Welcome to Kanazawa

It is my great pleasure to host the 17th International Symposium on Molecular Cell Biology of Macrophages 2009. This symposium was initiated here in Kanazawa in 1991 by Professor Kouji Matsushima to promote the research in the biology of macrophage and its related cells, ranging from signal transduction mechanisms to pathophysiological roles in host defense and inflammation as well as inflammatory diseases. Since then, this meeting has been held almost annually in Japan.

This year, we planned to have three sessions, "Macrophage-related cells in physiology and pathology", "Tissue injury and regeneration", and "Inflammation and cancer". Seventeen researchers are invited to speak from United States, Australia, Britain, France, China, and Japan. We hope you will enjoy by participating in the symposium, sharing exciting ideas, and nourishing mutual friendship.

Naofumi Mukaida, MD, PhD
Conference Chairperson

Conference Chairperson:
Naofumi Mukaida
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Makoto Naito (Niigata University)
Yasuyuki Imai (University of Shizuoka)
Kensuke Miyake (University of Tokyo)
Motohiro Takeya (Kumamoto University)
Kenjiro Matsuno (Dokkyo Medical University)

General Information

Date:	July 3 (Fri) to July 4 (Sat), 2009
Venue:	KKR Hotel Kanazawa (http://kkrhotelkanazawa.gr.jp/)
	2-32 Oote-machi, Kanazawa 920-0912, Japan
Official Language:	English

Reception Party	(Fee JPY 2,000)
Date and Time:	July 3 (Fri) 19:00-20:30
Venue:	KKR Hotel Kanazawa

Registration

Registration desk will be open at noon on July 3 and at 9:00 am on July 4 in front of the conference room.

Registration

Member (Before June 25)	JPY	5,000
Member (After June 26 or On site)	JPY	7,000
Non-member	JPY	10,000
Student	JPY	3,000

Presentation

Oral Session (Invited speakers)

An LCD projector will be provided. Please bring your own laptop computer (mini D-Sub 15-pin I/F or its adapter). For backup purpose, please bring your Power Point file in USB memory stick of CD-ROM. In order to avoid technical problems, we ask you to kindly bring your Power Point Presentation at least 30 min prior to the session.

Short oral presentation for Young Investigator Award

This award was established to encourage young investigators who have made significant contributions to this symposium. The committee will select two awardees from the first authors of the posters, who are graduate students or were awarded PhD degree withing five years. The person eligible for this award will send the PDF file summarizing his/her paper in one page to the Conference Chairperson by e-mail (naofumim@kenroku.kanazawa-u.ac.jp) by June 30 and will present his/her paper with this slide in one minute in Session 2 in the evening of July 3. The awardees are kindly asked to make a short oral presentation of their accomplishment shown in their posters (8 min talk and 2 min discussion). The organizing committee asks the persons eligible for this award to bring a Power Point file (USB memory or CD-ROM) for oral presentation. Awardees of the first and the second place will receive a certificate and prize money (JPY 100,000 and JPY 50,000, respectively).

Poster Session

Poster session will be held in a room adjacent to the conference room.

Poster Set Up	July 3 (Fri)	12:00-13:30
Poster Session	July 3 (Fri)	18:00-19:00
Poster Removal	July 4 (Sat)	14:50-15:30

The dimensions of the poster board is 100-cm wide and 150-cm high. We will provide the poster number only. Please prepare the title (including names and affiliations of authors) of the poster by the authors.

Acknowledgments

This symposium is partly supported by International Training Program (Rearing of medical scientists and physician-scientists, who can develop cancer diagnosis and treatment) and The Invitaion Fellowship for Research in Japan (Short-term), both of which are sponsored by Japan Soceity for the Promotion of Science. The organizer sincerely appreciates the generous support for and participation in the 17th International Symposium on Molecular Cell Biology of Macrophages 2009 by the following foundations.

第 17 回マクロファージ分子細胞生物学国際シンポジウムの開催に際して、日本学術振興会より、若手研究者インターナショナルトレーニングプログラム（がんの診断・治療法の開発を担う医科学研究者の育成）ならびに外国人招へい研究者（短期）の補助を受けています。また、下記の財団よりのご援助、ご寄付、ご協力を頂いております。ここに厚く御礼申し上げます。

SPONSORS AND CONTRIBUTORS　(2007 年 6 月 1 日現在)

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Kowa Life Science Foundation
内藤記念科学振興財団
The Naito Foundation

Program

July 3 (Friday)

12:00 Registration and Poster Setting

13:00 Opening Remarks **Naofumi Mukaida** (Kanazawa University)

Session 1: Macrophage-related cells in physiology and pathology

Chairpersons: **Kouji Matsushima** (University of Tokyo)
Motohiro Takeya (Kumamoto University)

13:10–13:55 Modulation of macrophage numbers during chronic inflammation
John Hamilton (University of Melbourne, Australia)

13:55-14:40 The bone marrow: an unexpected site of neutrophil clearance
Sara M. Rankin (Imperial College London)

14:40-15:05 Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis.
Masako Kohyama (Washington University)

15:05-15:20 **Coffee Break**

15:20-15:40 Macrophage mediated inflammatory cascades in ischemic kidney injury
Kengo Furuichi (Kanazawa University)

15:40-16:00 The complex role of chemokine receptor signal on macrophage in ocular neovascularization
Peirong Lu (Soochow University)

16:00-16:20 Role of saturated fatty acid/TLR4 signaling in macrophages in obesity-induced adipose tissue inflammation
Takayoshi Suganami (Tokyo Medical and Dental University)

16:20-17:05 PPARs and macrophage activation in health and disease
Ajay Chawla (Stanford University)

Session 2. Short Presentations from Posters

Chairperson: **Toshikazu Kondo** (Wakayama Medical University)

17:15-18:00 Oral presentation from posters
Young Investigator Award Competition

18:00-18:20 Business Meeting

18:20-19:00 Poster viewing

19:00- Get-together

July 4 (Saturday)

Session 3: Tissue injury and regeneration

Chairpersons: **Akihiro Matsukawa** (Okayama University)
Teizo Yoshimura (National Cancer Institute-Frederick)

9:00-9:45 TNF-TNFR2 interactions expand T regulatory cells
Joost J. Oppenheim (National Cancer Institute-Frederick)

9:45-10:15 Regulation of inflammatory responses by TLR-inducible proteins
Osamu Takeuchi (Osaka University)

10:15-10:35 **Coffee Break**

10:35-10:55 Regulation of skin wound healing by cytokines and chemokines
Yuko Ishida (Wakayama Medical University)

10:55-11:15 Identification of nepmucin as a phosphatidylserine receptor
Eiji Umemoto (Osaka University)

11:15-12:00 Epigenetic regulation of the macrophage and dendritic cell phenotype
Steven Kunkel (University of Michigan)

12:00-13:00 **Lunch Break**

13:00-13:20 **Young Investigator Award Presentation**

Session 4: Inflammation and cancer

Chairpersons: **Tatsuro Irimura** (University of Tokyo)
Masanobu Oshima (Kanazawa University)

13:25-14:10 'Re-educating' macrophages in infection and cancer by targeting NF-kappaB
Toby Lawrence (CNRS-INSERM-Universite de la Mediterranee)

14:10-14:30 Milk-fat globule-EGF8 released from myeloid cells is a critical mediator to facilitate tumor invasion and metastasis
Masahisa Jinushi (University of Tokyo)

14:30-14:50 Blockade of a chemokine, CCL2, reduces chronic colitis-associated carcinogenesis in mice
Boryana Popivanova (Kanazawa University)

