12th International Symposium on Molecular Cell Biology of Macrophages 2003

Utsunomiya, Japan; June 19 and 20, 2003

Organized by Japanese Society for Molecular Cell Biology of Macrophages

Under the Auspices of SORST, Japan Science and Technology Corporation (JTSC)

Welcome to Utsunomiya

It is our great pleasure to host the 12th International Symposium on Molecular Cell Biology of Macrophages 2003. The Japanese Society for Molecular Cell Biology of Macrophages was founded in 1991 in order to promote basic science as well as clinical research on macrophage biology. This meeting has been held annually and made a large contribution to the world-wide advance of this research field.

This year's meeting will address the three focused themes of "Role of macrophages/DC in allergy and autoimmune diseases", "Role of spleen in the host defense", and "Trafficking of macrophages and DCs and immune responses". The symposium will last two full days and consist of lectures by invited speakers followed by extended discussions. We hope to create a platform of mutual interactions and friendship among all speakers and attendants from abroad and Japan during the meeting. We sincerely hope that you will enjoy the symposium and Utsunomiya.

Kenjiro Matsuno
Conference Chairperson

http://macro.dokkyomed.ac.jp/mp2003/

Conference Chairperson:
Kenjiro Matsuno, MD.
Dept. Anatomy (Macro),
Dokkyo Univ. Sch. Med.
Phone: [81]-282-86-6238
Fax: [81]-282-96-6229
E-Mail: kenjiro@dokkyomed.ac.jp

Organizing Committee:
Kouji Matsushima
The University of Tokyo

Tadashi Kasahara
Kyoritsu College of Pharmacy

Makoto Naito
Niigata University

Kenjiro Matsuno
Dokkyo University

Shizuo Akira
Osaka University

Yoshiro Kobayashi
Toho University

Masanobu Kawakami
Jichi Medical College

GENERAL INFORMATION

REGISTRATION

Symposium will be held at Tochigi Prefectural Culture Center, Subhall (TochigiKen Sougou Bunnka Center, 1-8 Hon-cho, Utsunomiya, Tochigi, phone 028-643-1000). Registration desk will be open 8:30 am on 6/198 (Thurs) and 6/20 (Fri) in the front of the hall.

REISTRATION FEES

Participants: Member 5,000 yen, Member student 3,000 yen, Nonmember 8,000 yen, Nonmember student 5,000 yen.

PRESENTATION

We will provide the necessary equipment for power point presentations. Bring your own laptop computer, CD-ROMs, or MO. In order to avoid technical problems during your presentation, speakers are requested to contact as early as possible, at least 60 min prior to the presentation. The type of the computer should be informed beforehand.

POSTER SESSION

Poster Session with cheese and wine will be held at a lobby in front of the Subhall (free drinks and snacks). You may enjoy discussion and wine under a relaxed atmosphere.

POSTER AWARDS

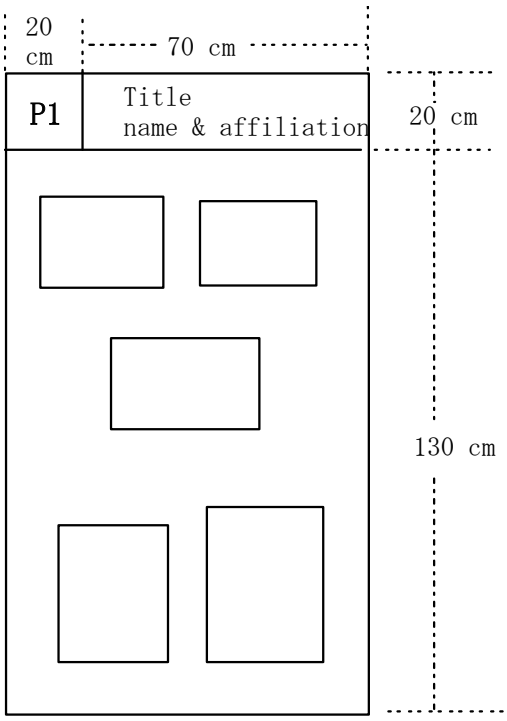
This new award is intended for deserving investigators who have made significant contributions to the poster session of this Symposium. The committee will select two awardees among all posters during the poster session in the evening of the first day. Awardees of the competition will receive a certificate of commendation and 100,000 yen (First Place) or 50,000 yen (Second Place) at the first session of the second day afternoon. All top authors who submit the poster abstract will automatically become the candidates of the competition. We strongly encourage you to submit the poster and enjoy the challenge.

The awardees must be members of The Japanese Society for Molecular Cell Biology of Macrophages at the time of award. Two awardees are kindly asked to make a short oral presentation of their posters: 10 min for talk and 5 min for discussion. Accordingly, all candidates should prepare for the oral presentation and be ready for talk in case being

selected. Please bring your Power Point files for talk to the meeting.

POSTER PRESENTATION

The size of the poster board is 0.9 m wide, and 1.8 m high. Panels should be within the area of 90 cm wide, and 150 cm high including a title panel (70 cm wide and 20 cm high). All abstracts will be posted during the 2-day session period.



GET TOGETHER PARTY

To be held from 18:00 to 20:00, 6/19 (Thur) in Rehearsal Room (**B2F**) at Tochigi Prefectural Culture Center just after poster session. Wines and draught beer will be also served. Booking at the time of registration will be highly recommended. Cost: **3,000** yen.

Program

June 19 (Thursday)

8:30 -	**Registration and Poster Mounting**
9:00 - 9:05	**Opening Remarks** **Kenjiro Matsuno** (Dokkyo Univ, Tochigi)

Session 1: Role of Macrophages/DCs in Allergy and Autoimmune Diseases

Organizers: **Susumu Ikehara** and **Richard Burt**

9:10-9:50 (12 p-)	Autoimmune diseases as stem cell disorders: Abnormalities of hemopoietic cells including dendritic cells **Susumu Ikehara** (Kansai Medical Univ, Osaka)
9:50-10:30 (14 p-)	Pathogenesis of Guillain-Barré syndrome subsequent to *Campylobacter jejuni* enteritis **Nobuhiro Yuki** (Dokkyo Univ, Tochigi)
10:30-10:50	**Coffee Break**
10:50-11:30 (16 p-)	Embryonic stem cells as an alternative donor marrow source and adoptive cellular (e.g. .. macrophage) source **Richard Burt** (Northwestern Univ Medical Center, Chicago, USA)
11:30-12:10 (17 p-)	The plasmacytoid dendritic cell and CpG oligonucleotides in immunotherapy **Güenther Hartmann** (Ludwig-Maximilians-Univ Munich, Germany)
12:10-13:10	**Lunch and Poster Viewing**

Session 2: Role of Spleen in the Host Defense

Organizers: **John Kearney and Kenjiro Matsuno**

13:10-13:50 (18 p-)	Still unresolved mysteries of the spleen **Kenjiro Matsuno** (Dokkyo Univ, Tochigi)
13:50-14:20 (19 p-)	Structure of mouse spleen investigated by hyper-spectral imaging **Hiromichi Tsurui** (Juntendo Univ, Tokyo)

14:20-14:40 **Coffee Break**

14:40-15:20 Signals and cells for memory CD4 T cells
(21 p-) **Peter Lane** (Birmingham Univ, UK)
15:20-16:00 Marginal zone B cells: Development and Function
(22 p-) **John Kearney** (Univ Alabama at Birmingham, USA)

16:00-16:15 **Coffee Break**

16:15-16:55 The spleen response to *Leishmania donovani*: a model for examining the
(23 p-) varied roles of macrophages and DC in infectious disease
Paul M. Kaye (London School of Hygiene and Tropical Medicine, UK)

16:55-17:05 **Business Meeting**
17:00-18:00 **Poster Session with Cheese and Wine** (Free Drinks)
18:00-20:00 **Get-Together Party**

June 20 (Friday)

Session 3: Trafficking of Macrophages and DCs in Immune Responses 1

Organizers: **Kouji Matsushima and Gordon MacPherson**

9:00-9:40 Visualizing the movement of developing T cells in the thymus
(25 p-) **Yousuke Takahama** (Tokushima Univ and RIKEN RCAI)
9:40-10:20 Leukocyte and monocyte trafficking in inflammation and atherosclerosis
(26 p-) **Klaus Ley** (Univ Virginia Health System, Charlottesville, USA)

10:20-10:40 **Coffee Break**

10:40-11:20 Dendritic cell migration in lymph from peripheral tissues
(28 p-) **Gordon MacPherson** (Oxford Univ, UK)
11:20-12:00 The *in vivo* trafficking, relationship, and function of myeloid and
(29 p-) plasmacytoid DC precursors
Hiroyuki Yoneyama (Tokyo Univ, Tokyo)

12:00-13:00 **Lunch and Poster Viewing**

13:00-13:20 **Honorary Lecture**
(30 p-) History of Macrophage Research
Kiyoshi Takahashi (Kumamoto Univ, Kumamoto)

13:20-13:50 **Poster Awards** (1st and 2nd Places) to be elected

Session 4: Trafficking of Ma34crophages and DCs in Immune Responses 2

Organizers: **Tatsuro Irimura and Teunis Geijtenbeek**

13:50-14:30 A fatal attraction: *Mycobacterium tuberculosis* and HIV-1 Target DC-
(32 p-) SIGN to escape immune surveillance
Teunis Geijtenbeek (Amsterdam Free Univ, The Netherlands)

14:30-14:50 **Coffee Break**

14:50-15:20 Heparanase at the invasive edge of migrating macrophages
(34 p-) **Nobuaki Higashi** (Tokyo Univ, Tokyo)
15:20-16:00 Transcriptome analysis of monocyte/macrophage differentiation and
(36 p-) monocyte/endothelial cell interaction
Tatsuhiko Kodama (Tokyo Univ, RCAST, Tokyo)

16:00-16:15 **Concluding Remarks** **Kouji Matsushima** (Tokyo Univ, Tokyo)

Poster presentation (38 p-)

P1

Visualizing of antigen-trafficking from skin both in steady and active state using hyperpigmented mice

Miya Yoshino, Hidetoshi Yamazaki and Shin-Ichi Hayashi

Div. Immunology, Dept. Molecular and Cellular Biol., Sch. Life Science, Fac. Med., Tottori Univ., 86 Nishi-Machi, Yonago, Japan

P2

Aberrant B1 cell trafficking in a murine model for SLE: Possible roles for autoantibody production by B1 cells and for breakdown of central tolerance in the thymus.

Sho Ishikawa, Taku Sato, Toshihiro Ito, Kenji Akadegawa, Hideaki Yurino, Masahiro Kitabatake, and Kouji Matsushima

Department of Molecular Preventive Medicine, School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan

P3

Regulation of Kupffer cell accumulation by chemokines in murine liver metastasis model

Naofumi Mukaida, Yoko Nemoto-Sasaki, and Xiaoqing Yang

Div. Molec. Bioregulation, Cancer Research Institute, Kanazawa University, Kanazawa, 920-0934, Japan

P4

Functional analysis of tumor-infiltrating plasmacytoid dendritic cells in head and neck cancer

E. Tuma[1], S. Rothenfusser[3], O. Gires[1], B. Wollenberg[2], G. Hartmann[3]

(1) Dept. of Otorhinolaryngology, Großhadern Medical Center, Ludwig-Maximilians-University Munich, Germany (2) Dept. of Otorhinolaryngology, University of Lübeck, Germanyand (3) Clinical Pharmacology, Ludwig-Maximilians-University of Munich, Germany

P5

Changes in macrophage number and distribution in the rat thymus after X-ray irradiation

Nobuko Tokuda, Keiko Hamasaki, Noriko Mizutani, Yamini Arudchelvan, Yasuhiro Adachi, Tomoo Sawada, *Yoshihisa Fujikura and Tetsuo Fukumoto

Dept. Human Science, Yamaguchi Univ. School of Medicine, Ube, Yamaguchi 755-8505, Japan, *Dept. Anatomy, Oita Medical Univ., Oita, Japan

P6

Prognostic importance of activated macrophage-derived soluble CD163 in fulminant hepatitis

Atsushi Hiraoka [1], Norio Horiike [1], Sk. Md. Fazle Akbar [1], Takami Matsuyama [2], Morikazu Onji [1]

[1] The Third Department of Internal Medicine, Ehime University School of Medicine, Ehime, Japan. [2] Department of Immunology, Kagoshima University School of Medicine, Kagoshima, Japan

P7

Development of a novel optical assay system for measurement of cellular chemotaxis

Shiro Kanegasaki, Nao Nitta, Yuka Nomura, Shuichi Akiyama and Takuya Tamatani

Effector Cell Institute in RCAST, Univ. of Tokyo, Tokyo 153-8904, Japan

P8

Characterization of murine grancalcin expressed in neutrophils and macrophages and its possible role in the defense against bacterial infections

Fengzhi Liu, Hiroto Shinomiya, Teruo Kirikae, Yoshihiro Asano

Department of Immunology and Host Defenses, Ehime University School of Medicine, Ehime; International Medical Center of Japan, Tokyo; Japan

P9

Sinomenine inhibits dendritic cells maturation and migration leading to promotion of allograft acceptance

Quanxing Wang[1,2], Baomei Wang[1], JianchunWu[1],Yushan Liu[1], Wenji Sun[1], Minhui Zhang[1], Min Zhang[1], Jianli Wang[1] , Xuetao Cao[1], Xiaokang Li[2]

1 Institute of Immunology, Second Military Medical University, Shanghai 200433, P.R.China; 2 National Medical research Institute for Child health, Tokyo 154-8567, Japan

P10

BCG induced Hepatic Granuloma Formation in TACO transgenic mice

Takayuki Hirose[1], Yuusuke Ebe[1], Takashi Yamamoto[1], Jean Pieters[2] and Makoto Naito[1]

[1]Department of Cellular Function, Division of Cellular and Molecular Pathology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

[2]Department of Immunology, Basel University, Basel, Switzerland

P11

Cell parking of GFP positive bone marrow cells after intra-portal injection in rats

Yuka Igarashi[*], Yuki Sato[*], Yoji Hakamata, Takashi Kaneko, Takashi Murakami, Masafumi Takahashi, and Eiji Kobayashi

[*]The first two authors were equally contributed.

Division of Organ Replacement Research and Animal Resource Project, Center for Molecular Medicine, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi, Kawachi, Tochigi, Japan

P12

Identification of Sialoadhesin as a Ligand for MGL1 in Murine Lymph Nodes: Potential Roles in the Interaction between Two Types of Macrophages within Subcapsular Sinus

Yosuke Kumamoto, Nobuaki Higashi, Koji Sato, Kaori Denda-Nagai, Makoto Tsuiji, *Paul R. Crocker, and Tatsuro Irimura

Laboratory of Cancer Biology and Molecular Immunology, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan, * The Wellcome Trust Biocentre, School of Life Sciences, University of Dundee, Dundee, UK

P13

Presentation of Glycosylated Antigens taken up by the Macrophage Galactose-type C-type Lectin on Bone Marrow-Derived Immature Dendritic Cells

Kaori Denda-Nagai, Satoshi Aida, Makoto Tsuiji, Stephen M. Hedrick* and Tatsuro Irimura

Laboratory of Cancer Biology and Molecular Immunology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan and *the Cancer Center and the Division of Biology, University of California, San Diego, La Jolla, California 92093-0687

P14

Selective expression of chemokines in the *Salmonella*-infected spleen

Hiroto Shinomiya[1], Fengzhi Liu[1], Teruo Kirikae[2], Asano Yoshihiro[1]

[1]Department of Immunology and Host Defenses, Ehime University School of Medicine, Ehime; [2]International Medical Center of Japan, Tokyo; Japan

P15

Localization of marginal zone macrophages is regulated by CCR7 signalling.

Manabu Ato, *Hideki Nakano, *Terutaka Kakiuchi, and Paul M. Kaye

Department of Infectious Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, the United Kingdom; *Department of Immunology, Toho University School of Medicine, Tokyo 143-8540, Japan

P16

K-567 cells lack MHC Class II expression due to an alternatively spliced CIITA transcript with a truncated coding region

Noel E. Day[1,2], Hideyo Ugai[2], Albert T. Ichiki[1] and *Kazunari K. Yokoyama[2]

[1]Department of Medical Genetics, Graduate School of Medicine, The University of Tennessee Medical Center, Knoxville, Tennessee 37920, USA; [2]Gene Engineering Division, Department of

Biological Systems, BioResource Center, RIKEN (The Institute of Physical and Chemical Research), Tsukuba, Ibaraki 305-0074, Japan.

