD4⁺ and CD8⁺ T cell recoveries were assessed by detection of interferon-gamma (IFN-g) producing cells with CMV antigen stimulation using intracellular cytokine staining or tetramers for CMV pp65 in whom HLA-A0201, -A0206 or -A2402 positive patients. The positive was defined as >0.03% IFN-g positive cells among CD4⁺ or CD8⁺ T cell population and >0.01% positive in tetramer assay.

CMV-reactive (IFN-g positive) CD4⁺ T cells were detected in 65% at 1 month, 88% at 2 months, 92% at 3 months, 92% at 4 months and 95% at 6 months after CBT, which were comparable to CMV-positive age-adjusted healthy control (100%). CMV-reactive (IFN-g positive) and CMV-specific (tetramer-positive) CD8⁺ T cells were detected in 53% and 5% at 1 month, 71% and 44% at 2 months, 68% and 36% at 3 months, 75% and 50% at 4 months and 65% and 50% at 6 months (39% and 67% in the control). Next, we looked the effect of HLA disparity (HLA-DR for CD4⁺ and HLA-A/-B for CD8⁺ T cell) in graft-versus-host direction with low resolution typing (LRT) and in high resolution typing (HRT). CMV-reactive CD4⁺ T cells were detected in 94% with matched (0MM), 81% with one antigen mismatched (1AMM) in LRT and 100% with 0MM, 89% with 1AMM, 80% with 2AMM in HRT at 2 months. CMV-specific CD8⁺ T cells were detected in 33% with 0MM, 38% with 1AMM, 56% with 2AMM in LRT and 38% with 1AMM, 50% with 2AMM, 67% with 3AMM in HRT at 2 months, respectively.

Postthymic naive T cells in cord blood might obtain memory and effector function in vivo with antigen-specific manner during early phase of posttransplant independent on effect of HLA compatibility. When we evaluated the impact of positive antigenemia on clinical outcomes of CBT, HLA disparities were not affected to high frequency of positive CMV antigenemia results. Significant longer hospitalization was needed in high-frequent CMV-reactivated patients after transplantation; however, cumulative incidences of neutrophil and platelet recoveries, of GVHD, of relapse and of nonrelapse mortality were not affected by high-frequent CMV positivity of post-CBT.

*Satoshi Takahashi
E-mail: radius@ims.u-tokyo.ac.jp

The immunobiology of hematopoietic stem cell transplantation

Anders Fasth*
Department of Pediatrics, University of Gothenburg, Gothenburg, Sweden; Department of Pediatrics, The Queen Silvia Children’s Hospital, Göteborg, Sweden

Citation: Microbial Ecology in Health & Disease 2012, 23: 17462 - http://dx.doi.org/10.3402/mehd.v23i0.17462
Hematopoietic stem cell transplantation (HSCT) is a unique situation in the sense that a foreign immune system is introduced into the host with the ultimate goal to replace the host’s hematopoiesis including its immune system. This to either allow repair of a faulty hematopoietic system, such as in case of congenital immunodeficiencies and hemoglobinopathies, or the cure of malignancies through a combination of high dose cytostatic treatment (conditioning therapy) and immunologic rejection of the malignant clone.

Two immune systems in one host open for many complications and have consequences for immune reconstruction after the transplantation.

The most serious complications are rejection of the transplanted cells or the rejection of the host itself, a process called graft-versus-host reaction (GvH), by the incoming T cells. As only a limited amount of stem cells together with more mature cells are transplanted, the balance would favor rejection of the transplanted cells if the host’s bone marrow is not ablated, immunosuppressed or, as in case of severe combined immunodeficiency, lacks the capacity to mount an immunoreaction. The most important risk factor for GvH is human leukocyte antigens (HLA) disparity between the host and the donor. HLA is an enormously varied set of cell surface molecules with billions of variants, whose genes are all closely clustered in chromosome 6. There is a 25% chance that siblings are HLA identical.

The pathophysiology of GvH is complicated. Some examples: (1) it involves also the immune system of the patient. For example, the inflammatory process elicited by the cytostatic treatment before the HSCT upgrades HLA class II expression on many cell surfaces, making them an optimal target for T cells in the incoming graft. (2) Also, the inflammatory process with production of proinflammatory cytokines activates the adaptive immune system of the graft.

(3) Not only T cells are important but also NK cells and their specific set of natural killer immunoglobulin-like inhibitory and activating receptors (KIR). (4) An important target of the GvH is the thymus. The damage to the thymus will prolong the immune reconstitution of the patient and might leave her with a life-long immunodeficiency. (5) Furthermore, factors such as host and donor age, ongoing infections, cell dose, stem cell source and others are important.

Finally, we must accept that GvH is an important part of the graft-versus-leukemia/tumor effect (GvL or GvT). GvH is a double edge sword that on one hand causes destruction and much suffering to the patient but, on the other hand, diminish the risk for relapse of the malignancy. To harness the GvH and separate it from GvL is a goal that is the subject for intense research.

The immune reconstitution post-HSCT takes long time, and as a consequence, infectious complications are common and contribute significantly both to morbidity and mortality. At the time of the transplantation, the patient is severely immunodeficient due to the conditioning regimen and/or the underlying disease and its treatment. The immune reconstitution after HSCT occur through expansion of T cells in the graft and more efficient through the egress of new T cells that have been expanded and educated in the host’s thymus.

As thymus starts to involve after puberty, the efficacy of thymus education and formation of new naïve T cells will diminish with increasing age and increase the risk for infectious complication in older patients. Experimentally, it has been shown that epithelial growth factor 7 has a protective role. Much research is devoted to protect the thymus from the damage of conditioning and GvH and to increase the output of new T cells from the thymus to enhance and speed up immune reconstitution. Other attempts to treat or prevent infections are the production of specific cytotoxic T cells directed to infectious agents such cytomegalovirus (CMV) and Epstein-Barr virus (EBV).

A swift establishment of the new stem cells also diminishes the risk for relapse of malignancy, even without overt GvH, pointing to a GvL effect separate from GvH. One important factor for early immune reconstitution is the cell dose. A high cell dose is important, and the low stem cell number, together with the naïve status of the T cells, is a major drawback of umbilical cord blood as source of stem cells.

*Anders Fasth
E-mail: anders.fasth@gu.se

Microbial metabolic functions in Crohn’s disease patients

Elisabeth Norin*

Department of Microbiology Tumor and Cell Biology (MTC), Karolinska Institutet, Stockholm, Sweden

The American physician Burrill Crohn described in 1932 a clinical and pathological entity in some patients with abdominal GI symptoms, and this disease has later been given the name Crohn’s disease (CD) (1). The disease occurs anywhere from the mount to anus, however, most often in the terminal ileum and/or colon, and it is characterized pathologically by transmural inflammation, deep linear ulceration and often granulomas.

In Sweden, about 25,000 patients are diagnosed as CD patients – approximately 400 persons get the diagnose yearly and those are equally distributed within males and females, most of them 20–30 years old, but the disease is also diagnosed in some few children.

There is no clear cause of the etiology of the disease – however, increasing evidence suggests that a combination of host genetics and the composition/function of the gut
microbiota are factors at work (2). Several bacteria species have previously been claimed to be involved, but current hypotheses includes the theory that an unbalanced antigenic microbial stimulation could be one reason for the disease development. A dysbalance of the intestinal microflora do influence on, e.g., the host immunological response, thus causing mucosa alterations.

In the 1980s, we and other demonstrated a decreased inactivation of intestinal trypptic activity (3, 4), and we hypothesized, there is a reduced amount or absence of trypsin-degrading microbes. Since then, data indicating that alterations in composition and function of the intestinal microbiota together with impaired epithelial barrier functions are involved in the disease (5, 6).

As it is known that the host genotype partly determines the microbial community composition in man, our aim has been to apply different ways of attach the question of intestinal flora composition and function in four patients with diagnosed CD. Our group at Karolinska Institutet in the ‘2 kg feces party’ has investigated some microflora-associated characteristics (7) in fecal samples from these patients. Other members of our party investigated both biopsies and fecal samples from the same patients using a culture-independent technique based on molecular biology methods (8) and traditional microbiological culturing techniques.

By applying the different techniques, we found a significant difference in the inactivation/degradation of trypptic activity in the CD patient fecal samples. Results from the other groups in our party using other techniques found that the CD patients have lower number of bacteroides in their intestinal and fecal flora. Thus, a lack of functionally active Bacteroides distasonis most probably is one factor involved in the disease development, as it previously has been shown that bacterial strains belonging in the Bacteroides from man as well as animals are able to break down trypsin, both in vivo and in vitro (9). We conclude that the altered pattern of fecal trypctic activity indicate either absence of functionally active bacteroides, lack of other trypsin-degrading bacteria or alterations in intestinal production of microbial or pancreatic secretory trypsin inhibitors (PSTI), thereby indicating the possibility that CD might be due to absence of some metabolically active microbes; in contrast to a more general opinion that presence of some specific microbes are involved in the pathogenesis of CD.

In conclusion, our observations of high levels of fecal trypctic activity found in CD patients could indicate a lack of bacterial-driven breakdown of trypsin – however, the small number of patients so far studied does not allow us to draw any strong conclusions of to what extent these alterations play a role in the etiopathogenesis of CD. Our findings in the present materials indicate that CD patients have functional alterations in their intestinal microbiome in parallel with an immunological dysfunction.

The 2 kg party is an interdisciplinary network at Karolinska Institutet dedicated to explore involvement of various host–intestinal microbial interactions in human health and disease.

References
1. Crohn BB. Granulomatous disease of the small and large bowel. A historical survey. Gastroenterology 1967; 52: 767–72.
2. Sartor RB. Mechanisms of disease: pathogenesis of Crohn’s disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006; 3: 390–407.
3. van der Meerve JP, Mol GJJ. Levels of trypsin and a-chymotrypsin in feces from patients with Crohn’s disease. Digestion 1982; 24: 1–4.
4. Bergstrand LO, Gustafsson BE, Holstrom B, Norkin KE. The physiological activity of human ileal flora in patients with Crohns disease and ulcerative colitis evaluated by determination of germfree animal characteristics. Acta Chir Scand 1981; 147: 707–9.
5. Willing B, Halfvarson J, Dicksved J, Rosenquist M, Järnerot G, Engstrand L, et al. Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn’s disease. Inflamm Bowel Dis 2009; 15: 653–60.
6. Dicksved J, Halfvarson J, Rosenquist M, Järnerot G, Tysk C, Aparajahti J, et al. Molecular analysis of the gut microbiota of identical twins with Crohn’s disease. ISME J 2008; 2: 716–27.
7. Norin E, Midtveld T. Born germfree – microbial dependent. In: Ouwehand A, Vaughan EE, eds. Gastrointestinal microbiology, Taylor & Francis; 2006. p. 273–84.
8. Zabarovsky E, Petenko L, Protopopov A, Vorontsova O, Kutsenko AS, Zhao Y, et al. Restriction site tagged microarrays (RST): a novel technique to identify the species composition of complex microbial systems. Nucleic Acids Res 2003; 31(e95): 1–8.
9. Ramare F, Haufert I, Verhe F, Calam J. Influence of inflammatory bowel disease on the distribution and concentration of pancreatic secretory trypsin inhibitor within the colon. Am J Pathol 1995; 146: 310–6.

*Elisabeth Norin
E-mail: thibi@z5.keio.ac.jp
Application of metabolomics approaches to study energy metabolism and reveals the hepatic glycogen accumulation in germ-free mice

Hsiao-Li Chuang¹, Yen-Te Huang¹, Chia-Chung Hou²,* and Chi-Chang Huang²,*

¹Germfree & Gnotobiotic Section, Technical Services Division, National Laboratory Animal Center, National Applied Research Laboratories, Taipei 11529, Taiwan, ROC; ²Sports Nutrition and Biochemistry Laboratory, Graduate Institute of Sports Science, National Taiwan Sport University, Taoyuan, 33301 Taiwan, ROC

Current nutrition research is focusing on health promotion, disease prevention and performance improvement for individuals and communities around the world. The provision of humans with required nutritional ingredients depends on both how well the individual is provided with balanced foods and what state of gut microbiota the host has. Studying the mutually beneficial relationships between gut microbiome and host is drawing ever-increasing attention in biomedical science. Increasing metabolome-based evidences show that gut microbiota can affect host energy balance, especially fat deposition and lipid metabolism, by microbial metabolites such as short-chain fatty acids. Very recently, we used a gas chromatography-mass spectrometry (GC-MS)-based metabolomics approach to reveal the metabolic profile in gut microbiota- lacking mice and suggested that increased gluconeogenesis and glycogenesis lead to glycogen accumulation in the liver (Chuang et al., 2011; J Nutr Biochem). Our findings also shed light on a new perspective of the role of gut microbiota in energy metabolism and will be useful to help study interactions between gut microbiota and host metabolism.

*Chia-Cung Hou/Chi-Chang Huang
E-mail: john5523@mail.ntsu.edu.tw

Fig. 1. Metabolomics for gnotobiotic research: from phenotype to pathophysiology.
Microbial ecology and shelf life of ready-to-eat pomegranate arils packaged under modified atmosphere

Athanasios Alexopoulos¹, Elisabeth Chatzivassiliou², Stavros Plessas¹, Maria Alexakoudi¹, Irene Theodoridou¹, Maria Fournomiti¹, Ioanna Mantzourani¹, Chryssa Voidarou¹, Elizabeth Stavropoulou³ and Eugenia Bezirtzoglou¹*

¹Democritus University of Thrace, Faculty of Agricultural Development, Laboratory of Microbiology, Biotechnology and Hygiene, Orestiada, Greece; ²Agricultural University of Athens, Laboratory of Phytopathology, Athens, Greece; ³Democritus University of Thrace, Medical School, Alexandroupolis, Greece

The pomegranate (Punica granatum) is a fruit tree of great adaptability to adverse climatic conditions; it is able to support severe colds, salinity soils, tolerate droughts and grow in semi-arid zones. These facts, along with the special dietary characteristics of pomegranate, revive its cultivation during the recent years worldwide and also in Greece. Pomegranates are consumed as fresh fruits, as a juice or as syrup in cocktails and pastries (grenadine). Some parts of the fruit are used in tannery due to the increased content in tannins or in the manufacturing process of dyes and coloring pigments. In food industry, the leading product is the minimally processed and modified atmosphere packaged (MAP) ‘ready-to-eat’ arils. Most of the Greek arils production is dedicated for exporting, and thus it is essential to be properly preserved in order to avoid any losses from spoilage and even more to prolong the shelf life and stability leading to competition advantages. Ways of minimal processing and preservation are the individually quick frozen, drying (natural or vacuumed) and modified atmosphere packaging, alone or in combination with sanitizing agents washing, pH modification, use of antioxidants and temperature control (hurdle technology).

In this study, the shelf life and microbiological safety of packaged pomegranate arils of the Wonderful variety was evaluated under different atmosphere compositions, with or without prior sanitizing (sodium hypochlorite solution), antioxidants (ascorbic acid) and pH modifier (citric acid).

As our results showed, arils packaged in PET trays without MAP and stored at 5°C were spoiled after a period of 8 to 12 days or when the total microbiological content (cfu/g) reached 6 logs. In contrast, the optimum results were obtained after initial sanitation of the arils (100 ppm sodium hypochlorite solution), pH modification with citric acid and packaging under MAP (15% CO₂/5% O₂). Those conditions if combined with an increased quality of the fruit, low postharvest injury incidence and low temperature preservation (~5°C) could extend the shelf life of the product to 18–20 days.

*Elisabeth Bezirtzoglou
E-mail: empezi@agro.duth.gr

Germfree animal studies lead to the revelation of new functions of vitamin K

Michio Komai* and Hitoshi Shirakawa

Laboratory of Nutrition, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

Phylloquinone (vitamin K1, VK1) and the menaquinones (MK-n, or vitamin K2, VK2) are naturally occurring forms of vitamin K. Most of the menaquinone analogs are synthesized by microorganisms, but we have reported that MK-4 is unique in being synthesized by the conversion of orally ingested VK1 or menadione (VK3) in the major tissues of germfree rats and mice, which lack their intestinal microflora. According to our previous studies with germfree animals, we could negate Martius’ theory that described the participation of bacterial enzyme of the intestinal flora to this conversion. Last year, another group (1–3) revealed the enzyme responsible for the menadione (VK3) conversion into MK-4, and this is UBIAD1 (Nature, 2010). However, this enzyme cannot catalyze VK1 conversion into MK-4, which means that UBIAD1 is not the actual enzyme for VK1 conversion into MK-4. Thus, we have just restarted efforts to reveal the true enzyme for the naturally occurring VK1 conversion into MK-4. The result of this study will be presented in the near future.

In addition to the in vivo conversion study, MK-4 has been attracting the attention of researchers due to its specific physiological action such as apoptotic activity on osteoclast cells and leukemia cells, etc. We also discovered new functions of MK-4 by using feeding vitamin K-deficient diet model in mice and rats. One outcome of MK-4 is the anti-inflammatory action.
action, and the other is the steroidogenic effect in the testis through the regulation of Cyp11a.

1. MK-4 enhances testosterone production in rats and testis-derived tumor cells.

Vitamin K is essential for the posttranslational modification of various Gla proteins. Although it is widespread in several organs, including the testis, the function of vitamin K in these organs is not well characterized. In this study, we investigated the function of vitamin K in the testis and analyzed its role in steroidogenesis.

Eight-week-old male Wistar rats were fed a diet supplemented with MK-4 (75 mg/kg diet), one of the predominant K2 vitamins present in the testis, for 5 weeks. In vivo testosterone levels of the rats’ plasma and testes were measured by enzyme-linked immunosorbent assay, and in vitro testosterone levels of testis-derived tumor cells (I-10 cells) maintained in Ham’s F-10 medium with 10% fetal bovine serum were measured following treatment with MK-4 (0 to 100 mM) at several time points. Testosterone and cellular protein levels were analyzed with respect to their effects on steroidogenesis.

Testosterone levels in the plasma and testes of MK-4-fed rats were significantly increased compared to those of control rats, with no obvious differences in plasma luteinizing hormone levels. Secreted testosterone levels from I-10 cells were elevated by MK-4, but not by vitamin K1, in a dose-dependent manner independent of cAMP treatment. Western blot analysis revealed that expression of CYP11A, the rate-limiting enzyme in steroidogenesis, and phosphorylation levels of protein kinase A (PKA) and the cAMP response element-binding protein were all stimulated by the presence of MK-4. Enhancement of testosterone production was inhibited by H89, a specific inhibitor of PKA, but not by warfarin, an inhibitor of g-glutamylcarboxylation.

2. Vitamin K suppresses the lipopolysaccharide (LPS)-induced expression of inflammatory cytokines in cultured macrophage-like cells.

We previously found that vitamin K suppresses the inflammatory reaction induced by LPS in rats and human macrophage-like THP-1 cells. In this study, we further investigated the mechanism underlying the anti-inflammatory effect of vitamin K by using cultures of LPS-treated human- and mouse-derived cells. All the vitamin K analogs analyzed in our study exhibited varied levels of anti-inflammatory activity. The isoprenyl side chain structures, except geranylgeraniol, of these analogs did not show such activity; warfarin did not interfere with this activity. The results of our study suggest that the 2-methyl-1,4-naphthoquinone ring structure contributes to express the anti-inflammatory activity, which is independent of the Gla formation activity of vitamin K. Furthermore, MK-4, a form of vitamin K2, reduced the activation of nuclear factor kB (NFkB) and inhibited the phosphorylation of IKKa/b after treatment of cells with LPS. These results clearly show that the anti-inflammatory activity of vitamin K is mediated via the inactivation of the NFkB signaling pathway.

References

1. Nakagawa K, et al. Nature 2010; 468(7320): 117–21.
2. Ohsaki Y, et al. J Nutr Biochem 2010; 21(11): 1120–6.
3. Ito A, et al. Lipids Health Dis 2011; 10: 158–166.

*Michio Komai
E-mail: mkomai@biochem.tohoku.ac.jp

Blood stream infections in allogeneic hematopoietic stem cell transplant recipients: reemergence of Gram-negative rods and increasing antibiotic resistance

M. Mikulska, V. Del Bono, A.M. Raiola, B. Bruno, F. Gualandri, D. Occhini, C. di Grazia, A. Bacigalupo, Francesco Frassoni* and C. Viscoli

Centro Cellule Staminali e Terapia Cellulare, Divisione Ematologia, Divisione Malattie Infettive, University of Genoa, San Martino Hospital Genoa, Italy

Blood stream infections (BSI) are a well-known cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) patients. The aim of this study was to analyze etiology and microbial resistance of BSI in patients undergoing allogeneic HSCT in a single center over a 4-year period (2004–2007). There were 168 episodes of BSI in 132 patients (median 10 days after HSCT) and 182 pathogens were isolated. Gram-positive bacteria (GPB) accounted for 57% of 182 isolates. Gram-negative rods (GNR) accounted for 37% and fungi for 6%. All patients received routine fluoroquinolone prophylaxis. There was a significant decrease in GPB/GNR ratio over time, from 2.4 in 2004 to 1 in 2007 (p = 0.043). Among GPB, staphylococci decreased from 37 of 68 (64%) in 2004–2005 to 8 of 35 (23%) in 2006-2007 (p < 0.002). The Enterococcus faecalis/E. faecium ratio decreased from 4.5 in 2004 to 0.33 in 2007 (p = 0.006), whereas the total number of enterococcal strains per year did not change. The incidence of Escherichia coli among GNR increased from 3 of 15 (20%) in 2004 to 13 of 21 (62%) in 2007 (p = 0.003). Fluoroquinolone-resistance was common, both among GPB and GNR (81% and 74%, respectively). Mortality
rate at 7 days after BSI was 11% (19 of 168), reaching 39% for *Pseudomonas aeruginosa* BSI (7 of 18). BSI remains a frequent and potentially life-threatening complication of allogeneic HSCT, the causative organism influencing 7- and 30-day mortality rate. BSI etiology may change rapidly, requiring implementation of new empirical-therapy schemes.

**Fungal infection in HSCT**

Shinichiro Mori*

Division of Hematological Malignancy, St. Luke's International Hospital, Japan

Hematopoietic stem cell transplant (HSCT) recipients have the highest risk of acquiring invasive fungal infection (IFI) that may be associated with significant morbidity and mortality. Prevention and early recognition of IFI is crucial in improving outcomes in HSCT recipients.

HSCT recipients have a unique nature of their immunocompromised status. Profound and long-lasting neutropenia and qualitative deficits in phagocyte function during early posttransplant period (first month, in general) are among the risk factors for all kinds of fungal species infection. During this period, HSCT recipients also have mucosal damage, which allows tissue invasion of enteric fungi, principally *Candida* species.

Deficiencies of T-cell immunity arising from lack of donor-derived T-cell function, immunosuppressive agents for prevention and treatment of Graft-versus-Host disease (GvHD), GvHD itself and corticosteroid use persist for longer period (> 6 months) after HSCT. Since T-cell have a major role for protection against fungal pathogens, susceptibility for various IFIs still persists. However, as prevention and management of yeast infection improves, the peak of invasive yeast infection appears to be shifting to the later posttransplant period. Environmental control measures, such as HEPA-filtered room, and prophylactic usage of antimold agents are the mainstay of prevention. Treatment of invasive aspergillosis (IA) is also improved. Historically, mortality rate of IA among HSCT recipients have exceeded as high as 80%, although recent epidemiological studies suggest that outcomes appear to be improving. Surprisingly, a recent prospective epidemiological study conducted by a North America group (PATH Alliance) revealed that the 6-week survival rate was significantly better for HSCT recipients with IA, followed by those with invasive candidiasis and those with zygomycoses or other mold.

Humoral immunity is also compromised after HSCT. Especially, recipients with chronic GvHD have long-lasting humoral immunity deficiency. Since opsonization with antcapsular antibody have a role for protecting against *Cryptococcus neoformans*, recipients with chronic GvHD and functionally splenectomized patients have high risk of acquiring cryptococcosis. However, as a result of broad usage of azole prophylaxis, cryptococcosis following HSCT seems to be very rare.

As I described above, epidemiology, morbidity and mortality of IFI after HSCT have been dramatically changing. These changes are results from improvement in prophylaxis, early diagnosis and treatment with newer antifungal agents.

In this review presentation, I would like to summarize the historical changes in IFI after HSCT and then, make it clear what are our current problems to be solved and future perspectives.

**Infectious complications following reduced-intensity cord blood transplantation**

Shuichi Taniguchi*

Department of Hematology, Toranomon Hospital, Tokyo, Japan

The number of UCBT has been increasing progressively, and >1000 UCBT was performed in 2010 in Japan, which is comparable to related- and unrelated bone marrow (BM) and peripheral blood (PB) transplantation. Rapid availability and less stringent HLA match requirement are the main reasons of expanding UCBT. In Japan, 40% of UCBT were
performed in elderly patients (>40 years) using reduced-intensity conditioning. Relatively more urgent transplants in elderly patients and related donor unavailability due to donor’s older age. UCB presents opportunities of allogeneic transplant to these aged patients.

Most serious complications in UCBT have been supposed to be infectious complications due to neutrophil and immunological recovery delay compared to BM and PB stem cell transplantation. Actually, neutrophils recovery delays by almost 7 days after UCBT, which might increase the incidence of severe bacterial infection. Immunological recovery, especially antigen-specific cytotoxic T-lymphocyte (CTL), has been reported to delay significantly in UCBT compared to PB and BM. Various viral infections occur more frequently and severely. Neutrophil recovery delays mainly due to low total cell and CD34+ number in cord blood, and immunological recovery delay was explained by dominant naïve T cells in transfused cord blood.