14:50-15:10 **Coffee Break**

15:10-15:45 Gastric tumorigenesis caused by cooperation of inflammation and oncogenic activation
Masanobu Ohshima (Kanazawa University)

15:45-16:30 CXC chemokines in the regulation of angiogenesis relevant to cancer
Robert M. Strieter (University of Virginia)

16:30–16:40 Closing Remarks **Kouji Matsushima** (University of Tokyo)

Poster Session

P1. The contribution of inflammatory macrophages, and CD8$^+$ effector T cells to adipose tissue remodeling, inflammation and insulin resistance in obesity

Satoshi Nishimura[1,2,5], Ichiro Manabe[1,2,5], Mika Nagasaki[1,3], Koji Eto[6], Takashi Kadowaki[2,4], and Ryozo Nagai[1,2]

Department of Cardiovascular Medicine, [2]TSBMI, [3]Computational Diagnostic Radiology and Preventive Medicine, [4]Department of Metabolic Diseases, The University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-8655, Japan.[5]PRESTO, Japan Science and Technology Agency [6]Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, The University of Tokyo

P2. Regulation of T cell responses by a distinct subset of resident splenic macrophages

Daisuke Kurotaki[1], Junko Morimoto[2] and Toshimitsu Uede[1,2]

[1]Division of Matrix Medicine, [2]Division of Molecular Immunology, Institute for Genetic Medicine, Hokkaido University, Sapporo, Japan

P3. The role of chemokine receptors, CCR2 and CX3CR1, in arthritis in IL-1 receptor antagonist-deficient mice

Hiroshi Fujii, Tomohisa Baba, Ryoko Hamano, Mitsuhiro Kawano, Masakazu Yamagishi and Naofumi Mukaida

Division of Rheumatology, Department of Internal Medicine, Kanazawa University Graduate School of Medicin, Division of Cardiology, Department of Internal Medicine, Kanazawa University Graduate School of Medicine, Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University Division of Molecular Bioregulation

P4. Pathogenic Roles of the CX3CL – CX3CR1 Intercations in Macrophage recruitment and function in Dextran Sodium Sulfate-Induced Acute Colitis in Mice

Feodora I. Kostadinova, Mohamed M. Shamekh, and Naofumi Mukaida

Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan

P5. Critical roles of NK and CD8$^+$ T cells in central nervous system listeriosis

Toshiyuki Hayashi[1,2], Shigenori Nagai[1,4], Hideki Fujii[1], Yukiko Baba[1,4], Eiji Ikeda[3], Takeshi Kawase[2], and Shigeo Koyasu[1]

[1]Deparment of Microbiology and Immunology, [2]Department of Neurosurgery and [3]Department of Pathology, Keio University School of Medicine, Tokyo 160-8582, Japan. [4]Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Tokyo 102-0075, Japan

P6. Cigarette smoke condensate extracts induce proinflammatory cytokines in synovial cells and exacerbate collagen-induced arthritis in mice

Satomi Chujo[1], Shosuke Okamoto[1], Ryohei Sunahara[1], Yuka Itoh[1], Hidetoshi Hayashi[1], Takemasa Takii[1], Kazuichi Hayakawa[2] and Kikuo Onozaki[1]
[1]Department of Molecular Health Sciences, Graduate School of Pharmaceutical Sciences, Nagoya City University, Mizuho, Nagoya 467-8603, Japan and [2]Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

P7. 5-hydroxytryptamine 4 receptor stimulus of myenteric neuron induces anti-inflammatory actions via alpha7nACh receptor of muscularis macrophages in postoperative ileus.

Masatoshi Hori [1)], Yasuaki Tsuchida [1,2)], Fumihiko Hatao [2)], Takahisa Murata [1)], Yasuyuki Seto [2)] and Hiroshi Ozaki [1)]
[1)]Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, [2)] Department of Metabolic Care and Gastrointestinal Surgery, Graduate School of Medcine, The University of Tokyo, Tokyo 113-8657, Japan

P8. Staphylococcal superantigen-like protein 5 (SSL5) inhibits the activity of matrix metalloproteinase-9 from human neutrophils

Saotomo Itoh[1,2], Ryosuke Yokoyama[1], Eri Hamada[2], Go Kamoshida[2], Kana Takeshita[2], Teruaki Oku[2], Takemasa Takii[1], Kikuo Onozaki[1] and Tsutomu Tsuji[2]
[1]Department of Molecular Health Sciences, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan.[2]Department of Microbiology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

P9. Stat3 mediates the cell-cell interaction of macrophages and tumor cells in human glioblastoma.

Yoshihiro Komohara, Yukio Fujiwara, Motohiro Takeya
Department of Cell Pathology, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University

P10. Crucial contribution of thymic Sirpα+ conventional dendritic cells to central tolerance against blood-borne antigens

Tomohisa Baba and Naofumi Mukaida
Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, Kanazawa 920-0934, Japan

P11. *Glycyrrhiza inflata*-derived chalcones, Licochalcone A, Licochalcone B and Licochalcone D, inhibit phosphorylation of NF-κB p65 in LPS signaling pathway

Jun-ichi Furusawa[1, 2], Megumi Funakoshi-Tago[1], Kenji Tago[3], Hideo Inoue[4], Yoshiko Sonoda[1], Shigeo Koyasu[2] and Tadashi Kasahara[1 1] Department of Biochemistry, Keio University School of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan.
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of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan.
[4] Research Laboratory of Minophagen Pharmaceutical Co., Zamashi, Kanagawa, 228, Japan

P12. PROTECTIVE ROLE OF CX3CR1-MEDIATED SIGNAL PATHWAY IN *CLOSTRIDIUM DIFFICILE* TOXIN A-INDUCED ENTERITIS.

Masanori Inui[1], Yuko Ishida[2], Akihiko Kimura[2], Naofumi Mukaida[3], Toshikazu Kondo[2]
[1]Department of Molecular Immunology, Institute of Advanced Medicine and [2]Department of Forensic Medicine, Wakayama Medical University, Wakayama, Japan, [3]Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, Kanazawa, Japan

P13. Presence of Acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1) on late endosome (LE) in cholesterol-rich macrophages: efficient cholesterol esterification on LE and possible therapeutic strategy for Niemann-Pick disease type C1

XiaoFeng Lei* Naomi Sakashita* Catherine CY Chang¶ Ta-Yuan Chang¶ Motohiro Takeya*
From the *Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Japan, and ¶Department of Biochemistry, Dartmouth Medical School, USA

P14. Draper-mediated phagocytosis of *Staphylococcus aureus* by *Drosophila* hemocytes

Yumi Hashimoto[1], Yukichika Tabuchi[2], Kenji Kurokawa[3], Takeshi Awasaki[4], Kazuhisa Sekimizu[3], Yoshinobu Nakanishi[1, 2], and Akiko Shiratsuchi[1, 2]
[1]Graduate School of Medical Science, and [2]Graduate School of Natural Science and Technology, Kanazawa University, Ishikawa, Japan, [3]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan, [4]Department of Neurobiology, University of Massachusetts Medical School, Massachusetts, USA

P15. Role of Pretaporter as a ligand for Draper in phagocytosis of apoptotic cells by *Drosophila* hemocytes

Yu Fujita[1], Yukiko Nakagawa[2], Takayuki Kuraishi[1], Yumi Hashimoto[1], Ryo Okada[1], Akiko Shiratsuchi[1,2], and Yoshinobu Nakanishi[1,2]
[1]Graduate School of Medical Science, and [2]Graduate School of Natural Science & Technology, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan.