P17

Possible involvement of muscularis resident macrophages in the impairments of interstitial cells of Cajal and myenteric nerve systems in Crohn's colitis model

Kazuya Kinoshita[1], Kazuhide Horiguchi[1], Shigeru Yamato[2], Masahiko Fujisawa[1], Koichi Sato[1], Masatoshi Hori[1] and Hiroshi Ozaki[1]*

[1] Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

[2] International Medical Center of Japan, Toyama 1-21-1, Shinjuku-ku, Tokyo 162-8655, Japan

P18

Interleukin-10-induced CCR5 expression in macrophage-like HL-60 cells: involvement of Erk1/2 and STAT-3

Yoshiko Sonoda, Yoko Makuta, Daisuke Yamamoto, Eriko Aizu-Yokota, and Tadashi Kasahara

Department of Biochemistry, Kyoritsu College of Pharmacy, Shibakoen 1-5-30, Minato-ku, Tokyo 105-8512

P19

TGF beta down-regulates IL-1-induced functional Toll like receptor (TLR) 2 expression in murine hepatocytes

Hayashi Hidetoshi[1], Takayuki Matsumura[1,2], Takemasa Takii[1], Jun-ichiro Inoue[2], Kikuo Onozaki[1]

[1]Dept. Mol. Health Sci., Grad. Sch. Pharm. Sci., Nagoya City Univ., Nagoya 467-8603, Japan, [2]Div. Cell. Mol. Biol., Inst. Med. Sci., Univ. Tokyo, Tokyo 108-8639, Japan

P20

A high endothelial venule secretory protein, mac25/angiomodulin, interacts with multiple high endothelial venule-associated molecules including chemokines

Daisuke Nagakubo[1,2], Toshiyuki Murai[1], Toshiyuki Tanaka[1], Takeo Usui[1], Masanori Matsumoto[1], Kiyotoshi Sekiguchi[3] and Masayuki Miyasaka[1]

[1]Lab. Molecular and Cellular Recognition, Osaka Univ. Graduate School of Medicine, Suita, Japan, [2]Dept. Microbiology, Kinki Univ. School of Medicine, Osaka-Sayama, Japan, [3]Div. Protein Chemistry, Institute for Protein Research, Osaka Univ., Suita, Japan.

P21

MEC/CCL28 Has Dual Functions in Mucosal Immunity as a Chemoattractant with Broad-Spectrum Antimicrobial Activity

Kunio Hieshima, Yuri Kawasaki, Takashi Nakayama, and Osamu Yoshie

Department of Microbiology, Kinki University School of Medicine, Osaka 589-8511, Japan

P22

The B cell chemokine CXCL13 B-lymphocyte chemoattractant (BLC) is expressed in the high endothelial venules of lymph nodes and Peyer's patches and affects B cell trafficking across high endothelial venules

Yukihiko Ebisuno[1], Toshiyuki Tanaka[1], Naotoshi Kanemitsu[1], Hidenobu Kanda[1], Kazuhito Yamaguchi[2], Tsuneyasu Kaisho[3], Shizuo Akira[3] & Masayuki Miyasaka[1]

[1]Laboratory of Molecular and Cellular Recognition, Osaka University Graduate School of Medicine, Suita, 565-0871, Japan, [2]Institute of Laboratory Animals, Yamaguchi University School of Medicine, Ube, 755-8505, and [3]Institute of Microbial Diseases, Osaka University, Suita, 565-0871.

P23

GM-CSF INDEPENDENT DEVELOPMENT OF DENDRITIC CELLS (DC) FROM BONE MARROW CELLS (BMC) IN GM-CSF RECEPTOR DEFICIENT MOUSE

Hua Yan, *Robb L, Hajime Hikino, Tohko Miyagi, Saito Hirohisa, Daniel P. Gold, Xiao-K Li,., Hiromitsu Kimura.

National Research Center for Child Health and Development, Tokyo, Japan

*Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia. Dept. of Research Surgery,

P24

CYTOKINE REQUIREMENT FOR THE GENERATION OF A LARGE NUMBER OF RAT DENDRITIC CELLS (DC) BY IN VITRO CULTURING OF BONE MARROW CELLS AND IT'S SELECTION OF CD161 (NKR-P1A)

Eigo Satoh*, Hua Yan, Tohko Miyagi, Saito Hirohisa, Daniel P. Gold, Xiao-K. Li, Masayuki Fujino, Kenichi Teramoto*, Shigeki Arii*, Hiromitsu Kimura.

Department of Research Surgery, National Research Center for Child Heath and Development, Tokyo, Japan *Department of Surgery, Tokyo Medical and Dental University, School of Medicen, Tokyo, Japan

P25 **Recruitment and involvement of cells expressing a macrophage galactose-type C-type lectin 1/2 (MGL1/2) in the antigen-specific inflammatory tissue formation**

Kayoko Sato*, Yasuyuki Imai[#], Nobuaki Higashi*, Yosuke Kumamoto*, and Tatsuro Irimura*

*Laboratory of Cancer Biology and Molecular Immunology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan and

[#]Department of Microbiology and COE Program in the 21st Century, University of Shizuoka School of Pharmaceutical Sciences, Shizuoka 422-8526, Japan

Autoimmune Diseases As Stem Cell Disorders: Abnormalities of Hemopoietic Cells Including Dendritic Cells

Susumu Ikehara

First Department of Pathology, Transplantation Center, Regeneration Research Center for Intractable Diseases, Kansai Medical University, Osaka 570-8506, Japan.

Bone marrow transplantation (BMT) is becoming a powerful strategy for the treatment of hematologic disorders, congenital immunodeficiencies, metabolic disorders and also autoimmune diseases. Using various animal models for autoimmune diseases, we have previously found that allogeneic BMT (not autologous BMT) can be used to treat autoimmune diseases[1]. In contrast, we have found that the transplantation of T-cell-depleted bone marrow cells or partially purified hemopoietic stem cells (HSCs) from autoimmune-prone mice to normal mice leads to the induction of autoimmune diseases in the recipients[2]. These findings have recently been confirmed even in humans. Owing to these findings, we have proposed that autoimmune diseases are "stem cell disorders."

Indeed, there have been many reports indicating that various abnormalities exist in T cells, B cells, and macrophages of both autoimmune-prone mice and patients with autoimmune diseases.

We have very recently found that the number of $CD11c^{+}CD3^{-}B220^{-}$ cells is increased in the various organs, including the peripheral blood, of autoimmune-prone mice such as (NZW x BXSB)F1 (W/BF1), (NZB x NZW)F1(B/WF1) and NZB mice in comparison with normal mice. In addition, we have found that the injection of $CD11c^{+}CD3^{-}B220^{-}$ cells from old W/BF1 mice to young W/BF1 mice transiently induces autoimmune disease (thrombocytopenia)[3]. This suggests that DCs play a crucial role in the induction and acceleration of autoimmune diseases. Thus, the abnormalities of autoimmune-prone mice originate in the hemopoietic stem cells. This being the case, BMT from normal donors should be carried out.

We have very recently established new strategies for BMT. We developed, using cynomolgus monkeys, a "Perfusion Method" (PM), which can replace the conventional aspiration method for collecting bone marrow cells (BMCs) and thus minimize the contamination of BMCs with T cells form the peripheral blood[4].

We injected the BMCs collected this way directly into the bone marrow cavity of recipients (intra-bone marrow [IBM]-BMT) that had received fractionated irradiation.

This "IBM-BMT" was found to be effective in treating autoimmune diseases in chimeric-resistant and radiation-sensitive MRL/lpr mice[5]. In addition, this strategy was found to be applicable for the transplantation of organs such as the skin and pancreas islets in mice and rats.

We are now carrying out allogeneic BMT using "PM + IBM-BMT" in monkeys in preparation for human application.

We believe that this method heralds a new transplantation era in BMT, gene therapy, organ transplantation and regeneration therapy, since "PM + IBM-BMT" can efficiently recruit

not only donor-derived pluripotent hemopoietic stem cells but also donor-derived mesenchymal stem cells into recipients.

References

1. Ikehara S, Good RA, Nakamura T, Sekita K, Inoue S, Maung Maung Oo, Muso E, Ogawa K, and Hamashima Y: Rationale for bone marrow transplantation in the treatment of autoimmune diseases. Proc. Natl. Acad. Sci. 82:2483-2487, 1985.
2. Ikehara S, Kawamura M, Takao F, Inaba M, Yasumizu R, Soe Than, Hisha H, Sugiura K, Koide Y, Yoshida TO, Ida T, Imura H, and Good RA: Organ-specific and systemic autoimmune diseases originate from defects in hematopoietic stem cells. Proc. Natl. Acad. Sci. 87:8341-8344, 1990.
3. Adachi Y, Taketani S, Toki J, Ikebukuro K, Sugiura K, Oyaizu H, Yasumizu R, Tomita M, Kaneda H, Amoh Y, Ito T, Okigaki M, Inaba M, and Ikehara S: Marked increase in number of dendritic cells in autoimmune-prone (NZW x BXSB)F1 mice with age. Stem Cells 20: 61-72, 2002.
4. Kushida T, Inaba M, Ikebukuro K, Nagahama T, Oyaizu H, Lee S, Ito T, Ichioka N, Hisha H, Sugiura K, Miyashima S, Ageyama N, Ono F, Iida H, Ogawa R, and Ikehara S: A new method for bone marrow cell harvesting. Stem Cells 18: 453-456, 2000.
5. Kushida T, Inaba M, Hisha H, Ichioka N, Esumi T, Ogawa R, and Ikehara S: Intra-bone marrow injection of allogeneic bone marrow cells: A powerful new strategy for treatment of intractable autoimmune diseases in MRL/lpr mice. Blood 97: 3292-3299, 2001.

Figures

Pathogenesis of Guillain-Barré syndrome subsequent to *Campylobacter jejuni* enteritis

Nobuhiro Yuki

Department of Neurology, Dokkyo University School of Medicine, Japan

The term Guillain-Barré syndrome (GBS) defines a recognizable clinical entity characterized by rapidly evolving symmetrical limb weakness and loss of tendon reflexes. Since the near-elimination of poliomyelitis throughout the world, GBS ranks as the most frequent cause of acute flaccid paralysis, the annual incidence being 1 or 2 cases per 100 000 populations. GBS had been considered a single disorder, acute inflammatory demyelinating polyneuropathy. However, the existence of primary axonal GBS, acute motor axonal neuropathy (AMAN), was established. Some form of infection precedes nearly two-thirds of GBS cases. In developed countries, *Campylobacter jejuni* is a leading cause of acute gastroenteritis. A prospective case-control study showed evidence of recent *C. jejuni* infection in 26% of the patients with GBS as compared with 2% of the household controls. This study established an epidemiological association between *C. jejuni* infection and GBS. The median interval from the onset of diarrhea to the onset of limb weakness is 9 days. In contrast with severe limb weakness, cranial nerve is less frequent. Sensory impairment is less common. The electrodiagnosis is AMAN. In AMAN, IgG is deposited on the axolemma of the spinal anterior roots. This indicates that IgG, which binds effectively with complements and macrophages, is the most important factor in the development of AMAN.

In 1989 I had the first GBS case for me. A 25-year-old policeman who had experienced rapidly progressive weakness of the limbs was admitted. He had had watery diarrhea before the onset of muscle weakness. He showed tetraplegia and areflexia without any sensory signs. Results of repeated electrophysiological examinations suggested the predominant process was axonal degeneration of the motor nerves, not demyelination. A few years ago, Dr. Latov's group found that serum from a patient with motor neuron disease contained IgM antibody to GM1 ganglioside. Patients with GBS frequently show sensory impairment of the glove-and-stocking type, whereas our patient showed pure motor dysfunction. This raised the possibility that, as with this patient with motor neuron disease, anti-GM1 antibody might be involved. As expected, his serum IgG strongly reacted with GM1. We found samples of deep-frozen sera from 9 patients with GBS, and showed that one of them contained anti-GM1 IgG antibody. The anti-GM1 IgG antibody titers in both patients decreased concurrently with the clinical course. Another patient also showed pure motor dysfunction. Electrophysiologically AMAN was diagnosed. Interestingly, the antecedent symptom of this patient was watery diarrhea as well. We determined the preceding *C. jejuni* infection serologically. We reported that 2 patients who developed AMAN subsequent to *C. jejuni* enteritis had anti-GM1 IgG during the acute phase of the illness. In contrast, patients with *C. jejuni* enteritis who did not have a neurological disorder did not have the autoantibody. Subsequent studies showed that having had a previous *C. jejuni* infection is closely associated with anti-GM1 antibody and AMAN.

Gangliosides extracted from bovine brain were used to treat various neurological disorders. Reports of patients developing GBS after ganglioside administration were recorded in Italy, Germany, and Spain. These observation gave us the idea that antecedent infectious agent has ganglioside epitope. Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria. We investigated whether GM1 epitope is present in the LPS of *C. jejuni* that had been isolated from a patient with GBS. The terminal structure of this LPS was identical to that of the terminal tetrasaccharide of the GM1 ganglioside. We found the existence of molecular mimicry between GM1 and the *C. jejuni* LPS.

To reveal the pathogenic role of anti-GM1 antibodies, we inoculated rabbits with GM1. The rabbits developed flaccid paresis of the limbs. The rabbits' IgGs strongly bound to GM1. IgG was deposited on axons of the peripheral nerves. In the cauda equina, macrophages were present in the periaxonal space. Electron microscopy confirmed that these macrophages directly attack the axon. The sciatic nerves showed Wallerian-like degeneration with rare demyelination. No lymphocytic infiltrations were present in any of the sciatic nerve regions. GM1 was expressed on the axons of peripheral nerves in rabbits as well as in humans.

To establish the molecular mimicry theory, we sensitized rabbits with the GM1-like LPS. Some rabbits developed flaccid paresis. The clinical, pathological, and immunological features of these rabbits were very similar to those of human AMAN. Possible pathogenesis of AMAN subsequent to *C. jejuni* enteritis associated with anti-GM1 IgG is shown (**Figure**). (1) Infection by *C. jejuni* bearing the GM1-like LPS induces anti-GM1 IgG production in patients with certain immunogenetic backgrounds. (2) Anti-GM1 IgG binds to the nodal axolemma, which fixes complement. (3) Anti-GM1 IgG and activated complement enter into the periaxonal space of the internode, which guide macrophage to the space. (4) Anti-GM1 IgG antibody-dependent, complement- and macrophage-mediated cytotoxicity induces primary axonal degeneration.

Ref. Yuki N. Infectious origins of, and molecular mimicry in Guillain-Barré and syndromes. Lancet Infect Dis 2001;1:29-37.

Figure

Embryonic stem cells as an alternative donor marrow source and adoptive cellular (e.g. .. macrophage) source

Richard K Burt, M.D. and Larissa Verde Ph.D., M.D.
Northwestern University Medical Center, Chicago Ill, USA, email rburt@nwu.edu

Hematopoietic stem cells (HSCs) have been considered unique in their ability to reconstitute hematopoiesis following transplantation into immunocompromised hosts. Ex vivo expansion of human HSCs without differentiation has not yet been realized.

We have investigated the possibility of murine embryonic stem cells (ESCs) to be maintain ex vivio and to form marrow and blood in lethally irradiated mice.

The 129/SvJX129/SV-CP F1 (MHC H2b) hybrid 3,5 day mouse blastocyst derived embryonic stem cell line, R1 was cultured on gelatinized tissue culture dishes in HG-DMEM supplemented with 15% FBS, LIF (1000U/ml). Irradiated primary embryonic fibroblasts were used as a feeder layer for a long-term culture of R1 ESCs. To induce of differentiation toward HSC in vitro, the embryoid bodies formation method in methylcellulose cultures and culturing EB-derived cells in methylcellulose-based hematopoietic differentiation medium (with various cytokines including SCF, IL-3, IL-6, EPO/GM-CSF) were used. After 3-4 weeks of culture phenotypic analysis of cultured cells showed that ~5% cells were CD45+, including ~1% CD34+ cells.