We have been involved in more than 700 UCBT from 2003 to 2011. Our patients are relatively older patients (mean age, 58–59 years) with advanced disease (80%). For these reasons, reduced-intensity conditioning was used in majority of UCBT (90%). In this case cohort, we analyzed T cell and B cell recovery (Fig. 1) and immunoglobulin production in CBT and compared with PB and BM patients. T cell subset recovery was relatively rapid and B and NK cell tend to recover earlier than unrelated BM. Immunoglobulin supplement to maintain IgG above 500 mg/dl was compared among related PB, unrelated BM and UCB. Although the onset of chronic graft-versus-host disease (cGVHD) and intensification of immunosuppression are the major determinant of IG production, IG supplement was less and shorter after transplant in UCB group compared to RPB and UBM group.

We also analyzed the incidence and species of bacteremia, viral infection (CMV, HHV6, EBV and ADV) and fungal infection. In symposium, I will discuss these results.

*Shuichi Taniguchi
E-mail: taniguchi-s@toranomon.gr.jp

**Contribution of intestinal flora to homeostasis and ibd pathogenesis**

Toshifumi Hibi*, Tadakazu Hisamatsu and Takanori Kanai

Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan

Inflammatory bowel disease (IBD) is classified into two typical phenotypes, ulcerative colitis and Crohn’s disease. Although the precise etiologies of IBD remain unclear, several studies have indicated that dysfunction of the mucosal immune system plays important roles in its pathogenesis. Especially, recent studies spotlight the cross talk between host and intestinal flora. The gut has $10^{10–12}$ bacteria through entire digestive tract. It means that gut is always exposed to enteric bacteria and food antigens; however, gut maintains its homeostasis without development of chronic inflammation in normal situation. Recent studies have demonstrated that (1) gut microbiota may contribute to gut immunological homeostasis, (2) gut has protective mechanisms such as intestinal macrophages system to suppress excess immune response to those foreign antigens and (3) the disruption of those regulatory systems may cause IBD.

One of the most important concepts of IBD pathophysiology is that the homeostasis of gut immune system to enteric flora becomes discordant. Intestinal macrophage is a key player for not only elimination of bacteria by phagocytosis...
but also intestinal immune homeostasis. We have revealed a functional role of intestinal macrophages for gut homeostasis and the fact that disregulation of intestinal macrophages to commensals lead to chronic intestinal inflammation in Crohn's disease.

Crohn's disease (1) is characterized by the Th1 dominant chronic intestinal inflammation. We identified that number of CD14⁺CD33⁺CD68⁺ unique intestinal macrophages were increased in lamina propria (LP) in the patients with IBD, especially Crohn's disease. These cells showed typical macrophages morphology, but they expressed some DC markers and they had antigen-presenting function. CD14⁺ intestinal macrophages induced both Th1 and Th17 cells from peripheral blood naïve T cells. Intestinal bacteria enhanced Th17 polarization through IL-1β and IL-6 produced by CD14⁺ intestinal macrophages, while IL-23 enhanced Th1 immunity. Thus, CD14⁺ intestinal macrophages may be involved in the pathogenesis of Crohn's disease as antigen presenting cells (APCs) (2).

In local immunity, these intestinal Mfs produce large amount of TNFa and IL-23, which are key cytokines for Crohn's disease pathogenesis, in response to commensal bacteria. IL-23 enhanced production of IFNγ by LP mononuclear cells. We identified that the source of IFNγ are CD4⁺CD8 T cells and mucosal natural killer cells. In addition, TL1A cooperating IL-23 may synergistically enhance IFNγ and IL-17 production by LP CD4⁺ T cells (3).

In conclusion, CD14⁺ intestinal macrophages play the central roles in the pathogenesis of Crohn’s disease by regulating local immunity and inducing both Th1 and Th17 immunity by APCs.

References

1. Kamada N, Hisamatsu T, Hibi T, et al. Retinoic acid contributes to the induction of IL-12-hypoproducing dendritic cells. Immunol 2009; 15: 1548-56.
2. Kamada N, Hisamatsu T, Hibi T, Kobayashi T, Chinen H, Kitazume MT, et al. Human CD14⁺ macrophages in intestinal lamina propria exhibit potent antigen-presenting ability. J Immunol 2009; 183: 1724-31.
3. Kamada N, Hisamatsu T, Hibi T, Chinen H, Kobayashi T, Sato T, et al. Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-gamma axis. J Clin Invest 2008; 118: 2269-80.

*Mitsufumi Hibi
E-mail: thibi@sc.ltc.keio.ac.jp

Mucosal decisions for immunity to co-habitation of microflora

Hiroshi Kiyon*

Division of Mucosal Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Digestive tract is covered by a single layer of mucosal epithelial cells, which is continuously exposed to infinite antigenic challenges in handling its day-to-day duties. The intestinal tract is thus equipped with the mucosal immune system (MIS) offering the first line of innate and acquired defense forces against invasion of pathogens and, hence, required to induce a prompt and robust immune response in order to prevent invasion of infectious agents. At the same time, the MIS also exposed to an enormous number and volume of innocuous and/or instructive antigens, which need to be appropriately ‘ignored’. Mounting an immunologically harmonized response, therefore, represents a key decision-making process of active and/or quiescent immune responses by the MIS. The mucosal surface covering the digestive tract represents a complex immunological network structured to execute the immunologically harmonized regulation of the two opposite immune responses. For the understanding of the harmonized MIS, it is essential to elucidate the molecular and cellular mechanisms of crosstalk system between the MIS and commensal flora. Our recent results suggested that gut-associated lymphoid tissues (GALT) including Peyer’s patches play a critical role in the creation of cohabitation niche between the host and commensal bacteria. Our recent studies have now thus provided new evidence for the intratissue habitation of commensal flora in the organized lymphoid structure associated with mucosa (e.g., GALT).

*Hiroshi Kiyono
E-mail: kiyono@ims.u-tokyo.ac.jp

Control of immune responses by commensal bacteria during acute gastrointestinal infections

Liliane M. dos Santos¹,²*, Timothy Hand², Nicolas Bouladoux² and Yasmine Belkaid²

¹Laboratory of Gnotobiology and Immunology, Federal University of Minas Gerais, Brazil; ²Laboratory of Parasitic Diseases, National Institutes of Health, USA

Citation: Microbial Ecology in Health & Disease 2012, 23: 17462 - http://dx.doi.org/10.3402/mehd.v23i0.17462
Physiological role of indigenous Lactobacilli/\textit{Helicobacter pylori} in the stomach

Yasuhiro Koga*

Laboratory for Infectious Diseases, Tokai University School of Medicine, Isehara, Japan

The gastrointestinal tract harbors a rich variety of microbiota consisting of hundreds of different bacterial species containing high densities of living bacteria, which achieve concentrations of up to $10^{11}$ or $10^{12}$ cells/g of luminal contents. The role of such indigenous microbiota of the gut in health and disease is well known to include metabolic activities, trophic effects on intestinal epithelia and the immune system and protection of the colonized host against invasion by alien microbes.

On the other hand, the stomach contains only a few species of bacteria in the human if it is free from infection with \textit{Helicobacter pylori}. During fasting, the gastric juice contains only small numbers of bacteria, approximately, $10^{2}$ to $10^{3}$/ml, which include \textit{Streptococcus}, \textit{Lactobacillus} and \textit{Veillonella}. However, these bacteria are considered nonresidents that are just in transit from the oral cavity and throat. The scarcity of such bacteria in the human stomach appears to be because of the high acidity of the luminal medium.

While \textit{H. pylori} is a well-known pathogenic bacterium that causes peptic ulcers and cancer in the human stomach, \textit{Helicobacter} species have also been proposed to belong to the indigenous gastric microbiota of humans from our earliest times. That hypothesis is supported by the fact that \textit{H. pylori} is acquired in early childhood and thereafter remains stably colonized in the stomach for decades in substantial numbers. This raises questions about the role of the indigenous bacteria of the stomach in the physiological development and function of this organ. However, it is difficult to clarify the answers to this question by an infection study using \textit{H. pylori}, because various pathogenic factors of \textit{H. pylori} such as CagA, vacuolating toxins, urease and its metabolites induce chronic pathological inflammation in the gastric tissue, which thus obscures the physiological role of \textit{H. pylori} as an indigenous bacterium.

In a previous study, we found an indigenous microbiota, which predominantly consists of lactobacilli, in the stomach of specific pathogen-free mice. The lower acidity in the stomach of mice was thought to enable the lactobacilli to colonize the stomach. Moreover, no evident inflammatory changes occurred in the stomach of the mice. In a recent study, a microarray analysis was performed to investigate the role of these innate lactobacilli in the development of physiological functions of the stomach using germfree (GF) and lactobacilli-associated gnotobiotic mice.

In this DNA microarray analysis, GF BALB/c mice were orally inoculated with $10^{9}$ CFU lactobacilli and their stomachs were excised after 10 days to extract RNA. As a result, lactobacilli-associated gnotobiotic mice showed a dramatically decreased expression of the gastrin gene in comparison to germfree mice. The mean of the log2 fold change of gastrin gene was $-4.3$. Immunohistochemistry also demonstrated the number of gastrin$^+$ cells to be significantly lower in the lactobacilli-associated gnotobiotic than in the GF mice. Moreover, oral inoculation of heat-killed lactobacilli to GF mice also decreased the gastrin$^+$ cell number. However, there was no significant difference in the number of somatostatin$^+$ cells in these groups of mice. Consequently, gastric acid secretion also decreased in the mice colonized by lactobacilli. While an increase in the expression of the genes related to the muscle system showed decreased inflammatory responses and lower parasite load. Studies using germfree mice were also carried out. Germfree mice infected with \textit{T. gondii} displayed less severe pathology and reduced parasite burden. Dysruption of intestinal homeostasis during \textit{T. gondii} infection led to systemic translocation of gut bacteria and temporal changes in the diversity of the gut microbial community. Three different bacteria that were abundant in the gut of \textit{T. gondii}-infected mice at the peak of infection were isolated and used for investigation of specific immune responses against commensal bacteria. We showed that \textit{T. gondii} acute infection induces specific antibody responses toward antigens from the microbiota.

* Liliane M. dos Santos  
E-mail: liliane_pucminas@yahoo.com.br

\section*{Abstracts}
Development of novel methods for the search of antibiofilm agents

Reiko Kariyama* and Hiromi Kumon

Department of Urology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

Over the last 20 years, we have been investigating biofilm infections in the urinary tract. For the prevention and control of biofilm infections, many basic and clinical studies are currently in progress worldwide. The important step in studying biofilm infections is establishing good experimental models and methods of evaluation. It is necessary to make a major breakthrough toward the development of novel therapies, medical devices and innovative antibiofilm agents. In this presentation, our recent experimental approaches by using real-time imaging in vitro and in vivo models to search antibiofilm agents are reviewed.

A capillary flow cell system as an in vitro model of complicated urinary tract infections (UTI) is utilized. Pseudomonas aeruginosa OP14-210 isolated from a patient with catheter-associated UTI was used and a green fluorescent protein (GFP)-producing strain, P. aeruginosa OP14-210 (pMF230), was constructed. Biofilms were grown in glass capillary tubes under continuous flow conditions with artificial urine and were observed by confocal laser scanning microscopy. To evaluate the effects of potential antibiofilm agents, levofloxacin (LVFX 10 times the MIC: 80 µg/ml), ulitloxacin (UXF 10 times the MIC: 20 µg/ml) and fosfomycin (FOM 3 times the MIC: 192 µg/ml) were tested. When both LVFX and FOM were added to the system 2-h after inoculation with the GFP-producing strain, very weak fluorescence signal indicating no biofilm formation was observed after 3 days. The GFP-producing 1-day biofilm after 72-h treatment with FOM alone was similar to that seen with no treatment. The irregular detached biofilm was observed by the treatment with LVFX alone. In combination of LVFX and FOM, the irregular detached biofilm was much thinner than that with LVFX alone. BacLight staining was applied to assess the effects of treatment on the number of live and dead cells and their distribution in biofilms. A higher proportion of dead cells was observed in the 2-day biofilms after 18-h treatment with either UFX alone or in combination of UFX and FOM compared with either LVFX alone or in combination of LVFX and FOM. The quantitative analysis of the intensity of green and red signals confirmed the increased bactericidal effect by the combination of UFX and FOM compared with LVFX and FOM.

More recently, we started to develop a new type of microdevice to screen antibiofilm agents efficiently. We designed the new type of microdevice, which might be set on microscope stages and evaluated the effects of many samples simultaneously. The specification of a model of microdevice with porous media is a double structure with a layer of biofilm formation and a layer of drug supply. The specification of a model of microdevice without porous media is three-step slopes, which are able to observe changes in biofilm phenotype by a different flow rate. Our previous findings by using a capillary flow cell system regarding the synergy between LVFX and FOM were confirmed using the new type of microdevice (the latest model without porous media). The basic design of a new type of microdevice for efficient screening of antibiofilm agents was almost established by continuous improvements. By using the new type of microdevice, it is possible to screen novel compounds and the combination of possible antibiofilm agents for the treatment of biofilm infections.

On the other hand, we assessed a noninvasive, real-time imaging technology (IVIS Lumina system. It was thought that this problem might be addressed by using novel bioluminescent strains engineered.

Most recently, we have found that a quorum sensing inhibitor of P. aeruginosa would be a strong anti-Pseudomonas agent combined with biapenem in a murine gastric acid secretion without affecting somatostatin secretion in mice.

*Yasuhiro Koga
E-mail: yasuhiro@is.isc.u-tokai.ac.jp
(male, 5 to 6 weeks old) neutropenic thigh infection model of a bioluminescent *P. aeruginosa* Xen 5 strain by using the IVIS® Lumina system. We confirmed that biapenem exhibits an antimicrobial effect with the time above MIC (T > MIC) in pharmacokinetic and pharmacodynamic (PK/PD) parameters. We also demonstrated a good correlation between photon count imaging and viable counts in vivo. By using *in vitro* models described above, the quorum sensing inhibitor of *P. aeruginosa* was found to inhibit biofilm formation of *P. aeruginosa*. Our experiments intended to establish the applicability of some animal models in efficacy assessments of therapeutic agents for treatment of chronic infections by bacterial biofilms in the future.

**Reference**

1. Kurosaka Y, Ishida Y, Yamamura E, Takase H, Otani T, Kumon H. A non-surgical rat model of foreign body-associated urinary tract infection with *Pseudomonas aeruginosa*. Microbiol Immunol 2001; 45: 9–15.

*Reiko Kariyama
E-mail: kariyama@md.okayama-u.ac.jp

---

**Two-component signal transduction systems and biofilm formation of *Staphylococcus epidermidis***

Di Qu1*, Yang Wu1, Shiqing Han2, Xu Shen3 and Hualiang Jiang3

1Key Laboratory of Medical Molecular Virology of Ministries of Education and Health, Institute of Medical Microbiology and Institutes of Biomedical Sciences, Shanghai Medical College of Fudan University, Shanghai, China; 2College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing, China; 3Drug Discovery and Design Center, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

Coagulase-negative *Staphylococcus epidermidis* has emerged as one of the most important nosocomial pathogens. The pathogenicity of *S. epidermidis* is mostly due to its ability to form a thick, multilayered biofilm on polymeric surfaces of implanted medical devices. Treatment of *S. epidermidis* infection has become a troublesome problem as biofilm-associated bacteria exhibit enhanced resistance to antibiotics and to components of the innate host defense. Based on the two complete genome sequences of *S. epidermidis* (ADCC12228 and ADCC35984), we have analyzed two-component signal transduction systems (TCSs) in *S. epidermidis*, which consists of 16 or 17 pairs TCSs. Each TCSs consists of a signal ligand responsive histidine kinase (HK) and a response regulator transcription factor present in bacteria. Some of them have been identified to regulate diverse functions of bacteria including biofilm formation, virulence, cell wall biosynthesis, quorum sensing, etc. Our research is mainly focused on the roles of TCSs in *S. epidermidis* biofilm formation. We have investigated the effects of *lytSR*, *saeRS*, *arlRS* and *srrBA* knocking out on biofilm formation and their regulated genes. We found that biofilm formation was enhanced in *ΔlytSR, ΔsaeRS* strains, while decreased in *ΔarlRS* and *ΔsrrBA* strains. The mechanisms of *lytSR*, *saeRS*, *arlRS* and *srrBA* in regulation of biofilm formation are under studying.

In another aspect, we are trying to discover inhibitors targeting to HK domain in YycG protein of *S. epidermidis*. Using structure-based virtual screening (SBVS) from a small molecular lead-compound library, followed by experimental validation, the inhibitors of YycG that we discovered displayed bactericidal effects on both planktonic and biofilm cells of *S. epidermidis*. To improve the inhibition and bactericidal activities of one of compounds (compound 2) on *S. epidermidis* biofilm, a series of the derivatives were synthesized by cyclization, aldol condensation, substitution and hydrolyzation reactions. The six derivatives out of 46 synthesized new compounds inhibited phosphoryl transfer activity of YycG histidine kinase and were proven to kill bacteria in both immature and mature biofilms of *S. epidermidis* more effectively than the leading compound. However, the structures of those YycG inhibitors need to be improved for increasing the potential application as antibiofilms agents.

*Di Qu
E-mail: dqu@fudan.edu.cn

---

**Biofilm formation of *Helicobacter pylori***

Hideo Yonezawa* and Shigeru Kamiya

Department of Infectious Disease, Kyorin University School of Medicine, Tokyo, Japan

---

In another aspect, we are trying to discover inhibitors targeting to HK domain in YycG protein of *S. epidermidis*. Using structure-based virtual screening (SBVS) from a small molecular lead-compound library, followed by experimental validation, the inhibitors of YycG that we discovered displayed bactericidal effects on both planktonic and biofilm cells of *S. epidermidis*. To improve the inhibition and bactericidal activities of one of compounds (compound 2) on *S. epidermidis* biofilm, a series of the derivatives were synthesized by cyclization, aldol condensation, substitution and hydrolyzation reactions. The six derivatives out of 46 synthesized new compounds inhibited phosphoryl transfer activity of YycG histidine kinase and were proven to kill bacteria in both immature and mature biofilms of *S. epidermidis* more effectively than the leading compound. However, the structures of those YycG inhibitors need to be improved for increasing the potential application as antibiofilms agents.

*Di Qu
E-mail: dqu@fudan.edu.cn

---

**Biofilm formation of *Helicobacter pylori***

Hideo Yonezawa* and Shigeru Kamiya

Department of Infectious Disease, Kyorin University School of Medicine, Tokyo, Japan

---

Citation: Microbial Ecology in Health & Disease 2012, 23: 17462 - http://dx.doi.org/10.3402/mehd.v23i0.17462
Biofilms are surface-bound communities of bacterial cells that are implicated in their survival. Recently, some reports have indicated that Helicobacter pylori forms biofilm in vitro and in vivo; however, biofilms of H. pylori have not been well characterized. We attempted to analyze the ability of H. pylori strains to form biofilms in vitro and characterized the underlying mechanisms of H. pylori biofilm formation in Brucella broth supplemented with 7% fetal calf serum (FCS). Strain TK1402 showed strong biofilm forming ability relative to the other strains under these conditions. There were no significant differences in the aggregation, motility and hydrophobicity of strain TK1402 compared with the other strains. The strong biofilm forming ability of TK1402 reflected the thickness of the biofilms. In addition, the biofilm formation was correlated strongly with the production of outer membrane vesicle (OMV) in strain TK1402. SEM analysis indicated that OMV was detected within the matrix of only the TK1402 biofilms. Strain TK1402 did not form thick biofilms in Brucella broth supplemented with 0.2% β-cyclodextrin; however, the addition of the OMV-fraction collected from TK1402 could enhance biofilm formation. Taken together, these results suggest that the TK1402 is a strongly biofilm-forming strain in Brucella broth supplemented with 7% FCS, and the OMV production from this strain play an important role in biofilm formation.

Next, we analyzed the comparison of the urease production between biofilm cells and planktonic cell. In Western blot analysis and quantitative RT-PCR with TK1402 biofilm cells, the level of the urease expression was increased in biofilm cells compared to planktonic cells. These suggested that the biofilm formation might be associated with virulence of this microorganism.

*Hideo Yonezawa
E-mail: yonezawa@ks.kyorin-u.ac.jp

Immune response of the gnotobiotic mouse model induced mycoplasmal pneumonia and evaluation of antimicrobials using the model

Haruhiko Taguchi1*, Satoshi Kurata2, Ken Arase1, Tsuyoshi Onogawa1 and Shigeru Kamiya2

1Department of Immunology, Kyorin University Faculty of Health Sciences, Tokyo, Japan; 2Department of Infectious Diseases, Kyorin University School of Medicine, Tokyo, Japan

Mycoplasmas are the smallest and self-replicating microorganisms. They are primarily mucosal pathogens, residing with epithelial surfaces extracellularly. Mycoplasma pneumoniae is an atypical surface bacteria that lacks a cell wall and is recognized as an important etiologic agent of acute lower respiratory infection in children and young adult. In Japan, approximately 30% to 40% of community-acquired pneumonia cases are due to M. pneumonia infection. Mycoplasmal pneumonia is considered to be a relatively benign disease that is improved by appropriate treatment with antimicrobials in many cases, even though it presents symptoms such as high fever, persistent cough and dyspnea. However, in some cases, it becomes severe by presenting various clinical features such as the development of bronchial asthma and complication by extrapulmonary lesions. Histopathologically, in mycoplasmal pneumonia, the bronchial and bronchiolar lumina are characteristically filled with polymorphonuclear leukocytes, and their walls have a mononuclear infiltration with plasma cells.

Although the mechanism by which primary atypical pneumonia is caused by M. pneumoniae has not been clarified, it is hypothesized that indirect injury through immune responses of the host besides the direct pathogenic factors of M. pneumoniae is important in the infection. Several animal models have been developed to examine the pathogenesis of mycoplasmal pneumonia; however, the importance of host immune response related to the pneumonia is not revealed. The traditional animal model of mycoplasmal pneumonia, the Syrian hamster, is limited by an inability to study the host cytokine response due to a lack of host-specific reagents. Thus, establishment of a murine model is extremely important to help elucidate the host immune response to M. pneumoniae infection and to better understand the pathogenesis of mycoplasmal pneumonia. On the other hand, gnotobiotic animal offer a well-defined model to study the pathogenicity of bacteria because it is possible to study the interaction of the bacterium with the host without the influence of any other bacteria. So, we attempted to establish an animal model using germfree mice for M. pneumoniae infection, and the immunological, histopathological and bacteriological studies were performed.

In this presentation, we would like to show the host immune response of the gnotobiotic mouse model induced mycoplasmal pneumonia and the efficacy of some anti-M. pneumoniae drug possessing immunomodulation activity independent of its antimicrobial activity.

*Haruhiko Taguchi
E-mail: taguchi@ks.kyorin-u.ac.jp
Epidemiology, diagnosis, medication and vaccination of avian mycoplasmosis in Taiwan

Michael M.Y. Lin*

Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, Neipu, Pingtung, Taiwan

Cultivation of variant serotypes of avian mycoplasmas with Frey’s medium from the tracheal swabs of chickens, ducks, geese and English sparrows in Taiwan during 1985–1995 provided 47.8%, 5.6%, 18.6% and 0.03% positive of isolation, respectively. Most of the poultry flocks in Taiwan were infected with mycoplasmas.

Rapid identification of variant serotypes of avian mycoplasmas using nested and serotype-specific polymerase chain reaction (PCR) for amplification of the rRNA gene and identification of the serotype relevant sizes of generated amplicons under agarose gel electrophoresis was established in this laboratory since 2001. Molecular identification of avian mycoplasmas comes to be more scientific than used to be done by fluorescent antibody technique.

Electrophoresis and immunoelectrophoresis of variant serotypes and strains of avian mycoplasma cellular and membrane proteins provided that different serotype or strain of avian mycoplasmas has its unique pattern of cellular or membrane protein profile, which can be used for serotyping or strain characterization.

Simple immunodot system (SIM) implying the reading of simple immunodot blotting assay (SIDBA) by Amersham computer software system was used to evaluate the intensity of the dot density in numerical scale. SIM assay provided high sensitivity and moderated specificity in the detection of MG antibody in chickens.

Inhibitory activity of all the commercial available antimicrobials against the standard strains and the 1987, 1994 and 1999 Taiwanese isolates of avian mycoplasmas had been evaluated in this laboratory. Most of the mycoplasmas were found with gradually developing their resistance against the antimicrobials recently used in the poultry farms in Taiwan. Only around 10 commercial antimicrobials were found with MIC90 level <10 µg/mL that are good to be used in the poultry clinic for treatment of avian mycoplasmosis in Taiwan.

Evaluation of five attenuated strains of MG as live vaccine in young chickens. Five trials were conducted to evaluate the virulence and the vaccination efficacy of the F, R, S6 and A5969 strains of MG at different in vitro passage levels. Vaccination was done by eye-drop or aerosol, and the efficacy was evaluated in terms of airsac lesion scoring level after aerosol challenge with the R strain of MG. Continuing the medium passage of these strains of MG resulted in gradual attenuation. Aerosol vaccination with highly attenuated MG at 21 days of age was more effective in stimulating the development of immunity than was eye-drop vaccination at 7 days but inducing higher airsac lesions. Aerosol vaccination with high-passaged F or S6 strain provided good immunity against MG challenge. Both low- and high-passaged (250p) F and S6 strains were found still virulent for turkey pouls, but only the high-passaged F strain significantly (p <0.05) less affected the body weight gaining. The MG-F strain was finally chosen as live vaccine production strain. The MG-F strain low passage has been marketed worldwide by Schering-Plough Co. Ltd. for more than 10 years. The MG-F strain high passage has been intended to be introduced in the market.