P16. Activation of Wnt signaling in gastric cancer cells by inflammatory macrophages

Keisuke Oguma[1], Hiroko Oshima[1], Makoto M. Taketo[2], and Masanobu Oshima[1]
[1]Division of Genetics, Cancer Research Institute, Kanazawa University, 13-1, Takaramachi, Kanazawa, 920-0934, Japan and [2]Department of Pharmacology, Kyoto University Graduate school of Medicine, Yoshida-konoé-cho, Sakyo-ku, Kyoto, 606-8501, Japan

P17. *MafB* and *c-Maf* are required for clearance of apoptotic cells in macrophages

Michito Hamada, Motochika Hattori, Megumi Nakamura, Naoki Morito, Hisashi Oishi, Kazuteru Hasegawa, Takashi Kudo and Satoru Takahashi

Department of Anatomy and Embryology, Biomolecular and integrated Medical Sciences, Graduate School of Comprehensive Human Science,
University of Tsukuba 1-1-1, Tennodai, Tsukuba, IBARAKI, JAPAN

P18. Lymphocyte recruitment to nasal-associated lymphoid tissues is regulated by two sulfotransferases

Jotaro Hirakawa[1], Yukari Ohmichi[1], Yasuyuki Imai[1], Minoru Fukuda[2], and Hiroto Kawashima[1,3]
[1]Laboratory of Microbiology and Immunology, and Global COE Program, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan, [2]Tumor Microenviroment Program, Cancer Research Center, Burnham Institute for Medical Research, La Jolla, CA 92037, USA, [3]PRESTO, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

P19. Involvement of IFN-γ and IL-6 in severe invasive group A *streptococcus* infection

Takayuki Matsumura[1], Tadayoshi Ikebe[2], Haruo Watanabe[2], Kazuo Kobayashi[1], and Manabu Ato[1]
[1]Department of Immunology and [2]Department of Bacteriology, National Institute of Infectious Diseases, Tokyo 162-8640, Japan

P20. MafB is indispensable for the development of atherosclerosis

Megumi Nakamura[1], Michito Hamada[1], Takashi Kudo[1], Satoru Takahashi[1]
[1]Department of Anatomy and Embryology, Life System Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan

P21. Involvement of a core protein of *Drosophila* Perlecan in the phagocytosis of apoptotic cells

Ryo Okada[1], Kaz Nagaosa[2], Junko Manaka[1], Kazuki Takeuchi[2], Kunizo Arai[2], Istvan Ando[3], and Yoshinobu Nakanishi[1, 2]
[1] Graduate School of Medical Science, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan
[2] Graduate School of Natural Science ＆ Technology, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan [3] I Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szegel 521, Hungary

P22. Relevance of long pentraxin 3 expression and release in macrophages and neutrophils *in vivo* and *in vitro*

Alexander S. Savchenko[1], Akira Inoue[1], Riuko Ohashi[1], Kenji Inoue[2], Shuying Jiang[1,3], Go Hasegawa[1], Makoto Naito[1]
[1]Department of Cellular Function, Division of Cellular and Molecular Pathology, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951-8510, Japan
[2]Department of Cardiology, Juntendo University Nerima Hospital, Tokyo 113-8431, Japan
[3]Perseus Proteomics Inc., Tokyo 153-0041, Japan

P23. Role of bone marrow-derived cells on repair of mucosal damage caused by either irradiation or anticancer drugs

Junji Takaba[1, 2], Kiyohiko Hatake[2], Tadashi Kasahara[1]

[1] Department of Biochemistry, Graduate School of Pharmaceutical Sciences, Keio University, Tokyo 105-8512, Japan

[2] Department of Clinical Research, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo 135-8550, Japan

P24. Identification of CCR7^{+}MHC class II^{hi} dendritic cells in mouse tumor

Satoshi Ueha[1], Yusuke Shono[1,3], Jun Abe[1], Makoto Kurachi[1], Kazuhiro Kakimi[2] and Kouji Matsushima[1]

[1]Department of Molecular Preventive Medicine, and [2]Department of Immunotherapeutics (Medinet), Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan. [3]Department of Hematology and Oncology, Graduate School of Medicine, Hokkaido University, Hokkaido 060-8638, Japan.

P25. Essential role of macrophages in the *in vitro* production of IL-4 or nonspecific IgE or IgG antibody by lymphocytes of submandibular lymph node from mice sensitized intranasally once with cedar pollen

Masayo Hirao,[1,2,3] Hiromi Ogita-Nakanishi,[1,2] Junko Tashiro-Yamaji,[1] Yumiko Kimoto-Yamamoto,[1] Kanji Sakurai,[1] Masako Miyoshi-Higashino,[1] Ryuji Kato,[3] Yoshio Ijiri,[3] Kazuhiko Tanaka,[3] Atsuko Kanazawa,[2] Hiroshi Takenaka,[2] Takahiro Kubota,[1] Ryotaro Yoshida[1]

Departments of [1]Physiology and [2]Otorhinolaryngology, Osaka Medical College, Takatsuki 569-8686, Japan; [3]Department of Clinical Pharmacy, Osaka University of Pharmaceutical Sciences, Takatsuki 569-1094, Japan

P26. High Mobility Group Protein-1 (HMGB1) and Receptor for Advanced Glycation End-Products (RAGE) Expression and Function in Human Inflamed Dental Pulp Cells and Macrophages: Possible role in dental pulpitis during pregnancy

Salunya Tancharoen [1,3], Tassanee Tengrungsun [2], Yuko Nawa [3], Ko-ichi Kawahara [3], Masayuki Tokuda [4], Teruto Hashiguchi [3], Theeralaksna Suddhasthira [5]&Ikuro Maruyama[3] Department of Pharmacology [1],Department of Hospital Dentistry [2], Faculty of Dentistry, Mahidol University, Bangkok, Thailand. Department of Laboratory and Vascular Medicine, Cardiovascular and Respiratory Disorders [3], Department of Restorative Dentistry and Endodontology [4], Kagoshima Graduate School of Medical and Dental Science, Kagoshima, Japan. Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mahidol University, Bangkok, Thailand [5]

P27. Nuclear protein nucleophosmin released from macrophages acts as an alarmin

Yuko Nawa,* Ko-ichi Kawahara,* Salunya Tancharoen,† Teruto Hashiguchi, * Ikuro Maruyama*

*Department of Laboratory and Vascular Medicine Cardiovascular and Respiratory Disorders Advanced Therapeutics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

†Department of Pharmacology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

P28. Peroxisome proliferator-activated receptor-γ contributes to peritoneal fibrosis through regulation of fibrocytes

Hiroyuki Yamauchi*, Norihiko Sakai**,Satoshi Kokubo*, Akinori Hara*, Kiyoki Kitagawa*, Kengo Furuichi**, Syuichi Kaneko*, Takashi Wada***
*Disease Control and Homeostasis, and ***Department of Laboratory Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Faculty of Medicine, Kanazawa University, Ishikawa, Japan **Division of blood purification, Kanazawa University Hospital, Ishikawa, Japan

P29. TAXIScan technology, used for analyses of chemotaxis of leukocytes from lung disease patients and for mast cell degranulation

Shiro KANEGASAKI,, Akira YAMAUCHI*, Shinichiro OKAMOTO, Mikasko DEGAWA-YAMAUCHI, Ryoko AOKI, Yasushi ISOKAWA and Tomoko TSUCHIYA
Central Laboratory, ECI Inc., Meguroku, Tokyo 153-0042

P30. MGL2$^+$ dermal dendritic cells are sufficient to initiate contact hypersensitivity *in vivo*