The suspension of those cells (1x10^6/0,1ml) was injected intra bone marrow. Irradiated (TBI 5,5 Gy) 6 weeks old BALB/c mice (MHC H2d) were used as recipients of ECS-derived cells. The analysis of peripheral blood mononuclear cells was based on immmunophenotypic characteristics of cells using anti-mouse H-2Kb/H-2Db, CD45, B220, CD11b, and CD3 monoclonal antibodies. Four to eight weeks after ESC-derived cells injection the reconstitution of lymphocytes (~15%), monocytes/granulocytes (~30%) was observed. Embryonic stem cells successfully give rise to blood cells in vivo and might be used as an alternate hematopoietic and macrophage donor source.

The plasmacytoid dendritic cell and CpG oligonucleotides in immunotherapy

Günther Hartmann, MD

Department of Internal Medicine, Division of Clinical Pharmacology
Ludwig-Maximilians-University of Munich, Ziemssenstraße 1, D-80366 München

Abstract

The vertebrate immune system has established TLR9 to detect microbial DNA based on unmethylated CG dinucleotides within certain sequence contexts (CpG motifs). In humans, the expression of TLR9 is restricted to B cells and plasmacytoid dendritic cells (PDC). The PDC is characterized by the ability to rapidly synthesize large amounts of type I IFN (IFN-α and IFN-β) in response to viral infection. In contrast to other dendritic cell subsets which express a broad profile of TLRs, the TLR profile in PDC is restricted to TLR7 and TLR9. So far, CpG DNA is the only defined microbial molecule recognized by PDC, while other microbial molecules such as LPS or poly I:C, due to the lack of the corresponding TLRs, do not stimulate PDC. An intriguing feature of PDC is its ability to simultaneously produce the two major Th1-inducing cytokines in humans, IFN-α and IL-12, both at high levels. The ratio of IFN-α versus IL-12 and the quantity of these cytokines are regulated by T helper cell-mediated costimulation via CD40 ligation. The ratio also depends on the differentiation stage of the PDC at the time of stimulation and the type of CpG ODN used. We propose a model in which the PDC functions as a switchboard for regulating Th1 versus Th2/Th0 responses: in the presence of appropriate microbial stimulation (such as CpG DNA), PDC trigger a Th1 response; in the absence of appropriate microbial stimulation PDC promote an unbiased T helper cell response (Th0) or Th2. Novel techniques are now available for the isolation of purified PDC in sufficient quantities from leukapheresis products. This allows to use PDC in combination with CpG ODN for immunotherapy of cancer. First animal studies provide evidence that large murine tumors can be cured by CpG ODN and dendritic cells. In conclusion, the CpG ODN represent a unique microbial stimulus for PDC. The PDC is a promising candidate for cell-based vaccine protocols currently under evaluation for the treatment of cancer.

Figure

Still unresolved mysteries of the spleen

Kenjiro MATSUNO

Dept. Anatomy (Macro), Dokkyo University, School of Medicine, Tochigi 321-0293, Japan

The spleen serves as a complex filter interposed in the circulation to clear the blood of particulate matter and senescent blood cells. It is also concerned with immune defense against blood-borne antigens. The spleen, however, remains one of the few organs with a still enigmatic structure and an obscure function. Even for the senior immunologists, it is not so easy to capture the whole view of the spleen. In my talk, I will briefly introduce microanatomical structure of the rat spleen and show immune responses and trafficking of lymphocytes and dendritic cells within splenic microstructure by using antibody response and organ transplantation models. Next, as a common interest in this session, I raise several questions concerning this organ. These will be addressed in advance to all the relevant speakers in this symposium as well as to some investigators not attending but being familiar with this field. As a chairperson, I will summary the answers and provide a time for extended discussion after each talk.

Questions are:

1. What are homing receptors for lymphocytes to enter the white pulp? These are distinct from those used for interactions between lymphocytes and the high endothelial venule.
2. What is a role of marginal zone B cells? Relevance to B1 B cells?
3. What is a role of marginal zone macrophage and marginal metallophilic macrophages?
4. Why splenectomy enhances immune response?
5. Why the draining blood enters the portal vein? Are there any crucial interactions between the spleen and liver?
6. The red pulp macrophages are considered to be scavengers of senescent erythrocytes, but erythrophagocytosis is scarcely observed.
7. Is the spleen really not necessary for host defense? Splenectomy causes a serious illness in human malaria infection.

Legends for the cover page pictures

Upper left: Compartment of T cell (black) and B cell (red) in the rat spleen by a double immunoenzyme staining (Anat. Int., in press).

Lower right: T cell proliferative response in the rat splenic PALS during heart allotransplantation (J. Leukoc. Biol. 69: 705-712, 2001). Note cluster formation between host dendritic cells (blue) and proliferating T cells (red), which suggests indirect sensitization pathway. Brown cells are B cell and macrophages.

Structure of mouse spleen investigated by hyper-spectral imaging

Hiromichi Tsurui
Department of Pathology, Juntendo Univ. Sch. Med.

We have developed a hyper-multicolor (more than 7 color) fluorescence imaging method based on Fourier spectroscopy and singular value decomposition (Tsurui et al. 2000). This method enables to detect multiple cell subsets simultaneously which are characterized by the expression of two or three molecules, or to figure the detailed structure consisting of many kind of components.

We applied this method to the investigation of the structure of mouse spleen, especially focusing on the distribution and function of the subsets of macrophages (Mfs) and dendritic cells (DCs).

Mfs in spleen are heterogeneous. They are classified as red pulp macrophage, marginal zone macrophage (MZM), marginal matallophilic macrophage (MMM), macrophage in follicle and that in PALS. MMMs are located along the border of follicle and marginal zone, just inside the marginal sinus. MZMs distribute just outside the marginal sinus. MMM and MZM are specifically stained with MOMA1 and ER-TR9, respectively. The role of these cells played in immune response has been clarified to some extent. However, the characterization of other macrophage-subsets is rather obscure due to the lack of their specific markers. To examine the phenotype of those macrophage subsets in detail, frozen sections of mouse spleen were simultaneously stained with 4 general macrophage markers, F4/80, Mac3, BM8 and MOMA2. In unstimulated spleen, red pulp macrophage showed F4/80+ve, BM8+ve, Mac3-ve, MOMA2-ve phenotype, whereas macrophages in white pulp, both in follicle and PALS, showed F4/80-ve, BM8-ve, Mac3+ve, MOMA2+ve phenotype. The meaning of the correlation of location, function and the phenotype in those Mfsubsets are not clear.

The functions of Mfs and DCs such as removing pathogens, transferring the structural information of antigens to other immunocompetent cells, and scavenging tissue debris, are performed through their phagocytosis and macropinocytosis. So, the method which provides the information about those activities is quite useful to elucidate the roles of those cells in immune response. The autofluorescence having strong correlation in location and intensity with phagocytosis or macropinocytosis was investigated. The substance which emits this autofluorescence seems, by its spectral feature, to consist of multiple components. The cell subsets which shed this autofluorescence are under investigation.

CD8+ve DCs, not CD8-ve DCS, have been said to engulf dying cells and to present antigens contained in it to some T cell subsets. To determine what kind of cells actually engulf the dying cells, we examined the spleen from mice injected with CFSE-labeled apoptosis-induced cells. Most of signals from CFSE were detected in the macrophages in red pulp, follicle and PALS. All the DCs that engulfed CFSE-labeled cells were CD8+ve cells and no CD11b+ve DC showed signal of CFSE. A little but significant portion of CFSE-labeled cells

were engulfed by MZM and MMM, even though MMM has been said to have scarce phagocytotic activity. MZM and MMM extended their process in a follicle, forming a fine meshwork with local close adhesion. CFSE's signal and phagocytosis specific autofluorescence were sometimes observed in those close adhesion region. In rat, it was reported that MMM plays important role in producing antibody forming cells against T-independent antigen. The distribution of CFSE's signal and phagocytosis specific autofluorescence may suggest that MZM and MMM play some roles in producing T-dependent antibodies.

When mice were injected with GFP-labeled E.coli, most of it was ingested by macrophages in red pulp, follicle and PALS. DCs in the spleen showed very low phagosytotic activity for the bacteria and all the DCs engulfed it were CD11b+ve.

When mice were injected with Alexa 350-labeled Zymosan (BioPaticle, Molecular Probe, Inc.), a fairly good portion of the particles were ingested by DCs, and it was CD11b+ve DCs that engulfed this substance.

Figures

Signals and cells for memory CD4 T cells

Peter J.L. Lane*, Mi-Yeon Kim[+], Fabrina M.C. Gaspal[+], Helen E. Wiggett[+], Fiona M. McConnell and Chandra Raykundalia
From the MRC Centre for Immune Regulation, University of Birmingham, Vincent Drive, Birmingham B152TT email: p.j.l.lane@bham.ac.uk

Using confocal microscopy to track the migration of CD4 T cells in lymphoid tissue, we have been able to identify the key cellular interactions that occur during priming of CD4 T cells. These studies have shown that T cells are initially primed on conventional $CD11c^+$ dendritic cells (DCs). A schematic representation of the surface costimulatory interactions that occur during this priming phase are shown in the diagram below left.

Figures

Subsequent to this initial priming phase, primed T cells migrate to the outer T zone to the interface between the B and T cell areas. Here they interact with an accessory cells distinct from conventional DCs that prime T cells. These cells costimulate T cells through CD30 and OX40, two members of the tumor necrosis family of receptors, but do not express significant amounts of CD80 and CD86 that are expressed on conventional DCs. We show that whereas CD80 and CD86 promote proliferation of T cells, OX40 signals from these accessory cells are implicated in survival and maintenance of T cell memory for help for longlived antibody responses.
These findings are described in our recent publication(1).

1. Kim, M., Gaspal FMC, H. Wiggett, F. McConnell, A. Gulbranson-Judge, C. Raykundalia, L. Walker, M. Goodall, and P. Lane. 2003. Identification and characterization of a distinct CD4+CD3- accessory cell which co-stimulates primed CD4 T cells through OX40 and CD30 at sites where T cells collaborate with B cells. *Immunity* In press.

Marginal Zone B cells: Development and Function

John Kearney, Kalaya Attanavanich, Woong-Jai Won, Flavius Martin, and Mercedesz Balazs, The University of Alabama at Birmingham, Birmingham, AL US 35294-3300

Marginal zone (MZ) and B1 lymphocytes can be distinguished from the bulk of recirculating naïve B cells, typified by follicular (FO) B cells, by a number of newly discovered membrane markers and distinct functions. MZ and B1 cell participate jointly in the early immune response against T independent (TI) particulate antigens by interacting with $CD11c^{lo}$ immature dendritic cells which capture and transport bacteria from blood to the spleen. $CD11c^{lo}$ DC provide critical survival signals, which can be inhibited by TACI-Fc, to antigen specific MZ B cells and promote their differentiation into IgM secreting plasmablasts. In the absence of soluble TAC1-ligands, Ag-activated MZ and B1 derived B cell blasts lack survival signals and undergo apoptosis, resulting in severely impaired antibody responses. In addition to their involvement in T-independent antibody responses MZ B cells are potent antigen-presenting cells. In comparison to FO B cells HEL specific MZ B cells from the MD4 transgenic mouse provide strong signals for CD4 HEL specific T cells to proliferate and produce cytokines early in the anti-HEL response. (NIH Grant AI14782)

The spleen response to *Leishmania donovani* infection: a model for macrophage and dendritic cell function in chronic infectious disease

Paul M. Kaye, Mattias Svensson, Christian Engwerda and Manabu Ato
Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT U.K.
Email: paul.kaye@lshtm.ac.uk

Much of the information that we have about the immunological function of macrophages and dendritic cells comes from studies of cells isolated from naïve animals and / or cultured in vitro. These studies, whilst of great value in setting benchmarks of immunological function, nevertheless do not take into account the pleiotropic responses of these mononuclear phagocytes to inflammatory and immunological stress. Most immunological responses result in changes to the local environment in which macrophages and DC may have to operate. Whilst these changes might be minor in nature, in the case of infectious disease, they more often than not result in major changes to tissue microenvironments. One of the major aims of research in this laboratory is the evaluation of how changes in microenvironment modify the behavior of macrophages and DC and their subsequent roles in host protective immunity. Our principal model, murine visceral leishmaniasis caused by Leishmania donovani infection, lends itself readily to these studies. Immune responses to this parasite are compartmentalized, with a protective and curative response generated in the liver, a persistent chronic infection generated in the spleen and bone marrow, and relative lack of involvement of peripheral lymph nodes. In this presentation, I will discuss our studies of the splenic microenvironment, and the dynamic nature of macrophage and dendritic cell responses that are associated with progression to a chronic state of infection.

The splenic MZ comprises two distinct macrophage sub-populations; marginal zone macrophages (MZM) located at its outer boundary adjacent to the red pulp, and marginal metalophils (MMM) located at its inner boundary adjacent to the white pulp. The mechanisms underlying this precise anatomical location are at present unknown, though preliminary data from our laboratory indicates that the steady state positioning of MZM is dependent upon the chemokine CCR7-CCL21 interactions. MZM, which express mSIGNR1, have been suggested to play a role in the induction of marginal zone B cell responses to T-independent antigens and to facilitate T cell trafficking into the white pulp. The role of MMM in immune responses is less clear, though they may facilitate antigen traffic into the white pulp. The MZ also contains a variety of DC populations and blood-borne pre-DC have ready access to this compartment. In the first part of this presentation, I will discuss recent data that sheds light on two key issues in the early inductive phase of immunity to L. donovani. First, do MZM or MMM have any role(s) to play in regulating DC-mediated T cell priming? Second, at what site in the spleen (i.e. MZ vs. white pulp) do productive DC-T cell interactions occur?

The progression of L. donovani infection to its chronic phase is associated with gross microarchitectural changes, recently reported by us (Ato et.al. *Nature Immunol*. **3:**1185-1191 2002; Engwerda et.al. *Am. J. Path*. **161**:429-437 2002). Most evident are: loss of MZM and associated depression in T cell migration; loss of T zone stromal cells and associated T zone production of CCL21 and CCL19; and deficient T zone migration of DC, resulting from TNFα-dependent, IL-10-mediated inhibition of CCR7 expression. Of the many outstanding issues that these observations raised, one that we have recently focussed on is the source of the elevated number of splenic DC with this migratory phenotype. We had previously noted that the spleen is a site of extensive myelopoiesis and that Leishmania parasites can be found infecting various stromal cell populations (Cotterell et.al. *Blood*. **95**: 1642-51 2000; *Infect Immun*. **68**: 1840-8 2000). In the second part of this presentation, I will discuss a new model for studying DC development in the chronically infected spleen. In this model, we isolate stroma from chronically infected mice and co-culture this with progenitor cells derived from bone marrow. Stroma from infected mice, but not uninfected mice, has the capacity to support DC development in the absence of exogenous cytokines. Optimal development of DC requires adherence between myeloid progenitors and stromal cells, as judged by transwell experiments. The DC that develop under these conditions have an immature $CD11c^{lo}$ phenotype and uniformly express CD11b. We are currently analyzing the function of these $CD11c^{lo}CD11b^{hi}$ DC, in comparison to other defined DC subsets and those found in the course of chronic infection. Furthermore, microarray analysis of stromal cell populations is being conducted to try to identify the key determinants of DC development under these conditions.

Together, our studies demonstrate the dynamic nature of macrophages and DC behavior in response to this chronic infection. Although our studies are performed using L. donovani as a model infection, similar changes in microenvironment can be seen in a variety of infectious and non-infectious conditions. Whilst the mechanisms for microenvironmental changes may be varied, the functional consequences may be similar. Understanding how such changes occur may lead to new approaches to therapeutic intervention.