Field application of MG-F strain live vaccine in Taiwan. MG-F strain live vaccine has been tried with only good for applying in leghorn layers but not good for broilers, native hybrid broilers or any kind of broiler breeders. Most of the layers originated from mycoplasma-free breeder flocks were supposed to be vaccinated at 10 to 15 weeks of age. Most of the layers originated from mycoplasma-infected breeder flocks were supposed to be vaccinated at 2–3 weeks of age. All the chicks subjected to be vaccinated supposed to be treated with some antibiotic (tiamutin, etc.) for 3 days first and then skipped off any antibiotic treatment at least for 7 days before vaccination. MG-F strain for eye drop vaccination shall be under 30th passage level, for aerosol vaccination shall be at around 250th passage level. Eye drop vaccination of MG-F strain can be done in combination with ND and IB live vaccines simultaneously using MG vaccine as diluent.

Preparation of MG-R strain bacterin and its application in broiler breeder in Taiwan. Hybrid broiler breeders were intramuscularly immunized (0.5 ml/ml) twice with MG-R bacterin at 7 and 15 weeks of age. MG-R bacterin provided some improved egg production (4.5 more egg production during first 82 days laying in comparison with the nonimmunized control.

Diagnosis of Mycoplasma pneumoniae and other respiratory tract infections using gene amplification method ‘loop-mediated isothermal amplification (LAMP)’

Yoshinori Ota, Manabu Yoshino, Toshimitus Annaka, Tsugunori Notomi and Hidetoshi Kanda

Biochemical Research Laboratory, Research & Development Division, Eiken Chemical Co., Ltd., Tochigi, Japan
There are several kinds of bacteria that are frequently isolated as the causative pathogens of community-acquired pneumonia, for example, Streptococcus pneumoniae, Haemophilus influenzae, as well as atypical pneumonia pathogens Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella spp.

Especially, the pneumonia caused by M. pneumoniae or Legionella spp. sometimes increases in severity. Since a delay in an early treatment is associated with increased mortality, an appropriate therapy of these pneumonias must be done immediately. However, beta-lactam antibiotic, which is usually a first choice for bacterial pneumonia treatment, does not work against these atypical pathogens. For an effective treatment, it is significantly important to choose the antibiotics based on a judgement whether a patient is suffering from the bacterial pneumonia or the atypical pneumonia.

For an identification of these causative bacteria, the most reliable method is a culture test. But the method requires a special technique for its operation, and further, it takes a longer period to obtain the results, which will not be used as a guide for an early treatment but just a diagnosis after the treatment. Due to those reasons, a relatively small number of the culture test has been conducted as an ordinary clinical laboratory test.

In order to provide a solution to these problems, we developed a nucleic acid tests for a detection of M. pneumoniae and Legionella spp., using LAMP method, and studied its clinical usefulness.

LAMP, which was invented by Eiken Chemical Co., Ltd. in 2000, is the nucleic acid amplification technique that rapidly amplifies and detects DNA. The LAMP method employs four unique primers and DNA polymerase possessing the strand-displacement activity, which enables a consecutive DNA amplification reaction under an isothermal condition (60–65°C).

The LAMP reaction proceeds through a repetition of both a self primed elongation, which initiates from a stem-loop structure of an amplified product, and a strand-displacement DNA synthesis, which starts from inner primer annealing to a loop structure portion of the amplicon.

Because LAMP method runs consecutive gene amplification reaction, its gene amplification efficiency is enormously high, which allows for a rapid amplification of a target gene (< 1 h). Its reaction can be monitored by means of a turbidity caused by a large amount of by-product generated during the gene amplification reaction, which enables a single tube reaction of whole process from a gene amplification to a detection.

For our clinical diagnosis kits, we used SDC gene, which is a part of the repeated gene sequences involved in host cell adhesion, as target site for the detection of M. pneumoniae and 16S rDNA for Legionella spp.

The clinical study of our kits showed a good agreement with those of the conventional method as well as PCR. We concluded that our kits are the useful tools to identify M. pneumoniae or Legionella spp., differentiate them from other respiratory tract infections and help medical treatment.

Further, we will introduce our approach to a Point of Care Testing using LAMP method that makes the nucleic acid test for other respiratory tract infections (Mycobacterium tuberculosis or influenza virus) more feasible in a usual clinical laboratory.

Yoshinori Ota
E-mail: yoshinori_ota@eiken.co.jp

Autologous hematopoietic stem cell preservation in nuclear plant workers

Shuichi Taniguchi*
Department of Hematology, Toranomon Hospital, Tokyo, Japan

The 9.0 magnitude earthquake and following tsunami on March 11, 2011, destroyed many coastal cities in the northeastern part of Japan. It swamped emergency generators at the Fukushima Daiichi nuclear power plant operated by the Tokyo Electric Power Company in Fukushima prefecture, disabling the cooling systems. Since these catastrophic events, hundreds of nuclear workers have been trying to remove the radioactive water from the tsunami ravaged nuclear compound and restart the regular cooling systems for the overheated nuclear fuel. On April 12, the Nuclear and Industrial Safety Agency of Japan decided to raise the severity level of the crisis to 7—the highest level and equal to the 1986 disaster at Chernobyl in the former Soviet Union. We emphasize the need to predict potential scenarios in Fukushima and to prepare medical care providers for how to respond in cases of accidental high radiation exposure, since this operation is estimated to take months to years.

Generally, rapidly dividing cells, such as intestinal-tract and hemopoietic cells, are most vulnerable to radiation. Radiation accidents can result in localized or whole-body exposure and in internal or external deposition of radioactive materials. On March 24, three workers at the Fukushima nuclear power plant were exposed accidentally to high localized radiation while standing in contaminated water. Fortunately, this accident did not cause major injuries, but the danger of a future accidental radiation exposure is not passed, since there has been a series of serious after-shocks even this April.

A clinically significant hemopoietic syndrome can occur after whole body doses of 2 Gy or higher as a result of bone-marrow depression. If the hemopoietic cells are not completely damaged, a recovery phase can be enhanced through the use of hemopoietic growth factors. In cases of radiation exposure of more than 5 Gy, hemopoietic stem cell rescue is
Enterococci as probiotics or autoprobiotics in treatment of the gastrointestinal diseases

Alexander Suvorov1*, Vladimir Simanenkov2, Elena Ermolenko1, Viktoria Kolodjiyeva1, Anna Tcapieva1, Natalia Zaharova2 and Olga Solovieva2

1Institute of Experimental Medicine RAMS, St. Petersburg, Russia; 2Medical Academy of Postgraduate Studies, St. Petersburg, Russia

Objectives
Enterococci are lactic acid bacteria which can be both the cause of serious infection diseases and the potent and clinically effective probiotics. The goal of the study was to analyze some differences between clinical and probiotic strains and to evaluate the possibility of usage of enterococcal indigenous strains as autoprobiotics.

Methods
Probiotic strain *Enterococcus faecium* L3 (L3) was analyzed employing various molecular biological approaches and compared with the strains causing enterococcal infections. Autoprobiotics were isolated from human feces, tested for virulence genes and used for making milk fermented product.

Results
Probiotic strain L3 in comparison with the clinical strains was free from virulence factor genes and carried the genes of two potent bacteriocins (enterocins A and B), which could be induced by the pheromone. Pheromone induction had been proved employing synthetic pheromones.

In addition, L3 was able to induce IL10 and decrease IL8 production in the epithelium. High antibacterial activity of L3 allowed the treatment of *Helicobacter pylori*-associated gastritis. Genomic comparison of the strain L3 with the clinical strain showed significant differences in size and genome organization. L3 strain prepared as milk fermented product was tested for the treatment of patients with IBS in comparison with autoprobiotic enterococci. Both types of
bacteria (autobiotics and L3) showed significant improvement of the condition of the patients in double blind placebo controlled study.

**Conclusion**
Probiotic enterococci and enterococcal autobiotics have a good potential for the treatment of gastrointestinal disorders.

**Host/bacteria interaction and influence of beneficial bacteria on mucosal immune responses against opportunistic/enteric pathogens: possible cellular-molecular mechanisms and practical approaches**

Nadiya Volodymyrivna Boyko*

Uzhhorod National University, Faculty of Medicine, Uzhhorod, Ukraine

Until recently, identification and application of probiotic microbes had been serendipitous and without a sound scientific basis for selection. This was largely due to our not yet having a broad understanding of the mechanisms and effects operative upon probiotic microbe/host interactions. Clearly, some probiotic candidates have proven to be effective in increasing human and farm animal resistance to frank, nosocomial and opportunistic pathogens. The use of germfree or gnotobiotic mice should provide a set of models that will be informative about possible beneficial microbial stimulation of elements of the immune system generally classified as being at the intersection of the innate and specific adaptive immune responses: ‘natural antibodies’, whose expression may be stimulated polyclonally or oligoclonally by microbial ‘mitogens’ and/or TI-1 and TI-2 antigens; ‘natural’ killer cells and subsets of T cells that may be polyclonally stimulated via toll-like receptors or costimulated in this fashion along with microbial antigens. We believe that the effects caused by the above mechanisms may be associated with resistance to microbial translocation, colonization and pathogenesis following subsequent challenge with pathogens. We have actively pursued in detail beneficial mechanisms should our model systems provide appropriate clues.

Our initial in vivo analysis of humoral and cellular mechanisms by which different commensal bacteria and bacterial antigens (T-dependent, TI-1 and TI-2) can specifically influence the host mucosal immune response and resistance to potential nosocomial pathogens currently had been recently extended. It allows us to examine and characterize their protective and/or therapeutic properties against opportunistic agents. Monoassociated, genetically different mouse models are very useful for studying the role of various subsets of innate and adaptive immune cells in the generation of specific immune responses to bacterial antigens and pathogenic agents at the mucosal surface and should provide a new insight into the mechanisms of vaccine and probiotic protection and treatment of some infectious pathologies of human and/or animals.

Since it has become generally accepted that probiotics might be an alternative to an antibiotic method of prevention and treatment of opportunistic infections, we suggest that some innocuous gut colonizers will be appreciated for their roles in possibly ameliorating particular infections via stimulation of the natural GALT and NALT mucosal immune system, i.e., probiotic microbes have good prospects to be used as a complement to specific immunization.

We have been successful in studying host/bacteria local immune responses and the role of B-1 vs. B-2 cells in generating total IgA in NALT and GALT. Using formerly germfree (GF) C.B-17 SCID mice colonized by *Haemophilus influenzae* mutant strains with and without PC in their LPS structure as recipients for adoptively transferred, sorted B-1 or B-2 cells from the peritoneal cavity (PeC) of mice bearing different Ig allotypes (BALB/c of a-allotype and C.B-20 of b-allotype), we have shown that both B-1 cells and B-2 cells can develop IgA mucosal responses in draining lymph nodes (LNs) in NALT. Strikingly, IgA were specific to PC+ type 1 thymus-independent Ag (TI-1). Moreover, our data indicated that ‘bystander’ T cells are required for this local immune response. Only mice colonized with PC+ *H. influenzae* strain and reconstituted with a mixture of B-1 cells and non-B-1 PeC cells were able to develop a specific mucosal response to PC. The use of transferred donor cells with different Ig allotypes allowed us to examine contributions of B-1 vs. B-2 cells to local IgA production. Contribution of B-1 cells to anti-PC IgA response was significantly greater than of B-2 cells. Thus, activation of B-1 cells by PC was the first direct evidence of their selective stimulation by Ag via BCR.

We have also found that formerly GF BALB/c imcomp mice colonized with *Lactobacillus salivarius* developed both systemic and local [lamina propria (LP), mesenteric LN (MLN) and Peyer’s patches (PP)] natural IgA responses. Interestingly, these bacteria showed an ability to translocate – disseminate into the internal parenchymal organs such as lungs, spleen, kidneys and liver after oral inoculation of GF IgA knockout (k.o.) mice, but not of their wild-type counterparts. Thus, we have demonstrated that probiotics might be an alternative to an antibiotic method of prevention and treatment of opportunistic infections.

*Alexander Suvorov*
E-mail: alexander_suvorov1@hotmail.com
Abstracts

(W/T) B6 counterparts. However, colonization with Schaedler’s Escherichia coli or Morganella morgani did not result in their translocation in either IgA k.o. or W/T mice and even protected against translocation of L. salivarius upon its subsequent oral inoculation. Increased level of autocrine growth factor Reg III $\beta$/$\gamma$ expression was observed primarily in the caecum of colonized IgA deficient mice as compared to GF or E. coli monoasssociated mice and gene expression of surfactant protein D (SP-D) had been induced in the lung of IgA deficient mice, inoculated by both bacteria and Schaedler’s E. coli alone, and the number of macrophages and neutrophils were greatly increased in the bronchoalveolar lavage (BAL) of IgA-deficient mice colonized with L. salivarius on the third day after inoculation, as compared to GF mice or those monoassociated with E. coli. RegIII has been considered as an autocrine growth factor, but recently, this product found in secretory granules of Paneth cells has been shown to have in vitro bactericidal effects preferentially vs. gram-positive microbes.

The most conspicuous compartments of the cellular mucosal immune system of the gut include the intraepithelial (IEL) spaces, PP and the gut LP. Little is known about the role of gut microflora in the development and activation of elements of the cellular immune response compared to those of the humoral immune response. Generally, the approaches being used compare neonates and adults as well as GF and conventionally (CNV) reared adults, prior to and following their deliberate colonization with commensal microbes. We observed changes in total cell numbers and in the proportions of subsets of cells with different phenotypes and functions in the IEL compartment during normal development and after colonization of formerly GF adult mice with gut microbes. Monoassociation of GF SCID mice with Schaedler’s E. coli led to an increase in the proportion of natural killer (NK) cells in the IEL spaces, compared to the cohort in Ag free (AF) incomp mice, but below the level of those in CNV SCID mice. The functional ability and numbers of NK cells in the IEL compartment of GF SCID mice have not yet been reliably determined. It is likely that normal gut microbes can be partly responsible for the activated state of NK cells in the IEL compartment, compared to those in spleen and peripheral LNs. Further analysis is needed to investigate the mechanisms of humoral and cellular local and systemic responses of B-1 vs. B-2 cells to TI-1 and TI-2 Ags, such as PC (associated with polysaccharide carriers such as teichoic acids) and polyribitol phosphate (PRP) of encapsulated H. influenzae, type b. Anti-PRP IgM, but not IgA response was locally detectable in mice colonized with either PC$^+$ or PC$^-$ strains of H. influenzae after transfer of sorted and purified B-1 and CD4$^+$ T cells into SCID recipients. In contrast, anti-PRP IgA was found in NALT fragment culture supernatants of recipients of unseparated PeC cells or mixtures of B-1 and non-B-1 PeC cells when mice were colonized with a PC$^+$ strain of H. influenzae. Additional studies are required to determine the role of commensal bacteria in activation of APCs, T cells and NK cells as elements of protective immunity against opportunistic pathogens.

Since cytokine production is most informative evidence of regulating probiotic protective/therapeutic resistance to pathogens and bacterial translocation, we had investigated their possible roles in managing bacterially idiosyncratic/different stimulation of DCs. The level of anti-inflammatory IL-10 was induced by the treatment with Bacillus subtilis only; whereas the level of IL-12 had been stimulated by the treatment with Schaedler’s E. coli. IL-1b was increased by the treatments of both LPS-containing strains: Schaedler’s E. coli and M. morganii.

Selection of promising representatives of commensal bacteria, their antigenic structures and mutant strains with strong antibacterial, anti-inflammatory or immunomodulatory properties to be used for the construction of new bacterial preparations and/or targeted mucosal vaccines is important. In particular, it has been shown that the selected commensal bacteria exhibit a specific effect on opportunistic pathogens isolated in the clinical units. L. salivarius, Schaedler’s E. coli, M. morganii and B. subtilis demonstrated both synergic stimulatory effect on host immune system functions and high inhibitory properties against Klebsiella pneumoniae, Enterobacter cloacae and Staphylococcus aureus, but not to Proteus mirabilis or Pseudomonas aeruginosa.

*Nadiiya Volodymyrivna Boyko
E-mail: nadiya.boyko@gmail.com

Partially Purified Bacteriocin and Molecular Characterization Using 16S rRNA From Endemic Tropical Fruit Fermentation of Yellow Marquisa (Passiflora edulis var. flavicarpa) In Indonesia

Sumaryati Syukur1*, Habibi Hidayat2 and Endang Purwati3

1Laboratory of Biotechnology, Departement of Chemistry, Faculty of Math and Natural Sciences, University of Andalas, Padang, Indonesia, 25163; 2Graduate Studies, Department of Chemistry, Faculty of Math and Natural Sciences, University of Andalas, Padang, Indonesia, 25163; 3Laboratory of Nutrition, Faculty of Animal Husbandary, University of Andalas padang, Indonesia, 25163
Yellow Marquisa (*Passiflora edulis var. flavicarpa*) is a healthy tropical fruit with high nutrition contains, good taste and aromas. During spontaneous fermentation for 24 h, the pH was lower. Our investigation focuses to isolate potential Lactic Acid bacteria (LAB), and partially purify antimicrobial bacteriocin, and molecular species determination using 16S rRNA. The medium of de Man, Ragosa, and Sharpe (MRS) were used to screen LAB, and 63 colonies were found. The screening of isolates based on LAB survival growth in acid pH ranges (2, 3, 4, and 5). Antimicrobial experiments were used using bacterial pathogen indicator such as *E. coli* NBRC 14237, *Staphylococcus aureus* NBRC 13276, *Bacillus subtilis* BTCCB, *Salmonella thyphii*, and *Listeria monocytogenes* (Unv. Andalas Collection). Six isolate were confirmed as antimicrobial pathogen and one isolate M4, selected as strong potential antimicrobial bacteriocin with diameter of inhibition zone reach (18 to 28 mm). Partially purified bacteriocin of potential M4 isolate, precipitated with 80% ammonium sulphate saturation and further purification by DEAE-Cellulose. The molecular weight determination by SDS-PAGES showed average of 10 kDa protein. The molecular determination were used primer of 27F: AGAGT TTGATCMTGGCTAG and R 1525: AAGGAGGTGWTCARCC. The M4 isolate showing closely related with an homology of 98% with *Weissella cibaria* II-I-59 with accession no NR 036924.1, after complete sequence of PCR products (1427 bp). There is no report so far obtaining *Weissella cibaria* in yellow marquisa resistant low pH with highly antimicrobial bacteriocin.

**Keywords**

Lactic acid bacteria, Bacteriocin, gene of 16S rRNA, Yellow Marquisa (*Passiflora edulis var. Flavicarpa*), *Weissella cibaria*.

---

**The University Of Chicago Gnotobiotic Research Animal Facility (GRAF): a new member in the gnotobiotic community**

Betty R. Theriault*, Christina Olivares, Alan Vest and George P. Langan

Animal Resources Center, The University Of Chicago, Chicago, IL, USA

**Introduction**

Gnotobiology has a rich history of fluidity in providing technology for researchers to dissect complex biological questions. The University Of Chicago recently found itself on the crest of a new wave of research interests questioning existing paradigms in autoimmunity, allergy and gastrointestinal disorders. To assist researchers in their ability to dissect the multifactorial influences contributing to these disease pathways, we developed a Gnotobiotic Research Animal Facility (GRAF). The purpose of this abstract is to share The University Of Chicago’s experience in developing a gnotobiotic research animal facility, lessons learned in establishing the facility and the future direction in research interests this technology is positioned to support.

**Approach**

The University Of Chicago has extensive experience in using and maintaining semirigid isolators; however, in evaluating the request to maintain germ free mice, it soon became apparent that we would have to invest in flexible film isolators, the equipment to deliver autoclaved supplies to the isolators and learn the technology required to sustain mice in a germ free environment. We were generously provided training by a collaborator at another institution. After preparation, we received our first shipment of germfree Swiss Webster breeding pairs from a commercial source. Within 30 days of arrival, our first cohort of mice required euthanasia for what was later determined to be clinical signs resulting from hypovitaminosis. Recognizing we had maintained sterility of our first isolator, but had overlooked other measures of quality control and standard operating procedures, we set about gathering more knowledge and training before maintaining additional mice. Synergizing many of the principals of practice gained at three facilities visited, we started over. Our second shipment of germfree Swiss Webster mice was received, and in short order, our first litter of mouse pups were born (Fig. 1). We continued to encounter success and expand our program from one isolator to three in the first year and seven operational isolators during the second. By the fourth year of operation, we had outgrown the initial space allocated to gnotobiotic/axenic isolators within our Gordon Center for Integrative Sciences (GCIS) and began preparation to construct a larger dedicated facility within our new Knapp Center for Biomedical Discovery (KCBD). Since initiating operation of our new facility, we have encountered a 30% increase in isolator usage with projected increase of 100% during the next year (Fig. 2).

Although we have encountered three isolated bacterial contaminations over the course of our 4-year history, each contamination was traced to a source, and in each case,
practices were modified in an effort to prevent repeat contaminations of the same nature. As advancing technologies become available in the fields of immunology, genetics, proteomics, metabolomics and human medicine, gnotobiotic technologies are expected to allow for novel and specific in vivo dissection of multifaceted components of complex diseases such as food allergy, immune-mediated diabetes, inflammatory bowel disease and celiac disease.

Summary
The University Of Chicago has benefited from the tradition initiated by Dr. Philip Trexler in June 1960 by generously having gnotobiotic technology shared with us by institutions already established in this specialty field. The implementation of this technology required purchase of specialized equipment, training of specialized staff and implementation of programmatic practices. Although hurdles and setbacks were encountered, refinement and enhancements to the gnotobiotic program at The University Of Chicago have resulted in success and continued steady growth. Gnotobiotic technology is permitting several of our investigators to conduct research in areas of interest in human health and disease.

*Betty R. Theriault
E-mail: btheriault@bsd.uchicago.edu

Fig. 1. Photographs detailing the initial phases of success in the maintenance of germfree mice at The University Of Chicago. (a) (top left) Example of the single level flexible film isolators in use at The University Of Chicago. (b) (bottom left) First Swiss Webster breeding colony maintained at The University Of Chicago. (c) (bottom right) First Swiss Webster litter born at The University Of Chicago.
Somatic cell nuclear transfer for genetic modification and preservation of gnotobiotic miniature pigs

Hoon Taek Lee*
Department of Animal Biotechnology, Bio-Organ Research Center/Animal Resources Research Center, Konkuk University, Seoul, Korea

Transgenically modified, gnotobiotic miniature pig is the most suitable organ donor for xenotransplantation in human. Introduction of complex genetic modification in these organisms can be best achieved by somatic cell nuclear transfer (SCNT). However, incomplete understanding of oocyte maturation mechanism, limited availability and high cost of receiving cytoplast are some of the barriers in the efficient application of SCNT to miniature pigs. Here, we show that recipient cytoplast from commercial Landrace pigs could successfully remodel and reprogram the donor nucleus from miniature pigs to form cloned embryos at a comparable efficiency. Transgenic embryos, expressing enhanced green fluorescent protein (EGFP) or LacZ, could also be produced from xenogenic nuclear transfer of cattle, mice and chicken cells into the enucleated oocytes of Landrace pigs, albeit at a lower frequency that depended on the phylogenetic distance between the donor and recipient species. Introduction of transgene, however, did not affect the in vitro development competence of the cloned embryos.

To further improve the efficiency of transgenic cloned animal production, we generated the stem-like cells from miniature pigs’ skin and testes. Testes-derived male germ-line stem cells (spermatogonial stem cells; SSC) could be maintained in culture for more than 2 months and could be reprogrammed to cloned embryos by SCNT. Although use of SSC did not improve the reprogramming efficiency and in vitro development of cloned embryos, it opens a new avenue for genetic manipulation of pigs. Furthermore, skin-derived stem-like cells may have application in tissue engineering. Pig embryos could also be successfully produced by SCNT of pig fibroblast or intracytoplasmic injection (ICSI) of boar sperm that were lyophilized and stored refrigerated at 4°C for long-term. The in vitro development of SCNT embryos could further be improved by inhibiting histone deacetylase in the cloned embryos for 24 h. Taken together, developments in SCNT technology may improve its utility for genetic modification and preservation of gnotobiotic miniature pigs to produce bio-organ for xenotransplantation in human.

*Hoon Taek Lee
E-mail: htl3675@konkuk.ac.kr

Fig. 2. Gnotobiotic Research Animal Facility (GRAF) and GRAF growth chart with projections. (a) (upper left) GCIS component of GRAF with single level isolator tables only. (b) (upper right) KCBD component of GRAF. Isolator tables depicted in red are double level tables. Isolator tables in gray are also double level tables and not yet assigned. (c) (lower left) U of C Growth chart, which demonstrates history of germfree operations at the U of C, current space allocation and future projections.
Establishment of gnotobiotic miniature swine for xenotransplantation research program in Konkuk University, Seoul, Korea

Jeong Ho Hwang*, Sung Han Jung, Sang Eun Kim, Yoon B. Kim and Hoon Taek Lee

Department of Bioscience and Biotechnology, Bio-Organ Research Center, Konkuk University, Seoul, Korea

To overcome shortages of human donor organs for allotransplantation for many organ failure patients, we made commitment to develop gnotobiotic miniature swine for alternative organ donor source for xenotransplantation program.