Kaori Denda-Nagai, Yosuke Kumamoto, Satoshi Aida, Nobuaki Higashi, and Tatsuro Irimura
Laboratory of Cancer Biology and Molecular Immunology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan

Session 1: Macrophage-related Cells in Physiology and Pathology

Chairpersons:

Kouji Matsushima and Motohiro Takeya

Speakers:

John A. Hamilton

Sara M. Rankin

Masako Kohyama

Kengo Furuichi

Peirong Lu

Takayoshi Suganami

Ajay Chawla

Modulation of macrophage numbers during chronic inflammation

J. A. Hamilton

Arthritis and Inflammation Research Centre, The University of Melbourne, Department of Medicine, Royal Melbourne Hospital, Victoria, Australia

Elevated macrophage numbers at a site of chronic inflammation, such as a rheumatoid synovium, can correlate with poor prognosis. In addition to altered trafficking into and from such a site, enhanced macrophage survival or even local proliferation could contribute to such an increase in their number. Three possible mechanisms for shifting the balance to favour macrophage survival over death will be discussed, namely a colony stimulating factor (CSF) network, uptake and persistence of poorly degradable "particulates", and hypoxia. The evidence for a possible contribution of enhanced glucose uptake and glycolysis to this shift towards the survival response to these stimuli will be presented.

The bone marrow: an unexpected site of neutrophil clearance

Sara M. Rankin

Leukocyte Biology Section, NHLI Division, Faculty of Medicine, Imperial College London SW7 2AZ, UK

In man 10^{11} neutrophils are released from the bone marrow each day. These cells have a half life in the circulation of only 6.5h. Homeostasis therefore requires efficient mechanisms for both neutrophil clearance and replenishment. We have previously shown that as neutrophils age they dramatically up-regulate their cell surface expression of CXCR4 and gain the capacity to migrate towards CXCL12. Furthermore our *in vivo* experiments have shown that senescent $CXCR4^{hi}$ neutrophils preferentially home back to the bone marrow in a CXCR4-dependent manner (1). The aim of the current study was to determine the relative contribution of the bone marrow, spleen and liver to the homeostatic clearance of senescent neutrophils from the circulation and investigate the molecular mechanisms underlying this process. We show here that the bone marrow accounts for the clearance of >30% of neutrophils from the circulation under homeostatic conditions. Trafficking of neutrophils back to the bone marrow is pertussis toxin-sensitive, but independent of the adhesion molecules, P-selectin, CD49d and CD18. Tracking senescent neutrophils by fluorescent microspheres we show by FACS, light and electron microscopy that *in vivo* senescent neutrophils are phagocytosed by bone marrow macrophages. Further, in vitro studies show that the uptake of apoptotic neutrophils by bone marrow macrophages, but not peritoneal/inflammatory macrophages, stimulates their production of G-CSF. This suggests that neutrophil clearance via the bone marrow indirectly regulates neutrophil production in the bone marrow, suggesting a finely tuned homeostatic mechanism for regulating neutrophil numbers in the blood.

(1) Martin *et al.* Immunity 2003; 19:583-59
(2) Furze and Rankin FASEB 2008 (2008) 22:3111-9

This work was funded by The Wellcome Trust

Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis

Masako Kohyama
Department of Pathology and Immunology, Washington University School of Medicine

Tissue macrophages comprise a heterogeneous group of cell types differing in location, surface markers and function. Red pulp macrophages are a distinct splenic subset involved in removing senescent red blood cells. Transcription factors such as PU.1 and C/EBPα play general roles in myelomonocytic development , but the transcriptional basis for producing tissue macrophage subsets remains unknown. Here we show that Spi-C, a PU.1 related transcription factor, selectively controls the development of red pulp macrophages. Spi-C is highly expressed in red pulp macrophages, but not monocytes, dendritic cells or other tissue macrophages. Spi-$C^{-/-}$ mice exhibit a cell-autonomous defect in the development of red pulp macrophages that is corrected by retroviral Spi-C expression in bone marrow cells, but have normal monocyte and other macrophage subsets. Red pulp macrophages highly express genes involved in capturing circulating hemoglobin and iron regulation. Spi-$C^{-/-}$ mice show normal trapping of red blood cells in the spleen, but fail to phagocytose these red blood cells efficiently, and develop an iron overload localized selectively to splenic red pulp. Thus, Spi-C controls development of red pulp macrophages required for red blood cell recycling and iron homeostasis.

References:

1: **Kohyama M**, Ise W, Edelson BT, Wilker PR, Hildner K, Mejia C, Frazier WA, Murphy TL, Murphy KM.

Role for Spi-C in the development of red pulp macrophages and splenic iron homeostasis.

Nature. 2009; 457(7227):318-21.

Macrophage mediated inflammatory cascades in ischemic kidney injury.

Kengo Furuichi
Division of Blood Purification, Kanazawa University Hospital
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Ischemia-reperfusion is one of main causes of acute kidney injury, and the acute kidney injury is a poor prognostic factor in various clinical settings. Tubular necrosis is a characteristic pathologic finding of acute ischemic kidney injury and various types of inflammatory cells, including marcophages, infiltrate into the injured kidney. The main necrotic area would be repaired with regenerated tubular epithelial cells after the injury. Moreover, some parts of the injured kidney progress interstitial fibrosis, which is a characteristic pathologic change of irreversible advanced kidney injury. However, specific molecular mechanisms and precise contributions to pathology have not been fully delineated for macrophages. Here, we provide the hypothesis that macrophage mediated inflammatory cascades contribute to specific pathologic changes after renal ischemia-reperfusion injury.

To reveal these mechanisms of cascades, we used a mouse model of renal ischemia-reperfusion injury. A mouse left kidney was clamped and allowed to be reperfused. A large number of chemokines were upregulated after ischemic injury, and chemokine receptor expressing inflammatory cells were attracted by these chemokines. Genetic or molecular modulating experiments in the mouse model have begun to dissect the key players and their specific roles at the phases of inflammation, regeneration and fibrosis. Among these chemokies/chemokine receptors, our data indicated CCR2 mediated macrophage infiltration in early phase of the injury mainly affected to tubular necrosis after ischemic acute kidney injury. In contrast, interferon-gamma inducible protein (IP)-10 producing macrophage participates in regeneration of tubular epithelial cells. Finally, CX3CR1 mediated macrophage and platelet infiltration and aggregation in the late phase of the injury play some roles in interstitial fibrosis.

These findings revealed that specific chemokines and cognate chemokine receptors mediating macrophage subsets participate in specific pathologic changes at specific time points after renal ischemia-reperfusion injury. These chemokines and cognate chemokine receptors on infiltrating inflammatory cells would be novel clinical markers as well as targets for therapeutic intervention.