Figures

Visualizing the movement of developing T cells in the thymus

Yousuke Takahama

Division of Experimental Immunology, Institute for Genome Research, University of Tokushima, and Laboratory of Immune System Development, RIKEN Center for Allergy and Immunology, Japan

The development of T lymphocytes involves dynamic cellular movement into, within, and out of the thymus. Lymphocyte precursor cells that are capable of generating T cells immigrate into the thymus from hematopoietic organs such as fetal liver and bone marrow. Within the thymus, developing thymocytes migrate along repertoire selection through the cortex to the medulla. Newly matured T cells emigrate out of the thymus to the circulation. It is unclear, however, how cell migration is controlled during the T cell development. We have recently devised a time-lapse visualization system to directly evaluate the migration of developing thymocytes. Based on the visualization method, and supported by molecular genetic approaches, we have recently identified that CCR7 ligands play a major role in the emigration of mature thymocytes to the circulation in newborn mice. Furthermore, by time-lapse visualization within intact thymus architecture using two-photon laser microscopy, we show that immature T cells newly generated in the cortex are dormant in motility, and that TCR signal is essential for gaining their motility, suggesting that TCR-mediated self-recognition in the cortex directly contributes to initiating the relocation of positively selected T cells out of the cortex to the medulla.

Leukocyte and monocyte trafficking in inflammation and atherosclerosis

Klaus Ley
Cardiovascular Rsearch Center and Department of Biomedical Engineering, University of Virginia. Charlottesville, VA 22908, USA

Although only a handful of adhesion molecules and a few dozen chemokines are involved in regulating leukocyte trafficking in the organism, exquisite specificity is achieved through combinatorial processes t each step of the adhesion cascade, i.e., capture, rolling, deceleration (slow rolling), adhesion and transmigration. Here, I will focus on the processes that allow rolling leukocytes to arrest. Interestingly, these processes appear to be fundamentally different for neutrophils versus other leukocytes.

Adhesion molecules Of the three selectins, only L-selectin is expressed on leukocytes, whereas E-selectin is restricted to inflamed endothelium and P-selectin to inflamed endothelium and platelets. Of the four known β2 integrins, only LFA-1 (αLβ2) and Mac-1 (αMβ2) have been shown to be relevant for leukocyte adhesion under flow. Both bind to InterCellular Adhesion Molecule-1 (ICAM-1) and other ligands on inflamed endothelial cells. Of the 10 known β1 integrins, only α4β1 has been shown to participate in the arrest of monocytes, eosinophils, lymphocytes and (under conditions of chronic inflammation) neutrophils. α4β1 binds Vascular Cell Adhesion Molecule-1 (VCAM-1) on the inflamed endothelium and can also bind a form of fibronectin that can be immobilized on the endothelial surface. α4β1 is involved in slow rolling of monocytes and also in their arrest. α4β1, like other integrins, can rapidly change its conformation in response to cellular activation and bind ligands with higher affinity. In addition, integrin molecules can cluster in groups and support increased adhesion (avidity regulation). Homing of certain lymphocytes to gut-associated lymphatic tissues is mediated by α4β7 integrin, which binds to Mucosal Addressin Adhesion Molecule-1 (MAdCAM-1) on the endothelium of intestinal organs. Many other adhesion molecules have been proposed to participate in leukocyte adhesion under flow, but their functional has not been rigorously demonstrated.

Chemokines are small, soluble or surface-bound peptides, that bind to G-protein coupled receptors on various classes of leukocytes. In the context of leukocyte arrest, only surface-bound chemokines are relevant, because soluble chemokines cannot produce a stable gradient in the blood stream and are immediately diluted and washed away. CXCL8 (interleukin-8), CCR5 (RANTES) and CCR2 (MCP-1) can be bound by the glycocalyx of inflamed endothelial cells and be displayed on the surface. These chemokines then bind their cognate receptors on rolling leukocytes and trigger an integrin affinity increase. This affinity increase can occur extremely rapidly in the case of T cells and monocytes or more slowly in the case of neutrophils.

Neutrophils and inflammation. When neutrophils enter a site of inflammation, they attach to the wall of postcapillary venules at the site where capillaries enter these venules. The reason for this very limited location is not the expression of adhesion molecules suitable for rolling, because E- and P selectins are expressed all along the venular tree. However, the endothelium is covered by a thick glycocalyx layer (500 nm) that dwarfs the adhesion molecules (20-50 nm). Therefore, attachment is very unfavorable, except at the exit of capillaries, where the deforming neutrophil is squeezed against the vessel wall and squishes down the glycocalyx layer so that leukocyte adhesion molecules, mainly P-selectin Glycoprotein Ligand-1, can engage endothelial P- and E-selectin to start the rolling process. Very little attachment is seen elsewhere, and attachment that is seen occurs through secondary capture, i.e., leukocyte-leukocyte interactions . Rolling neutrophils also leave behind fragments (tethers and microparticles) that facilitate the attachment

of more neutrophils and other inflammatory cells. Neutrophil recruitment to sites of inflammation is extremely efficient. In severe, TNF-α-induced inflammation, almost 100% of all neutrophils transported in the blood initiate rolling and 95% of these make it to firm attachment.

Neutrophils must roll for a long time (1-3 minutes) and a long distance (several hundred micrometers) before they can firmly attach through 2 integrins (LFA-1 and Mac-1). In fact, both LFA-1 and Mac-1 are engaged with ICAM-1 and other ligands during slow rolling in inflamed venules. It is not clear why neutrophils take so long to arrest, but it is plausible that they may scan the surface and integrate activating signals from chemokines, other chemoattractants, and adhesion molecule engagement. Consistent with this hypothesis, intracellular free calcium concentrations increase as rolling leukocytes slow down to stop. However, the details of the signaling pathways involved in neutrophil activation are not understood. If exposed to a high concentration of chemoattractant, rolling neutrophils can stop immediately, but it is not known whether such high local concentrations occur during inflammation in vivo.

Monocytes and atherosclerosis. Monocyte arrest under flow has been studied in various in vitro systems using endothelial cells and recombinant adhesion molecules as substrates. In atherosclerosis, blood monocytes are recruited into the layers of the vessel wall under extremely unfavorable conditions of very high shear stress and lack of capillary-size geometry. In 1999, we developed a model to study monocyte recruitment to atherosclerotic lesions in carotid arteries of apolipoproteinE-/- mice. When perfused at sub-physiological levels of shear stress (2 to 3 dyn/cm^2), monocytes can roll on atherosclerosis-prone endothelium by engaging endothelial P-selectin through PSGL-1. This rolling is also supported by α4β1 integrin interacting with VCAM-1, because blocking either α4 or VCAM-1 increases rolling velocity by about 50%. The chemokines KC (CXCL1), RANTES and MCP-1 are expressed on the vascular endothelium overlying the atherosclerotic lesion, but not elsewhere. Blocking MCP-1 has no effect, but blocking RANTES or KC signaling reduces monocyte arrest by about 50% each.A similar effect can be achieved by blocking G protein coupled receptor signaling through Gαi using pertussis toxin. This suggests that the receptors for these chemokines require signaling through Gαi to up-regulate integrin affinity and avidity in rolling monocytes. In this model, all arrest is mediated by α4β1 integrins binding to VCAM-1 with no detectable involvement of β2 integrins or ICAM-1.However, the β2-ICAM-1 pathway may be important for maturation of adhesion and for sustained adhesion under the high shear stresses (up to 100 dyn/cm2) observed in vivo.

Although the P-selectin- and α4-dependnet adhesion pathway is functional at lower shear stresses, monocyte adhesion under in vivo conditions is rarely observed. Using stroboscopic epifluorescence video microscopy, the carotid artery of atherosclerotic mice can be visualized. To see the monocytes, we use mice that express green fluorescent protein under a monocyte-specific endogenous promoter. However, even when observing for hours, no monocyte adhesion to the atherosclerosis-prone site is seen, highlighting how inefficient the process is. This changes when activated platelets are injected into the blood stream. Activated platelets strongly promote monocyte adhesion to atherosclerotic lesions through at least three mechanisms. First, they deposit RANTES on the surface of monocytes, which leads to G protein dependent signaling and increase α4β1 affinity. Second, activated platelets transiently interact with the endothelium and deposit RANTES and possibly other chemokines on the endothelial surface, which in turn can activate rolling monocytes. Third, activated platelets can physically bridge between monocytes and the endothelium. Platelet P-selectin is required for all of these processes. It interacts with PSGL-1 on the monocyte surface and with unknown ligands on the endothelium. The result of these enhanced interactions is an increased delivery of blood monocytes to atherosclerotic lesions. When activated platelets are injected into apoE-/- mice for 12 weeks, the resulting atherosclerotic lesions are twice as large as those in control mice. Injecting P-selectin deficient platelets or supernatant of activated platelets has no effect.

Dendritic cell migration in lymph from peripheral tissues

Gordon MacPherson, Emma Turnbull, Ulf Yrlid, Lesley Cousins and Simon Milling
Sir William Dunn School of Pathology
South Parks Rd., Oxford OX1 3RE UK

All peripheral lymph so far examined contains populations of migrating dendritic cells (DCs). This migration is constitutive and occurs in absence of any overt inflammatory stimuli. My group has studied the migration of DCs in pseudo-afferent intestinal lymph in the rat. DCs enter the intestine from blood, spend a short time therein and migrate via afferent lymph to mesenteric nodes. This traffic is constitutive and these steady-state DCs can acquire enteric antigens during their residence in the gut. Migrating DCs comprise at least 2 populations identified by expression of CD4 and OX41. Migrating DCs are MHC Class II^{hi}, $CD86^{int}$, but $CD80^{lo}$ and $CD40^{lo}$. However these DCs stimulate very strong allogeneic MLRs. CD4+/OX41+ DCs are the stronger antigen-presenting cells but are excluded from T cell areas of mesenteric nodes. CD4-/OX41- DCs transport apoptotic enterocytes to T cell areas of mesenteric nodes, suggesting a role in self-tolerance.

Pro-inflammatory stimuli (i.v. LPS, intra-intestinal Salmonella typhimurium or E. coli heat-labile toxin, but **NOT** intra-intestinal TNBS) dramatically increase DCs exit from the gut into lymph but stimulate only minor increases in expression of CD40 and B7 compared to the effects of maturation stimuli given to the same cells in vitro. I.v. LPS (not known for other stimuli) alters migration of DCs within the mesenteric node, stimulating migration of CD4+/OX41+ DCs into T cell areas.

The significance of these results will be discussed in relationship to the generation of tolerogenic responses versus active immunity.

The *in vivo* trafficking, relationship, and function of myeloid and plasmacytoid DC precursors

Hiroyuki Yoneyama*, Kenjiro Matsuno#, Kouji Matsushima

*Department of Molecular Preventive Medicine, Graduate School of Medicine & SORST, The University of Tokyo, #Department of Anatomy (Macro) & SORST, Dokkyo University School of Medicine

Migration of dendritic cells (DCs) to lymph nodes (LNs) from peripheral tissues is pivotal to the establishment of immune responses. It is not known, however, if DCs migrate directly from the circulation into LNs. We show here the existence of two subsets of murine circulating DC precursors with distinctive homing potentials by adoptive transfer experiments. $B220^{-}CD11c^{+}$ cells are tissue-seeking myeloid DC precursors that are remobilized into draining LNs through afferent lymphatics. In contrast, $B220^{+}CD11c^{+}$ cells are plasmacytoid DC precursors that directly bind to and transmigrate across high endothelial venules of inflamed LNs.

In a granulomatous liver disease model induced by a causing bacterium of sarcoidosis, *Propionibacterium* (*P. acnes*), we previously reported that numerous $CCR1/5^{+}$ myeloid DC precursors rapidly appear in the circulation and migrate into peri-sinusoidal space in responses to CCL3/MIP1α produced by Kupffer cells to participate in granuloma formation. The first immune response occurs at the inflammation-associated lymphoid tissue appears at the portal area (termed, PALT). We also showed directly that T helper cell 1 (Th1) polarization occur at the draining LNs and CXCL10/IP-10 produced by migrated mature DCs in the paracortex area regulates DC-Th1 cluster formation and controls the exit of effector/memory T cells from the LNs to home to the inflamed liver to complete granuloma formation. These studies have established that chemokines pivotally regulate inflammation and immunity, and chemokine-recruited DCs tightly link innate and acquired immunity.

In addition, the mechanism of rapid mobilization of two subsets of DC precursors into circulation, and the fate of *in vivo* trafficking of plasmacytoid DC precursors into the secondary LNs will be presented.

Figure

Honorary lecture: History of Macrophage Research

Macrophage Development and Differentiation: Historical Review and Current Concepts.

Kiyoshi Takahashi, Kumamoto University, Kumamoto, Japan

Since the first designation of large mononuclear phagocytic cells as macrophages by Metchnikoff in 1892, the origin and differentiation of macrophages have been seriously debated. In 1924, Aschoff proposed the concept of the reticuloendothelial system (RES) and included macrophages (histiocytes), a major member of the sytem, together with reticulum cells and reticuloendothelia (phagocytic endothelia). The RES theory was based on the results of his studies and his co-workers, particularly vital staining studies with Kiyono in 1914~1918 and studies of cholesterol metabolism with Landau and McNee in 1914. Before Aschoff's postulate of RES concept, Kiyono (1918) proposed the concept of histiocytic cell system, which included histiocytes, reticulum cells, and reticuloendothelia (phagocytic endothelia), essentially identical with that of RES. According to the RES concept, Aschoff and Kiyono considered that reticulum cells become flattened and show a shape like that of endothelial cells, when they are located at sites facing the blood and lymph stream. These phagocytic endothelial cells were called "reticuloendothelia" and the reticulum cells and the reticuloendothelia were thought to transform into histiocytes as free round mononuclear phagocytic cells. This concept had been widely accepted for about fifty years until the end of 1960s. In 1952, Akazaki, a pupil of Aschoff, revised the RES concept of Aschoff and Kiyono, concluding that RES was composed of two different groups of cells, 1) reticuloendothelia and 2) reticulum cells or histiocytes, and that the reticuloendothelia had an entdothelial cell origin, which was thus different from that of reticulum cells or histiocytes. Afterward, Kojima, a pupil of Akazaki, also actively studied the relationship between reticulum cells and histiocytes (tissue macrophages), concluded that histiocytes differed from reticulum cells in cell morphology, function, and origin, and proposed histiocytic cell system by grouping tissue macrophages in various organs and tissues under a normal steady state condition. The essential idea of the Aschoff's concept of RES was disintegrated on the basis of the results of studies by Akazaki, Kojima, and their co-workers.

Against the RES, Sabin and her co-workers (1925) and Ebert and Florey presented the monocytic origin of macrophages and Amano (1948) also maintained that macrophages were derived from monocytes migrating from peripheral blood into tissues and that monocytes developed via promonocytes from monoblasts originating from progenitor cells in bone marrow. In 1972, van Furth and his co-workers proposed the concept of mononuclear phagocyte system (MPS) that not only all macrophages in various tissues under a normal steady state condition but also those in inflammatory foci were derived from monocytes, which differentiated through promonocytes from monoblasts originating in bone marrow, and that macrophages were non-dividing, short-lived, terminal cells of MPS and were supplied from blood monocytes alone. This concept has been widely accepted and monocyte/macrophage population is important to

participate in inflammatory foci or stimulated conditions. However, results conflicting with the MPS concept have been presented.

In our ontogenetic studies, we found that macrophages developed before the development of monocytic cell series in the early ontogeny, particularly yolk sac hematopoiesis. In osteopetrotic (op) mice, we confirmed that M-CSF-independent immature macrophages develop in various organs and tissues, derive from the stage of differentiation before monocytic cell series, and differentiate into tissue macrophages due to administration of M-CSF into the mutant mice. In PU.1-deficient mice, PU.1-independent large macrophages develop in tissues such as liver and bone marrow, when the mutant mice are rescued by treatment of antibiotics immediately after birth and survive for more than two weeks. All these studies indicate the development and differentiation of early hematopoietic progenitor cells into macrophages in the postnatal life as well as in the early ontogeny. Our studies with ^{89}Sr-induced, severely monocytopenic mice demonstrated that tissue macrophages can proliferate and are sustained by their own self-renewal without supply of blood monocytes from peripheral blood. Furthermore, we found in viable motheaten mice that CD5-positive macrophages develop in the peritoneal cavity, spleen, and lymph nodes and are derived from B cell progenitor cells. Based on the results of these studies, it is reasonable that macrophages are not a single lineage system advocated by the MPS concept and are developed through multiple pathways of differentiation or from hematopoietic progenitor cells at different stages of differentiation.