For this, we have constructed absolute barrier-sustained gnotobiotic facility at Konkuk University, Bio-Organ Research Center, Seoul, Korea, and applied germfree technology. Gnotobiotic miniature swine was originally developed by Dr. Yoon B. Kim of Rosalind Franklin University of Medicine and Science, Chicago Medical School, North Chicago, IL, and donated to Seoul National University College of Medicine (SNU), Seoul, Korea. Pregnant SNU sows were procured from SNU, and germfree piglets were obtained by aseptic hysterectomy. These piglets were maintained in germfree isolators for about 4 weeks, were deprived of colostrums and were fed sterilized soybean milk by gamma-irradiation and they were associated with anaerobic di-flora, Lactobacillus sp. and Streptococcus sp., confirmed successful associations by rectal swab cultures and transferred into gnotobiotic facility aseptically. They are maintained on Hepa filtered air in and out, maintaining constant pen room air pressure of 0.9±0.1 in., sterile water, and sterilized diet. In 10 sessions of hysterectomy, 18 male and 33 female piglets were obtained of which 8 male and 8 female piglets died within 5 days after birth. Among live piglets, 6 male and 15 female piglets were confirmed to be germfree by microbial monitoring before the association of di-flora. Genotyping for swine leukocyte antigen (SLA) showed 2 males and 7 females to be homozygous for MHC class II (DRB, DQB), 0301, and 1 male and 1 female were homozygous for MHC class II (DRB, DQB), 0201. Molecular genetic and immunological research projects for further development of genetic modifications for humanized and inbred miniature swine for ideal organ donor source for xenotransplantation program are progressing.

*Jeong Ho Hwang
E-mail: hjh5847@hotmail.com

Lactobacillus monoassociated mice might be downregulated by the serum antibody levels induced by dietary antigen compared with Bacteroides monoassociated mice

Yuji Hamamoto¹*, Akira Hosono¹, Masato Tsuda¹, Daiki Kamoi¹, Satoshi Hachimura², Yoshika Momose³, Kikuji Itoh³, Kazuhiro Hirayama³, Kyoko Takahashi¹ and Shuichi Kaminogawa¹

¹Food and Physiological Functions Laboratory, Department of Food Bioscience and Biotechnology, Nihon University, Kanagawa, Japan; ²Research Center for Food Safety, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo, Japan; ³Department of Veterinary Public Health, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo, Japan

It is thought that colonization of the gut by commensal bacteria modulates the induction of oral tolerance and food allergy. However, it is not known which genera of intestinal commensal bacteria modulate food allergy induced by oral antigens. Therefore, we examined whether the distinct intestinal commensal bacteria have different immunomodulatory effects for food antigen-specific immune responses.

In order to investigate this theme, we used ovalbumin-specific T cell receptor transgenic (OVA23-3) mice under the condition that controlled bacterial environment.

We generated monoassociated mice by inoculating germ-free (GF) mice of OVA23-3 with either Lactobacillus johnsonii 129 (LJ) or Bacteroides acidifaciens type A43 (BA) isolated from murine intestine. For monoassociation...
with BA or LJ, these mice were orally inoculated with approximately 1 × 10^9 CFU of BA or LJ at 4–5 weeks of age. Thereafter, BA, LJ and conventional (CV) mice were administered a 20% OVA-containing diet or a casein-containing diet for 4 weeks. Serum antibody titers were measured by ELISA.

There was a tendency for total IgE and OVA-specific IgE levels to be higher in the serum of BA mice fed with the OVA diet than those of CV mice fed the same diet. The OVA-specific IgG2a levels in the serum of BA mice were significantly higher than that of CV mice. However, LJ mice showed similar levels of each serum antibody titers compared with CV mice. These data suggest that distinct intestinal commensal bacteria have different immunomodulatory effects, and Lactobacillus monoassociated mice might be downregulated by the serum antibody levels induced by dietary antigen compared with Bacteroides monoassociated mice.

Yuji Hamamoto
E-mail: hamayapple@yahoo.co.jp

High incidence of radiation-induced cavernous hemangioma in long-term survivors who underwent hematopoietic stem cell transplantation with radiation therapy during childhood or adolescence

Takashi Koike1, Noriharu Yanagimachi2, Hiroyuki Ishiguro1, Hiromasa Yabe3, Miharu Yabe4, Tsuyoshi Morimoto1, Takashi Shimizu2, Hiromitsu Takakura1 and Shunichi Kato3

1Department of Pediatrics, Tokai University School of Medicine, Isehara, Japan; 2Department of Radiology, Tokai University School of Medicine, Isehara, Japan; 3Department of Cell Transplantation & Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan; 4Department of Laboratory Medicine, Tokai University School of Medicine, Isehara, Japan

Radiation-induced cavernous hemangioma (RICH) is a late complication of cerebral radiation therapy. Long-term surviving hematopoietic stem cell transplant (HSCT) recipients who have received radiation therapy could be at risk of RICH.

We investigated records for 68 patients who underwent HSCT during childhood or adolescence. Magnetic resonance (MR) imaging including T2*-weighted image of the brain was performed annually for 5 years, over a range of 6 to 29 years after HSCT.

Furthermore, we developed a scoring and grading system for CH in order to monitor the process and the progress of radiological changes. Among the 68 patients, 28 (41.2%) were diagnosed as CH.

All CH cases received total body irradiation as a conditioning treatment for HSCT and/or cranial radiation therapy prior to HSCT as part of the treatment for their primary disease. CH was found in none of those without radiation (N = 19), in 46.2% of those who received 6–12 Gy (N = 39) and in all of those who received 18–36 Gy (N = 10). Total CH scores were correlated with higher radiation doses. Careful and long-term evaluation with MR imaging including T2*-weighted imaging is necessary for HSCT recipients who received radiation therapy prior to and/or during HSCT.

Problems in chimerism analysis using buccal mucosa after hematopoietic stem cell transplantation

Osamu Hyodo, Humiko Tsuchida, Tatsuya Sugimoto, Kaoru Sato, Hiromasa Yabe, Fumiaki Yoshida and Shunichi Kato*

Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan

Purpose and Background: Chimerism analysis is important for evaluation of engraftment after hematopoietic stem cell transplantation (HSCT). Our department provides routine examination of chimerism by short tandem repeat (STR).

Oral buccal swab specimen is used as the host DNA sample source when pretransplant blood or bone marrow samples of the patient are absent. They are used because of the safety and the technical easiness to extract DNA.
Characteristic of Candida from fungoid-bacterial associations at inflammatory disorders of the upper respiratory tract

Anatoliy P. Godovalov1,2*, Liliya P. Bykova1, Anatoliy I. Morgunenko1 and Sergey Gris2

1Acad. E.A. Wagner Perm State Medical Academy, Perm, Russia; 2Medical Unit of Municipal Department of Internal Affairs on Perm region, Perm, Russia

In recent years, the world has seen an increase in the number of diseases caused by fungi. According to the World Health Organization, they suffer every fourth inhabitant of the planet, regardless of age, among the many varieties of fungi in the lead Candida. According to European Research, the presence of fungi on the surface of any body or organ is abnormal and should be prevented and treated intensively. These fungi can easily take root and take already populated habitats. Of great interest are the features of their existence in microbiocenosis upper respiratory tract and connection between microbes with the action of antibiotics.

In this regard, the purpose of work was characterization of fungi of the genus Candida, isolated from the fungal–bacterial association of patients with inflammatory diseases of upper respiratory tract. Isolation of microorganisms was carried out using classical bacteriological method to the identification of species grown on cultural and biochemical characteristics. Determination of the sensitivity of microbial associations. The greatest stability of the genus Candida of the fungus Candida albicans is registered in association with species of the genus Streptococcus. Latest in this case are sensitive to most antibiotics.

Thus, it was found that the adhesive properties of fungi Candida, causing inflammatory diseases of upper respiratory tract, are of medium severity, and the mechanical impact on their adhesion to buccal epithelium does not alter the virulence properties of yeast-like fungi. Stability of the fungus Candida to antifungal drugs varies in microbial associations. The greatest stability of the genus Candida is registered in association with species of the genus Streptococcus. Latest in this case are sensitive to most antibiotics.

*Anatoliy P. Godovalov
E-mail: AGodovalov@gmail.com
The role of sugars in the minor correction of immune status of beings

Olga Sergeevna Korneeva* and I.V. Cheremuskina
Voronezh State Technological Academy, Russia

The formation of the immune response is significantly affected by normal flora of the gastrointestinal tract. One of the ways to maintain is the use of prebiotics. Substances with prebiotic activity, being not digested carbohydrates in an unmodified form, easily reach the colon, where they become the promoters of normal flora. There exists an evidence that prebiotics can modulate various properties of the immune system, including the properties of lymphoid tissue. The most common factors are bifidogenic amino and neutral sugars, which include mannose and fucose.

An evaluation of prebiotic activity in in vitro tests revealed a tendency of positive effect of various concentrations of mannose, fucose and oligosaccharides on the growth of bifidobacteria. Marked by the ability of mannose- and fucose-containing components to increase the resistance of bifidobacteria cells in the presence of broad spectrum antibiotic, as well as the ability to increase symbiotic relationship between B. bifidum and E. coli, confirm their prebiotic properties.

The bifidogenic and lactogenic activity of mannose and manno-oligosaccharides was revealed in in vivo experiments. Their application in experimental dysbiosis, led to increasing of the number of bifidobacteria and stimulated the growth of lactic acid bacteria.

Studies of immune status of experimental animals, when introduced into their diet mannose-contained components, revealed a tendency for the positive effect of mannose to indicators of nonspecific immunity of experimental animals. Oral consumption of mannose and manno-oligosaccharides has a stimulating effect on the functional activity of macrophages, increasing absorptive capacity of the cells and digested. It was found a significant increase in activity of neutrophils in an experimental dysbiosis relatively intact animals, as well as the ability to induce the expression of mannose pro- and anti-inflammatory cytokines.

The introduction of fucose in the diet of mammals has a positive effect on the degree of oocyte membrane fucosilation and reproduction of experimental animals.

Studies of immunoglobulins synthesized with different carbohydrate diet showed that the qualitative composition of immunoglobulins is significantly changed after the application of mannose diet. The introduction of fucose to the diet of mammals provided the stimulating effect on antibody production.

Taking into account all the data, there is no doubt that an effective supplement on the basis of mannose and fucose-containing components will be a prerequisite for the restoration of biochemical processes in the body and improve the immune status.

The research was done under the Federal target program ‘Research and pedagogical staff or innovative of Russia’ (SC Nos. P260 and P1074).

*Olga Sergeevna Korneeva
E-mail: korneewa-olga@vmail.ru

Characteristics of interaction between tick-born encephalitis virus and human immune system

Liliya P. Bykova¹, Rafael Z. Kuziaev¹, Anatoliy P. Godovalov¹,²* and Sergey Gris¹
¹Acad. E.A. Wagner Perm State Medical Academy, Perm, Russia; ²Medical Unit of Municipal Department of Internal Affairs on Perm region, Perm, Russia

The tick-borne encephalitis is the neurovirus infection, which is widespread on the Western Ural territory, the natural center of tick-borne infections. The tick-borne encephalitis infection develops owing to transmissible or alimentary introduction of neurotropic virus and its interaction with a human body. This interaction is defined by properties of the originator and reactance of a macroorganism. Many researchers believe that tick-borne encephalitis severity depends on its ability to get through a blood-brain barrier with brain lesion or without it. The nervous system lesion is characterized by polymorphism of implications. Feature of the infection pathogenesis is the ability of tick-borne...
encephalitis virus to remain active for a long time, even against treatment of this disease. According to clinical data, frequency of infection transformation into the chronic form is 3–11% to total number of cases of acute type disease. The immune response begins with activity of innate immunity factors, including phagocytosis. Further virus neutralization is connected with the activity of antibodies and specific cellular factors. Period of virus conservation in an organism and a condition of patient’s immune system are of interest.

The research objective is to define RNA of the tick-borne encephalitis virus and to study the cellular and humoral immune response of the patient with chronic form of disease. We observed 33 people with different disease terms and clinical implications of chronic tick-borne encephalitis. The blood and serums received from patients were research material. We used PCR to define RNA of the virus and ELISA to define immunoglobulins of M and G classes. Assessment of phagocytic activity of blood neutrophils was carried out by a method with formalinized sheep erythrocytes.

Researchers have found that 60% of patients had RNA of the tick-borne encephalitis virus in their blood. Thus, disease terms varied from 1 to 15 years. Phagocytic reaction was shown by raised activity and made 65.4±4.1%. Study of phagocytic reaction in the presence of specific viral antigen showed a negligible increase in the activity of phagocytosis (p > 0.05). 70% of examined people had immunoglobulins. People with RNA of the virus had IgM in 57% of cases and IgG in 40% (Fig. 1). Research studies have taped ineffective activation of a cellular and humoral link of the immune response in chronic form of tick-borne encephalitis. Detection of RNA of the tick-borne encephalitis virus in late disease terms shows the possibility of long persistence of the virus. It can be persistent during humoral immune response but it is ineffective in protection against the tick-borne encephalitis virus. The inefficiency of phagocytic response is possibly connected with the fact that the virus damages phagocytic system. These are the mechanisms allowing the virus to escape action not only of humoral protection but also of cellular one. Fluctuations of researchers’ results can be connected with specific features of the patients’ immune status and strain virus characteristics. PCR could be included into a complex of methods for studying tick-borne encephalitis as an additional way to diagnose chronic tick-borne encephalitis.

Changes in phenotypic and functional properties of mononuclear leukocytes as a result of bacterial vaccine application under induced immunosuppression

Olga V. Lebedinskaya1*, Elena A. Lebedinskaya1, Nelly K. Akhmatova2, Anatoliy P. Godovalov1*, Sergey Gris1

1E.A. Wagner Perm State Medical Academy, Perm, Russia; 2I.I. Mechnikov Scientific Research Institute of Vaccines and Sera, RAMS, Moscow, Russia

Treatment of malignant tumors is accompanied by the suppressive action on the immune system that is related to numerous factors and chemotherapeutic preparations in particular. Examination of immunological parameters while using cytostatics is attractive for the purpose of searching an adequate method of correction of subsequent alterations. The aim of work was the study of the effect of bacterial vaccine Immunovac (VP-4) on the immune phenotype and the functional activity of murine spleen lymphocytes against a background of cyclophosphane (CP)-induced immunosuppression. Investigations involved the use of preparations: CP and multicomponent vaccine Immunovac (I.I. Mechnikov SRIVS, PAMS, Moscow) that is composed of antigenic components of *Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris* and *Escherichia coli*. Alongside with its typical neutropenic action, CP being introduced into mice provoked marked immunosuppression that was manifested in significant lowering in absolute lymphocyte number in murine peripheral blood and spleen. Lymphopenia was primarily expressed in the reduction of helper lymphocytes (CD4+), B-cells and NK-cells (Fig. 1). In addition, CP immunosuppressive action was demonstrated via decrease in the NK-cell functional capability that was not recovered even by the fifth day of the experiment (Table 1).

Such alterations could result in insufficiency of innate and adaptive components of immunity. Native immune modulator VP-4 was found to level the CP-induced lymphopenia, as well as alterations in lymphocyte subpopulation composition and their functional state.

![Fig. 1. The amount of specific immunoglobulins M and G to the virus (geometric titer) and the number of tick-borne encephalitis patients in whom viral RNA was found (%).](image-url)
The advantage of combined application of CP and given immune modulating preparation appears to be faster approaching the normalizing effect as compared with the cytostatic action. This fact seems to be crucial for prevention of infectious complications occurring in cancer patients with chemopreparation-induced immunosuppression. Considering earlier observations of the VP-4 ability to promote anti-infective and antitumor immunity, it seems expedient to carry out clinical trials of this preparation for the prevention of the immune status disorders in cancer patients after the chemotherapy treatment. This work was supported by RFBR Grant No11-04-96037 rural and administrative body of Perm Region.

*Anatoliy P. Godovalov
E-mail: AGodovalov@gmail.com

Table 1. Effect of VP-4 on NK-activity of mice spleen cells against CP-induced immunodeficiency

| Time after administration, h | Group number | Preparation | 1:05   | 1:02   | 1:01   |
|-----------------------------|--------------|-------------|--------|--------|--------|
| 0                           | 1            | CP          | 73.4±5.6| 39.3±1.2| 27.9±0.8|
| 48                          | 2            | CP          | 34.1±3.7*| 20.1±2.2*| 14.5±0.6*|
|                             | 3            | CP+VP-4     | 63.2±2.9**| 40.8±2.7**| 22.3±1.2**|
| 96                          | 4            | CP          | 39.6±1.2*| 27.1±3.1*| 9.3±0.1*|
|                             | 5            | CP+VP-4     | 57.3±2.4**| 35.4±1.8| 12.9±0.1*|
| 168                         | 6            | CP          | 52.7±3.1*| 43.6±0.9| 35.4±1.5|
|                             | 7            | CP+VP-4     | 49.5±1.9*| 41.4±2.0| 33.9±2.7|

*p <0.05 compared to control.
**p <0.05 compared to CP.

Fig. 1. The expression of surface molecules CD19/CD8, CD25/CD4 and DX5/CD3 on mice spleen mononuclear leukocytes. Double vital staining with monoclonal antibodies.
Anti-inflammatory properties of *Bifidobacterium* spp. strains isolated from healthy infant feces

Ekaterina V. Khokhlova¹, Boris A. Efimov¹, Lyudmila I. Kafarskaik¹, Svetalana I. Pavlova² and Andrei N. Shkoporov¹*

¹Department of Microbiology and Virology, Russian State Medical University, Moscow, Russian Federation; ²Department of Pharmacology, Russian State Medical University, Moscow, Russian Federation

Several groups have reported recently that certain probiotic *Bifidobacterium* strains are able to inhibit inflammatory response in intestinal epithelial cells *in vitro* and *in vivo*. However, neither chemical nature nor precise mechanism of action of putative anti-inflammatory agents has been determined. In addition, it is unclear whether such immunomodulating activity is a property of some specific *Bifidobacterium* strains or is common for the whole genus. Here, we report on partial characterization of similar anti-inflammatory properties in several *Bifidobacterium* strains, isolated from healthy infant feces. It was found that conditioned media (CM) of all tested strains was capable to attenuate, albeit to varying extent, TNF-α- and LPS-induced inflammatory response in HT-29 colon epithelial cell line. In contrast, neither killed bifidobacterial cells nor cell extracts possess such activities. The active substance present in bifidobacterial CM was found to be resistant to protease and nuclease treatment, heat-labile, nonlipophilic and having molecular weight of less than 3 kDa. The anti-inflammatory effect of bifidobacterial culture supernatants was dose- and time-dependent and was accompanied by inhibition of IkB phosphorylation and NF-κB-dependent promoter activation. *Bifidobacterium* CM were found to be nontoxic to HT-29 cells and did not influence gene expression pattern in the absence of proinflammatory stimuli. However, combined treatment of cells with CM and LPS or TNF-α resulted in upregulation of TGFβ1, IkBζ and p21CIP mRNAs.

*Andrei N. Shkoporov*
E-mail: a.shkoporov@gmail.com

---

Physiological changes of liver induced by fluoroquinolones and hepatoprotective effect of CAP18/LL-37 synthetic peptide in mice

Shiaki Takagi¹*, Yoko Sugawara¹, Katsuya Inada², Hiroshi Isogai³ and Emiko Isogai¹

¹Department of Animal Microbiology, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan; ²Iwate University, Iwate, Japan; ³Division of Animal Experimentation, Sapporo Medical University, Hokkaido, Japan

**Objectives**: It has been reported that patients with severe bacterial diarrhea sometimes showed hepatic dysfunction after antibiotic treatment. Antibiotic treatment is also reported to induce lipopolysaccharide (LPS) from the infectious bacteria. The upregulation of TNF-alpha expression has been reported as pathogenic symptoms in human hepatitis. In this study, we examined whether hepatic physiological changes related to LPS release and TNF-alpha production occurs *in vivo* when fluoroquinolones were administered to SPF mice with intestinal flora. We also examined the hepatoprotective effect of CAP18/LL-37 synthetic analogue peptide, which has strong LPS-binding activity, on LPS-inoculated mice.

**Methods**: Sitaflaxacin (STFX) and levofloxacin (LVFX) were used as antibiotics. ICR mice (SPF, male, 5-weeks-old) were used. In the first experiment, the antibiotics (5 or 20 mg/kg/day) were administered to the mice once a day for 5 days intraoral or abdominal administration. In the second experiment, LPS (*Shigella flexneri* serotype 1A) was administered 1 h after the administration of the peptide for neutralization of LPS. In both experiments, blood, liver and spleen were collected from the mice. The concentration of ALT, AST, TNF and LPS were measured.

**Results**: In the first experiment, ALT and AST increased after antibiotic administration. LPS in the blood plasma was detected from 1 to -8 days after STFX or LVFX. TNF-alpha was detected in the plasma from the mice treated with STFX and in liver from the mice treated with STFX (or LVFX). On eighth day, TNF-alpha was not detected in any mice. In second experiment, ALT and AST increased after inoculation of LPS but not in the mice treated with the synthetic peptide. TNF-alpha also did not detect in the peptide treated mice.
**Conclusion:** We showed increase in AST and ALT after treatment of STFX or LVFX in normal SPF mice. CAP18/LL-37 synthetic analogue peptide can protect mice/human from the released-LPS after antibiotic administration.

*Shiaki Takagi
E-mail: petit-toma@p2223.nsk.ne.jp

**Role of IL-10 and IL-17 in the *Mycoplasma pneumoniae* experimental pneumonia model**

Satoshi Kurata\(^1\)*, Haruhiko Taguchi\(^2\), Takako Osaki\(^1\), Tomoko Hanawa\(^1\), Hideo Yonezawa\(^1\) and Shigeru Kamiya\(^1\)

\(^1\)Department of Infectious Diseases, Kyorin University School of Medicine; \(^2\)Department of Immunology, Kyorin University Faculty of Health Sciences, Tokyo, Japan

*Mycoplasma pneumoniae* is a common cause of community-acquired respiratory tract infection, and the participation of the excessive reply of the host immunity is thought to be involved in mycoplasmal pneumonia and the onset pathogenic mechanisms of the various complications. Recently, several studies showed that Th17 cells as the third positive effector T cell, characterized by production of IL-17, IL-21 and IL-22 have been implicated in the onset of autoimmune diseases. On the other hand, Treg cells have been reported to contribute to suppress a hyper immune response, and T cell subsets except Th1 and Th2 cells were inferred to be involved in pathogenic mechanisms of mycoplasmal pneumonia and the complications.

In this study, we established an experimental pneumonia mouse model by the use of *M. pneumoniae* sonicated crude antigens and performed pathological and immunological analyses to examine the induction mechanisms of Th17 cells. Additionally, we have examined the specificity of Th17 cell inducibility by using mouse splenocytes. **In vivo** analysis indicates that mRNA expression levels of IL-17 and IL-10 in the lung were increased by the high concentration and high frequency of *M. pneumoniae* antigens sensitization. *M. pneumoniae* antigens sensitization caused the lung inflammation with infiltration of neutrophils and macrophages. **In vitro** analysis indicates that *M. pneumoniae* antigens induced IL-17 release from mouse splenocytes in a concentration-dependent manner.

These results suggested that effective sensitization of *M. pneumoniae* antigens induced Th17 cells differentiation, and this immune response was suppressed by Treg cells producing IL-10.

*Satoshi Kurata
E-mail: kura@ks.kyorin-u.ac.jp

**Wild deer have antibodies recognizing tick defensin with tertiary structure**

Emiko Isogai\(^1\)*, Nayuta Isogai\(^2\), Kazuki Sato\(^1\), Hiroshi Yoneyama\(^1\), Tomoichi Fukuda\(^1\), Yoshitake Deguchi\(^3\), Kazuei Matsubara\(^4\), Hideo Murata\(^5\), Takamitsu Tuboi\(^5\), Makoto Haritani\(^5\), Toru Miyamoto\(^5\) and Hiroshi Isogai\(^2\)

\(^1\)Department of Animal Microbiology, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan; \(^2\)Division of Animal Experimentation, Sapporo Medical University, Hokkaido, Japan; \(^3\)Faculty of Agriculture, Iwate University, Iwate, Japan; \(^4\)Faculty of Life Science, Kyoto Sangyo University, Kyoto, Japan; \(^5\)National Institute of Animal Health, Ibaraki, Japan

**Objectives:** *Borrelia burgdorferi, B. garinii* and others, the aetiological agents of the tick-borne zoonosis Lyme disease, circulate between ticks and vertebrate hosts. In the transmission and maintenance cycle of the spirochetes, it is important to know the density of vector ticks. After tick bite, antibodies against Lyme disease spirochetes have been detected in human and animals. Vector competence for Lyme disease spirochetes has been confirmed for tick species. **Abstracts**
Tick defensins, small cysteine-rich cationic proteins, are active against various bacteria but not against Lyme disease spirochetes. Therefore, we examined antibodies against tick defensins in the sera from wild deer in different ecology (with different tick species). The importance of the tertiary structure of tick defensin in antibody recognition in wild deer was also examined.

Methods: Blood of the wild sika deer was collected from three distinct areas, Hokkaido, Aomori and Iwate, in Japan. We synthesized the tertiary and linear peptides on the information of the sequence in defensin originated from the taiga tick, *Ixodes persulcatus*. Antibody detection was done by ELISA.

Results: Both tertiary and linear synthetic peptides reacted with antibodies from wild deer. The antibody titer against tick defensin with tertiary structure was significantly higher than that against linear one. The antibodies from wild sika deer recognized the tertiary structure. Tick defensin shows different amino acid sequence. In Hokkaido, 98.8% of sika deer sera are positive against tick defensin from *I. persulcatus*. The positive percent of the deer in Aomori and Iwate was significantly lower than that in Hokkaido (major habitat: *I. persulcatus*). In Aomori and Iwate, deer has antibodies against *Haemaphysalis longicornis*. No antibodies against *Ornithodoros moubata* were detected in deer.

Conclusion: The tertiary structure of tick defensin is important to recognition in antibody response in wild deer. We can know vector potential by detection of antibodies against the tick-species specific defensin. The wild animal monitoring system is useful to get information of zoonotic diseases such as Lyme disease.