1. **K. Furuichi**, J. L. Gao, R. Horuk, T. Wada, S. Kaneko and P. M. Murphy: Chemokine receptor CCR1 regulates inflammatory cell infiltration after renal ischemia-reperfusion injury. *J Immunol*, 181(12), 8670-6 (2008)
2. **K. Furuichi**, T. Wada, S. Kitajikma, T. Toyama, T. Okumura, A. Hara, H. Kawachi, F. Shimizu, T. Sugaya, N. Mukaida, S. Narumi, K. Matsushima and S. Kaneko: IFN-inducible protein 10 (CXCL10) regulates tubular cell proliferation in renal ischemia-reperfusion injury. *Nephron Exp Nephrol*, 109(1), c29-38 (2008)
3. **K. Furuichi**, S. Kaneko and T. Wada: Chemokine/chemokine receptor-mediated inflammation regulates pathologic changes from acute kidney injury to chronic kidney disease. *Clin Exp Nephrol* (2008)
4. **K. Furuichi**, T. Wada, S. Kaneko and P. M. Murphy: Roles of chemokines in renal ischemia/reperfusion injury. *Front Biosci*, 13, 4021-8 (2008)

5. **K. Furuichi**, J. L. Gao and P. M. Murphy: Chemokine receptor CX3CR1 regulates renal interstitial fibrosis after ischemia-reperfusion injury. *Am J Pathol*, 169(2), 372-87 (2006)
6. **K. Furuichi**, T. Wada, Y. Iwata, S. Kokubo, A. Hara, J. Yamahana, T. Sugaya, Y. Iwakura, K. Matsushima, M. Asano, H. Yokoyama and S. Kaneko: Interleukin-1-dependent sequential chemokine expression and inflammatory cell infiltration in ischemia-reperfusion injury. *Crit Care Med*, 34(9), 2447-55 (2006)
7. **K. Furuichi**, T. Wada, Y. Iwata, K. Kitagawa, K. Kobayashi, H. Hashimoto, Y. Ishiwata, N. Tomosugi, N. Mukaida, K. Matsushima, K. Egashira and H. Yokoyama: Gene therapy expressing amino-terminal truncated monocyte chemoattractant protein-1 prevents renal ischemia-reperfusion injury. *J Am Soc Nephrol*, 14(4), 1066-71 (2003)
8. **K. Furuichi**, T. Wada, Y. Iwata, K. Kitagawa, K. Kobayashi, H. Hashimoto, Y. Ishiwata, M. Asano, H. Wang, K. Matsushima, M. Takeya, W. A. Kuziel, N. Mukaida and H. Yokoyama: CCR2 signaling contributes to ischemia-reperfusion injury in kidney. *J Am Soc Nephrol*, 14(10), 2503-15 (2003)

The Complex Role of Chemokine Receptor Signal on Macrophage in Ocular Neovascularization

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Corneal neovascularization (CNV) can arise from various causes, including corneal infections, misuse of contact lens, chemical burn and inflammation and frequently leads to impaired vision. Preceding the onset of alkali injury-induced CNV, leukocytes infiltrate into cornea. Although neutrophils are presumed to be the predominant leukocyte population, which infiltrate to the injured corneas in the early stages, we proved that alkali injury-induced CNV can occur independently of neutrophil infiltration.

There is increasing evidence that implicates a critical role for macrophages in ocular neovascularization. Mouse monocytes/macrophages express a distinct set of chemokine receptors such as CCR1,CCR2,CCR5 and CX3CR1 on their cell surface. To investigate the role of chemokine receptor signal on infiltrating macrophages in the development of experimental CNV. CNV was induced by alkali injury in mice deficient in a macrophage-tropic chemokine receptor, CCR1, CCR2, CCR5 or CX3CR1. CCR2-KO mice exhibited reduced alkali-induced CNV with reduced macrophage infiltration, whereas CX3CR1-KO mice developed a more severe form of alkali-induced CNV with reduced macrophage infiltration. CCR5-KO and CCL3-KO mice, but not CCR1-KO mice, exhibited reduced alkali-induced CNV 2 weeks after injury, the infiltration of F4/80-positive macrophages was significantly attenuated in CCL3-KO mice compared with WT mice. Alkali injury induced a massive increase in the intraocular mRNA expression of a potent angiogenic factor, vascular endothelial growth factor (VEGF) in WT mice, whereas these increments were severely retarded in CCL3-KO mice. The intracorneal mRNA expression of angiogenic factor, VEGF, was enhanced to similar extents in WT and CX3CR1-KO mice after the injury. The mRNA expression of anti-angiogenic factors, ADAMTS-1, thrombospondin (TSP)-1, and TSP-2, was enhanced in WT mice but the enhancement was attenuated in CX3CR1-KO mice. Moreover, in vitro experiment indicated CCL3 and CCL2 enhanced VEGF expression by murine peritoneal macrophages. Wherease, CX3CL1 enhanced antiangiogenic factors (ADAMTS-1 and TSP-1) but not VEGF expression by murine peritoneal macrophages. Furthermore, a double immunofluorescence analysis demonstrated that F4/80-positive cells expressed CX3CR1 and ADAMTS-1, and ADAMTS-1 was detected in CX3CR1-positive cells. Topical application of CX3CL1 inhibited CNV at 2 weeks, along with enhanced intraocular expression of ADAMTS-1 and TSP-1.

Macrophage can either promote or inhibit neovascularization process depending on the chemokine receptor signal or different macrophage subset. It is important to further elucidate those factors that alter macrophage-mediated regulation of neovascularization. Chemokine receptor signal on macrophage may be a therapeutic target of ocular neovascularization.

References:

1. Lu P, Li R, Mukaida N, Zhang X. Alkali-induced Corneal Neovascularization Occurs Independently of CXCR2-mediated Neutrophil Infiltration. Cornea. 2007, 26:199-206.
2. Lu P, Li L, Kuno K, Wu Y, Baba T, Li YY, Zhang X, Mukaida N. Protective Roles of the Fractalkine/CX3CL1-CX3CR1 interaction against Alkali-induced Corneal Neovascularization through Enhanced Anti-angiogenic Factor Expression. J Immunol. 2008,180:4283-4291.
3. Lu P, Li L, Wu Y, Mukaida N, Zhang X. Essential contribution of CCL3 to alkali-induced corneal neovascularization by regulating vascular endothelial growth factor production by macrophages. Molecular Vision. 2008,14:1614-1622.
4. Lu P, Li L, Liu G, Rooijen Nv, Mukaida N, Zhang X. Opposite Roles of CCR2 and CX3CR1 Macrophages in Alkali-induced Corneal Neovascularization. Cornea. In press.

Role of saturated fatty acid/TLR4 signaling in macrophages in obesity-induced adipose tissue inflammation.

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Known as the metabolic syndrome, the cluster of risk factors for cardiovascular diseases; visceral fat obesity, impaired glucose metabolism, atherogenic dyslipidemia, and blood pressure elevation, is an increasing health problem worldwide. The pathophysiology underlying the metabolic syndrome is not fully understood and visceral fat obesity appears to be an important component. There is considerable evidence that obesity is a state of chronic low-grade inflammation, which may play a critical role in the pathophysiology of the metabolic syndrome. Obese adipose tissue is markedly infiltrated by macrophages, suggesting that they may participate in the inflammatory pathways that are activated in obese adipose tissue. Using an *in vitro* co-culture system composed of adipocytes and macrophages, we have provided evidence that a paracrine loop involving saturated fatty acids (FAs) and TNFα derived from adipocytes and macrophages, respectively, establishes a vicious cycle that augment the inflammatory change in obese adipose tissue.

Free FAs represent an important energy source mobilized from triglycerides stored in the adipose tissue, particularly during periods of starvation, but recent evidence has suggested the pathophysiologic roles other than the supply of nutrients in times of fasting or increased energy demand. We have demonstrated that saturated FAs, which are released from adipocytes via the macrophage-induced lipolysis, serves as a naturally occurring ligand for TLR4 to induce NF-κB activation in macrophages. We also showed that TLR4 plays an important role in adipose tissue inflammation. Moreover, we have recently identified activating transcription factor 3 (ATF3), a member of the ATF/CREB family of basic leucine zipper-type transcription factors, which is markedly induced in macrophages through TLR4 in response to saturated FAs *in vitro* and in obese adipose tissue *in vivo*. Furthermore, we elucidated the potential role of ATF3 as a transcriptional repressor of saturated FAs/TLR4 signaling in macrophages using transgenic mice overexpressing ATF3 specifically in macrophages. Interestingly, *n-3* polyunsaturated FAs (*n-3*PUFAs) such as eicosapentaenoic acid (EPA) effectively inhibited saturated FAs-induced inflammatory changes in macrophages.