References

1) Takahashi, K: Development and differentiation of macrophages and related cells: Historical review and current concepts. J Clin Exp Hematopathol 41:1~31, 2001

2) Takahashi K, Naito M, Takeya M: Development and heterogeneity of macrophages and their related cells through their differentiation pathways. Pathol Int 46:473~485, 1996

Figures

A Fatal Attraction: *Mycobacterium tuberculosis* and HIV-1 Target DC-SIGN to Escape Immune Surveillance

Teunis B.H. Geijtenbeek

Department of Cell Biology & Immunology, Faculty of Medicine, Vrije Universiteit
van der Boechorststraat 7, 1081BT Amsterdam, The Netherlands

Antigen presenting cells, such as dendritic cells (DCs) and macrophages, are localized in essentially every tissue, where they operate at the interface of innate and acquired immunity by capturing pathogens and presenting pathogen-derived peptides to T cells. C-type lectins are important pathogen recognition receptors and the DC-specific C-type lectin DC-SIGN is unique in that, in addition to pathogen capture, it regulates adhesion processes such as DC trafficking and T cell synapse formation (Figure 1).
Although DCs are vital in the defense against pathogens, it is now becoming evident that some pathogens subvert DC functions to escape immune surveillance. HIV-1 targets DC-SIGN to hijack DCs for viral dissemination (Figure 2). HIV-1 binding to DC-SIGN protects HIV-1 from antigen processing and facilitates its transport to lymphoid tissues, where DC-SIGN promotes HIV-1 infection of T cells. Recent studies demonstrate that DC-SIGN is a more universal pathogen receptor that also recognizes Ebola, cytomegalovirus and mycobacteria. *Mycobacterium tuberculosis* targets DC-SIGN by a mechanism that is distinct from that of HIV-1, leading to inhibition of the immunostimulatory function of DC and pathogen survival.
We have isolated a murine homologue of DC-SIGN, called mSIGNR1, to obtain a better understanding of the role of DC-SIGN in both trafficking and pathogen interactions *in vivo*. mSIGNR1 is expressed by medullary and subcapsular macrophages in lymph nodes, and by marginal zone macrophages (MZM) in spleen. Strikingly, these MZM are in direct contact with the blood stream and efficiently capture specific polysaccharide-antigens present on the surface of encapsulated bacteria. We have investigated the *in vivo* function of mSIGNR1 on MZM in spleen. We have demonstrated here that mSIGNR1 functions *in vivo* as a pathogen recognition receptor on MZM that captures blood-born antigens, which are rapidly internalized and targeted to lysosomes for processing. Moreover, the antigen-capture is completely blocked *in vivo* by the blocking mSIGNR1-specific antibodies. Thus, mSIGNR1, a murine homologue of DC-SIGN, is important in the defense against pathogens and this study will facilitate further investigations into its *in vivo* function.

Figure 1

Figure 1. DC-SIGN controls many functions of DC to elicit immune responses. The egress of precursor DC from blood into tissues is partly mediated by DC-SIGN. The DC-SIGN-ICAM-2 interaction facilitates both rolling and trans-endothelial migration of DC-SIGN$^+$ precursor DC, whereas arrest is mediated by integrin mediated interactions. DC-SIGN also functions as an antigen receptor. DC-SIGN rapidly internalizes upon binding soluble ligand and is targeted to late endosomes/lysosomes, where antigens are processed and presented by MHC class II molecules. Moreover, initial DC-T cell clustering, necessary for an efficent immune response, is mediated by transient interactions between DC-SIGN and ICAM-3. This interaction facilitates the formation of low-avidity LFA-1/ICAM-1 interaction and scanning of the antigen-MHC repertoire.

Figure 2

Figure 2. HIV-1 hijacks DC through DC-SIGN to infiltrate lymphoid tissues. DC-SIGN is expressed by both immature DC in mucosal tissues and by DC precursors in blood. HIV-1 is captured by DC-SIGN on DC precursors in blood after infection or on immature DC at mucosal entry sites during sexual transmission. DC-SIGN-bound HIV-1 is protected intracellular during migration to the lymphoid tissues. Once arrived, DC-SIGN transmits HIV-1 to CD4$^+$ T cells *in trans*, resulting in a productive HIV-1 infection of the CD4$^+$ T cells.

Heparanase at the invasive edge of migrating macrophages

Nobuaki Higashi*, Norihiko Sasaki*, Motowo Nakajima[#], and Tatsuro Irimura*

* Laboratory of Cancer Biology and Molecular Immunology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan

[#] Tsukuba Research Institute, Novartis Pharma, K.K., Tsukuba 300-2611, Japan

Macrophages and related cells play pivotal roles in the immune system. Monocytes, precursors of tissue macrophages and related cells of myeloid origins, are known to circulate throughout the body, extravasate through the endothelial lining of the blood vessel wall and enter the underlying tissue in response to local inflammation. During these processes, monocytes pass through vascular basement membrane that is composed of type IV collagen, laminin, and heparan sulfate proteoglycans (HSPGs). HSPGs not only support the mechanical integrity of basement membranes but also serve as reservoirs of growth factors and cytokines. Therefore, HSPG degradation should be a critical step in the regulation of monocyte and macrophage functions. Heparanases, heparan sulfate-specific endo-β-glucuronidases, have been shown to be a key enzyme in invasion and matrix degradation by melanoma cells and suggested to play a role in the extravasation of leukocytes including monocytes. Therefore, as a part of coordinated events during the trafficking, heparanases in monocytes and macrophages should be regulated more strictly than in melanoma cells to achieve efficient migration without tissue damage. To explore unique potentials of heparanase as an enzyme, which might determine the distribution of monocytes and macrophages, we examined regulation of hepanarase during monocyte differentiation and transmigration using human promonocytic leukemia U937 and THP-1 cells.

Phorbol myristate acetate (PMA)-treated but not untreated cells degraded [^{35}S]-labeled HS in endothelial extracellular matrix into fragments of an approximate molecular weight of 5 kDa. The degradation by the intact cells upon differentiation does not seem to be due to the gene expression levels or by the amounts of active enzymes. PMA-treated differentiated cells expressed the heparanase on the cell surface as shown by cytochemical methods with anti-heparanase mAb. After adhesion of the PMA-treated cells, the enzymes were shown to move into a restricted domain. During the chemotaxis induced by fMLP, cell surface hepanarase redistributed along the gradient and toward the higher concentration of the chemoattractant, suggesting that the heparanase is concentrated to the leading edge of the migration. Addition of anti-heparanase mAb significantly inhibited in vitro invasion of Matrigel by PMA-treated cells, indicating that the heparanase on the cell surface is directly involved in monocyte extravasation.

Relocalization of heparanase to cell surface and capping of this enzyme seem to

represent two distinct events, because methyl-β-cyclodextrin, known to deplete cholesterol from the plasma membranes and disrupt lipid microdomains, did not inhibit the cell surface expression but the capping of heparanase. Heparanase appeared to be localized into lipid microdomains after PMA-induced differentiation judging from colocalization with GM1. Microdomains, isolated by sucrose density gradient centrifugation as a low density membrane fraction, contained the 50 kDa (active form) heparanase. Therefore, the GM1-rich microdomain should be potential site of invasive edge of migrating macrophages. In the same lipid microdomain, integrins including α6β1 laminin receptor and membrane bound matrix degradation enzyme MT1-MMP were shown to colocalize by cytochemical analysis with specific antibodies. The functional and coordinated colocalization of the adhesion molecules and degradation enzymes would be suitable for macrophages to achieve effective transmigration without tissue damage.

Figure

Transcriptome analysis of monocyte/macrophage differentiation and monocyte/endothelial cell interaction

Tatsuhiko Kodama
Laboratory for Systems Biology and Medicine, The University of Tokyo

The completion of human genome project enables us to monitor the global RNA expression profile during various cell biological events. We at LSBM of University of Tokyo established a comprehensive normal human tissue RNA expression data base about more than 30000 genes in 40 human organs and 60 cultured human cell lines. These data can be accessible from our web site, HTTP://www.lsbm.org. From these data, we can identify the genes specifically expressed in human monocyte and macrophage. The expression profiles in normal human cells are useful tool to define cell biological event specific genes. Several important methods and tips are established during these analysis.

One of the most important point is that careful identification of each cell's state will be necessary. When studied using DNA microarray, various cell lines indicate quite different profiles from our expectation. THP-1 cells dose not have RNA expression profile similar to human blood borne monocyte, although several similarity to macrophage differentiation can be observed during PMA forced differentiation. From systmems biological stand point cells are considered as "state machine", and the definition of original state is critical.

Second important point is the importance of time course. During human monocyte macrophage differentiation, genes induced early stage (30-120 min) and late stage (1 to 3days) are quite different. If cells with different time course are mixed, we may miss important information concerning about the signaling mechanism. This is one of the reason why DNA microarray analysis is effective when using chemicals (agonist and antagonist, inhibitors and ligands) as compared with transgen or knockout approach because cells are easily synchronized.

Third point is the usefulness of cyclohexamide to dissect transcriptional cascade. For example, TNF signal cause a series of transcriptional cascade in human vascular endothelial cell. Addition of CHX helps to define primary response (e.g. ICAM1 upregulation)from secondary response (e.g. VCAM1 upregulation).

In this symposium I would like to report the data about gene changes in gene expression profile in following biological events;

1. Time course analysis of gene expression profile during M-CSF and GM-CSF forced

differentiation of human CD14 positive monocyte to macrophage

2. Induction and Activation of the Transcription Factor NFATc1 (NFAT2) Integrate RANKL Signaling in Terminal Differentiation of Osteoclasts.

3. Transcriptome analysis of vascular endothelial cell activation induced by TNFα

P1 Visualizing of antigen-trafficking from skin both in steady and active state using hyperpigmented mice

Miya Yoshino, Hidetoshi Yamazaki and Shin-Ichi Hayashi

Div. Immunology, Dept. Molecular and Cellular Biol., Sch. Life Science, Fac. Med., Tottori Univ., 86 Nishi-Machi, Yonago, Japan

Antigen capturing and its trafficking into regional lymph nodes (RLN) initiate immune responses. In this study, employing melanin granule (MG) as an easily traceable antigen in two transgenic mouse strains with melanocytosis in epidermis (*Mgf-Tg*) or in dermis (*Hgf-Tg*) respectively, we investigated mechanisms of antigen-trafficking from skin. MGs captured in epidermis or dermis were accumulated in the RLN, but not other tissues. Since either transgenic mice were not observed inflammatory regions, our developing system enables us to investigate constitutive capturing and trafficking of insoluble antigens in the steady state. Both dendritic cells (DC) and macrophages laded MGs in the RLN. To determine which cells traffic antigens, we prepared double mutants carrying the transgenes and lacking transforming growth factor (TGF)-beta1, which are reported to be deficient of Langerhans cells (LC). Few MGs in the RLN of these double mutant mice were observed. This result indicates that antigens in both epidermis and dermis are trafficked into RLN by only TGF-beta1-dependent cells, which may be a DC lineage. Impaired DC-trafficking into RLN has been reported in the *plt/plt* mice that lack the chemokines, CCL19 and CCL21-Serine. However, MGs trafficked into RLN of *plt/plt Mgf-Tg* double mutants in the steady state. Interestingly, while LPS injection induced LC migration from the skin in both of *plt/plt* and their normal littermates, only DC reached to the RLNs in normal littermates. Taken together, the mechanisms of antigen-trafficking can be classified; one not dependent on CCR7-CCL21-Serine in the steady state; another lacking in the *plt/plt* mutants in the active state. The former may regulate the immune-tolerance induced by an autoantigen-stimulation in the steady state, and the latter may associates acquired immune responses.
Collaborators: Drs. Hideki Nakano and Terutaka Kakiuchi (Dept. Immunology, Toho Univ.)

corresponding author: M. Yoshino, e-mail: myoshi@grape.med.tottori-u.ac.jp

P2 Aberrant B1 cell trafficking in a murine model for SLE: Possible roles for autoantibody production by B1 cells and for breakdown of central tolerance in the thymus.

Sho Ishikawa, Taku Sato, Toshihiro Ito, Kenji Akadegawa, Hideaki Yurino, Masahiro Kitabatake, and Kouji Matsushima[1]

Department of Molecular Preventive Medicine, School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan

We previously reported that B lymphocyte chemoattractant (BLC/CXCL13) was highly and ectopically expressed by dendritic cells in aged BWF1 mice developing lupus nephritis and that B1 cells were preferentially chemoattracted towards BLC(Ishikawa S. et al. J. Exp. Med. 193:1393-1402, 2001). We also demonstrated that $CD11b^{+}CD11c^{+}$ DCs were increased in the circulation in BWF1 mice before the onset of proteinuria and that BLC was induced in these peripheral blood DCs and bone marrow derived myeloid DCs in the presence of TNF-α as well as IL-1α (Ishikawa S. et al. Eur. J. Immunol. 32:1881-1887, 2002).

In the present study, we found that B1 cells failed to home the peritoneal cavity in aged BWF1 mice when injected i.v. and that they were preferentially localized in the cellular infiltrates in the target organs including the kidney, lung and thymus. $CD11b^{high}F4/80^{high}$ cells with macrophage morphology were the major cell source for BLC in the peritoneal cavity both in young and aged BWF1 mice. The number of BLC producing-peritoneal macrophages was markedly decreased in aged BWF1 mice. The serum levels of T15 Id^{+} IgM antibodies produced by B1 cells was significantly lower in aged BWF1 mice than those in young BWF1 mice. On the other hand splenic CD4 T cells from aged BWF1 mice enhanced IgG antibody production by B1 cells. Furthermore, co-culture of thymocytes with B1 cells resulted in an escape of particular TCR $V\square^{+}$ T cells from clonal deletion.

These results suggest that the decreased number of BLC producing-peritoneal macrophages together with ectopic high expression of BLC in aged BWF1 mice result in abnormal B1 cell trafficking which leads to decreased natural antibody production, enhanced IgG autoantibody production, and breakdown of central tolerance in the thymus during the development of murine lupus.

Corresponding author: Sho Ishikawa, mail: yamasho@m.u-tokyo.ac.jp

P3 Regulation of Kupffer cell accumulation by chemokines in murine liver metastasis model

Naofumi Mukaida, Yoko Nemoto-Sasaki, and Xiaoqing Yang

Div. Molec. Bioregulation, Cancer Research Institute, Kanazawa University, Kanazawa, 920-0934, Japan

Liver metastasis is one of the most important factors, which affect the prognosis of patients with gastrointestinal cancer. Kupffer cells have been presumed to have crucial roles in host defense mechanism in liver but it still remains elusive on the regulatory mechanism of their trafficking in liver metastasis. Hence, we investigated the traficking of Kupffer cells and the gene expression of chemokines with potent chemotactic activities for Kupffer cells, in murine liver metastasis model.

Liver metastasis was induced by injecting a colon adenocarcinoma cell line, colon 26 (5 x10^4 cells/mouse) into tail vein of 8-week old female BALB/c mice. Liver was removed for the histological analyses and the extraction of total RNA for RT-PCR at various time intervals.

Micrometastasis foci were observed at 6 days after the injection, and the numbers and the sizes of foci were increased progressively, thereafter. Simultaneously with the appearance of micrometastasis foci, F4/80-positive macrophages/Kupffer cells accumulated in liver, particularly inside the metastatic foci. Among chemokines with chemotactic activities for macrophages/Kupffer cells, monocyte chemoattractant protein (MCP)-1/CCL2 gene expression was enhanced from 6 days, while macrophage inflammatory protein (MIP)-1α/CCL3 and RANTES/CCL5 gene expression was increased from 10 days after the tumor injection. Moreover, immunohistochemical analysis demonstrated the presence of MCP-1/CCL2 proteins in endothelial cells as well as tumor cells, 10 days after the tumor injection. These observations suggest the potential roles of MCP-1/CCL2, MIP-1α/CCL3, and RANTES/CCL5 in Kupffer cell accumulation in the process of liver metastasis.