*Emiko Isogai*
E-mail: emiko@bios.tohoku.ac.jp

Gut indigenous microbiota and epigenetics

Boris A. Shenderov*

Moscow Research Institute of Epidemiology and Microbiology after G.N. Gabrichevsky, Moscow, Russia

Any phenotype is the result of complex interactions between genotype, epigenome and environment. Epigenetics focuses on processes that regulate how and when certain genes are turned on and turned off, while epigenomics pertains to analysis of epigenetic changes across many genes in a cell or entire organism. The epigenome combines molecular events that integrate genotype with phenotype. Epigenetics is defined as gene-regulating activities that do not connect with alteration of the base sequence. In recent years, it has become increasingly clear that epigenetic regulation of gene expression is critical during all stages of living being life, and perturbations in the epigenetic mechanisms can result in mammalian health disturbances. Epigenomic effects are connected with the covalent attachment of different chemical groups to DNA, histones, chromatin and to different other associated proteins during posttranscription period. Biochemical basis of epigenetics are methylation, acetylation, phosphorylation, ubiquitylation, ADP-ribosylation, biotinylation, sumoylation, repeat-induced gene silencing, microRNA interferences and some other processes. Epigenomic reprogramming of cell genome and posttranscription modification of gene expression are essential mechanisms of the development, regeneration and postnatal life of higher eukaryotic organisms (gene expression regulation, cell proliferation, cellular stress events, aging and DNA repair, lifelong circadian drifts, equilibrium between mitosis and apoptosis, modification of bacterial and host cell quorum sensing, host–bacteria cross-talk and so on). All these epigenetic modifications can be causally involved in a broad spectrum of human condition including various monogenic and multifactorial disorders (metabolic syndrome, type II diabetes, schizophrenia, autoimmune diseases, cancer, autism and so on). Epigenics may help explain some features of complex diseases such as late onset, gender effects, fluctuation of symptoms, phenotypic differences between monozygotic twins and others. Epigenetic code can be individual, tissue and cell-specific and may change over time because of aging, disease or environmental factors (e.g., nutrition, life style and toxin exposure).

However, the epigenetic reprogramming and posttranslational mechanisms remain unclear in details. The mammalian gastrointestinal tract is colonized by the vast majority of symbiotic microorganisms. Indigenous microbiota has the intriguing diversity and produce extremely important contributions to human physiology, biochemistry and behavior. In the recent years, it has been established indigenous microbiota produce multiple low molecular weight (LMW) substances (lacton-like autoinducers, peptide pheromones, protein autoinducers, stress proteins, SCFA and other organic acids, various enzymes, amino and nucleic acids, nucleotides, vitamins, amines, polyamines, hormone-similar substances, polysaccharides, oligosaccharides, peptidoglycans, lipoteichoic acid, glycopeptides, lipopolysaccharides, antimicrobial compounds, lectins, biosurfactants and many others), which can quickly be distributed along human organisms and interact with different targets in the host cells and tissues. These LMW compounds are considered to be potential autoinducers regulating prokaryotic and eukaryotic growth and development, bacteria–bacteria and host–bacteria relationships. Recent genomics-, epigenomics- and
metabolomics-based studies have permitted to provide insights into how indigenous microbiota (including probiotics) sense and adapt to the gastrointestinal tract environment and regulate gene expression and posttranslational modification of gene determined final products in prokaryotic and eukaryotic cells in and outside host intestinal tract. It is known that gene expression governs human existence. Above-mentioned microbial LMW compounds may be one of the key endogenous environmental factors to which human genes are exposed throughout life. Indigenous microbiota, in turn, governs the concentration of different eukaryotic cell producing proteins in different organs by functioning as regulators of stability DNA and RNA, gene transcription and translation, RNA processing, mRNA degradation, posttranslational modification and so on. The intensity of microbial signal and the subsequent response can vary with the composition and number of certain intestinal microorganisms. The physiological condition of the individual also may determine which human genes are influenced by indigenous gut microbiota that can serve both as source of substrates, cofactors or coenzymes for different metabolic processes and as a donor of various chemical groups (methyl-, acetyl-, phosphate-, etc), modified bases, signaling molecules or enzymes participating in the processes connected with epigenomic regulation, co-factors of these enzymes and as a donor of microbiially generated metabolites from certain food components. All of them are able to interfere in the host metabolism, genomic and epigenomic processes. Besides, some LMW molecules of microbial origin can activate or inhibit epigenomic regulation through interfere to activity of enzymes (methyltransferases, deacetylases, deacetyltransferases, phosphatases and so on) that are participants in epigenomic reprogramming and/or post-translational modification histone and other proteins. LMW substances and metabolites produced by pregnant woman gut microbiota can penetrate to fetus resulting in permanent effects its development programming, cognitive function, metabolism and body composition in the natal and postnatal periods of life through activation or suppression of gene expression, or turning genes on or off. Integration of genomics, epigenomics, nutrition and microbiota permits to understand how LMW molecules of diet and microbe origin affect gene expression and posttranslational modification. These observations can become the basis for development of novel approaches in designing new medicines and diagnostic tests for different pathological syndromes having epigenetic components.

The author substantiates the necessity to create International Project ‘Human Gut Microbiota and Epigenetics’. The goals of this project will be to facilitate interdisciplinary collaborations among scientists engaged in host microbial ecology, metagenomics, epigenomics, nutrition, health support, diseases prevention and treatment researches. The main goal of such investigations will be the establishment of the profile of microbe-connected LMW substances, which characterize the role of human indigenous microbiota in the epigenomic regulation of genome and microbiome activity and protein posttranslational modification. Midterm goal is to provide metabolic databases that could be used for selection of microbe-associated biologically active LMW molecules participating in the epigenomic processes relevant to human health and metabolic diseases.

*Boris A. Shenderov  
E-mail: shenderof@yandex.ru

Impact of dihydrodaidzein-producing *Clostridium*-like intestinal bacterium, strain TM-40 on *in vitro* metabolism of daidzein by equol-producing bacterium and fecal microbiota from human

Motoi Tamura*, Sachiko Hori, Yukie Kurusu and Hiroyuki Nakagawa

National Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Japan

Much attention has been focused on the biological effects of equol, a metabolite of daidzein produced by intestinal microbiota. However, little is known about the role of isoflavone metabolizing bacteria in the intestinal microbiota. Recently, we isolated a dihydrodaidzein (DHD)-producing *Clostridium*-like bacterium, strain TM-40, from human feces. We investigated the effects of strain TM-40 on *in vitro* daidzein metabolism by equol-producing bacterium TM, human fecal microbiota from a male equol producer and two male equol nonproducers. Adding strain TM-40 to the incubation solution of equol-producing strain TM greatly increased equol production from daidzein *in vitro*. In the fecal suspension from the male equol nonproducer and DHD producer, DHD was detected in the *in vitro* fecal incubation of daidzein by adding TM-40. The DHD concentration increased as the concentration of strain TM-40 increased. In the fecal suspension from the equol producer, the fecal equol production was increased by adding strain TM-40. The occupation ratios of *Bifidobacterium* and *Lactobacillales* were higher in the equol nonproducers than in the equol producer. Adding isoflavone-metabolizing bacteria to the equol-producing bacterium or fecal microbiota used in our investigation should facilitate the estimation of the metabolism of the isoflavonoids by the fecal microbiota.

Citation: Microbial Ecology in Health & Disease 2012, 23: 17461 - http://dx.doi.org/10.3402/mehd.v23i0.17461

(page number not for citation purpose)
Studies on the interactions between the equol-producing microbiota and DHD-producing bacteria might lead to clarification of some regulating mechanisms of equol production by fecal microbiota.

*Motoi Tamura
E-mail: motota@affrc.go.jp

Microflora of pregnant women and methods of its correction at disbiosysis

Taras M. Lyaskovsky*

Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kiev, MSP, Ukraine

Quantitative and genus stuff of vaginal microflora and intestine of pregnant women have been examined. At the result of the study, main taxonomic groups of microorganisms present in the vaginal canal and in the intestine have been isolated. Main pathogens, which cause microflora disbalance and as a result provoke vaginal disease, were examined. They are mainly represented by Gardnerella vaginalis, Escherichia coli, anaerobic bacteria, Candida sp. and so on.

Lactic acid bacteria were represented by Lactobacillus acidophilus, L. plantarum, L. fermentum, L. vaginalis; Enterococcus faecium, E. faecalis, E. durans; Streptococcus thermophilus and Lactococcus lactis.

Identification of probiotic strains of lactic acid bacteria based on phenotype characteristics (API-testing) and molecular biology techniques with the use of specific primers confirmed the genus of S. thermophilus, E. faecium and L. plantarum.

The antagonistic properties of probiotic cultures of lactic acid bacteria toward referent strains of pathogenic microorganisms have been shown. Antagonistic activity depends on the genus and strain of lactobacilli as well as conditions of cultivations. Adhesive activity of probiotic strains of lactic acid bacteria to vaginal epithelium has also been shown. For the first time, the change of antibiotics sensitivity of lactic acid bacteria on the model of lymphoblastoid cells has been indicated. The ability of probiotic strains to induce alfa and gamma interferons, as well as to inhibit flu and genital herpes viruses activity, has been exhibited. Probiotic substances have been proposed for the purpose of use as prophylactic and curing preparations for vaginal and intestinal dysbacteriosis treatment.

Keywords
Lactic acid bacteria; identification; antagonistic properties; adhesive activity; antibiotic sensitivity; interferonogenic and antivirus activity of probiotic strains

*Taras M. Lyaskovsky
E-mail: taras.lyaskovsky@gmail.com

Comparative analysis of gastric bacterial microbiota in Mongolian gerbil after long-term infection with Helicobacter pylori

Takako Osaki1*, Cynthia Zaman1, Takahiro Matsuki2, Takashi Asahara2, Hideo Yonezawa1, Tomoko Hanawa1, Satoshi Kuruta1, Fuhito Hojo1, Haruhiko Taguchi3 and Shigeru Kamiya1

1Department of Infectious Diseases, Kyorin University School of Medicine, Japan; 2Yakult Central Institute for Microbiological Research, Yaho Kunitachi Tokyo, Japan; 3Department of Immunology, Kyorin University Faculty of Health Sciences, Japan

The quantitative (qt) real-time PCR using 16SrDNA primers is useful for determination of bacterial composition in Mongolian gerbil’s gastric microbiota. The aim of the present study was to determine the differences of the gastric microbiota after long-term infection with Helicobacter pylori. At after 1 year inoculation with H. pylori, five gerbils were determined as H. pylori-positive and 6 gerbils were H. pylori-negative by culture and real-time qt PCR methods. In the gastric mucus of Mongolian gerbils, DNA of Atopobium cluster, Bifidobacterium spp., Clostridium coccoide group, Clostridium leptum subgroup, Eubacterium cylindroides group, Enterococcus spp., Lactobacillus spp. and Prevotella spp. were detected. The numbers of C. leptum subgroup, C. coccoide group and Bifidobacterium spp. in gastric mucus of H. pylori-negative Mongolian gerbils were significantly lower than those in noninfected gerbils and E. cylindroides group and Prevotella spp. were detected only in H. pylori-negative gerbils. The composition of the...
Gut colonization shapes the host’s metabolism

Sandrine P. Claus1*, Léa Maître2, Steve Robinette2, Sunil Kochhar3 and Jeremy K. Nicholson2

1Department of Food and Nutritional Sciences, The University of Reading, Whiteknights, PO Box 226, RG6 6AP, Reading, United Kingdom; 2Biomolecular Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, SW7 2AZ, London, United Kingdom; 3Nestlé Research Centre, NESTEC Limited, Vers-chez-les-Blancs, Lausanne, Switzerland

Gut microbiota are an essential component of the host metabolism, providing nutrients as well as stimulating the host immune system. This symbiosis is the consequence of a long co-evolution, which results in a relatively low level of diversity compared to most soil ecosystems. This implies that the host adapts its metabolism to the presence of microbial symbionts, an adjustment that starts at birth in parallel to the beginning of gut colonization. Simultaneously to this major event, many fundamental modifications occur such as immune development and brain maturation.

In a recent study, we characterized the metabolic phenotype of germfree mice by a 1H NMR-based metabonomic approach coupled with pattern recognition methods (1). We further examined the global effects of gut colonization on the host metabolism in the same germfree mouse model monitored during 20 days (n = 35/group, 5 time-points). Acquisition of the gut microbiota was associated with rapid increase in body weight (4%) over the first 5 days of colonization with parallel changes in multiple pathways in all compartments analyzed (i.e., urine, plasma, liver, kidney and colon). The colonization process stimulated glycogenosis in the liver prior to triggering increased in hepatic triglyceride synthesis. These changes were associated with modifications of hepatic Cyp8b1 expression and the subsequent alteration of bile acid metabolites, including taurocholate and tauromuricholate, which are key regulators of the global hydrophobicity of the bile acid pool and consequently control cholesterol and lipid absorption. Activity and expression of the drug metabolizing enzymes Cyp3a11 and Cyp2c29 were also significantly altered in germfree animals. Finally, correlations between the hepatic metabolotype and microbiota composition analyzed by 16S-pyrosequencing revealed strong associations of the Coriobacteriaceae (genus Eggerthella) with the hepatic triglyceride levels, energy metabolites and Cyp3a11 activity, the equivalent of the human CYP3A4, a major constituent of drug mono-oxygenation (2).

We then extended this study by analyzing the brain metabolite signature over the colonization process using 1H high resolution magic angle spinning NMR spectroscopy concomitantly with the 1H NMR metabolic profile of fecal waters in the same animals. The metabolic profiling of fecal water at several time-points revealed the rapid appearance of SCFAs (acetate followed by butyrate) concurrently with the disappearance of complex oligosaccharides as a result of their fermentation by colonic bacteria. Protein breakdown also occurred early as demonstrated by the rapid appearance of branched-chain amino acids (valine, leucine and isoleucine) and aromatic amino acids such as phenylalanine, tyrosine and histidine. Altogether, these results gave insights into the biochemical reactions that occurred in the intestine during gut microbial acquisition. Simultaneously, we observed a considerable impact of the lack of gut microbiota on brain metabolism in the two investigated sections (i.e., cerebellum and cortex). In particular, a remarkable increase in scyllo-inositol concentration was consistently observed in germfree animals. This was accompanied by higher levels of choline and aspartate in the germfree cortex and higher levels of fumarate and glutamate in the germfree cerebellum. All of these metabolic abnormalities were normalized 10 days postcolonization, indicating a rapid recovery of brain metabolism driven by the presence of gut bacteria. These results support recently published data highlighting differential gene expressions in the cortex and the cerebellum of germfree animals compared to their conventional counterparts (3).

Altogether, these results give new insights into the biochemical mechanisms associated to the multiple metabolic and behavioral perturbations reported in germfree animals. They also provide a basis to further develop novel strategies in the alteration of the host-microbiota relationship to beneficially modulate the host metabolism.

References

1. Claus SP, Tsang TM, Wang Y, Cloarec O, Skordi E, Martin F-P, et al. Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. Mol Syst Biol 2008; 4: 219.
2. Claus SP, Ellero SL, Berger B, Krause L, Bruttin A, Molina J, et al. Colonization-induced host-gut microbial metabolic interaction. MBio 2011; 2: e00271–10.
3. Heijtz RH, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci USA 2011; 108: 3047.

*Sandrine P. Claus
E-mail: s.p.claus@reading.ac.uk
Features of intestinal microbial biocenoses in dysbiotic individuals

Elena F. Zavgorodnyaya* and Lyudmila A. Stashkevich

Khabarovsk Research Institute of Epidemiology and Microbiology, Federal State Institution of Science under the Federal Service for Supervision in the Field of Consumer Right Protection and Human Welfare, Russian Federation

Incidence as well as certain biological properties of opportunistic pathogenic bacteria (OPB), isolated from microbial associations and monocultures of individuals with intestinal dysbioses, were analyzed.

Colonic microflora of 1,690 individuals, including 1,587 two-month- through three-year-old children and 103 adults, as well as their OPB isolates, was examined.

Dysbacteriosis was found in 98.8% of children and 93.2% of adults. An increase of OPB quantity to numbers exceeding an age-appropriate standard was responsible for dysbioses in 75.5% of children and 69.9% of adults.

Klebsiella bacteria were prevalent in the structure of intestinal microbial biocenoses in both children and adults, accounting for 80.0% and 68.5%, respectively. Staphylococcus aureus accounted for 12.0% in children and 3.7% in adults. Other Enterobacteriaceae were much more infrequent and accounted for 9.7% of children’s biocenoses and 11.1% of adults’ biocenoses.

Candida fungi were isolated from 65.6% of children and 34.4% of adults. In terms of species structure, C. albicans (61.9% of all fungi isolated from children and 54.6% isolated from adults) and C. glabrata (33.3% and 45.5%, respectively) were prevalent; C. tropicalis and C. krusei were isolated in much lesser quantity and accounted for 9.7% of children’s biocenoses and 11.1% of adults’ biocenoses.

In recent years, the number of dysbioses caused by microbial associations has increased. Associations of various OPB with bacteria of genus Klebsiella are most common. Thus, said microorganisms were isolated from associations in 57.6% of children under 1 year of age, 49.3% of children under 2 years of age, 40.0% of children under 3 years of age and in 32.7% of adults. Klebsiella + S. aureus association was most common in children, accounting for 72.9% in children under 1 year of age, 46.4% in children under 2 years of age and 35.7% in children under 3 years of age. Said association was found in 30.0% of adults, while associations of Klebsiella + fungi of genus Candida were most common (55%). Hemolytic E. coli ranked second among S. aureus’ associates in all the groups examined (in 66.3% of children and 10.0% of adults, in the average), while, as a monoculture, it was isolated from 50.3% of all the children and 1.9% of all the adults examined.

It was discovered that Klebsiella + S. aureus association in 82.8% of individuals included Klebsiella strains of great hemolytic activity, while monocultures displayed hemolytic properties in 17.2% of cases.

About 18.8% of phage-sensitive strains were only isolated among Klebsiella organisms, which is due to significant occurrence of K. oxytoca in intestinal biocenoses. It should be emphasized that circulation of K. oxytoca strains tends to increase and now exceeds the number of K. pneumoniae strains isolated. 94.3% of phage-resistant Klebsiella strains were isolated from associations.

Among the strains of S. aureus, 27.4% were found to be resistant to staphylococcal and 42.5% to polyvalent pyobacteriophages. Associate strains were found to demonstrate much greater degree of resistance manifestation (85.0% and 78.0%, respectively).

The study of antagonistic activity of associate strains (Klebsiella + S. aureus) revealed a high degree of antagonistic activity. Thus, associate Klebsiellae yielded 76.7% of highly active strains isolated, while monocultures, 63.6%. Similar figures for S. aureus were 72.5% and 57.1%, respectively.

Thus, in dysbioses, OPB appear prevalent in microbial biocenosis of children and adults; isolation of these microorganisms from associations proves to be much more frequent; microbial association strains appear to have more marked antagonistic activity and phage-resistance than monocultures.

E-mail: elenzav@mail.ru

Evaluation of sterility tests for cord blood banking in the Tokai University Cord Blood Bank

Tatsuya Sugimoto, Hitomi Sasaki, Rie Ando, Sachiyu Sakama, Chie Nakashioya, Fumiko Tsuchida, Kaoru Sato and Shunichi Kato*

Tokai University Cord Blood Bank, Tokai University Hospital, Isehara, Japan

Citation: Microbial Ecology in Health & Disease 2012, 23: 17461 - http://dx.doi.org/10.3402/mehd.v23i0.17461
Cord blood has been increasingly used as the source of hematopoietic stem cell transplantation. Sterility test (ST) is important for quality assurance of cell products. However, the material and method for ST is not unified among 11 cord blood banks of the Japan Cord Blood Bank Network (JCBBN).

ST had been performed using only a part of the final product by BACT/ALERT culture previously in our bank, but a part of red cell concentration was added as material, and Oxioid SIGNAL Blood Culture System Medium (Oxioid method) was employed as method other than BACT/ALERT culture in August 2009.

Incidence of bacterial positivity was compared between October 2008-July 2009 (group 1) and August 2009-May 2010 (group 2). Bacterial positivity was 1.3% in group 1 and 3.0% in group 2 (p = 0.052). Although the difference was marginally significant, some bacteria that could not be found in the previous method were identified by the newly employed method.

These results suggest that this new ST could contribute to the safer cord blood product.

*Shunichi Kato
E-mail: skato@is.icc.u-tokai.ac.jp

---

The study of the bacterial contamination in the spray water of electronic toilets and in the gluteal and inguinal regions due to splashing following spray water

Hideki Katano1,2*, Kumi Yokoyama3, Yasushi Takei4, Hideaki Matsuki1, Mami Tsukiji5 and Seiki Tazume1,2

1School of Health Sciences, Tokai University, Tokyo, Japan; 2Venex Research Institute, Kanagawa, Japan; 3Tokyo Healthcare University, Tokyo, Japan; 4Gifu University of Medical Science, Seki, Japan; 5Kanagawa Prefectural University of Human Service, Yokosuka (in original Yokohama), Japan

Objective: The function of the lukewarm water spray feature of electronic toilets is to wash away contamination following defecation and to maintain hygiene. However, there have been few studies reporting the bacterial contamination in the spray water and in the gluteal and inguinal regions due to splashing following such spray washing. We have thus conducted a study to investigate the viable counts in the spray water and on the gluteal and inguinal regions following spray washing.

Material and Methods: 1. Viable count in spray water: Spray water was collected from ordinary houses and public toilets. Each sample of 0.1 mL was smeared on an individual culture medium, and cultured for 48 h under aerobic conditions at 37°C. The samples were then left for 6 days at room temperature, and colony numbers were counted. 2. Viable counts on the gluteal and inguinal regions following spray washing: The sample was collected from regions around 5 cm from the anus (gluteal region) and 10 cm anterior to the anus (inguinal region). The areas were cleaned with ethanol for disinfection and dried, and the sample was collected from the skin surface of the gluteal and inguinal regions using the XM-G agar medium for E. coli and coliform bacteria with the stamp method (control). Following spray washing, the sample was collected from the skin surface of the gluteal and inguinal regions using the same medium with the stamp method. The collected samples were cultured for 24 h under aerobic conditions at 37°C, and the colony numbers were counted.

Results and Discussion: 1. Viable count in spray water: The viable counts in the spray water of the ordinary houses, where toilets are used less frequently, were found to be 310.7 ± 156.9 cfu/0.1 mL (n = 80). In public toilets, which are more frequently used, the count was found to be 109.5 ± 62.9 cfu/0.1 mL (n = 28). These results show that regardless of the frequency of use, the spray water of toilets was contaminated by bacteria. Moreover, the viable counts in the spray water of the ordinary houses were higher than those of the public toilets. These results suggest that the spray water of the lukewarm water spray feature of electronic toilets, which had always been considered hygienic, was in fact contaminated by bacteria. It is conjected that this occurs due to the heating of mains water used for spraying, which volatilizes the chlorine in the spray tank and induces the growth of bacteria. 2. Viable counts on the gluteal and inguinal regions following spray washing: the E. coli and coliform viable count was 479 ± 272 cfu (n = 62) from the gluteal region and 169 ± 9.9 cfu (n = 16) from the inguinal region. These results suggest that the splashing from spray washing results in spreading of the E. coli and coliform bacteria in stool to the gluteal and inguinal regions.

Therefore, it is conjected to be possible that the use of water contaminated with bacteria at the time of spray washing, and also the spreading of E. coli and coliform bacteria after washing, increase infectiousness. Thus, it is necessary to understand the risk of bacterial infection, review hygiene management and look into measures for blocking pathways of bacterial transmission.

*Hideki Katano
E-mail: katano@tokai-u.jp
Molecular epidemiological analysis of methicillin-resistant Staphylococci in a neonatal intensive care unit

Yasushi Takei1,2*, Kumi Yokoyama3, Hideki Katano4, Mami Tsukiji5, Takayuki Ezaki1 and Seiki Tazume6

1Department of Microbiology, Gifu University, Graduate School of Medicine, Gifu-shi Gifu, Japan; 2Juntendo University School of Health Sciences and Nursing, Mishima-shi, Shizuoka, Japan; 3Tokyo Healthcare University, Shinagawa, Tokyo, Japan; 4Venex Research Institute, Atsugi-shi, Kanagawa, Japan; 5Kanagawa Prefectural University of Human Service, Yokosuka-shi, Kanagawa, Japan; 6Tokai University, Isehara-shi, Kanagawa, Japan

To investigate contamination and transmission of MRSA/MRCNS in the NICU of Hospital A (old NICU) and the relocated NICU (new NICU), we isolated and evaluated staphylococci from nurses’ palms, towels under the heads of neonates, infant incubators (including portholes and infant covers) and room air. Detection rates of MRSA/MRCNS isolated from different sample locations were 52.6% in the old NICU and 51% in the new NICU, which demonstrates that the nurses’ palms in both the old and new NICUs and indoor environment were contaminated with MRSA/MRCNS. In the old NICU, numerous MRSA and MRCNS strains (log 3.17±0.19 cfu/10 cm²) were identified in towels, and the implementation of improvement plans resulted in a decrease in the number of MRSA/MRCNS isolates (log 1.95±0.57 cfu/10 cm²) on the towels used in the new NICU. A homology study of MRSA/MRCNS strains by PFGE DNA restriction patterns identified genotypes that showed similar patterns in the nurses’ palms, towels, infant incubators and room air.