Our study provides *in vitro* and *in vivo* evidence that the saturated FAs/TLR4 pathway plays a role in obesity-induced adipose tissue inflammation and *n-3*PUFAs and ATF3 antagonize the action of saturated FAs, which helps identify the therapeutic targets to reduce or treat obesity-related inflammation and the metabolic syndrome

PPARs and Macrophage Activation in Health and Disease

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Macrophages are immune sentinels that quiescently monitor the tissue milieu for early signs of infection or damage. In this role, the macrophage is responsible for sensing, integrating, and responding appropriately to a bewildering array of stimuli from its microenvironment. These pleiotropic responses are coordinated through distinct programs of macrophage activation, ranging from classical to alternative. Prior to my work, the transcriptional basis for maturation of alternatively activated macrophages was simply not known. We discovered that cooperative interactions between cytokine signaling pathways (IL-4 and IL-13) and metabolic regulators (PPARs) form the molecular switch that polarizes tissue macrophages to the alternative state. This is of particular interest because metabolic tissues, such as liver and adipose, are preferentially populated by alternatively activated macrophages. By generating mice deficient in alternatively activated macrophages, we demonstrated that these cells can ameliorate diet-induced metabolic disease. While deficiency of PPARγ in myeloid cells abolishes alternative activation of adipose tissue macrophages, Kupffer cells deficient in PPARδ display marked impairment in alternative activation. Strikingly, paracrine and/or endocrine actions of these cells regulate nutrient homeostasis in peripheral tissues. Together, our findings demonstrate that distinct transcriptional regulators control depot-specific maturation of alternatively activated macrophages, which exert beneficial effects on nutrient homeostasis, especially in the setting of obesity and type 2 diabetes.

References:

1. Vats, D., Mukundan, L., Odegaard, J.I., Zhang, L., Smith, K.L., Morel, C.R., Greaves, D.R., Murray, P.J., Chawla, A. Oxidative metabolism and PGC-1β attenuate macrophage-mediated inflammation. **Cell Metabolism**, 4, 13-24, 2006.
2. Odegaard, J.I., Ricardo-Gonzalez, R.R., Goforth, M.H., Morel, C.R., Subramanian, V., Mukundan, L., Vats, D., Brombacher, F., Ferrante, A.W., Chawla, A. Macrophage-specific PPARγ controls alternative activation and improves insulin resistance. **Nature**, 447: 1116-20, 2007.
3. Odegaard, J.I., Ricardo-Gonzalez, R.R., Red Eagle, A., Vats, D., Morel, C.R., Subramanian, V., Mukundan, L., F., Ferrante, A.W., Chawla, A. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. **Cell Metabolism**, 6: 496-507, 2008.
4. Odegaard, J.I., Chawla, A. Mechanisms of macrophage activation in obesity-induced insulin resistance. **Nature Clin Pract Endo Metab.**, 4: 619-26, 2008. Epub 2008 Oct 7.

Session 3: Tissue Injury and Regeneration

Chairpersons:
Akihiro Matsukawa and Teizo Yoshimura

Speakers:
Joost J. Oppenheim
Osamu Takeuchi
Yuko Ishida
Eiji Umemoto
Steven Kunkel

TNF-TNFR2 Interactions Expand T Regulatory Cells

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Our studies have revealed TNF to have biphasic proinflammatory and immunosuppressive effects. TNF stimulates the activation of T effector cells (Teffs) as well as T regulatory cells (Tregs). In vitro, TNF overcomes the anergic state of Tregs to TCR stimulation. In concert with IL-2, TNF selectively promotes proliferation of WT Tregs, but failed to stimulate TNFR2 KO Tregs. TNFR2 is preferentially expressed by mouse Tregs. In normal C57BL/6 mice, ~80% of thymic Tregs and ~40% of peripheral Tregs express TNFR2. TNFR2 expressing Tregs exhibit an activated phenotype with high levels of CTLA-4 expression (>80%) and ~6-fold more suppressive capacity than CD103+ Tregs, while $TNFR2^-$ Tregs show a naïve phenotype with low levels of CTLA-4 expression (~40%) and only have minimal suppressive activity.

In mouse 4T1 breast and LLC (Lewis Lung Carcinoma) tumors, 80~100% of Tregs present in tumor infiltrating lymphocytes (TILs) are highly suppressive TNFR2+ Tregs. TGFβ converts naïve CD4 cells into FoxP3-expressing Tregs, and TGFβ is considered responsible for the intratumoral accumulation of Tregs. We found that although anti-TGFβ Ab (1D11) markedly inhibits the growth of primary tumor, this antibody unexpectedly increases the proportion of FoxP3+ cells in the CD4 TILs. In contrast, anti-TNF Ab (XT22.11) decreases the proportion of FoxP3+ cells in CD4 TILs. In vitro, TGFβ potently inhibits TNF-induced proliferation of Tregs. Thus, TGFβ, as opposed to TNF, actually suppresses proliferative expansion of Tregs.

Although TNFR2 knockout mice express normal levels of functional $CD4^+FoxP3^+$ Tregs, by 1 and 2 days after induction of a septic episode due to cecal ligation and puncture (CLP), they fail to increase their level of Tregs and lack the immunosuppressive phase seen in normal mice. Thus increase in Treg levels in response to tumoral or septic inflammation appears to be TNFR2 dependent. Overall, these studies suggest TNFR2 plays an important role in the activation and expansion of potent suppressive Tregs under inflammatory conditions.

In normal healthy human peripheral blood (PB), $CD25^+TNFR2^+$ cells comprised 5~12% of CD4 lymphocytes. These human $CD4^+CD25^+TNFR2^+$ cells express high levels of FoxP3 and CTLA-4. Upon TCR stimulation, human PB $CD4^+CD25^+TNFR2^+$ cells were anergic and markedly inhibited the proliferation and cytokine production of co-cultured T responder cells. Thus the combination of CD25 and TNFR2 identifies more suppressive human Tregs and this may prove to have therapeutic benefit in cancer and autoimmune diseases.

References

Chen, X., M. Baumel, D. N. Mannel, O. M. Howard, and J. J. Oppenheim. 2007. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse CD4+CD25+ T regulatory cells. *J Immunol* 179:154-161.

Chen, X., J. J. Subleski, H. Kopf, O. M. Howard, D. N. Mannel, and J. J. Oppenheim. 2008. Cutting edge: expression of TNFR2 defines a maximally suppressive subset of mouse CD4+CD25+FoxP3+ T regulatory cells: applicability to tumor-infiltrating T regulatory cells. *J Immunol* 180:6467-6471.