Corresponding author: N. Mukaida, e-mail: naofumim@kenroku.kanazawa-u.ac.jp

P4 Functional analysis of tumor-infiltrating plasmacytoid dendritic cells in head and neck cancer

E. Tuma[1], S. Rothenfusser[3], O. Gires[1], B. Wollenberg[2], G. Hartmann[3]

(1) Dept. of Otorhinolaryngology, Großhadern Medical Center, Ludwig-Maximilians-University Munich, Germany (2) Dept. of Otorhinolaryngology, University of Lübeck, Germany and (3) Clinical Pharmacology, Ludwig-Maximilians-University of Munich, Germany

Background. The anti-tumor activity of IFN-α is well established. However, the role of the plasmacytoid dendritic cell (PDC), the major producer of IFN-α upon viral infection, in tumor biology is unknown. We sought to study the presence and function of PDC infiltrating head and neck squamous cell carcinoma. **Methods.** PDC were characterized in single cell suspensions of tumor tissue by flow cytometry. Functional activity of PDC was examined by using CpG motif containing oligonucleotides (CpG ODN), the only defined microbial stimulus for PDC (recognized via toll-like receptor-9, TLR9). TLR9 expression on isolated PDC was quantified by real time PCR. **Results.** Relatively high numbers of PDC were found to infiltrate the tumor tissue of head and neck squamous cell carcinoma. Interestingly, CpG-induced PDC activity within the tumor environment was differentially affected: while tumor-derived factors synergistically enhanced the CpG-induced upregulation of costimulatory molecules, CpG-induced IFN-α production in PDC was impaired. TLR9 was downregulated in PDC exposed to tumor cell supernatant. IL-10, known to inhibit IFN-α production in PDC, was increased in tumor tissue but was absent in the supernatant of tumor cell lines which also inhibited CpG-induced IFN-α production by PDC. CpG-induced IFN-α production was associated with activation of myeloid dendritic cells and of CD4 and CD8 T cells, and with increased levels of IFN-α and IP-10. **Conclusion.** These results show that tumors suppress the capacity of PDC to produce IFN-α. PDC which in the absence of appropriate stimulation have been reported to promote regulatory CD8 T cells, may contribute to an impaired T cell-mediated immune response in HNSCC.

P5 Changes in macrophage number and distribution in the rat thymus after X-ray irradiation

Nobuko Tokuda, Keiko Hamasaki, Noriko Mizutani, Yamini Arudchelvan, Yasuhiro Adachi, Tomoo Sawada, *Yoshihisa Fujikura and Tetsuo Fukumoto

Dept. Human Science, Yamaguchi Univ. School of Medicine, Ube, Yamaguchi 755-8505, Japan, *Dept. Anatomy, Oita Medical Univ., Oita, Japan

X-ray radiation severely affects lymphoid organs. Among the lymphoid organs, thymus is considered to be extremely sensitive to radiation. We have investigated the effects of various doses of radiation on rat thymus: After irradiation thymocyte number decreased and recovered. The recovering features appeared to be dependent on the radiation doses. In this study, we examined the effects of radiation focusing on the changes in the macrophage number and distribution.

[Materials and Methods] Nine-week-old, inbred, female DA (RT-1^{a}) rats were used. Each rat received whole body irradiation (0.5, 6, and 8 Gy). Eight-Gy irradiation was sublethal yet close to the maximum dose that left the animals alive. Immunohistochemical analysis on the thymus was conducted using anti-macrophage antibody (ED2).

[Results] [1] After irradiation, the rats lost body and thymus weights: In 0.5 Gy irradiated rats, thymus weight decreased to 60 % of normal control. In 8 Gy irradiated rats, the weight became about 15 % of normal control. The thymus weights then increased rapidly. [2] In H&E staining, after irradiation cellularity of thymocytes once decreased and then recovered: After 0.5 Gy-irradiation, the thymus structure showed decreased cellularity on day 1-2 and recovered on day 5. In 6 Gy irradiated rats, thymus showed the decrease in thymocytes number on day 3-5, and appeared to recover on day 7. In 8 Gy irradiated rats, reduced number of thymocytes was observed on day 3-7. On day 7 the cortico-medullary junction was unclear; on day 14, the structures seemed almost recovered. [3] In immunohistochemical staining for ED2 in normal control rats, positive cells were seen in the cortex and cortico-medullary region. The medulla was almost negative for ED2. After 0.5 Gy-irradiation, the ED2 positive cells increased 1-2 days after irradiation and returned to normal by day 5. In 6 Gy irradiated rats, the ED2 positive cells increased in number on day 3 after irradiation; then the positive cell number recovered on day 7. After 8 Gy-irradiation, the number of ED2-positive cells decreased in number and appeared to recover to normal on day 14.

[Discussion] From these results, ED2 positive macrophages at the cortical region increased once before the thymic structure recovered in both low and high dose irradiation while they once decreased and recovered after very high dose irradiation (8 Gy). The transient increase of macrophages in 0.5 and 6 Gy irradiated rats thymus may be important for clearing out the dead thymocytes and some humoral factors released from these macrophages may be important for the regeneration of thymocytes after irradiation.

corresponding author: N. Tokuda, e-mail: toku@yamaguchi-u.ac.jp

P6 Prognostic importance of activated macrophage-derived soluble CD163 in fulminant hepatitis

Atsushi Hiraoka [1], Norio Horiike [1], Sk. Md. Fazle Akbar [1], Takami Matsuyama [2], Morikazu Onji [1]

[1] The Third Department of Internal Medicine, Ehime University School of Medicine, Ehime, Japan. [2] Department of Immunology, Kagoshima University School of Medicine, Kagoshima, Japan

The initial symptoms of both acute and fulminant hepatitis (FH) are similar; however, some patients develop FH and progress to a rapidly downhill course. It is hard to predict which patient would develop FH in clinical situation. Again, there is paucity of information regarding the prognostic marker of FH. Activation of macrophages and the products of activated macrophages are implicated in the pathogenesis and progression of FH.

Accordingly, we checked the serum levels of a soluble form of CD163 (sCD163) in patients with liver diseases to evaluate if sCD163 has any prognostic importance in patients with FH.

sCD163 was assessed in 27 patients with FH, 19 patients with acute hepatitis (AH), and 24 patients with chronic hepatitis by an enzyme-linked immunosorbent assay. The levels of sCD163 were higher in patients with FH (781.5 ± 386.7 ng/ml) and AH (265.8 ± 169.0 ng/ml) compared to those of chronic hepatitis (169.8 ± 88.8 ng/ml) [$p<0.05$]. There was a good correlation between the levels of sCD163 in the sera and the prothrombin time ($r=-0.65$, $p<0.01$) and total bilirubin ($r=0.36$, $p<0.01$) levels. Kinetic study indicated that the levels of sCD163 decreased in patients with AH and in survivors of FH, whereas the levels of sCD163 progressively increased in nonsurvivors of FH. This study indicates that the levels of sCD163 in the sera may have prognostic importance to predict transition and fatality to FH.

Corresponding author: Norio Horiike, M.D., e-mail: horiike @ m.ehime-u.ac.jp

P7 Development of a novel optical assay system for measurement of cellular chemotaxis

Shiro Kanegasaki, Nao Nitta, Yuka Nomura, Shuichi Akiyama and Takuya Tamatani
Effector Cell Institute in RCAST, Univ. of Tokyo, Tokyo 153-8904, Japan

Chemotaxis is the directional movement of cells in their various roles or activities such as inflammatory response, homeostatic circulation, and development. It concerns a number of disorders including infectious and allergic diseases, wound healing, angiogenesis, atherosclerosis, and tumor metastasis. Learning more about the chemotactic processes of various cells including monocytes and macrophages should bring numerous benefits to the basic and applied medical sciences.

The conventional assay method currently used is the Boyden's chamber technique, which utilizes an upper and lower compartment containing cells and a chemoattractant, respectively, separated by a filter. To assay chemotaxis, the number of migrated cells on the lower surface of the filter membrane or in the lower compartment is counted, directly or indirectly. It is not possible to observe cell migration or to obtain sufficient information on the nature of gradients established. Additional serious limitation by the technique is the requirement of a large number of cells (5×10^4 or more) for each assay.

We have developed an optically accessible, horizontal chemotaxis apparatus consisting basically of an etched silicon substrate and a flat glass plate, both of which form two compartments and a microchannel in between. The device is held together with a stainless steel holder with holes for injecting cells and a chemoattractant to the different compartments. Migration of cells in the channel is traced with time-lapse intervals using a CCD camera.

By developing a method of aligning cells at the edge of the channel, we could successfully reduce the number of cells required for a chemotactic assay to 100 or less. To prevent ceaseless flow of contents between the adjacent compartments via the communicating microchannel, a space at the top end of the holder was filled with medium after aligning the cells. By using a fluorescent probe, we demonstrated experimentally that a stable concentration gradient could be maintained. Furthermore, we determined theoretical details of the gradient established using a model chemokine and a computational fluid dynamics code. Reproducible kinetic results of migration of human neutrophils toward IL-8 and human monocytes toward Mip-1α or MCP-1 were obtained as models.

Using the 48 channel apparatus, we have developed an instrument consisting of an automatic sample dispensers and the automatic monitoring system.

Corresponding author: S. Kanegasaki, e-mail:shirokan@ims.u-tokyo.ac.jp

P8 Characterization of murine grancalcin expressed in neutrophils and macrophages and its possible role in the defense against bacterial infections

Fengzhi Liu, Hiroto Shinomiya, Teruo Kirikae, Yoshihiro Asano

Department of Immunology and Host Defenses, Ehime University School of Medicine, Ehime; International Medical Center of Japan, Tokyo; Japan

The innate immune system initiates the first line of host defense by recognizing pathogen-specific molecules like lipopolysccharide (LPS) and coordinating inflammatory responses. Phagocytes such as neutrophils, macrophages, and immature dendritic cells are the key cellular components of the system. In order for these cells to execute their functions, it is of importance that they are able to recruit into inflamed or infected tissues. In this respect, we previously identified a 65-kDa protein (p65/L-plastin) containing a series of Ca^{2+}-, calmodulin-, and β-actin-binding domains that is phosphorylated in macrophages by stimulation with LPS. Interestingly, the protein proven to contribute to the regulation of integrin-mediated cellular adhesion and locomotion, which has prompted us to further investigate the p65/L-plastin-involved cellular events in more detail.

In the present study, we have characterized grancalcin that has recently been suggested to be a binding partner of p65/L-plastin in cells and examined its possible role in the host defense. We cloned the cDNA of murine grancalcin and analyzed its nucleotide sequences (accession No. AB088388). The gene encodes a 220 amino acid protein (MW = 24,700, pI = 4.74). According to the sequence, grancalcin belongs to the penta-EF-hand subfamily of Ca^{2+}-binding proteins with EF-hands, which also comprises sorcin (expressed in drug-resistant cancer cells) and ALG (apoptosis-linked gene)-2 (expressed in thymocytes). To investigate the biological functions of grancalcin, we expressed a recombinant (r) grancalcin in *E. coli* and found that r-grancalcin precipitated in the presence of Ca^{2+}, indicating that it goes important conformational changes upon binding of Ca^{2+}. Specific antibodies (Ab) against r-grancalcin were prepared and a flow cytometric assay using the Ab was developed, which enabled us to detect the intracellular grancalcin. Double staining analysis with the Ab, together with Abs to cell surface markers (CD3, B220, CD11b, and Ly-6G), revealed the grancalcin expression in each subpopulation. Phagocytes like macrophages and neutrophils highly express the protein, whereas the expression in lymphocytes is relatively small. Interestingly, the protein was particularly abundant in the cells infiltrated into the infected site. It was also observed by immunohistochemical analysis that the intracellular localization of grancalcin was extensively changed by bacterial stimulation. These findings suggest that grancalcin may play a crucial role in the cell-mediate defense mechanisms.

Corresponding author: H. Shinomiya, e-mail: hiroto@m.ehime-u.ac.jp

P9 Sinomenine inhibits dendritic cells maturation and migration leading to promotion of allograft acceptance

Quanxing Wang[1,2], Baomei Wang[1], JianchunWu[1],Yushan Liu[1], Wenji Sun[1], Minhui Zhang[1], Min Zhang[1], Jianli Wang[1] , Xuetao Cao[1], Xiaokang Li[2]

1 Institute of Immunology, Second Military Medical University, Shanghai 200433, P.R.China;
2 National Medical research Institute for Child health, Tokyo 154-8567, Japan

Sinomenine (SN) is a pure compound extracted from the medical plant *Sinomenium acutum* which has been successfully used for treatment of various immune disorders such as rheumatic diseases for many centuries in China. SN has been shown to be able to inhibit proinflammatory factor production by macrophages and impair proliferation of lymphocytes, but it is not clear whether SN can inhibit the maturation and functions of dendritic cells (DC). In the present study, we examined the effects of SN on the maturation and functions of DC. We found that SN significantly inhibits DC expression of CD40, CD86, MHC class II molecules and cytokines with or without the stimulation of LPS. SN reduces DC chemokine receptor expression and chemotasis activity, impairs their priming capacity and the allostimulatory activity, selectively suppress NF-κB and p38MAPK pathway. SN treated DC can effectively induce T-cell hyporesponsiveness. In vivo pretreatment of mice with immature DC, SN-treated DC or SN alone could prolong allograft survival to some degree but can not induce long-term allograft acceptance. However, pretreatment of the recipients with SN-treated DC in combination with SN administration prolonged allograft survival most significantly, resulting in long-term survival of 30% allografts. The decreased expression of Th1 and proinflammatory cytokines in the allografts and enhanced generation of microchimerism in the recipients may contribute to the promotion of allograft acceptance by SN. Our results suggest that SN potentiate the tolerogenicity of immature DC and combined use of SN and treated iDC can effectively induce immune tolerance.

Key word: sinomenine; dendritic cells; anti-inflammatory drug; maturation; allograft survival; tolerance

Corresponding author: Xiaokang Li, e-mail: kang1103@clubba.com

P10 BCG induced Hepatic Granuloma Formation in TACO transgenic mice

Takayuki Hirose[1], Yuusuke Ebe[1], Takashi Yamamoto[1], Jean Pieters[2] and Makoto Naito[1]

[1]Department of Cellular Function, Division of Cellular and Molecular Pathology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[2]Department of Immunology, Basel University, Basel, Switzerland

TACO is a murine macrophage-specific protein which represents a component of phagosome coat by live mycobacteria. Retaining of TACO on mycobacterial phagosome seems to prevent phagosomal maturation and thus these pathogens can escape host defense mechanisms. In this study, we examined BCG-induced granuloma formation in TACO transgenic mice to address potential involvement of TACO during the process of granulomatous inflammation *in vivo* and *in vitro*.

Live Mycobacterium bovis BCG ($1x10^7$CFU) was injected intravenously to TACO transgenic mice (TG) and wild-type (WT) mice. In TG mice, the number of hepatic granulomas was smaller than that in WT mice until 2 weeks after infection. And the number of TACO-positive granulomas was also smaller than that in WT mice. However, there was no difference between the sizes of granulomas in the both groups. In the mouse liver, expressions of TACO mRNA and IFN-gamma were decreased in TG mice in the early stage of infection. Because TACO is suggested to be involved in the endocytic and intracellular transport mechanism, we then analyzed the phagocytic ability of mycobacteria using thioglycollate-elicited exudate macrophages from TG and WT mice. However, there was no significant difference in the phagocytic capacity between two groups.

These findings suggest that TACO plays an important role in intracellular killing of mycobacteria, but not in phagocytosis of mycobacteria.