*Yasushi Takei
E-mail: ystakei@juntendo.ac.jp

Isolation of toxigenic and nontoxigenic Corynebacterium diphtheriae strains upon preventive examination of hospital patients

Irina Nicolaevna Alekseeva*, Svetlana Evgenievna Ivanova, Natalia Vladimirovna Kasiyanenko, Elena Vladimirovna Lovschuk, Natalia Igorevna Dorogovtseva and Gennadiy Frankovich Rakitskiy

Territorial Mental Hospital, State Institution of Public Health, Khabarovsk

Mass immunization of children and adults with diphtheria toxoid in Russia has resulted in a significant decrease in diphtheria incidence rate. As instructed by the Ministry of Public Health of Russia, epidemiological control over the diphtheritic infection has been routinely exercised in line with the infection prevention policy. For preventive purposes, the clinicodiagnostic laboratory of the Territorial Mental Hospital is engaged in bacteriological examination of all the patients hospitalized. The extent of examinations makes 800 people a month and 10,000 people a year. A significant portion of the inpatients belongs to an asocial group with decreased immunity and lacking vaccination against diphtheria.

The objective of the present research consisted in microbiological monitoring of circulation of toxigenic and nontoxigenic Corynebacterium diphtheriae strains.

Faucéal and nasal discharge was used as a material for examination. The material was inoculated onto dense Corynebacterium agar medium with 2% potassium tellurite solution added and incubated for 24–48 h at 37°C. Morphological, cultural and biochemical properties of isolated strains were examined. Toxigenic properties of C. diphtheriae were determined by a standard gel precipitation test. Biovariants and toxigenicity of the isolated strains were assessed. In recent years, we did not isolate any toxigenic C. diphtheriae strains. The rate of nontoxigenic strain isolation was low: 1–2 per 10,000 inpatients. However, from December 2010 to March 2011, we isolated 18 nontoxigenic strains of C. diphtheriae in 1,900 inpatients. Thus, the rate of isolation made 9.5 per 1,000 people examined—the figure agrees with the research data obtained at G.N. Gabichevsky Research Institute of Epidemiology and Microbiology (Moscow).

According to their biochemical and cultural properties, 14 strains of the 18 C. diphtheriae strains isolated were ranked as C. diphtheriae mitis (78%) and 4 strains as C. diphtheriae gravis (22%). In the course of research, we found a difference in cultural properties between the strains isolated from our
patients and the reference toxigenic C. diphtheriae strains obtained from L.A. Tarasevich Research Institute for Standardization of Control over Medical and Biological Specimens. According to the research carried out by G.N. Gabrichevsky Research Institute of Epidemiology and Microbiology (Moscow), 20.9% nontoxigenic C. diphtheriae strains, which were circulating in Russia in 1984–1998 and carrying a tox-gene, were isolated by PCR method. Results of the study evidence the potential role of the strains in generation of diphtheritic agent population. Moreover, there are strains of low toxigenicity level that may not be detected in routine research.

Thus, observations of C. diphtheriae strains isolated from patients during the preventive examination are an important component of epidemiological control over the diphtheritic infection. C. diphtheriae mitis was found to be prevailing (78%) among the isolated C. Diphtheriae strains. Diversity of cultural properties of the causative agent investigated requires a closer attention upon identification thereof. The strains isolated by the authors are subject to reidentification by PCR method to define if tox-gene carriage is likely. The data of microbiological monitoring should be used for prediction of epidemic and infectious development of diphtheritic infection processes.

*Irina Nicolaevna Alekseeva
E-mail: hgn49@mail.ru

Inhibition of biofilm formation and virulence factor expression via control of quorum sensing in pathogenic bacteria

Tsukasa Ikeda* and Tomohiro Morohoshi

Department of Material and Environmental Chemistry, Graduate School of Engineering, Utsunomiya University, Yoto, Utsunomiya, Japan

Quorum sensing is a well-known bacterial communication mechanism and several kinds of N-acyl-L-homoserine lactones (AHLs, Fig. 1) have been identified as one of the quorum sensing signal compounds in gram-negative bacteria (1). As a lot of bacteria species control the expression of genes responsible for bioluminescence, secretion of virulence factors, expression of pathogenicities or forming biofilm via quorum sensing, the control methods of quorum sensing have been extensively studied. Herein, report about several results about inhibition of quorum sensing in gram-negative bacteria.

Several kinds of AHL degrading enzymes have been isolated, and the use of them for inhibition of quorum sensing has been studied and showed good effects. Some kinds of synthetic AHL analogues can act as inhibitors of quorum sensing. Especially, N-acyl-cyclopentylamines (Cn-CPAs) showed inhibitory effects on quorum sensing including biofilm formation in Psuedomonas aeruginosa, Serratia marcescens and several kinds of gram-negative bacteria without effects on bacterial cell growth (2). Cyclodextrins (CDs), a series of oligosaccharide, can make a complex with AHLs in a bacterial culture medium, and quorum sensing in P. aeruginosa and S. marcescens was inhibited by adding CDs to a bacterial culture medium (3). For the development of this method, several kinds of modified CDs and CD-immobilized gel sheets were synthesized and showed strong inhibitory effects on quorum sensing.

These quorum sensing inhibition methods may one of the useful methods to inhibit biofilm formation and bacterial infectious disease without antibiotic-resistant bacterial problem.

References

1. Greenberg EP. Quorum sensing in gram-negative bacteria. ASM News 1997; 63: 371–7.
2. Morohoshi T, Shiono T, Takidouchi K, Kato M, Kato N, Kato J, et al. Inhibition of quorum sensing in Serratia marcescens AS-1 by synthetic analogs of N-acylhomoserine lactone. Appl Environ Microbiol 2007; 73: 6339-44.
3. Ikeda T, Inoue Y, Suehiro A, Ikeshoji H, Ishida T, Takiguchi N, et al. The effects of cyclodextrins on autoinducer activities of quorum sensing in Pseudomonas aeruginosa. J Incl Phenom Macro Chem 2002; 44: 381-2.

*Tsukasa Ikeda
E-mail: tikeda@cc.utsunomiya-u.ac.jp

Fig. 1. Structure of AHLs.
Deletion of alarmon synthetase altered physiology and biofilm formation of *Bordetella pertussis*

Kentaro Sugisaki1*, Tomoko Hanawa1, Hideo Yonezawa1, Takako Osaki1, Satoshi Kurata1, H. Kawakami2 and Shigeru Kamiya1

1Department of Infectious Diseases School of Medicine, Kyorin University, Tokyo, Japan; 2Department of Anatomy, School of Medicine, Kyorin University, Tokyo, Japan

The ability of the cell to adapt to nutrient limitation depends on its capacity to change its gene expression immediately when nutrients are depleted. One important mechanism for regulating this change in gene expression is the stringent response. The guanosine tetraphosphate (ppGpp) molecule, which is an effector of the stringent response, binds to RNA polymerase and regulates transcription of various genes. During infection in host, bacteria need to adjust rapidly to the environments such as amino acid starvation and/or other nutritional starvation. Stringent response in *Bordetella pertussis* has not been examined so far. To elucidate the role of stringent response, we constructed the deletion mutants of ppGpp synthetase and assessed the biofilm formation. According to the genome sequences of *B. pertussis*, relA and spoT were identified as the genes encoding ppGpp synthetase and assessed the biofilm formation. Therefore, *relA* and *spoT* double mutant of *B. pertussis* was constructed and characterized.

Inhibition of *Staphylococcus aureus* biofilm formation and nasal colonization by a commensal bacterium *Staphylococcus epidermidis*

Tadayuki Iwase1*, Yoshio Uehara2, Hitomi Shinji1, Akiko Tajima1, Hiromi Seo2, Sinya Sugimoto1, Toshihiko Agata3, Koji Takada4 and Yoshimitsu Mizuno3

1Department of Bacteriology, The Jikei University School of Medicine, Tokyo, Japan; 2Department of General Medicine, Kochi Medical School, Nankoku, Japan; 3Department of Environmental Health, The Jikei University School of Medicine, Tokyo, Japan; 4Department of Biochemistry, The Jikei University School of Medicine, Tokyo, Japan

Commensal bacteria are known to inhibit pathogenic colonization; however, complex host-microbe and microbe-microbe interactions have made it difficult to gain a detailed understanding of the mechanisms underlying the inhibition of colonization. In our study, by using a collaborating approach of bacteriology and epidemiology, we show that the serine protease Esp secreted by a subset of *Staphylococcus epidermidis* inhibits *Staphylococcus aureus* biofilm formation and nasal colonization.

Our findings are as follows:

1) A subset of *S. epidermidis* produced Esp, which inhibited biofilm formation and destroyed the preexisting biofilms formed by *S. aureus*, including those by multidrug resistant strains. 2) Epidemiological studies indicated that the presence of Esp-positive *S. epidermidis* in the nasal cavities of human volunteers correlates with the absence of *S. aureus*. 3) Esp enhanced the susceptibility of *S. aureus* in biofilms to human beta-defensin2 (hBD2), an antimicrobial peptide secreted by keratinocytes. Our recent data indicate that Esp also enhanced the susceptibility of multi-drug resistant strains in biofilms to hBD2. 4) *In vivo* studies have shown that purified Esp and Esp-positive *S. epidermidis* eliminated *S. aureus* nasal colonization. These findings imply that Esp hinders *S. aureus* colonization in vivo through a novel mechanism of bacterial interference; this knowledge could lead to the development of novel approaches for the prevention and treatment of *S. aureus* infections, including infections caused by multiple drug-resistant strains. Studies at the strain level and interdisciplinary approaches will further enhance our understanding of microbial ecology, including host-microbe interactions and the relationships between commensal bacteria and infectious pathogens.

*Kentarou Sugisaki*  
E-mail: kensugisaki@ks.kyorin-u.ac.jp

*Tadayuki Iwase*  
E-mail: iwase.tadayuki@jikei.ac.jp
Probiotics and innate immunity: regulation of anticancer activity

Vyacheslav Abramov¹, Valentin Khlebnikov¹, Irina Chikileva¹,², Vadim Sakulin¹, Igor Kosarev¹, Raisa Vasilenko¹, Mikhail Kiselevsky¹ and Vyacheslav Melnikov²*

¹The Institute of Immunological Engineering, Lyubuchany, Moscow Reg., Russia; ²Russian Oncological Centre, RAMS, Moscow, Russia; *International Science and Technology Center, Moscow, Russia

Probiotics improve innate anticancer defense capacity; however, the mechanisms of this activity are sill little studied. We gathered a collection of Lactobacillus and Bifidobacterium strains isolated from various regions of Russia and Armenia. Lactobacilli were identified using API 50 CHL techniques and sequencing of 16S gene RNA. Identification of bifidobacteria was performed by PCR. The strains effectively growing on nutrient medium and positively tested for their stability to gastric and intestinal stresses were selected for immunological studies. Bifidobacteria were additionally tested for their stability to oxygen. L. casei DN 114001 (Danone, Actimel) and B. animalis lactis DN 173001 (Danone, Activia) were used as controls. All the studied strains of lactobacilli and bifidobacteria did not induce the production of IL-4, key mediator of allergic reactions, in monocytes. At the same time, a stimulation of the synthesis of IL-6 responsible for B lymphocyte maturation and humoral immunity development was registered. These data indicated probiotic participation in regulation of Th1/Th2 balance and their preventive role in allergic disease appearances. Bifidobacterium strains under study inhibited IL-12 synthesis in immature dendritic cells (DCs) and stimulated IL-10 production blocking DCs maturation. The studied L. casei DN 114001, L. plantarum 191 strains displayed a stimulatory effect on the immune cells (NK, monocytes/macrophages and DCs) and antitumor effect on human erythroblast leukemia cells K562. Several studies established a key role of tumor cell-platelet interaction as one of the earliest processes favoring tumor metastasis. Therefore, we aimed to determine the effect of lactobacilli and their culture liquid (CL) on the adhesion of highly metastatic breast cancer cell line MCF-7 to a platelet-coated surface under static conditions in vitro. CL of L. casei DN 114001, L. plantarum 191 and L. plantarum 189 strains, as well as live bacteria, caused prominent inhibitory effect on the binding of tumor cells and platelets, which exceeded the effect of the fucoidan LS (positive control). Similar results were obtained in the tests with another tumor cell line DU-145 (human prostate cancer). The antimetastatic effect of CL of L. casei DN 114001 and L. plantarum 191 strains was tested in vivo using Balb/c-nude mice. Tumor cells were injected subcutaneously into animals to provoke melanoma B-16 in them. On the 28th day, this melanoma was removed surgically. On the 56th day, CL immunorestorative effect was observed in mice. After surgical removal of the tumor, intensive formation of metastases was observed in the lungs and pleura of the animals, which had not received CL. As a result, 100% of the animals from the control group had metastases. The number of the metastases varied from 8 to 15. The most mice that received CL had no metastases. These studies allowed one to ascertain for the first time CL antimetastatic effect of lactobacilli in conditions of tumor surgical removal. Isolation of surface factors of L. casei DN 114001 and L. plantarum 191 inhibited the interaction of the tumor cell with thrombocytes, and determination of the structure of these factors would enable to create new medicinal preparations for a complex therapy of oncological patients in postoperative period. A search for lactobacillus strains with upregulated activity to activate NK cells in a combination with effective metastasis inhibition and development on their base of functional nutrition products is a new and perspective direction in prophylaxis and treatment of cancer diseases.

*Vyacheslav Melnikov
E-mail: melnikov@istc.ru

Possible health promoting benefits of heat-killed Lactobacillus gasseri TMC0356, a new selected immune regulatory probiotics strain

Kenji Miyazawa, Fang He*, Kazutoyo Yoda, Manabu Kawase, Akira Kubota and Masaru Hiramatsu

Technical Research Laboratory, Takanashi Milk Products Co., Ltd, Yokohama, Japan

Lactobacillus gasseri TMC0356 (TMC0356) was a human original probiotic strain found in 1997. This bacterium can activate macrophage to secret both inflammatory and anti-inflammatory cytokines and induce IgA production from Peyer's patches apparently. Animal and human clinical studies found that TMC0356 can significantly improve allergic symptoms and the related immunity, protect host animal against virus infection and suppress the tumors growth (1).
Recently, emerging scientific evidence has revealed that inactive probiotic cells may confer apparent health benefits on host animals. Compared with living cells, inactive cells are easier and more convenient to handle and can be used in different food product lines in addition to fermented milk or yoghurt, which are popular probiotic foods.

In the present study, the heat-killed TMC0356 induced IL-12 production from macrophage-like cell line J774.1, inhibited the growth of transplanted tumors in mice and protected mice from influenza virus infection. Furthermore, the heat-killed TMC0356 also expressed the promoting anti-obesity effects and potent possibility to alter age-related immunosenescence in host animals.

These results suggested that heat-killed TMC0356 can contribute to the health of host animal as well as its living cells. Heat treatment could be considered as one of the effective and practical ways to expand the application of probiotics in various foods and nutrition supplements.

Reference

1. Miyazawa K, He F, Kawase M, Kubota A, Yoda K, Hiramatsu M. Enhancement of immunoregulatory effects of Lactobacillus gasseri TMC0356 by heat treatment and culture medium. Lett Appl Microbiol 2011; 53: 210–6.

*Fang He
E-mail: he-fang@takanashi-milk.co.jp

Effects of probiotics on gut microbiota composition in familiar Mediterranean fever and Crohn’s disease patients

Anahit M. Manvelyan1, Marine A. Balayan1, Elya S. Pepoyan1, Susanna S. Mirzabekyan1, Lena M. Malkhasyan1, Vardan V. Tsaturyan2 and Astghik Z. Pepoyan1*

1Food Security and Biotechnology Department, Armenian State Agrarian University, Teryan, Yerevan, Republic of Armenia; 2Yerevan State Medical University after M. Heratsi, Republic of Armenia

Background: The incidence of patients with Crohn’s disease (CD) and with familiar Mediterranean fever (FMF) disease has been increasing dramatically over the last few years in world. FMF is an inherited disorder that usually occurs in people of Mediterranean origin—including Sephardic Jews, Arabs, Armenians and Turks, but it may affect any ethnic group. The frequency of FMF patients in the Armenian population is 0.25%, unusually high. The ratio of obligate heterozygotes to healthy persons is 0.14. While there is no cure for this disorder, one may be able to relieve signs and symptoms of FMF—or even prevent them altogether—by adhering to a highly effective treatment: colchicine, 1–2 mg/day, for life. The drug’s mode of action is unknown, but this molecule is able to inhibit attacks or prolong the intervals between them. It may also prevent or delay the appearance of renal complications in 2/3 of the patients. While colchicine is effective in treating FMF, it is not without side effects: many patients taking colchicines complain of general gastrointestinal upset.

Objectives: The aim of these investigations was characterization and comparative analysis of gut bacterial diversities of FMF and CD patients with or without probiotics treatment.

Methods: 110 FMF (with or without colchicine treatment) and 20 CD volunteer patients were enrolled in our study at different hospitals in Yerevan, Armenia. The patients’ fecal bacterial diversities were investigated using culture-based method and 16S rDNA libraries construction.

Results: The results of our investigations showed that there is an obvious deviation in gut bacterial diversities of Armenian FMF and CD patients. Due to few numbers of patients with probiotic treatment, we did not found any association between gut bacterial composition and probiotic use of patients. Taking into account the results of investigations, we hypothesize that the corrective probiotics therapy may be recommended for the patients only based on their gut microbiota composition. This work was supported by ISTC A-732 and A-1227.

*Astghik Z. Pepoyan
E-mail: past@rambler.ru

Development of probiotic preparations and functional food products on the base of lactic acid bacteria

Nadiia K. Kovalenko*

Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kiev, MSP, Ukraine
New modern approach for probiotic strain selection has been described. Distribution of lactic acid bacteria (LAB) in nature have been demonstrated on the basis of experimental results. It has been revealed that optimal characteristics of LAB depend on their environment. New interpretation of LAB ecology has been presented.

The specificity of influence of environmental conditions of natural substrates is the subject of scientific interest in term of study taxonomy of LAB and their biological properties. The collection of LAB strains isolated from different ecological niches has been created. The identification of these strains has been conducted by the proposed scheme of successive steps of differentiation using both phenotypic and genetic analysis (PCR, RAPD PCR, RFIP and 16S rDNA sequencing).

It has been determined that selected strains possess probiotic activity, as well as manifest antiviral activity and the adaptive ability. The regulation of their activity in some ecosystems has been developed. Probiotics for protection human and animal organisms against acute gastrointestinal diseases have been manufactured. Among them are Lactosan, Bovilact, Streptosan, Litosil, Lactin and Gerosan. The functional food products based on probiotic cultures for elderly people have been developed (Gerolact and Lactogerovit).

The results obtained allow to produce probiotics and functional food products with new comprehensive method, considering modern requirements as for bacteria identification.

*Nadiia K. Kovalenko
E-mail: nkovalenko@serv.imv.kiev.ua

Beneficial effects of fermented milk containing Lactobacillus GG on DSS-induced colitis in mice

Kazutoyo Yoda1*, Fang He1, Kenji Miyazawa1, Manabu Kawase1, Akira Kubota1, Masaru Hiramatsu1 and Fang Yan2

1Technical Research Laboratory, Takanashi Milk Products Co., Ltd., Yokohama, Japan; 2Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN, USA

Probiotic bacteria are microorganisms that benefit the host by preventing and/or treating diseases. Lactobacillus GG (LGG), a well-known probiotic bacterium, secretes two functional proteins, P40 and P75, which suppress intestinal inflammation. The purpose of this study is to detect these functional proteins from a commercial fermented milk product containing LGG (LGG-milk) and investigate actions of LGG-milk on cultured epithelial cells and DSS-induced colitis in mice.

P40 and P75 were detected from supernatant of LGG-milk by Western-blotting analysis using anti-P40 and anti-P75 antibodies.

Young adult mouse colon (YAMC) cells were cultured with 1:100 to 1:2,000 diluted supernatant of LGG-milk for 2 h. Cellular lysates were prepared for Western-blotting analysis using anti-EGF receptor-Tyr1068 and anti-phosphorylated Akt antibodies to detect EGF receptor and Akt activation, respectively. LGG-milk activated EGF receptor and Akt in a concentration-dependent manner in the YAMC cells.

To evaluate the preventive effect of LGG-milk on DSS-induced colon epithelial injury and colitis, female C57BL/6 mice were treated with 3% DSS in drinking water for 4 days to induce colon injury and acute colitis. 500 μl of LGG-milk was gavaged to mice 6 days before and during DSS treatment. Inflammation and injury was assessed using a score system. DSS-induced colitis (score: 6.2 ± 0.84) was significantly decreased by LGG milk treatment (score: 3.4 ± 3.14). The shortening of the colon induced by DSS (6.38 ± 0.39 cm), as a marker for colitis, was prevented by LGG-milk treatment (7.48 ± 0.48 cm). These results indicate that LGG-milk exerts a preventive effect on DSS-induced colitis in mice.

*Kazutoyo Yoda
E-mail: k-yoda@takanashi-milk.co.jp

Influence of probiotic Enterococci on the gastrointestinal tract of rats before and after the treatment of antibiotic-associated dysbiosis

Alexander Suvorov1*, Ludmila Gromova2, Yuri Borschev2, Alena Karaseva3 and Andrei Gruzdakov2

1Institute of Experimental Medicine RAMS, St. Petersburg, Russia; 2 Pavlov Institute of Physiology RAS, St. Petersburg, Russia

Citation: Microbial Ecology in Health & Disease 2012, 23: 17461 - http://dx.doi.org/10.3402/mehd.v23i0.17461

[page number not for citation purpose] 47
Implementation of probiotics containing lactic acid bacteria (LAB) have been proved to be useful in many clinical conditions including antibiotic associated diarrhea. However, little is known about the specialties of their colonization in mammals and effects on the digestive system when LABs are used to prevent or treat the intestinal dysbiosis. The purpose of this study was to investigate the influence of probiotic Enterococci on the condition of the experimental animals, their microbiota and the activity of key digestive enzymes. Male Wistar rats were getting milk fermented products containing *E. faecium* L5 (ermB-labeled derivative of probiotic strain L3). Bacteria were introduced into the stomach of the healthy animals or the rats treated with antibiotics (ampicillin and metronidazole, 3 days). At the end of the experiment, samples of chyme and epithelium were taken from different parts of the gastrointestinal tract and studied by bacteriological and biochemical methods. It was shown that the administration of probiotic did not affect on the rat’s condition and behavior. Dysbiosis associated with the intake of antibiotics was confirmed by the changes in microbiota and by the appearance of dyspeptic symptoms. Pathological symptoms disappeared after the administration of Enterococci and the original intestinal microbiota was restored.

Usage of probiotic led for colonization of jejunum, ileum and all parts of large bowel with *E. faecium* L5. Moreover, erythromycin-resistant Enterococci were detected in the stomach of some animals. No bacteria were found in the spleen, liver, heart or the blood of rats. It was typical for all rats treated with probiotic: the reduction of maltase activity of the epithelium and the increasing of activity of alkaline phosphatase in the chyme of the small intestine. Rats after the consumption of antibiotics had lower values of maltase activity in the chyme of large bowel in contrast to healthy rats or animals with dysbiosis receiving probiotic therapy.

Strain *E. faecium* L5 was able to temporarily colonize the gastrointestinal tract of the animals and caused the disappearance of dysbiosis and changes in the activity of intestinal enzymes (in particular maltase activity in the cavity of the colon). The specific effects provided by strain *E. faecium* L5 (L3) on the activity of maltase and alkaline phosphatase should be considered when comparing this strain with other probiotic microorganisms.

*Alexander Suvorov*
E-mail: alexander_suvorov1@hotmail.com

---

**Development of the Caucasian lactic acid bacterial culture collection for the potential industrial use**

Nina Chanishvili*

Eliava Institute of Bacteriophage, Microbiology and Virology, Tbilisi, Georgia

The traditional Caucasian dairy products (such as Matsoni—the Caucasian yogurt like product, cheeses, etc.) remain almost unexplored. The commercial and, especially, health beneficial value of these products are poorly studied. At the same time, e.g., Matsoni is historically known as a supplement of the infant’s diet, remarkable remedy against intestinal disorders, burned wounds and dermatitis, in domestic cosmetics for improvement of skin and hair conditions, also as a base of special food-preserving solutions. Hence, it can be considered as a potential pool of the strains with antimicrobial activity against pathogens causing human and animal diseases, as well as food-spoilage micro-organisms.

Thus, the bacterial cultures isolated from the homemade dairy products may be considered as a potential pool of genes determining antibacterial activities against human and animal pathogens as well as food taste and quality spoilage microorganisms.

Traditional methods including chromatography (HPLC analysis of fermentation and products), API tests (bio Merieux) along with modern genetic techniques for identification of strains, such as PCR and DNA sequencing of 16S-23S rRNA intergenic regions have been applied for strain identification.

During the past years, about 50 homemade Matsoni samples and 20 cheese samples have been collected in different parts of Georgia. Approximately 400 lactic acid bacterial cultures have been isolated. The number of components in the homemade Matsoni samples were 2 at minimum and 7 at maximum, and diversity of lactic acid strains remarkably varied in accordance with geographical origin of the starter. In particular, presence of the following bacterial and yeast species have been determined: *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus delbrueckii* ssp. *lactis*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Lactococcus cremoris*, *Lactococcus lactis*, *Enterococcus sp.*, *Saccharomyces cerevisiae*, *Candida lucitaniae* [5, 6, 7]. It was demonstrated that only *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* are responsible for clot formation and taste; however, the taste of Matsoni is different from yogurt. Another bacterial species, *E. durans* is also frequently found in the Matsoni samples, which is an essential part of the Italian cheese Mozzarella. According to the present knowledge, *Enterococci* do not play significant role in Matsoni, but some of them are having distinguished antibacterial properties.