Regulation of inflammatory responses by TLR-inducible proteins

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Toll-like receptors (TLRs) recognize microbial components, and evoke inflammation and immune responses (1, 2). TLR stimulation activates complex gene expression networks that regulate the magnitude and duration of the immune reaction. Previously, we showed that IkBz, an TLR-inducible protein, played an important role in the positive regulation of a set of TLR-inducible genes (3). In the present study, we newly identified *Zc3h12a* as a potential immune response modifier by microarray screening (4). *Zc3h12a*-deficient (-/-) mice suffered from severe anemia, and most died within 12 weeks. *Zc3h12a*$^{-/-}$ mice also showed highly elevated serum immunoglobulin levels and autoantibody production together with a greatly increased number of plasma cells, as well as infiltration of plasma cells to the lung. Most *Zc3h12a*$^{-/-}$ splenic T cells showed effecter/memory characteristics and produced interferon-γ in response to T cell receptor stimulation. Macrophages from *Zc3h12a*$^{-/-}$ mice showed highly elevated production of IL-6 and IL-12p40, but not TNF, in response to TLR ligands. Although activation of TLR signaling pathways was normal, *Il6* mRNA decay was severely impaired in *Zc3h12a*$^{-/-}$ macrophages. Overexpression of Zc3h12a accelerated *Il6* mRNA degradation via its 3'-untranslated region (UTR) and destabilized RNAs with 3'-UTRs for genes including IL-6, IL-12p40 and the Calcitonin receptor. Zc3h12a harbors a putative N-terminal nuclease domain, and the expressed protein exhibited ribonuclease activity, consistent with a role in the decay of IL-6 mRNA. Together, these results indicate that Zc3h12a is an essential ribonuclease that prevents immune disorders by directly controlling the stability of a set of inflammatory genes.

References

1) Akira S., Uematsu, S. & Takeuchi, O. Pathogen recognition and innate immunity. Cell 124: 783-801, (2006)
2) Takeuchi O & Akira S. Signaling pathways activated by microorganisms. Curr Opin Cell Biol. 19: 185-91, (2007)
3) Yamamoto, M., Yamazaki, S., Uematsu, S., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Kuwata, H., Takeuchi, O., Takeshige, K., Saitoh, T., Yamaoka, S., Yamamoto, N., Yamamoto, S., Muta, T., Takeda, K. and Akira, S. Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IkBzeta. Nature 430: 218-222, (2004)
4) Matsushita K*, Takeuchi O*, Standley DM, Kumagai Y, Kawagoe T, Miyake T, Satoh T, Kato H, Tsujimura T, Nakamura H & Akira S (*equal contribution) Zc3h12a is an RNase essential for controlling immune responses by regulating mRNA decay. Nature 458: 1185-90, (2009)

Regulation of Skin Wound Healing by Cytokines and Chemokines

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Optimum healing of a skin wound requires a well-orchestrated integration of the complex biological and molecular events of cell migration and proliferation, and of extracellular matrix deposition and remodeling (1). All events of wound healing processes are controlled by a wide variety of cytokines, chemokines, as well as growth factors. We have demonstrated that the deficiencies of several proinflammatory cytokines profoundly affected wound healing. When excisional skin wounds were generated in IL-$6^{-/-}$ mice, the reepithelialization and granulation tissue formation were significantly delayed with attenuated leukocyte infiltration compared with WT mice (2). In contrast, TNF-$Rp55^{-/-}$ mice showed an enhanced angiogenesis and collagen accumulation, and eventually accelerated wound healing despite reduced leukocyte infiltration (3). By using IFN-$\gamma^{-/-}$ mice, we demonstrated that there was a crosstalk between IFN-γ/Stat1 and TGF-β/Smad signaling pathways in the wound healing process. The absence of IFN-γ augmented the TGF-β-mediated signaling and eventually accelerated wound healing with enhanced collagen deposition (4). We also revealed the crosstalk between IL-1-mediated NF-κB and TGF-β-mediated signals by survey of wound healing process in IL-$1ra^{-/-}$ mice (5). More recently, we demonstrated the multiple functions of individual chemokines during the phases of wound healing. The presence of chemokine receptors, particularly CX3CR1 (6) and CCR5, on BM-derived cells represents that chemokine/chemokine receptor interaction also contribute to the regulation of tissue remodeling, repair and regeneration. Collectively, inflammatory cytokines and chemokines are in an exclusive position to integrate inflammatory events and reparative processes and are important modulators of skin wound healing.

References

1. Singer AJ et al. Cutaneous wound healing. N Engl J Med. 341:738-46. 1999
2. Lin Z et al. Essential involvement of IL-6 in skin wound healing process as evidenced by delayed wound healing in IL-6-deficient mice. J Leukoc Biol. 73:713-21. 2003
3. Mori R et al. Accelerated wound healing in tumor necrosis factor receptor p55-deficient mice with reduced leukocyte infiltration. FASEB J. 16:963-74. 2002
4. Ishida Y et al. The essential involvement of crosstalk between IFN-γ and TGF-β in skin wound healing process. J Immunol. 172:1848-55. 2004
5. Ishida Y et al. Absence of IL-1 receptor antagonist impaired wound healing along with aberrant NF-κB activation and a reciprocal suppression of TGF-β signal pathway. J Immunol. 176:5598-606. 2006
6. Ishida Y et al. Chemokine receptor CX3CR1 mediates skin wound healing by promoting macrophage and fibroblast accumulation and function. J Immunol. 180:569-79. 2008

Identification of nepmucin as a phosphatidylserine receptor

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The surface exposure of phosphatidylserine (PS) is a hallmark of apoptotic cells, and recognition of the cell-surface PS is a critical signal for clearance of dead cells by phagocytes such as macrophages. Nepmucin/CLM-9 is a V-type Ig domain-containing sialomucin expressed in vascular endothelial cells including those of high endothelial venules (HEVs) in lymph nodes. We previously reported that nepmucin mediates lymphocyte binding and transmigration via its Ig domain. In a search for a nepmucin ligand(s) on lymphocytes, we found that nepmucin bound to apoptotic cells avidly. Flow cytometric analysis showed that the wild-type nepmucin-Fc and the mucin-domain lacking nepmucin-Fc chimera, but not the Ig-domain lacking nepmucin-Fc, bound to Annexin V^+ apoptotic cells. Analysis by solid-phase ELISA showed that nepmucin-Fc directly bound to PS, and that the binding to PS was specifically inhibited by anti-nepmucin antibodies that recognize the Ig domain of nepmucin. In addition, NIH/3T3 cells expressing nepmucin avidly phagocytosed apoptotic thymocytes in a manner dependent on nepmucin. We next asked the role of nepmucin *in vivo*. Whereas nepmucin is also expressed on certain myeloid cells such as peritoneal macrophages, uptake of apoptotic cells by the peritoneal macrophages was only modestly inhibited, if at all, by anti-nepmucin antibodies. We then examined the role of nepmucin in HEV endothelial cells that have been shown to phagocytose apoptotic leukocytes. Confocal microscopic analysis revealed that HEV cells isolated from lymph nodes took up apoptotic cells at readily detectable levels, which was inhibited by the addition of anti-nepmucin antibodies. Taken together, these findings indicate that nepmucin mediates uptake of apoptotic cells through the binding of PS via its Ig domain and that nepmucin may serve the role of removing apoptotic cells from the systemic circulation, thus allowing migration of only functional lymphocytes into lymphoid tissues.