Corresponding author: Makoto Naito E-mail;mnaito@med.niigata-u.ac.jp

P11 Cell parking of GFP positive bone marrow cells after intra-portal injection in rats

Yuka Igarashi*, **Yuki Sato***, Yoji Hakamata, Takashi Kaneko, Takashi Murakami, Masafumi Takahashi, and Eiji Kobayashi

*The first two authors were equally contributed.
Division of Organ Replacement Research and Animal Resource Project, Center for Molecular Medicine, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi, Kawachi, Tochigi, Japan

Background. Recent studies showed the evidence that hepatic stem cells exist in adult bone marrow. While bone marrow derived hepatic stem cells may provide an attractive new cell therapy to repair damaged liver, little is known about the early fate of bone marrow cells (BMCs) following their intra-portal administration. In the present study, we analyzed trafficking of transplanted donor BMCs after intra-portal injection using GFP Tg LEW rats, which was newly established from the our previously developed GFP Tg Wistar rats (BBRC 2001;286:779).
Methods. Whole BMCs (3-5 x 10^8 cells) from GFP Tg LEW rats were injected into the right liver lobe of naïve LEW rats via portal vein (n=3). Selective cell transplantation to the hepatic lobe was achieved by clamping the left side of portal vein and hepatic artery. Two days after transplantation, recipients were sacrificed and GFP positive cells obtained from various hemato-lymphoid organs were analyzed by flow cytometer. The FSC/SSC patterns of the GFP positive cells were also examined to detect cell lineages of lymphocytes or granulocytes.
Results. In the recipient non-ischemic liver (right lobe), 2.6% of total mononuclear cells were GFP positive cells and more than 20% of cells in granulocyte gating were GFP positive. In the spleen and the bone marrow from recipients, 6.4% and 3.3% of total cells were GFP positive, respectively. On the other hand, less than 1 % of GFP positive cells were found in ischemic liver (left lobe). The FSC/SSC patterns of the GFP positive cells which were accumulated in the liver showed more than half of the donor derived cells were belonged granulocyte gating. This pattern was quite different from that of obtained from the spleen.
Conclusions. The mechanism of entry and differentiation of the transplanted bone marrow cells within the liver is still unclear and some BMCs might enter the liver through the systemic circulation after intra-portal injection. However, our data suggest that intra-portal administration of BMCs into liver parehcyma might be a promising strategy to accumulate hepatic stem cells efficiently for liver regeneration.

Corresponding author: E. Kobayashi, e-mail: eijikoba@jichi.ac.jp

P12 Identification of Sialoadhesin as a Ligand for MGL1 in Murine Lymph Nodes: Potential Roles in the Interaction between Two Types of Macrophages within Subcapsular Sinus

Yosuke Kumamoto, Nobuaki Higashi, Koji Sato, Kaori Denda-Nagai, Makoto Tsuiji, *Paul R. Crocker, and Tatsuro Irimura

Laboratory of Cancer Biology and Molecular Immunology, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan, * The Wellcome Trust Biocentre, School of Life Sciences, University of Dundee, Dundee, UK

Migration of immune cells is a critical event in the immune responses. In contact hypersensitivity, epidermal Langerhans cells (LCs) and dermal macrophages (MØs) are known to migrate from the skin to lymph nodes (LNs) after percutaneous invasion by antigen. MGL (MØ galactose-type C-type lectin) is a family of C-type lectins consisted of MGL1 and MGL2 at least in mice and expressed on dermal MØs in skins. These MGL1/2$^+$ cells in the dermis are known to migrate into and accumulate within regional LNs after sensitization with FITC dissolved in acetone/dibutylphthalate (1/1) solution. The distribution of MGL1/2$^+$ cells in regional LNs after sensitization was immunohistochemically demonstrated to be highly restricted to the subcapsular and medullary sinus, interfollicular regions, and the boundary between T- and B-cell areas. This distribution of MGL1/2$^+$ cells is clearly distinct from that of LCs, which is known to migrate into T-cell areas in LNs after sensitization. The distribution of MGL1/2$^+$ cells was overlapped with that of the areas bound by recombinant MGL1 (rMGL1) in LNs. The results suggested that the unique distribution of MGL1/2$^+$ cells in LNs was determined by the interaction between MGL1 and its ligands. It is essential to identify the ligands for MGL1 in LNs for understanding the role of MGL1 in dermal MØ trafficking in contact hypersensitivity.

Affinity chromatography using immobilized rMGL1 was performed to separate lysates of mouse LNs. SDS-PAGE analysis of bound and eluted components indicated that the most prominent component migrated at 200-kDa. The component was sown to be sialoadhesin (Sn) by the MS-fingerprinting methods. Western blotting using two monoclonal antibodies confirmed this finding. In ELISA, the MGL1 binding was detected and was shown to be reduced after treatment of Sn with *N*-glycosidase F and by the presence of EDTA. *In vitro* cell adhesion assays revealed that MGL1$^+$ cells are capable of binding to Sn. Finally, immunohistochemical studies indicated that MGL1$^+$ cells colocalized with Sn in the subcapsular sinus of peripheral LNs.

In conclusion, we demonstrate that Sn, a marker for subcapsular sinus MØs in LNs, is a ligand for MGL1. Based on these data, we propose that the direct interactions between two unique MØ subsets regulate MØ trafficking in LNs.

corresponding author: Tatsuro Irimura, e-mail: irimura@mol.f.u-tokyo.ac.jp

P13 Presentation of Glycosylated Antigens taken up by the Macrophage Galactose-type C-type Lectin on Bone Marrow-Derived Immature Dendritic Cells

Kaori Denda-Nagai, Satoshi Aida, Makoto Tsuiji, Stephen M. Hedrick* and Tatsuro Irimura

Laboratory of Cancer Biology and Molecular Immunology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan and *the Cancer Center and the Division of Biology, University of California, San Diego, La Jolla, California 92093-0687

Dendritic cells (DCs) are known as professional antigen presenting cells (APCs). These cells capture antigens, process them to form MHC-peptide complexes, and activate naive T cells through these complexes. Recognition and internalization of antigens seem to be mediated by a variety of cell surface molecules. The macrophage galactose-type C-type lectin (MGL) is a type II transmembrane glycoprotein and potentially participates in the recognition and internalization of antigens having clusters of *O*-glycans. Recently, we identified novel MGL gene, mMGL2, which is highly homologous to mMGL1 previously called mMGL. The carbohydrate specificity of recombinant mMGL1 (Lewis X) was shown to be distinct from that of mMGL2 (GalNAc). In the present study, we examined the expression and function of mMGL1/2 by using bone marrow-derived DCs (BM-DCs) generated from wild-type C57BL/6 mice or *mMgl1* knock out mice and CHO cells expressing mMGL1 or mMGL2.

The surface expression of mMGL1/2 on BM-DCs was examined by flowcytometry using specific mAbs against mMGL1 or mMGL2. mMGL1/2 was expressed on immature DCs with low to moderate levels of MHC class II and not expressed on the majority of mature DCs with high levels of MHC class II. The binding and internalization of glycosylated antigens to BM-DCs were examined using α-*N*-acetylgalactosaminides conjugated to soluble polyacrylamide carriers (α-GalNAc polymers) as a model antigen. α-GalNAc polymers, but not β-*N*-acetylglucosaminide (β-GlcNAc) polymers, bound to immature DCs in Ca^{2+}- and GalNAc-dependent manners. By the use of CHO cells expressing mMGL1 or mMGL2, both mMGL1 and mMGL2 were shown to recognize α-GalNAc polymers. Binding and uptake of α-GalNAc polymers by BM-DCs generated from *mMgl1* knock out mice were approximately 50% of BM-DCs generated from wild-type mice. Rat anti-mMGL polyclonal antisera, which bound to both mMGL1 and mMGL2, inhibited the binding and uptake of α-GalNAc polymers by BM-DCs generated from *mMgl1* knock out mice and wild-type mice. Subsequently, we examined whether the antigens internalized through mMGL were presented to T cells. Lymph node T cells were prepared from mice immunized with streptavidin. BM-DCs were cultured overnight with bio-α-GalNAc polymer and streptavidin, bio-β-GlcNAc polymer and streptavidin, or streptavidin alone. Proliferation of streptavidin-primed T cells was more prominent when they were co-cultured with bio-α-GalNAc polymer/streptavidin-treated BM-DCs than control BM-DCs.

These results indicate that molecules with multiple GalNAc residues efficiently taken up through mMGL1/2 on BM-DCs are presented to antigen-specific T cells.

References

1. Tsuiji M., Fujimori M., Ohashi Y., Higashi N., Onami TM., Hedrick SM., and Irimura T. Molecular cloning and characterization of a novel mouse macrophage C-type lectin, which has a distinct carbohydrate specificity from mMGL1. J. Biol. Chem., 277, 28892-28901, 2002
2. Denda-Nagai K., Kubota N., Tsuiji M., Kamata M., and Irimura T. Macrophage C-type lectin on bone marrow-derived immature dendritic cells is involved in the internalization of glycosylated antigens. Glycobiology, 12, 443-450, 2002

corresponding author: Tatsuro Irimura, e-mail: irimura@mol.f.u-tokyo.ac.jp

P14 Selective expression of chemokines in the *Salmonella*-infected spleen

Hiroto Shinomiya[1], Fengzhi Liu[1], Teruo Kirikae[2], Asano Yoshihiro[1]

[1]Department of Immunology and Host Defenses, Ehime University School of Medicine, Ehime; [2]International Medical Center of Japan, Tokyo; Japan

The spleen serves two major critical roles in host protection against many types of pathogen ― it is a reticuloendothelial system that removes microorganisms from the bloodstream and it is a major secondary lymphoid organ where T and B cells are antigen-specifically stimulated. Some kinds of pathogenic bacteria like *Salmonella* can survive in the spleen of the nonimmune host, and the spleen needs to reinforce its defense mechanisms after infection. Indeed, marked splenomegaly that is thought to be a host-defensive response is induced 4-7 days after inoculation with *Salmonella* organisms as we previously reported. Immunohistochemical studies revealed that CD11b-positive cells greatly increased in the red pulp and that some CD11b- and/or CD11c-positive cells migrated into the white pulp. In order to maintain the functional integrity of the spleen, chemokines playing pivotal roles in the migration and the retetion of various leukocytes seem to be involved in this process. We thus examined the expression of chemokines (12 CXC, 19 CC, one CX3C, and one C chemokines) during the development of the splenomegaly by RT-PCR analyses. Interestingly, very selective chemokines were found to be expressed in the spleen of mice infected with *Salmonella*. These findings suggest that inflammatory and immune responses induced by splenic sequestration of bacteria are coordinated through the induction of a set of chemokines.

Corresponding author: H. Shinomiya, e-mail: hiroto@m.ehime-u.ac.jp

P15 Localization of marginal zone macrophages is regulated by CCR7 signalling.

Manabu Ato, *Hideki Nakano, *Terutaka Kakiuchi, and Paul M. Kaye

Department of Infectious Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, the United Kingdom; *Department of Immunology, Toho University School of Medicine, Tokyo 143-8540, Japan

The marginal zone of the spleen is known as an important site for capturing blood-borne pathogens and as a gateway for the trafficking lymphocytes into the splenic white pulp. We previously reported that during visceral leishmaniasis, a chronic infection caused by the parasite *Leishmania donovani*, is associated with changes to the structure of the marginal zone, notably a loss of marginal zone macrophages (MZMs). Loss of MZMs correlated with decreased lymphocyte traffic into the white pulp, leading us to suggest that these anatomical changes may contribute to the immunodeficiency that is characteristic of this disease. As a basis for investigating why MZMs are lost during chronic infection, we have investigated how these cells maintain their anatomical distribution in the steady state in normal, uninfected mice. Here we report that *plt/plt* mice, which lack functional CCL19 and CCL21, have far fewer MZMs compared with normal C56BL/6 mice. Moreover, administration of Pertussis toxin (PTx), an inhibitor of chemokine receptor signalling, to C56BL/6 mice resulted in MZMs exiting the marginal zone and becoming localized in the red pulp. Preliminary studies also indicate that MZMs can migrate in vitro in response to CCL21 but not CCL19. Collectively these data suggest that CCR7-mediated responses to CCL21 may play a role in the localization of MZMs in the spleen. Our previous observation that the stromal cell source of CCL21 in the spleen is dramatically reduced during chronic infection (Ato et. al. Nature Immunol 2002 3:1185-1191) thus provides one possible explanation for the loss of MZMs seen during this disease.
Corresponding author: manabu.ato@lshtm.ac.uk.

P16 K-567 cells lack MHC Class II expression due to an alternatively spliced CIITA transcript with a truncated coding region

Noel E. Day[1,2], Hideyo Ugai[2], Albert T. Ichiki[1] and *Kazunari K. Yokoyama[2]

[1]Department of Medical Genetics, Graduate School of Medicine, The University of Tennessee Medical Center, Knoxville, Tennessee 37920, USA; [2]Gene Engineering Division, Department of Biological Systems, BioResource Center, RIKEN (The Institute of Physical and Chemical Research), Tsukuba, Ibaraki 305-0074, Japan.

The focus of this study was to determine the functional capacity of CIITA. A regulatory factor of MHC class II genes, in K-562 cells. We show that CIITA mRNA is present in K-562 cells and the IFN-γ inducible CIITA promoter-IV exhibits low levels of basal activity, which is greatly enhanced upon treatment with IFN-γ. Further study revealed that the CIITA cDNA contains an insertion of genomic sequence, which introduces a stop codon. The truncated coding region of the CIITA transcript in K-562 cells provides a possible explanation for the absence of MHC class II molecules.

corresponding author: K. Yokoyama, e-mail: kazu@rtc.riken.go.jp

P17 Possible involvement of muscularis resident macrophages in the impairments of interstitial cells of Cajal and myenteric nerve systems in Crohn's colitis model

Kazuya Kinoshita[1], Kazuhide Horiguchi[1], Shigeru Yamato[2], Masahiko Fujisawa[1], Koichi Sato[1], Masatoshi Hori[1] and Hiroshi Ozaki[1]*

[1] Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan
[2] International Medical Center of Japan, Toyama 1-21-1, Shinjuku-ku, Tokyo 162-8655, Japan

We evaluated the changes in interstitial cells of Cajal (ICCs, pace maker cells) and myenteric nerve system in relation to the activity and number of muscularis resident macrophage at a level of myenteric nerve plexus in the Crohn's disease model treated with 2,4,6-trinitrobenzene sulfonic acid (TNBS). In the TNBS-treated rat colon, ICCs network and myenteric nerve system were damaged or disappeared in the inflamed region. The number of ED2-immunoreactive resident macrophage significantly increased where the ICCs or myenteric nerve systems changed. Although resident macrophage appeared morphologically ramified in control intestine, TNBS-treatment changed it to round shape, possibly an indication of functionally activated state. Physiological study indicated that the motility index, the amplitude and frequency of spontaneous contractions were significantly decreased in the TNBS-induced colitis intestine. Moreover, the index of peristalsis observed in whole proximal colon tissue was inhibited by the treatment with TNBS. Electron microscopic analysis indicated that the contour of the myenteric ganglia became irregular in TNBS-treated rat colon, and numerous macrophages were observed around the ganglia. In conclusion, the inhibition of spontaneous contractions and peristalsis in circular smooth muscle from TNBS-induced colitis rat colon may be attributable to the impairments of ICCs and myenteric nerve systems. Because of the indication of activation and the close correlation between the degeneration and macrophages accumulation suggest that these cells are somehow be involved in the degenerative pathology of intestinal motility.