A basic culture collection of the endemic dairy strains has been developed. Only 10–20% of the strains from this culture collection is fully or sometimes partially characterized by now. It appeared that about 30% of the tested
lactic acid bacteria are antagonistic to the various pathogens, while only 20% out of them showed the best biotechnology potential. The studies need to be continued for selection of the most efficient probiotics and the strains with the strong proteolytic activities.

Nina Chanishvili
E-mail: n_chanish.ibmv@caucasus.net

Probiotic ‘Lactovit-K’ fights against coccidiosis of poultry and honey bee diseases

Nina N. Gavrilova¹, Irina A. Ratnikova¹* and Vyacheslav G. Melnikov²

¹Institute of Microbiology and Virology, Almaty, Kazakhstan; ²International Science and Technology Center, Moscow, Russia

The coccidiosis of poultry is sometimes difficult to treat because of the associative character of the disease—coccidium aftereffects with enteropathogenic bacteria infection. It is not always taken into account. Secondary infection makes the primary disease much more complicated. Septic form of colibacteriosis often becomes a cause of the animal death. In order to fight against this secondary bacterial superinfection, we performed a probiotic preparation ‘Lactovit-K’, which contains lactobacilli, propionibacteria and vitamins B₃, B₈, B₁₂. The probiotic appeared to be capable of inhibiting growth of pathogenic E. coli and Salmonella in vitro.

Experiment was carried out using the livestock of 8,000 chickens with coccidiosis. The preparation was given to the chickens with forage during 10 days. In the end of treatment period, the general state of the chickens improved, and there was almost no loss of the birds. The viscera had no any pathological changes; mucous membrane of the bowel was normal. Pathogenic E. coli were not observed. In only some chickens, moderate amount of coccidium oocysts (3–5 ones in the microscopic field) was found. Chickens in the control group did not grow well; they had diarrhea, and their feathers were disheveled and dirty. Considerable loss of the chickens was seen. Laboratory examination of dead birds revealed inflammation of bowel mucous membrane, heavy coccidial and pathogenic E. coli infections. So, the research results have proved the possibility of the use of probiotics against coccidiosis.

Lactovit-K has showed its high effectiveness against honey bee diseases too. When using the preparation in combination with carbohydrate and protein feed, we observed the reduction of incidence of American foulbrood (bacterial disease of bees) by 97% and of Varroa jacobsoni (tick-born disease) by 74%. In combination with sugar syrup, the effectiveness of Lactovit-K regarding American foulbrood was 80%, and the incidence of V. jacobsoni was reduced by 70%.

*Irina A. Ratnikova
E-mail: iratnikova@almanet.kz

Biological active compounds from probiotic bacteria

Galina Novik¹*, Elena Kiseleva², Vyacheslav Melnikov³, Andrzei Gamian⁴, Yuriy Knirel⁵ and Estera Szwajcer Dey⁶

¹Institute of Microbiology BNAS, Minsk, Belarus; ²Institute of bioorganic chemistry BNAS, Minsk, Belarus; ³International Science and Technology Center, Moscow, Russia; ⁴Institute of Immunology and Experimental Therapy, PAS, Wroclaw, Poland; ⁵N.D. Zelinsky Institute of Organic Chemistry, RAS, Moscow, Russia; ⁶Lund University, Pure and Applied Biochemistry, Lund, Sweden

Probiotic bacteria are natural inhabitants of human gastrointestinal tract. Extensive investigations of biological active compounds from probiotic bacteria are aimed primarily at the development of highly effective therapeutic products. Among these substances, there are bacterial polysaccharides and polar lipids. Therefore, it is important to identify the biologically active compounds and to determine their structures. Chemical, NMR spectroscopic and serological studies revealed a significant structural heterogeneity of the polysaccharides, which in turn might influence their biological activity, namely the antigenicity. A polysaccharide of α1,4-glucan type was isolated from Bifidobacterium adolescentis 94-BIM. The major polysaccharide specific for strain B. bifidum BIM B-465 was found to be a branched glucoagalactan with a heptasaccharide repeating unit. B. longum BIM B-476D produced several polysaccharides,
including a glucosylated ribitol teichoic acid and a polymer with a branched pentasaccharide 1-phosphate repeating unit. Current research on biological active compounds from probiotic bacteria is aimed primarily at the elaboration of glycoconjugates products. By employing a new modification of supercritical fluid of carbon dioxide (scCO₂), polar lipids of lactic acid bacteria and bifidobacteria can be very effectively extracted. TLC analysis of scCO₂ fractions showed predominance of glycolipids and phospholipids. Glucose and galactose were detected as sugar components of isolated glycolipids. Glycolipids with high immunoreactivity were identified by enzyme linked immunosorbent assay (ELISA). Additionally we received data suggesting a possible role of probiotic bacteria of genera Bifidobacterium and Lactobacillus in pathogenesis of autoimmune thyroid disease (ATD). Using proteins and immunogenic peptides data-banks and relevant computer programs, homology was established between the amino acid sequences of thyroid peroxidase (TPO) and thyroglobulin (Tg), which are potential B- and T-cell epitopes of these autoantigens and of a number of proteins of Bifidobacterium and Lactobacillus. We also have found nonprotein components of cells of Bifidobacterium bifidum 791, B. adolescentis 94 BIM, B. longum B379M and Lactobacillus plantarum B-01 that cross-react with human serum autoantibodies (AAbs) to TPO and Tg and compete with relevant antigens for the binding of these AAbs in ELISA. Moreover, a three-fold difference was disclosed between the probability of detection of Abs to the antigens of L. plantarum B-01 and B. bifidum 791 in serum samples containing and not containing AAbs to TPO, which are serologic markers of ATD. On the whole, our data are arguments in favor of the assumption of a possible role of PM of genera Bifidobacterium and Lactobacillus in triggering ATD through the mechanism of molecular mimicry. In prospect, the scCO₂ isolation technology of probiotic glycoconjugates may be combined with metabolic engineering and immunological studies in technologies aimed at manufacturing highly effective therapeutic products.

The role of probiotics in rehabilitation of sportsmen after postmatch exertion

Gagik Hoveyan¹, Marine Badalyan¹, Ani Hoveyan², Elya Pepoyan³, Vardan Tsaturyan² and Astghik Pepoyan²*

¹Buniatian Institute of Biochemistry, National Academy of Sciences, Republic of Armenia; ²Yerevan State Medical University after M. Heratsi, Republic of Armenia; ³Food Security and Biotechnology Department, Armenian State Agrarian University, Republic of Armenia

One of the most important problems of modern biomedical science and clinical medicine is the revealing of the bimolecular mechanisms of wide scope of physical and emotional exertion and rehabilitation of sportsmen by administration of probiotics, prebiotics and food supplements. It is known that during and after sport fatigue, the inhibition of organism adaptation takes place. That is the universal biological process, by means of which the organism can get accustomed to the various changes of the external and internal mediums. In the process of adaptation, the time-depending organization of organism undergoes changes as well different nervous, hormonal and immunologic displacements occur bearing wave-like character. In the each state of adaptation, the homeostatic factors supporting the adaptive properties occurred in previous stages simultaneously neutralize the negative damaging properties of them. Adaptation needs coordination of regulation and integration of endocrine, nervous and immune systems, which regulate processes of metabolism, generation of energy, increase of antiradical defense and redistribution of substances in tissues of organism.

On the other hand, probiotics are considered as important factors not only for the restoration of commensal microbiota but also for increasing adaptation-compensatory possibilities of organisms. Probiotics possess antifree radical, immune stimulating activities and possibility to take part in processing, distribution and absorption of nutrients in intestine.

The aim of these investigations was the characterization and comparative analysis of gut bacterial diversities of sportsmen-groups. Thirty-five sportsmen were involved in our investigations. The fecal samples from sportsmen were gathered a week before and after the championships, and the fecal bacterial diversities by classical culture-based method were investigated.

The results of our preliminary investigations showed that there is a deviation in gut main bacterial groups depending on championship period. The role of probiotics as important agents of rehabilitation of sportsmen at physical exertion and overtraining is also discussed.

¹Astghik Pepoyan
E-mail: past@rambler.ru

*Galina Novik
E-mail: galina_novik@mbio.bas-net.by

Citation: Microbial Ecology in Health & Disease 2012, 23: 17461 - http://dx.doi.org/10.3402/mehd.v23i0.17461
Breakdown of *Bifidobacterium* flora by species in the process of intestinal biocenosis development in newborns and in dysbiotic individuals

Elena Fedorovna Zavgorodnyaya*

Khabarovsk Research Institute of Epidemiology and Microbiology, Federal State Institution of Science under the Federal Service for Supervision in the Field of Consumer Right Protection and Human Welfare, Russian Federation

Study was made of *Bifidobacteria* species, adhesive and antagonistic activity thereof in 25 pregnant women and their newborns (in the process of microflora acquisition from the 1st through the 30th day of life)—180 tests, as well as in dysbiotic cases of children over 3 years of age and adults—257 tests.

*Bifidobacterium* species are isolated from the gut culture of 64.0% of children since the first day of life in quantity of 3.3 lg/g; by the 5th day of life, the quantity thereof increases up to 8.3 lg/g. However, by the age of one month, *Bifidobacterium* quantity amounts to 9.0 lg/g in only 52.2% of children. Stable prevalence of *Bifidobacterium* in the biocenosis of infants aged up to 10 days of life was only registered in 20% of children, of *B. adolescentis* species, adhesive and antagonistic activity thereof in 25 pregnant women and their newborns (in the process of microflora acquisition from the 1st through the 30th day of life)—180 tests, as well as in dysbiotic cases of children over 3 years of age and adults—257 tests.

*Bifidobacterium* species are isolated from the gut culture of 64.0% of children since the first day of life in quantity of 3.3 lg/g; by the 5th day of life, the quantity thereof increases up to 8.3 lg/g. However, by the age of one month, *Bifidobacterium* quantity amounts to 9.0 lg/g in only 52.2% of children. Stable prevalence of *Bifidobacterium* in the biocenosis of infants aged up to 10 days of life was only registered in 20% of children, of infants aged 10 through 15 days—in 44.0% of children (it is these figures that are registered in the majority of newborns in European Russia); 24.0% of newborns demonstrated delay in bifidoflora colonization up to as late as the 20th–30th day of life, while 12.0% of infants were not dominated by *Bifidobacterium* even by the end of the first month of life, i.e., the fact of delay in bifidoflora development was established. Most likely, this was due to the transfer of *Bifidobacterium* of lower biological activity from the mother to the newborn, which results from the prevalence of dysbacteriosis in people, including pregnant women.

Intestinal microflora of pregnant women presented with dominance of *B. adolescentis* and *B. breve* (total of 86.4%). Newborns aged 1 through 5 days were also dominated by *B. adolescentis* (84.0%) and *B. breve* (16.0%). Thus, a second specific feature of bifidoflora development in newborns was identified, namely: child-specific term of an initial increase (from the first to the fifth day of life) and a subsequent decrease (more frequently on the fifth–fifteenth day) in both the total number of *Bifidobacterium* and their share in the intestinal microflora (45.4% and 63.6%, respectively).

It has been found out that, in the process of the decrease in the quantity of *Bifidobacterium* species, rearrangement thereof takes place, and in 80% of cases, *B. adolescentis* and *B. breve* are replaced by the so-called ‘suckling’ and other (*B. infantis, B. lactensis* and *B. bifidum*) species or associations thereof, which dominate in the biocenosis of healthy infants during the entire period of breast-feeding.

Species rearrangement of *Bifidobacterium* is preceded by a decrease in antagonistic and adhesive activity thereof, which was registered in 42.8% of *Bifidobacterium* strains isolated from infants aged 5–15 days. Apparently, species rearrangement of bifidoflora makeup occurs in stages and may be described as follows: *first stage*—decrease in a physiological activity of the prevalent species of *Bifidobacteria* (e.g., *B. adolescentis*); *second stage*—decrease in the number of *Bifidobacteria* of said species; *third stage*—increase in the number of *Bifidobacteria* of other species; (e.g., *B. lactentis*), with a gradual domination thereof in the biocenosis.

The same consistent pattern is also seen in intestinal dysbacteriosis development, but the succeeding *Bifidobacteria* species prove to be other than the above. Thus, *B. adolescentis* dominates in the large intestine of 67.7% healthy adults and children over 3 years old in all seasons, both in monocultures and in associations with other species. In dysbacteriosis cases, the same species remains prevalent but the quantity of genus bifidum proves significantly increased (14.3% in eubiotic condition and 25.6% in dysbacteriotic condition). That is, rearrangement of *Bifidoflora makeup* takes place in the course of development of intestinal dysbacteriosis.

From 81.8% of dysbacteriotic individuals, *Bifidobacteria* of lower antagonistic activity (in normocenosis—from 43.3% only) and lower adhesive activity (46.7% vs 78.2%) were most frequently isolated.

Thus, the process of rearrangement of makeup during bifidoflora development and dysbacteriosis development follows the same biological mechanism.

---

**Efficacy of the probiotic strain *Clostridium butyricum* MIYAIRI 588 on poultry and piglet zootechnical performance and prevention of necrotic enteritis**

Motomichi Takahashi¹,²,*, Miroslava Piskoriková³, Kaoruko Yuge¹, Kentaro Oka¹,², Koji Uno¹, Elinor McCartney³ and Shigeru Kamiya²

¹Miyarisan Pharmaceutical, Japan; ²Department of Infectious Diseases, Kyorin University School of Medicine, Japan; ³EU Pen & Tec Consulting, Spain

Citation: Microbial Ecology in Health & Disease 2012, 23: 17461 - http://dx.doi.org/10.3402/mehd.v23i0.17461
The 2006 EU ban on antibiotic growth promoters has stimulated research on alternative products to improve zootechnical performance and reduce problems such as necrotic enteritis (NE). *Clostridium butyricum* MIYAIRI 588 (CBM 588) is a viable spore probiotic strain, approved for human pharmaceutical products in Japan and Asia and as a feed additive in EU. From 2006 to 2010, we carried out four large field studies with broilers and five studies with weaned piglets in several EU countries.

Zootechnical data from all studies were analyzed, including daily weight gain, feed intake, feed efficiency (feed:gain) and mortality. Data were then tested for homogeneity and pooled for meta-analysis, where \( p \leq 0.05 \) was considered significant, and \( 0.05 < p \leq 0.10 \) a near-significant trend. We also tested the ability of CBM 588 to reduce NE lesions in a pilot study in broilers that modeled naturally occurring NE.

The meta-analysis of four broiler field trials (1–42 days of age) demonstrated a significant (2.3%) improvement in feed:gain (1.74 versus 1.70 for control and CBM 588 broilers, respectively, \( p = 0.0001 \)) and a 1.8% decrease in feed intake (95.7 versus 94.0 g/day for control and CBM 588 broilers, respectively, \( p = 0.0163 \)). No significant differences were detected in mortality.

In the pilot broiler NE study (0–35 days of age), there were no significant differences in performance (growth, liveweight, feed intake, feed efficiency, mortality and European Production Efficiency Factor) but a clear dose-response at 32 days of age related to reduction of the severity of NE lesions (\( P < 0.05 \)).

The meta-analysis of five piglet field studies demonstrated a significant (4.2%) improvement in feed:gain (1.60 versus 1.67 for CBM 588 and control piglets, respectively, \( p = 0.0019 \)) and a 4.8% increase in mean daily gain (380.55 versus 363.22 g/day for CBM 588 and control piglets, respectively, \( p = 0.0048 \)). No significant differences were detected in feed intake (602.97 versus 602.03 g/day for CBM 588 and control piglets, respectively, \( p = 0.9222 \)).

The meta-analysis data indicate that CBM 588 is an effective zootechnical feed additive in broilers and piglets and may help reduce NE.

---

**Reactivation of latent HIV-1 by a wide variety of butyric acid-producing bacteria**

Kenichi Imai*, Kiyoshi Yamada, Muneaki Tamura and Kuniyasu Ochiai

Department of Microbiology, Nihon University School of Dentistry, Chiyoda-ku, Tokyo 102-8310, Japan

**Background:** Latently infected cells harbor HIV-1 proviral DNA integrated in heterochromatins, allowing persistence of transcriptionally silent proviruses. Hypoacetylation of histone proteins by histone deacetylases (HDACs) is involved in maintaining the HIV-1 latency by repressing transcription from HIV-1 proviruses. Although it is known that a bacterial metabolite, butyric acid, is involved in reactivation of the ‘repressed’ chromatin, it is not known whether butyric acid-producing bacteria, such as periodontogenic *Porphyromonas gingivalis* and *Fusobacterium* in resident flora, are involved in activation of HIV gene expression. Here, we examined whether infection of these bacteria could facilitate progression of HIV infection by reactivating the latent HIV provirus.

**Methods:** Human ACH-2 and OM10.1 cells, latently infected with HIV-1, were incubated with culture supernatant of various bacteria. HIV-1 proteins were detected by immunoblot and ELISA. Luciferase and chromatin immunoprecipitation assays were employed to analyze the transcription factors involved in HIV transcription. Butyric acid and other short chain fatty acids were measured by gas chromatography.

**Results:** We found that the culture supernatant of periodontopathic bacteria, *P. gingivalis*, strongly induced HIV-1 replication via chromatin modification and these effects could be ascribed to butyric acid (1). Other bacteria producing butyric acid, such as *Clostridium*, *Fusobacterium*, and *Eubacterium* in the intestine, also accelerated replication of HIV-1. In addition, some anaerobic bacteria resident in vaginal mucous membrane such as *Peptomicrobium* and *Anaerococcus* could also induce HIV-1 replication. These bacteria produce high concentrations of butyric acid acting as a potent inhibitor of HDACs and induce histone acetylation, thus eventually leading to reactivation of HIV-1 in latently infected cells.

**Conclusions:** Our observations indicate that butyric acid-producing bacteria are generally involved in AIDS progression by reactivating the latent HIV provirus. Thus, it is concluded that elimination of such bacterial infection would prevent the clinical progression of HIV infection.

**Reference**

1. Imai K, Ochiai K, Okamoto T. Reactivation of latent HIV-1 infection by the periodontopathic bacterium *Porphyromonas gingivalis* involves histone modification. J Immunol. 2009 15;182:3688–95.

*Kenichi Imai*

E-mail: imai-k@dent.nihon-u.ac.jp

---

*Motomichi Takahashi*

E-mail: motomichi.takahashi@miyarisan.com

---

Citation: Microbial Ecology in Health & Disease 2012, 23: 17461 - http://dx.doi.org/10.3402/mehd.v23i0.17461
Extracellular HIV-1 TAT (ExTat) regulates bacterial superoxide dismutase (SOD) allowing upregulation of reactive oxygen species (ROS) in Porphyromonas gingivalis and Fusobacterium nucleatum

Marni Cueno, Muneaki Tamura, Kenichi Imai, Megumi Hamadate and Kuniyasu Ochiai*

Department of Microbiology, Nihon University School of Dentistry, Tokyo, Japan

Among AIDS patients, viral load has been detected in the saliva suggesting extracellular HIV-1 Tat (ExTat) also accumulates. ExTat is known to cause oxidative stress by regulating superoxide dismutase (SOD) activity, thereby allowing reactive oxygen species (ROS) to accumulate. The effects of ExTat is well studied in humans; however, its effects in the bacterial flora have never been elucidated. Here, we studied the effects of Tat on periodontal anaerobic bacteria. We compared the effects of commercial Tat (CommTat) and ExTat produced from plant cells on both Porphyromonas gingivalis ATCC 33277 and Fusobacterium nucleatum ATCC 25586. Bacterial isolates were grown in GAM broth supplemented with either CommTat or ExTat at a final concentration of 0.9 mg/mL. Both Tat-supplemented and control strains were grown under anaerobic conditions for 48 h at 37°C. Cells were lysed using 10% SDS solution. ROS and SOD levels were measured using commercially available assays. In both control and Tat-supplemented samples, we found no drastic changes in bacterial growth density; however, SOD levels were found to be down-regulated, which we attribute to Tat action. Subsequently, ROS levels were found to be higher among Tat-supplemented samples as compared to control samples. Interestingly, we found ExTat induced higher ROS as compared to CommTat. We attribute this observation to glycosylation since, unlike CommTat where Tat is unglycosylated, Extat, which was produced from plant cells, is glycosylated and, thus, is more room-temperature stable. Our results would suggest that, at least among anaerobic bacteria, particularly P. gingivalis and F. nucleatum, Tat downregulates SOD activity, which subsequently allows ROS to accumulate. This would imply two points: (1) ROS increase ascribable to ExTat is not unique to mammalian cells and (2) the bacterial flora of HIV-infected patients may similarly be affected by ExTat.

*Kuniyasu Ochiai
E-mail: ochiai@dent.nihon-u.ac.jp

Evaluation of survival and infectivity of bacteriophage isolates from environmental samples in function of temperature and pH

Nima Bahador*

Department of Microbiology, Islamic Azad University, Science and Research Branch, Fars, Iran

Twenty bacteriophages were isolated from Pavana river water and sewage samples (India). Out of all the isolates, two bacteriophages were characterized and identified (according to Ref. 1) as coliphage (Leviviridae) and staphylophage (Podoviridae). Then, effect of pH and temperature on survival and infectivity of the isolates were evaluated in order to achieve optimum pH and temperature for the isolates and use them in further study for eradication of biological pollutant in water. The buffers that have been used in this study were succinate, Tris and glycine. In addition, effect of different temperatures (20, 25, 30, 35, 40 and 45°C) on survival and infectivity of the phages have been evaluated. The results indicated that the optimum pH for both of the isolates was 7.0. The optimum temperature for survival and infectivity of the isolates were 37°C for coliphage (Ecp) and 27°C for the staphylophage (Sap). Overall, evaluation of optimum temperature and pH as significant character for eradication of microorganisms using bacteriophages are required.

Keywords
Bacteriophage; survival; infectivity; temperature; pH

Reference
1. Ackerman 1998.

*Nima Bahador
E-mail: nimabahador@yahoo.com

Citation: Microbial Ecology in Health & Disease 2012, 23: 17461 - http://dx.doi.org/10.3402/mehd.v23i0.17461
Coinfection of mammalian and tick cells with pathogenic and nonpathogenic spotted fever group rickettsiae

Tsuneo Uchiyama¹* and Hiromi Fujita²

¹Department of Microbiology, Institute of Health Biosciences, The University of Tokushima Graduate School, Kuramoto, Tokushima, Japan; ²Ohara Research Laboratory, Ohara General Hospital, Kamata, Fukushima, Japan

It is known that ticks sometimes possess multiple species of rickettsiae. However, it is still uncertain if coinfection of humans with these rickettsiae actually occurs. In this study, we intended to analyze the growth kinetics of pathogenic and nonpathogenic spotted fever group rickettsiae when they are coinfected in mammalian and tick cell lines.

*Rickettsia japonica was used as a pathogenic species of spotted fever group rickettsiae. *R. montanensis*, isolated from ticks in the northern and eastern USA, and *Rickettsia* sp. LON, isolated from ticks in Japan and genetically closely related to *R. japonica*, were used as nonpathogenic rickettsiae. Vero and HeLa cells derived from mammals and DALBE3 and ISE6 cells derived from ticks were inoculated with nonpathogenic strains. On the day three of inoculation, the infected cells were further inoculated with pathogenic *R. japonica*. Inoculation in the reverse order was also performed. The yields of each rickettsia were measured by means of plaque assay and immunostaining with species-specific monoclonal antibodies. These cells were solubilized every three days for Western blotting to clarify the participation of autophagy in the growth of these rickettsiae. Transmission electron microscopy was also performed to observe their morphological changes.

The mammalian and tick cells were persistently infected with *R. montanensis* producing low levels of rickettsiae. The elevation of the yields of *R. montanensis* occurred after the superinfection with *R. japonica* on the *R. montanensis*-infected cells. On the other hand, LON grew well in tick cells although it caused persistent infection in mammalian cells. After the superinfection with *R. japonica* on the LON-infected mammalian cells, the elevation of the yields of LON also occurred. The cause of the difference in the growth kinetics of *R. montanensis* and LON in tick cells is yet to be elucidated. Western blotting and the electron microscopy presented the evidences of autophagy occurred in the *R. montanensis*-infected cells. After superinfection with *R. japonica*, autophagy was rather restricted. It is suggested that the growth restriction of the nonpathogenic species *R. montanensis* at least partly due to the autophagy that occurs in the infected cells and that the pathogenic species *R. japonica* may secretes an autophagy-restriction factor(s). The factors are yet to be identified.

*Tsuneo Uchiyama  
E-mail: uchiyama@basic.med.tokushima-u.ac.jp

Leptotrichia genus bacteria biotopes in the human body

Natalia Viktorovna Strelnikova*, Alexandra Anatolievna Antonova and Elena Borisovna Polovova

Microbiology, Virology and Immunology Department, Far Eastern State Medical University, Khabarovsk, Russia

*Leptotrichia* are nonspore-forming gram-negative anaerobic rod and are normal commensals of the human oropharynx, gastrointestinal tract and female genital tract. *Leptotrichia buccalis* is the type species of *Leptotrichia* genus and since 2005 belongs to *Fusobacteriaceae* family.

*L. buccalis* is the species most frequently isolated from clinical samples. *Leptotrichia* species are considered an important component in mixed anaerobic infections. Infection caused by *L. buccalis* is rare.

Nevertheless, according to the literature over the past 20 years, the role of *Leptotrichia* genus bacteria in human pathology is increasing (1–15).

At present, *Leptotrichia* genus bacteria can be regarded as emergent pathogens in the infectious pathology of man (16, 17). The organism has been isolated mostly from opportunistic infections. The most frequently isolated bacteria from blood samples in cases of patients with lesions in the oral mucous membrane, in particular from patients with neutropenia and cancer. *Leptotrichia* has also been recovered from immunocompetent patients with severe pneumonia, hepatic abscess, endocarditis, bacterial vaginosis and other. Although their role in infections is poorly understood, they have been suggested as emerging pathogens.