Corresponding author: H. Ozaki, E-mail: aozaki@mail.ec.u-tokyo.ac.jp

P18 Interleukin-10-induced CCR5 expression in macrophage-like HL-60 cells: involvement of Erk1/2 and STAT-3

Yoshiko Sonoda, Yoko Makuta, Daisuke Yamamoto, Eriko Aizu-Yokota, and Tadashi Kasahara

Department of Biochemistry, Kyoritsu College of Pharmacy, Shibakoen 1-5-30, Minato-ku, Tokyo 105-8512

As an immunosuppressive and anti-inflammatory cytokine, IL-10 was recently reported to play roles in CCR5 expression in human monocytes. CCR5 promoter regions contain Oct-2, TCF-1, GATA, and STAT binding sites. Here, we studied the signals involved in the CCR5 expression in IL-10-stimulated cells using a HL-60 cell line. HL-60 cells were stimulated with PMA and differentiated to macrophage-like cells, then stimulated with IL-10. IL-10 induced significant expression of CCR5 protein and CCR5 mRNA in these cells. Induction of CCR5 by IL-10 was inhibited by a MEK-1 inhibitor, PD98059. In addition, IL-10 induced tyrosine (Tyr) phosphorylation of Erk, as well as serine (Ser) and Tyr phosphorylation of STAT-3. Tyr phosphorylation of Erk and Ser phosphorylation of STAT-3 were inhibited by PD98059, while Tyr phosphorylation of STAT-3 was not inhibited by PD98059. DNA binding activity of STAT-3 was observed by the stimulation with IL-10, which was inhibited by PD98059. These results first indicate that Erk1/2 and STAT-3 regulate CCR5 expression, and that Erk-mediated phosphorylation of Ser is required for full stimulation of STAT-3 in CCR5 expression.

correspondence author: T. Kasahara, e-mail: kasahara-td@kyoritsu.ph.ac.jp

P19 TGF beta down-regulates IL-1-induced functional Toll like receptor (TLR) 2 expression in murine hepatocytes

Hayashi Hidetoshi[1], Takayuki Matsumura[1,2], Takemasa Takii[1], Jun-ichiro Inoue[2], Kikuo Onozaki[1]

[1]Dept. Mol. Health Sci., Grad. Sch. Pharm. Sci., Nagoya City Univ., Nagoya 467-8603, Japan, [2]Div. Cell. Mol. Biol., Inst. Med. Sci., Univ. Tokyo, Tokyo 108-8639, Japan

We have previously reported that the pro-inflammatory cytokine IL-1α up-regulates functional TLR2 expression in primary cultured murine hepatocytes, and bacterial lipopeptide (BLP) is capable of signaling through TLR2 to induce serum amyloid A (SAA) expression in hepatocytes. In the present study, we investigated the effect of the anti-inflammatory cytokine TGFβ on TLR2 expression in primary cultured murine hepatocytes. At both the mRNA and protein levels, TGFβ up-regulated TLR2 expression, but inhibited TLR2 expression induced by IL-1α at 24hr. BLP-induced SAA promoter activity could be augmented by pretreatment with IL-1α, but not TGFβ or the combination of TGFβ and IL-1α. Both TLR2 promoter activity and NF-κB activation by IL-1α were inhibited by TGFβ treatment. Pretreatment with TGFβ strongly suppressed IL-1-induced TLR2 promoter activity and NF-κB activation, which was consistent with the down-regulation of type I IL-1 receptor (IL-1RI) mRNA expression. IL-1α up-regulated IL-1RI mRNA, but it was inhibited by the treatment with TGFβ. These results suggest that TGFβ suppr56sses the induction of TLR2 expression by IL-1α through down-regulation of IL-1RI expression. These results also demonstrate the disparity between IL-1α and TGFβ in regulating TLR2-mediated SAA production in hepatocytes.

Correspondence author: K.Onozaki, e-mail: konozaki@phar.nagoya-cu.ac.jp

P20 A high endothelial venule secretory protein, mac25/angiomodulin, interacts with multiple high endothelial venule-associated molecules including chemokines

Daisuke Nagakubo[1,2], Toshiyuki Murai[1], Toshiyuki Tanaka[1], Takeo Usui[1], Masanori Matsumoto[1], Kiyotoshi Sekiguchi[3] and Masayuki Miyasaka[1]

[1]Lab. Molecular and Cellular Recognition, Osaka Univ. Graduate School of Medicine, Suita, Japan, [2]Dept. Microbiology, Kinki Univ. School of Medicine, Osaka-Sayama, Japan, [3]Div. Protein Chemistry, Institute for Protein Research, Osaka Univ., Suita, Japan.

High endothelial venules (HEVs) are specialized venules that allow rapid and selective lymphocyte trafficking from the blood into the lymph nodes and Peyer's patches. During the course of a search for novel genes expressed specifically in HEVs, we identified mac25/angiomodulin (AGM) as a highly expressed secretory protein in mouse HEVs. Mac25/AGM selectively accumulates in the basal lamina of HEVs, but the biological significance of the localization of mac25/AGM remains unknown. To understand the biological role of mac25/AGM in the basal lamina of HEVs, we investigated whether mac25/AGM interacts with molecules that are present within or in the victinity of HEVs. Mac25/AGM interacts preferentially with certain molecules that are expressed in or around HEVs. In particular, mac25/AGM interacted with not only the extracellular matrix (ECM) proteins and glycosaminoglycans (GAGs) that are expressed in most blood vessels including HEVs, but also with some chemokines that are implicated in the regulation of lymphocyte trafficking, such as the secondary lymphoid-tissue chemokine (SLC; CCL21), IFN-γ-inducible protein 10 (IP-10; CXCL10), and RANTES (CCL5). The binding of mac25/AGM to SLC and IP-10 was dose-dependent and saturable. The binding to IP-10 could be inhibited by SLC but not by a non-mac25/AGM-binding chemokine, EBI1-ligand chemokine (ELC; CCL19). Interestingly, mac25/AGM failed to interact with 18 other chemokines, suggesting that it binds to certain chemokines preferentially. Immunohistochemical analysis indicated that mac25/AGM colocalizes at least partially with SLC and IP-10 at the basal lamina of HEVs. Upon binding with mac25/AGM, SLC and IP-10 retained all their Ca^{2+}-signaling activity *in vitro*, suggesting that mac25/AGM can hold and present chemokines in the basal lamina of HEVs. These results imply that mac25/AGM plays a multifunctional role, serving not only as an adhesion protein to interact with GAGs and ECM proteins but also as a molecule to present chemokines so that lymphocytes extravasating through HEVs receive further directional cues subsequent to the luminal encounter with lymphoid chemokines.

Corresponding author: M. Miyasaka, e-mail: mmiyasak@orgctl.med.osaka-u.ac.jp

P21 MEC/CCL28 Has Dual Functions in Mucosal Immunity as a Chemoattractant with Broad-Spectrum Antimicrobial Activity

Kunio Hieshima, Yuri Kawasaki, Takashi Nakayama, and Osamu Yoshie

Department of Microbiology, Kinki University School of Medicine, Osaka 589-8511, Japan

CCL28 is a CC chemokine selectively expressed by mucosal epithelial cells of exocrine glands (such as salivary glands and mammary glands), colon and trachea. We found that CCL28 is constitutively expressed and secreted by the salivary glands. It attracts $IgA^{+}B220^{lo}$ antibody secreting cells (ASC) expressing CCR10, receptor for CCL28. Furthermore, it is secreted into saliva and and has a potent antimicrobial activity.

Serous acinar cells of human and mouse salivary glands were strongly positive for CCL28 and human whole and parotid saliva contained CCL28 mRNA at high levels. We also noticed a sequence similarity between the C-terminal region of human CCL28 and histatin-5, a histidine-rich cationic peptide in human saliva with a potent candidacidal activity. We demonstrated that human and mouse CCL28 have a broad-spectrum antimicrobial activity against *Candida albicans*, Gram-negative bacteria and Gram-positive bacteria. We also demonstrated that the C-terminal 28-amino acid domain of human CCL28 has a selective candidacidal activity as histatin-5. On the other hand, we found no such antimicrobial activity by CCL27, which is most closely related to CCL28 and shares CCR10 with CCL28. The antimicrobial activity of CCL28 is sensitive to high salt concentrations, as those of most other antimicrobial peptides, and rapidly induces membrane permeability in target microbes.

We also demonstrated that $IgA^{+}B220^{lo}$ ASC from parotid glands and those from intestinal tissues including Peyer's patches, mesenteric lymph nodes, small intestine and colon express CCR10. These cells also expressed CCR9 and CXCR4 in addition to CCR10. Semi-quantitative RT-PCR analysis demonstrated that CCL28 is broadly expressed in large and small intestinal tissues in contrast to CCL25 (CCR9 ligand) which is selectively expressed in small intestine. CXCL12 (CXCR4 ligand) was expressed in various tissues including bone marrow and mesenteric lymph nodes. These and other findings establish these chemokines and their receptors are selectively involved in migration and target tissue homing of $IgA^{+}B220^{lo}$ ASC.

In conclusion, CCL28 has dual functions in mucosal immunity as a chemoattractant and as a secreted molecule with a broad-spectrum antimicrobial activity.

Corresponding author: K. Hieshima, e-mail: hieshima@med.kindai.ac.jp

P22 **The B cell chemokine CXCL13 B-lymphocyte chemoattractant (BLC) is expressed in the high endothelial venules of lymph nodes and Peyer's patches and affects B cell trafficking across high endothelial venules**

Yukihiko Ebisuno[1], Toshiyuki Tanaka[1], Naotoshi Kanemitsu[1], Hidenobu Kanda[1], Kazuhito Yamaguchi[2], Tsuneyasu Kaisho[3], Shizuo Akira[3] & Masayuki Miyasaka[1]

[1]Laboratory of Molecular and Cellular Recognition, Osaka University Graduate School of Medicine, Suita, 565-0871, Japan, [2]Institute of Laboratory Animals, Yamaguchi University School of Medicine, Ube, 755-8505, and [3]Institute of Microbial Diseases, Osaka University, Suita, 565-0871.

Chemokines direct the recruitment of leukocyte subsets to various tissues. Certain CC chemokines, such as CCL21/SLC and CCL19/ELC, which are expressed by high endothelial venules (HEVs), are critical for T cell trafficking into lymph nodes (LNs) and Peyer's patches (PPs). However, chemokine that regulates B cell trafficking across HEVs remained to be fully elucidated. Here we show that a B cell chemokine CXCL13/BLC is expressed by the majority of HEVs in the absence of deliberate antigenic stimulation. While CXCL13 transcripts were found at only marginal levels in HEVs, the CXCL13 protein was readily detected immunohistochemically in the HEVs of LNs and PPs. Systemically administered anti-CXCL13 antibody bound to the surface of approximately 50% of HEVs, but not to other types of blood vessels, indicating that CXCL13 is present in the lumen of HEVs. Furthermore, in CXCL13-null mice, B cells rarely adhered to HEVs whereas T cells did efficiently. Superfusion of CXCL13-null PPs with CXCL13 restored the luminal presentation of CXCL13 and also B cell arrest in PP HEVs at least partially. Collectively, these results indicate that the CXCL13 expressed in the HEV lumen plays a crucial role in B cell trafficking into secondary lymphoid tissues such as PPs.

corresponding author: Masayuki Miyasaka
E-mail: mmiyasak@orgctl.med.osaka-u.ac.jp

P23 GM-CSF INDEPENDENT DEVELOPMENT OF DENDRITIC CELLS (DC) FROM BONE MARROW CELLS (BMC) IN GM-CSF RECEPTOR DEFICIENT MOUSE

Hua Yan, *Robb L, Hajime Hikino, Tohko Miyagi, Saito Hirohisa, Daniel P. Gold, Xiao-K Li,., Hiromitsu Kimura.

National Research Center for Child Health and Development, Tokyo, Japan
*Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia. Dept. of Research Surgery,

It has been well established that granulocyte macrophage colony-stimulating factor (GM-CSF) is one of the potential cytokines that is able to support both growth and/or differentiation of dendritic cell (DC) precursor and its progeny. Nevertheless it has been also reported that both GM-CSF deficient mouse and GM-CSF receptor knock-out mouse bears typical DC in peripheral lymphoid tissues. Inasmuch as the most of, if not all, DC or DC-related APC are derived from hematogeneous organ such as BMC, it is important to determine what is a crucial cytokine(s) to induce cell growth, differentiation and migration of DC from its BMC progeny. Here, we provide evidence that employing GM-CSF receptor deficient mouse, DC precursor and/or its progenitor cells of hematopoietic stem cells, i.e., BMC, are able to grow and differentiate into DC. Our study demonstrated that unlike normal mouse or human systems, we were not able to obtain any evidence to support the idea that GM-CSF *per se* as a growth factor for DC precursor(s) in BMC regardless of the cytokine resources. However, we obtained the most efficient outgrowth of the DC precursors with either Flt3/Flk2 ligand *per se* or cytokine combinations, Flt3/Flk2 ligand and IL-6, not with c-kit ligand and/or IL-6. Thus fully myeloid DC was found to be obtained from GM-CSF independent differentiation pathway in GM-CSF receptor knockout mouse.

P24 CYTOKINE REQUIREMENT FOR THE GENERATION OF A LARGE NUMBER OF RAT DENDRITIC CELLS (DC) BY IN VITRO CULTURING OF BONE MARROW CELLS AND IT S SELECTION OF CD161 (NKR-P1A)

Eigo Satoh*, Hua Yan, Tohko Miyagi, Saito Hirohisa, Daniel P. Gold, Xiao-K. Li, Masayuki Fujino, Kenichi Teramoto*, Shigeki Arii*, Hiromitsu Kimura.

Department of Research Surgery, National Research Center for Child Heath and Development, Tokyo, Japan *Department of Surgery, Tokyo Medical and Dental University, School of Medicen, Tokyo, Japan

It has been reported that granulocyte macrophage colony-stimulating factor (GM-CSF) is one of the potential candidates that is able to support both growth and/or differentiation from dendritic cells (DC) precursor and its progeny in mouse BMC and peripheral blood, respectively. However, this is not the case with rat DC cultures. In addition to this it has also been reported that co-stimulatory factors such as tumor necrosis factor (TNF-alfa) and/or IL-4 are necessary for the enhancement of human DC induction from BMC culture. Inasmuch as the most of, if not all, DC or DC-related APC are derived from hematogeneous organ such as BMC, it is important to determine what is a crucial cytokine to induce cell growth, differentiation and migration of DC from its BMC progeny. Here, we provide evidence that unlike mouse or human systems, c-kit ligand and/or GM-CSF *per se* are not primary cytokines to induce growth and differentiation of rat DC from their progeny.

We provide evidence that the most efficient outgrowth of the DC precursors were observed for more than one month with an aid of cytokines, Flt3/Flk2 ligand and IL-6, not with c-kit ligand and IL-6. In addition, a potential cell surface marker, CD 161 (NKR-P1A) which is known to be a functional molecule of NK cells, is useful to final purification of rat mature DC rather than with OX62. Thus fully mature DC were obtained from a differentiation of the DC precursors in concert with additional cytokines of such as GM-CSF, TNF-alfa, and IL-4.

P25 **Recruitment and involvement of cells expressing a macrophage galactose-type C-type lectin 1/2 (MGL1/2) in the antigen-specific inflammatory tissue formation**

Kayoko Sato*, Yasuyuki Imai[#], Nobuaki Higashi*, Yosuke Kumamoto*, and Tatsuro Irimura*

*Laboratory of Cancer Biology and Molecular Immunology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan and
[#]Department of Microbiology and COE Program in the 21st Century, University of Shizuoka School of Pharmaceutical Sciences, Shizuoka 422-8526, Japan

Chronic inflammation was induced in a mouse air pouch model and the role of cytokines secreted by inflammatory tissue cells expressing mouse macrophage galactose-type C-type lectin 1 and 2 (MGL1/2) was investigated. Azobenzene arsonate-conjugated acetyl BSA used as an antigen was repeatedly introduced into an air pouch on pre-immunized mice. After performing an antigenic challenge twice, chronic granulation tissue was formed in hypodermic regions of the air pouch and persisted. The granulation tissue had two distinct layers. CD11b-positive, MGL1/2-negative inflammatory cells were abundant in the area close to the antigenic stimulus, while the area far from the antigenic stimulus was dominated by MGL1/2-positive/CD11b-negative cells. Flow cytometric analyses of enzymatically-isolated cells revealed a high-level expression of MHC class II on MGL1/2-positive cells. The MGL1/2-positive population was shown to be the major source of IL-1□ by RT-PCR. Both anti-IL-1α mAb treatment and anti-MGL1 mAb LOM-8.7 in the air pouch inhibited the granulation tissue. Furthermore, anti-IL-1α mAb treatment inhibited accumulation of MGL1/2-positive cells in the granulation tissue and suppressed MHC class II expression in these cells. Anti-MGL1 blocking mAb also blocked the accumulation of MGL1/2-positive cells. These results suggest that IL-1α produced by MGL1/2-positive cells recruits MGL1/2-positive cells and maintains the integrity of granulation tissue.

Corresponding author: Tatsuro Irimura, e-mail: irimura@mol.f.u-tokyo.ac.jp