In our study of *Leptotrichia* infections in adult and children, a total of 43 strains of *Leptotrichia* species were recovered from 229 (18, 8%) specimens. In one case, *Leptotrichia* has been isolated from blood culture of immunocompetent 19-year-old girl with rectal fistula. In one case, *Leptotrichia* has been isolated from plural hepatic abscesses culture of 53-year-old
woman. In a series of 24 patients with chronic tonsillitis, *Leptotrichia* and *Staphylococcus* were isolated in 13 cases and *Leptotrichia* and *Candida* in 6 cases; *L. buccalis* alone was isolated in only 5 cases.

As the findings show, *Leptotrichia* species were recovered from blood culture of females with bronchial asthma and fever. In addition, isolated *Leptotrichia* from urine samples in cases of patients with chronic cystitis, pyelonephritis and urolithiasis and in particular from patients with congenital renal pathology.

*Leptotrichia* are normal commensals of the human oral microbiota and, in Khabarovsk territory, are found in 95% of the population. Under certain conditions, *Leptotrichia* can cause inflammatory diseases of the oral mucosa, e.g., stomatitis, gingivitis, cheilitis and glossitis. The etiology of about 34% of the cases presented *Leptotrichia* and association of microbes: *Leptotrichia* with *Staphylococcus*, *Leptotrichia* with *Candida*, *Leptotrichia* with *Enterococcus*.

These data require a deeper study of condition immunity, as well as environmental factors affecting the state of oral microbiota in the population of the region.

Microbial biocenosis is important for maintenance of homeostatic balance in the human body (1). It is characterized by stability, which is due to Quorum sensing bacteria (2, 3), associated in a microbial-tissue complex, a biofilm (4). Respiratory tract microbiota accounts for the macroorganism’s colonization resistance, while its disbalance leads to a risk of pneumonia. Our own research has shown that the extreme biogeoclimatic environment in the Russian Far East contributes to an increase in respiratory diseases in children (5, 6), which, among other things, is due to the nasopharyngeal circulation of opportunistic bacteria in the regional children population.

The objective of the research consisted in the study of respiratory microbial biocenosis and its assessment thereof as a risk factor of community-acquired pneumonia in children.

Nasopharyngeal microbial biocenosis in children in the Amur River area of Russia

Galina Nikolaevna Kholodok* and Vladimir Kirillovich Kozlov

Research Institute of Mother and Child Health Care, Khabarovsk Branch of the Far-Eastern Research Center of Respiratory Physiology and Pathology under the Siberian Branch of the Russian Academy of Medical Sciences

Microbial biocenosis is important for maintenance of homeostatic balance in the human body (1). It is characterized by stability, which is due to Quorum sensing bacteria (2, 3), associated in a microbial-tissue complex, a biofilm (4). Respiratory tract microbiota accounts for the macroorganism’s colonization resistance, while its disbalance leads to a risk of pneumonia. Our own research has shown that the extreme biogeoclimatic environment in the Russian Far East contributes to an increase in respiratory diseases in children (5, 6), which, among other things, is due to the nasopharyngeal circulation of opportunistic bacteria in the regional children population.

The objective of the research consisted in the study of respiratory microbial biocenosis and its assessment thereof as a risk factor of community-acquired pneumonia in children.

Nasopharyngeal microbial biocenosis in 1,175 children was studied; 1,640 strains of indigenous and transitory opportunistic bacteria of 8 families, 14 genera and 32 species were isolated. To assess species diversity in a nasopharyngeal biotope, Simpson index, Margalef species richness index calculated as a ratio of the number of species to the total number of individuals in the biotope, Shannon (entropy) index providing for the number of species and the extent of their activity in the community and attaching greater importance to rare species were used. Pielou index admitting of diversity comparison among the samples with different number of species and assessment of the degree of community polydominance, Berger–Parker index representing the number of the most abundant species in the community were also used.

Analysis of the data obtained shows that Shannon index indicates to the stable composition of pharyngeal microbiota due to the presence of gram-positive bacteria, with their diversity (assessed by Simpson index) being less than that of gram-negative bacteria. Shannon index for gram-positive flora in the pharynx made 1.16, for gram-negative flora in the pharynx, 0.56 (i.e., 2 times as less) with Simpson’s index equal to 0.37 and 0.71 and Margalef index equal to 1.69 and 1.28, respectively. Berger–Parker index in the pharynx showed prevalence of genus *Neiseriae* (0.69) among gram-negative bacteria, genus *Streptococcus* (0.468) among gram-positive bacteria, and genus *Acinetobacter* (0.8) among nonfermentative bacteria. For nasal microbiota, gram-negative flora was of greater significance. Shannon index for gram-positive bacteria was found to be less than for gram-negative bacteria and made 0.97 and 1.02, with Pielou evenness indices being 0.49 and 0.73, respectively. Species diversity in nasal microbial biocenosis was greater for gram-positive bacteria. Simpson’s index made 0.52 and 0.45 and Margalef’s index, 2.6 and 1.89 for gram-positive and gram-negative bacteria, respectively. Berger–Parker indices in a nasal biotope were high for...
Abstracts

Staphylococci (0.7) and Neisseriae (0.61). Thus, a dysbiocenotic state of a nasopharyngeal microbiota was identified, which was caused by increased enterobacterial and staphylococcal vegetation. Nasopharyngeal carriage of Streptococcus pneumoniae and Haemophilus influenzae in organized children groups made 36.3% and 49%, and, in case of PCR detection of DNA, made as much as 92.5% and 70.2%, respectively. Staphylococcus aureus carriage was identified in 23.7% cases (up to 50% in some categories of children). Enterobacteria in a nasopharyngeal microbiota of the population are detected in 2.4±0.37% cases, which is significantly more rare than in pneumonia children—in 13.9±1.2% cases (p < 0.001). The level of nasopharyngeal carriage of S. pneumoniae and H. influenzae, estimated by bacteriological and PCR methods, was found to be 36% and 86.7% of cases and 92.5% and 70.2% of cases, respectively. Moraxella catarrhalis DNA was detected in 73% of cases. The nature of discovered changes in microbial biocenosis in children of the Amur River area evidences of low colonization resistance of respiratory tract microbiota and a high risk of bacterial inflammation in the respiratory system.

References

1. Kozlov VK, Rakitskaya EV, Krasnova MA, Uchakina RV. Actual issues of adolescent medicine/Collect. abstr. Scientific-prakt. Konf. with international participation “Actual problems of the health of women and children at this stage,” October 31-November 1, 2006. Khabarovsk. 2006, pp. 3-18.
2. Kozlov VK, Evseeva GP. Medical and demographic characteristics and health status of children in the Far Eastern Federal District/Mater. First Congress of pediatricians of the Far East, 20-21 May 2010, Khabarovsk, 2010, pp. 3-11.
3. Tkachenko EI, Uspensky YP. Nutrition, microbiocenosis and the human intellect/St. Petersburg.: SpetsLit, 2006, pp. 167-93.
4. Donlan RM. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002; 15: pp. 3-18.
5. Kievit TR, Iglevski BH. Bacterial quorum sensing in pathogenic relationships. Infect Immun 2000; 68: 2027-30.
6. Miller MB, Bassler BL. Quorum sensing in bacteria. Annu Rev Microbiol 2001; 55: 165-99.

Implication of the role of stringent response in the expression of adenylate cyclase toxin in Bordetella pertussis

Tomoko Hanawa*, Kentaro Sugisaki, Hideo Yonezawa, Takako Osaki, Satoshi Kurata, Cynthia Zaman and Shigeru Kamiya

Department of Infectious Diseases, School of Medicine, Kyorin University, Shinkawa Mitaka, Tokyo, Japan

Bordetella pertussis is an etiological agent of whooping cough and produces a lot of virulence factors such as adhesins and toxins. The transcriptions of major virulence factors such as adenylate cyclase toxin (ACT), pertussis toxin and filamentous hemagglutinin are regulated by BvgSA two-component system. It is modulated by concentration of MgSO4, nicotinic acid or unknown factor(s) in the host environment.

ACT is an essential virulence factor for B. pertussis and other Bordetella species. This toxin is a bifunctional protein exhibiting both adenylate cyclase and hemolysin enzymatic domains and able to invade target cells and to impair intracellular functions. ACT is encoded by cyaA, and in the same operon, there are three genes, cyaB, cyaD and cyaE, encoding the type I secretion machinery members. After synthesis of a 177-kDa precursor and palmitoylation by CyaC, ACT is secreted as a 216-kDa active form by type I secretion system.

Stringent response is a most important stress response to the nutritional starvation. When amino acids or carbon sources are depleted, ppGpp molecules are accumulated in the cell and regulate various genes transcriptions positively or negatively. In consequence, growth rate is repressed to avoid death by overgrowth under the limited nutrients. In the present study, we found the increase of both cellular and secreted ACT by deletion of ppGpp synthetases in B. pertussis. Our findings suggest the regulation of expression of ACT by stringent response in B. pertussis.

Examination of growth conditions and nitrogen fixation capability of legumes selected from various sources in Northern Greece

Stavros Kazakos1, Stavros Plessas1*, Athanasios Alexopoulos1 and Eugenia Bezirtzoglou1,2

1Democritus University of Thrace, Faculty of Agriculture Development, Laboratory of Microbiology, Biotechnology and Hygiene, Orestiada, Greece; 2Democritus University of Thrace, Faculty of Agriculture Development, Laboratory of Food Processing, Orestiada, Greece
Legumes are a very significant research field in the last years due to the great importance of nitrogen fixation. In the frame of the present research work, various legumes were collected: *Cicer arietinum*, *Glycine max*, *Lathyrus sativus*, *Lathyrus clymenym*, *Lens culinaris*, *Lupinus albus*, *Pisum sativum* and *Vicia faba*. Afterward, nitrogen-fixing bacteria were isolated from legumes, and the possibility of nitrogen fixation was studied in vitro using the appropriate synthetic liquid (Yeast extract mannitol), as well as the conditions of growth in various acidities conditions. The results showed that the symbiotic relation between nitrogen-fixing bacteria and nodules is of great importance for the function of nitrogen fixation and that this function does not seem to work in vitro. Regarding the acidity study, it was clear that nitrogen-fixing bacteria were cultured successfully and that they have the same demands to those that have in the case of soil, with some small differences. In general, it was revealed that they do not grow in acidic conditions and they prefer pH values near to 7. Nevertheless, in the case of *L. culinaris* and *G. Max*, the highest biomass production was determined at pH value of 5. The explanation is due to the kind of bacteria isolated in the laboratory, since there are cases of bacteria such as *Rhizobium loti* and *R. tropici* that are resistant in low levels of pH. In general and according to international studies, it has been supported that different kinds of the same genus may show different in different levels of acidities.

*Stavros Plessas*
E-mail: splessas@agro.duth.gr

---

Biochemical studies on the virulence factors of fungi associated with she-camel milk

Abd El-Aziz Mosaad¹, Ahmed El-Kirdasy², Mostafa Al-Sherif³, Said Fathalla⁴*, Abd El-Rahman M El-Bagory⁵ and Gamal Abd El. Gaber⁶

¹Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Minufiya University (Sadat Branch), Egypt; ²Department of Biochemistry, and Chemistry of Nutrition, Faculty of Veterinary Medicine, Minufiya University (Sadat Branch), Egypt; ³Department of Biology, Faculty of Art and Science, Al-Mergeb University, El-khoms, Libya; ⁴Department of Physiol., Faculty of Veterinary Medicine, Minufiya University (Sadat Branch), Egypt; ⁵Department of Food Hygiene & Control, Faculty of Veterinary Medicine, Minufiya University (Sadat Branch), Egypt; ⁶Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Mansoura University, Egypt

Milk is an ideal habitat for the growth and multiplication of microorganisms due to its nutritional constitution which contains protein, carbohydrate, mineral and vitamins. All these components support the growth of many forms of bacteria, fungi and yeasts. Raw milk aseptically drawn from a healthy animal usually contains a few bacteria. This work aimed to study the virulent factors of isolated yeasts in raw camel milk. A total of 40 samples were collected from camel milk from different localities in Egypt and were subjected for subclinical mastitis ‘California mastitis test’. Results revealed that four samples (10%) have subclinical mastitis and the rest of samples were negative to the test. From the results of fungal examination and isolation on specific media, we found that six samples were single infection (15%) and two were mixed infection (5%). Identification of these isolates were *Aspergillus niger* 1 (2.5%), *Aspergillus fumigatus* 1 (2.5%), *Rhizopus* 1 (2.5%), *Mucor* 1 (2.5%), *Penicillium* 2 (5%), and *Candida albicans* 4 isolates (10%). We found that all *C. albicans* have ability to produce germ tube by incubation on rabbit serum, Chlamydospore, and phospholipase enzyme by using PCR to detect this enzyme as a rapid and sensitive method, so we found that all isolates have ability to produce that enzyme and we can use PCR also as a direct method for Identification with high efficacy. From the results, it can be concluded that: low incidence of fungal agents associated with she-camel’s milk and the main common moulds and yeasts associated with mastitis were zygomycetes ‘*Rhizopus* 1 (2.5%), *Mucor* 1 (2.5%)’, *A. fumigatus* 1 (2.5%), *A. niger* 1 (2.5%), *Penicillium* 2 (5%) and *C. albicans* 4 (10%). Many virulent factors of *C. albicans* can be detected and produced such as germ tube, chlamydospores. Also, proteolytic and phospholipase activities can be detected by using PCR.

**Keywords**
Camel milk; yeast; yeast

*Said Fathalla*
E-mail: saidfathalla@yahoo.com

---

Citation: Microbial Ecology in Health & Disease 2012, 23: 17461 - http://dx.doi.org/10.3402/mehd.v23i0.17461
In vivo antibacterial activity of Phx-3 against Helicobacter pylori

Fuhito Hojo1*, Takako Osaki2, Tomoko Hanawa2, Akio Tomoda3 and Shigeru Kamiya1,2

1Institute of Laboratory Animals, Graduate School of Medicine, Kyorin University, Tokyo, Japan; 2Department of Infectious Diseases, Division of Medical Microbiology, Kyorin University, School of Medicine, Tokyo, Japan; 3Department of Biochemistry and Intractable Immune System Disease Research Center, Tokyo Medical University, Tokyo, Japan

Phx-3 (2-amino-phenoxazine-3-one), one of the phenoxazine derivatives, is reported to have inhibitory effect on several pathogens. We have demonstrated that Phx-3 has antibacterial activity against Helicobacter pylori in vitro, but the effect of Phx-3 on H. pylori in vivo have not been assessed. Then, we examined the in vivo antibacterial activity of Phx-3 by using the mice infected with H. pylori Sydney Strain 1 (SS1). In the present study, it has been shown that colony-forming unit of H. pylori isolated from stomach significantly reduced by Phx-3. In addition, histological analysis has shown that plasma cells and Russel bodies were observed in gastric submucosa of mice treated with Phx-3, indicating induction of humoral immunity.

*Fuhito Hojo
E-mail: f-hojo@ks.kyorin-u.ac.jp

Detection of Campylobacter is possible using their physical characters

Majid Baserisalehi*

Department of Microbiology, Islamic Azad University-Kazeroun branch, Kazeroun, Iran

The major aim of this study was introduced the novel device (Kapadnis–Baseri device) and method (prêt-Kapadnis Baseri) for isolation of Campylobacter spp without using selective media incorporated antibiotics. In the present study, detection of these bacteria was carried out using their physical characters (viz., motility and its activity at low temperature) instead of their antibiotic resistant character. To determine ability of these methods for isolation of Campylobacter, their isolation rate was evaluated in comparison with the conventional methods.

In total, 238 environmental samples (animal feces, viz., cow and sheep) were collected and 68 Campylobacter strains were isolated from them. Of all isolates, 39 and 19 strains were isolated by the new methods and conventional methods, respectively. Therefore, the rate of Campylobacter isolation using the new methods was high in comparison with conventional methods. Overall, the KB device and the prêt KB method were designed and developed on the basis of unique characters of Campylobacter, viz., motility and its activity at low temperature (6°C). It means that the KB device and the prêt KB method minimize population of competing bacteria and detect Campylobacter from the environmental samples without using antibiotics. Hence, based on foregoing evidence, the new device and the method will be useful for isolation and enumeration of Campylobacter from environmental samples. Besides, these methods would also detect antibiotic sensitive campylobacters, which are not detected by conventional methods.

*Majid Baserisalehi
E-mail: majidbaseri@hotmail.com

Bacillus probiotics: biological and clinical effects

Larisa A. Safronova*

Zabolotny Institute of Microbiology and Virology, Kiev, Ukraine

Production of effective and safe for human and environment biological preparations for medicine and agriculture on the basis of microorganisms is quickly growing area of biotechnological market. Use of such preparations promotes strengthening of human health and does not lead to negative consequences for an organism and an environment.

The bacteria of the genus Bacillus are widely occurred in the environment, including those objects, with which human and
the past two decades, *S. epidermidis* have emerged as one of the major pathogens in nosocomial infections. The primary pathogenicity trait of *S. epidermidis* is associated with its ability to form biofilms on surfaces of medical devices, limiting severely the efficacy of many conventional antibiotics, and biofilms may also protect the bacteria against attacks from the host defense system. Because, to date, there is no effective antibiotic to eradicate biofilm infection, the development of biofilm-preventing vaccines is necessary.

Biofilm formation by *S. epidermidis* requires accumulation-associated protein (Aap), which is considered one of the most important protein-based adhesins. Aap contains sequence repeats known as G5 domains. The zinc-dependent dimerization of G5 domains could be responsible for the function of Aap to mediate the intercellular adhesion of *S. epidermidis*. Antibodies against Aap have been reported to inhibit biofilm accumulation by *S. epidermidis*, indicating that Aap could serve as a vaccine candidate to prevent *S. epidermidis* biofilm infection. However, the full-length Aap is not considered to be a safe vaccine for systemic immunization because such bacterial antigens contain many antigenic determinants and may induce hypersensitivity reactions. A peptide that induces antibiofilm humoral immunity would be an optimal vaccine. The Aap C-terminal single B-repeat construct followed by the 79-aa half repeat (AapBrpt1.5) is the basic functional unit of Aap, which is necessary to mediate bacterial accumulation, suggesting that it harbors the epitopes that would guide the development of biofilm-preventing epitope-based peptide vaccines.

In the institute of Microbiology and Virology of NAS of Ukraine, a number of preparations containing live cultures of bacilli for medicine and agriculture are developed. High efficacy and safety of probiotic preparations have been shown. Probiotics biosporin and subalin are successfully used in medical practice.

*Larisa A. Safronova*  
E-mail: safronova_larisa@ukr.net

## Monoclonal antibodies against the accumulation-associated protein influence EPS biosynthesis and enhance bacterial accumulation of *Staphylococcus epidermidis*

Yang Wu, Jian Hu, Tao Xu, Huayong Liu, Youcong Wu and Di Qu*

Key Laboratory of Medical Molecular Virology of Ministries of Education and Health, Institute of Medical Microbiology and Institutes of Biomedical Sciences, Fudan University, Shanghai, China

*Staphylococcus epidermidis* is a normal inhabitant of human skin and mucous membranes that rarely causes pyogenic infections in healthy individuals. However, during the past two decades, *S. epidermidis* has emerged as one of the major pathogens in nosocomial infections. The primary pathogenicity trait of *S. epidermidis* is associated with its ability to form biofilms on surfaces of medical devices, limiting severely the efficacy of many conventional antibiotics, and biofilms may also protect the bacteria against attacks from the host defense system. Because, to date, there is no effective antibiotic to eradicate biofilm infection, the development of biofilm-preventing vaccines is necessary.

To locate the epitopes in Aap that induce antibiofilm humoral immunity, monoclonal antibodies (MAbs; MAb18B6, MAb25C11 and MAb20B9) against AapBrpt1.5 were prepared in the present study. All of the MAbs were generated bound to Aap antigens at extremely high affinities. However, only MAb18B6 was found to possess the weak but broad-spectrum activity necessary to inhibit biofilm formation by *S. epidermidis*, whereas MAb25C11 and MAb20B9 enhanced the biofilm formation by some strains of *S. epidermidis*, including RP62A. All of the MAbs strengthened the cell aggregation of planktonic *S. epidermidis*. The epitope of each MAb was identified by using protein truncation and immunoprecipitation. Epitope mapping of the MAbs revealed that the biofilm-inhibiting MAb18B6 recognized an identical area within all Aap-Brpt constructs from *S. epidermidis* RP62A, which was not shared by the other two MAbs. To explore the reasons for the biofilm-enhancing activities of the MAbs, EPS biosynthesis was studied by detecting Aap expression, extracellular DNA release and PIA synthesis in *S. epidermidis* RP62A co-cultured with the MAbs. All MAbs were found to affect EPS biosynthesis in *S. epidermidis* RP62A co-cultured with the MAbs. All MAbs were found to affect EPS biosynthesis in *S. epidermidis* and further enhance the bacterial accumulation. The different activities of the MAbs on biofilm formation could be related to the resultant effect of inhibition of Aap dimerization (inhibiting biofilm formation) and alteration of EPS biosynthesis (enhancing biofilm formation).

In addition to our novel findings on the altered EPS biosynthesis in *S. epidermidis* mediated by mouse MAbs against Aap, the epitope mapping of biofilm-affecting MAbs will, for the first time, contribute to a better understanding of staphylococcal biofilm formation and help develop epitope-peptide vaccines against staphylococcal infections.
Eubiotics in medicine

Nina N. Gavrilova and Irina A. Ratnikova*

The Institute of Microbiology and Virology, Almaty, Kazakhstan

**Plantafermin:** The increased incidence of dysbacteriosis observed in the last decade among more than 90% of population is associated with environmental degradation, uncontrolled use of antimicrobial drugs, stress, poor food quality and other factors due to which it can be considered a social phenomenon. Intestinal dysbiosis weakens body defenses, increases susceptibility to infectious diseases and causes allergic reactions (exudative diathesis, food allergy and seborrhea). Intestinal dysbiosis leads to protracted, recurrent course of diseases, development of complications, disturbance of digestion and absorption of food.

Lactic acid and bifidobacteria preparations are currently proposed for struggling with dysbacteriosis: Bifidumbacte-rine, Lactobacterine, Linex, etc. However, they are not much efficient possibly due to low range of probiotic bacteria antimicrobial activity.

The Institute of Microbiology and Virology of the Ministry of Education and Science of the Republic of Kazakhstan formulated a broad-spectrum therapeutic drug Plantafermin based on lactic acid bacteria and bifidobacteria. The eubiotic drug is effective against dysbacteriosis of various etiology in children from infancy and adults, inflammatory and infectious diseases; in treatment of gastric and intestinal dyspepsia, defecation disorders, inflammatory and infectious diseases of gastrointestinal tract, liver and bile passage diseases, inflammatory and functional disorders of pancreatic gland; in treatment of inflammatory and infectious urogenital diseases; in treatment of candidiasis of different localization, its prevention in the period of antibiotic treatment, hormonal, cytostatic and immunosuppressive drug administration; for prevention of dysbacteriosis in stress, increased mental and physical tension under unfavorable environmental conditions.

**Fermented Beet Juice:** Beet juices and drinks are useful in dietary nutrition due to the content of proteins, carbohydrates, fiber, free organic acids, potassium, sodium, vitamins B1, B2, PP, provitamin A and vitamin C in optimum ratios. The presence of polyphenols predetermines its use in irradiation injuries, cancerous, cardiovascular diseases and atherosclerosis.

We have developed a technology for production of beet juice fermented by specially selected strains of lactic acid bacteria. In the fermented juice: the content of coloring agent bethanidine is stabilized, and the quantity of essential amino acids and vitamins is increased—lysine by 25%, threonine by 60%, valine by 14%, isoleucine by 33%, leucine by 50% phenylalanine by 15%, vitamin B1 by 60%, vitamin B2 by 50%, vitamin C by 27%, water-soluble pectins by 1% with simultaneous reducing of nitrate content. Clinical trials carried out at the Nutrition Research Center of Kazakhstan showed that fortified fermented juice has positive therapeutic efficacy in treatment of chronic diseases: gastritis, enterocolitis, hepatitis, cholecystitis, and can be used as a curative drink.

*E-mail: iratnikova@almanet.kz*
**Purpose:** HHV-6 infection is one of the major morbidity and mortality after hematopoietic stem cell transplantation (HSCT), and early diagnosis of HHV-6 viremia and evaluation of specific cellular immunity are needed.

**Methods and Patients:** 1. HHV-6 viremia was assayed for 933 blood samples of 131 allogeneic HSCT recipients by a real-time PCR test, which was designed to specifically detect HHV-6.

2. HHV-6-specific cellular immunity was evaluated using peripheral blood mononuclear cells of 114 allogeneic HSCT patients by an LPR assay. Normal healthy volunteers served as positive controls and 11 cord blood donors as negative controls.

**Results:** 1. HHV-6 was frequently detected in the blood samples in the first 2 to 6 weeks after HSCT.

2. HHV-6-specific LPR became positive 4 to 8 weeks after HSCT following HHV-6 viremia, and HHV-6 viremia usually disappeared following positive LPR, but low number of HHV-6 copies could be detected in a small portion of patients for months after HSCT.

**Conclusion:** HHV-6 infection could be specifically and quantitatively in HSCT patients.

This work was supported in part by the Health and Labour Sciences Research Grant(s) for research on measures for emerging and reemerging infections.

*Shunichi Kato
E-mail: skato@is.icc.u-tokai.ac.jp

Citation: Microbial Ecology in Health & Disease 2012, 23: 17461 - http://dx.doi.org/10.3402/mehd.v23i0.17461