Epigenetic regulation of the Macrophage and Dendritic Cell Phenotype

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We have explored both static and dynamic epigenetic events that dictate, in part, the phenotype expressed by macrophages and dendritic cells (DCs). We have initially studied this phenomenon in dendritic cells (DCs) recovered from animals surviving severe peritonitis-induced sepsis. Immediately following the initiation of experimental sepsis the host mounts a cytokine storm characterized by a systemic inflammatory response syndrome. This syndrome results in a transient expression of extremely high, systemic levels of pro-inflammatory cytokines followed by a global depletion of various immune cells, including the depletion of DCs from the lung and spleen. In animals surviving this syndrome the bone marrow repopulates the distant organs with various immune cells. Interestingly, DCs recovered from long-term surviving animals exhibited a significant and chronic suppression of interleukin IL-12, a key host defense cytokine. The suppression of DC-derived IL-12 persists for weeks after recovery and was not due to the long-term expression of immunoregulatory cytokines, such as IL-10. Our studies demonstrate that the deficiency in DC-derived IL-12 was due to epigenetic alterations. Specifically, IL-12 expression was regulated by stable reciprocal changes in histone H3 lysine-4 trimethylation (H3K4me3) and histone H3 lysine-27 dimethylation (H3K27me2), as well as changes in cognate histone methyltransferase (HMT) complexes on the *Il12p35* and *Il12p40* promoters. These data implicate histone modification enzymes in suppressing DC-derived IL-12, which may provide one of the mechanisms of long-term immunosuppression. In addition, we have identified that the alternatively activated macrophage phenotype which are characterized by a unique phenotype, such as high expression of mannose receptor, FIZZ1, and YM1 is controlled by dynamic alterations in chromatin remodeling. While these cells appear to play an important role in disease chronicity, little is known regarding mechanisms that regulate their protein expression profile. Our studies demonstrate that this population of macrophage is dynamically influenced by the activity of both HMT and the demethylase JMJD3. Collectively these studies provide a mechanism whereby epigenetic changes can influence gene expression patterns by both dendritic cells and macrophages during the evolution of an immune response. Furthermore, they underscore the importance of the inflammatory environment, which dynamically influences the enzymatic machinery needed to remodel chromatin for gene activation or gene silencing.

Session 4: Inflammation and Cancer

Chairpersons:
Tatsuro Irimura and Masanobu Oshima

Speakers:
Toby Lawrence
Masahisa Jinushi
Boryana Popivanova
Masanobu Oshima
Robert M. Strieter

‘Re-educating’ Macrophages in Infection and Cancer by Targeting NF-kappaB.

Toby Lawrence

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Macrophages are critical regulators of inflammation and innate immunity and evasion of macrophage–mediated innate immunity is a feature of many human pathogens. Recent literature also shows an important role for macrophages in experimental models of cancer, which is in conflict with the intrinsic anti-tumour potential of these innate immune cells. Current evidence suggests the tumour-microenvironment subverts the anti-cancer activity of tumour-associated macrophages (TAM) promoting a phenotype that contributes to tumour-progression. The Nuclear Factor (NF)-kappaB pathway is thought to play a central role in inflammation and immunity. In response to pro-inflammatory cytokines and pathogen associated molecular patterns NF-kappaB activation is controlled by IkappaB kinase (IKK) beta. Using Cre/lox mediated tissue-specific gene targeting of IKKβ we have uncovered an unexpected role for IKKbeta in regulating macrophage activation in infection and cancer. IKKbeta inhibition specifically in macrophages conferred resistance to infection with gram-positive bacteria, group B streptococcus, and the fungal pathogen Crypotococcus neoformans. This was associated with increased activation of M1 macrophages and production of the protective cytokine interleukin (IL)-12. In parallel experiments we targeted IKKbeta in tumour-associated macrophages (TAM), that are known to have an anti-inflammatory (M2) phenotype. Specific inhibition of IKKbeta in TAM promotes and M1 phenotype and increases anti-tumour immunity, which is also associated with increased expression of IL-12, inducible nitric oxide synthase (NOS2) and MHC II. In vitro experiments show negative cross-talk between IKKbeta and interferon (IFN) signalling which inhibits the ‘classically’ activated or M1 macrophage phenotype in both infection and cancer. These studies have established a new role for IKKbeta in the regulation of macrophage activation with important implications for both innate immunity and cancer.

Hagemann, T. et al. “Re-educating” tumor-associated macrophages by targeting NF-kappaB. J. Exp. Med. 205:1261-68 2008.

Fong, C H. et al. An antiinflammatory role for IKKbeta through inhibition of ‘classical’ macrophage activation. J. Exp Med. 205:1269-76 2008.

Milk-fat globule-EGF8 released from myeloid cells is a critical mediator to facilitate tumor invasion and metastasis

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The pathogenesis of various malignancies involves the interaction of tumor cells with multiple host-derived cellular components. However, the underlying mechanisms leading to tumor progression has not been understood completely.

MFG-E8 mediates uptake of apoptotic cells by antigen-presenting cells and is a key mediator to determine GM-CSF-triggered tolerance and inflammation. MFG-E8 attenuates the vaccination activity of GM-CSF-secreting tumor cells, underscoring the negative effects of MFG-E8 in antitumor efficacy of tumor cell vaccines.

Interestingly, MFG-E8 is highly expressed in tumor-infiltrating macrophages in patients with melanoma at advanced stage compared to those at early stage or with pre-malignant melanocytes.MFG-E8-negative B16 melanomas displayed aggressive and metastatic growth phenotypes in the animals reconstituted with MFG-E8gene-transferred bone-marrow cells. In contrast, their growth was attenuated in the animals reconstituted with BMC transfected with MFG-E8 dominant negative gene. The detailed analysis of tumor-infiltrating lymphoid cells from bone marrow-chimeric mice showed that MFG-E8 facilitates the infiltration of $CD11b^{+}Gr\text{-}1^{-}$ and $CD11b^{+}Gr\text{-}1^{+}$myeloid cells but reduces $CD11c^{+}$dendritic cells at tumor sites. The genetic knockdown of MFG-E8 with siRNA rendered bone-marrow derived immature dendritic cells to facilitate IL-12 production and tumor-specific CTL induction.

Overall, myeloid cell-derived MFG-E8 plays a critical role in creating the immunosuppressive tumor microenvironments and facilitating tumor invasion and metastasis.

References:

1. **Jinushi M**, Nakazaki Y, Carrasco DR, Draganov D, Souders N, Johnson M, Mihm M & Dranoff G.. MFG-E8 promotes melanoma progression through coordinated Akt and Twist signaling in the tumor microenvironment. ***Cancer Res***, 68, 8889-8898, 2008.
2. **Jinushi M,** Hodi FS, & Dranoff G.. Enhancing the clinical activities of granulocyte-macrophage colony stimulating factor-secreting tumor cell vaccines. ***Immunol Rev.***, 222, 287-298, 2008.
3. **Jinushi M**, Nakazaki Y, Dougan M, Carrasco DR, Mihm M, & Dranoff G. MFG-E8 mediated uptake of apoptotic cells links the pro- and anti-inflammatory activities of GM-CSF. ***J. Clin. Inves.***, 117, 1902-1913, 2007.

Blockade of a chemokine, CCL2, reduces chronic colitis-associated carcinogenesis in mice

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Accumulating evidence indicates the crucial contribution of chronic inflammation to various types of carcinogenesis, as well as the association of tumor infiltrating macrophages with carcinoma development. However, it remains elusive on the roles of chemokines in intratumoral macrophage infiltration and subsequent carcinogenesis. Azoxymethane (AOM) administration followed by repetitive dextran sulfate sodium (DSS) ingestion recapitulates ulcerative colitis-associated colon carcinogenesis in mice. In the course of this carcinogenesis process, the expression of a macrophage-tropic chemokine, CCL2, was enhanced together with massive intracolonic infiltration of macrophages, which were a major source of cyclooxygenase (COX)-2, a crucial mediator of colon carcinogenesis. Mice deficient in CCL2-specific receptor, CCR2, exhibited less macrophage infiltration and lower tumor numbers with attenuated COX-2 expression. Moreover, analysis of bone marrow chimeric mice revealed that bone-marrow-derived, but not non-bone marrow-derived CCR2-expressing cells are indispensable for this colon carcinogenesis. Furthermore, CCL2 antagonists decreased intracolonic macrophage infiltration and COX-2 expression, attenuated neovascularization, and eventually reduced the numbers and size of colon tumors, even when given after multiple colon tumors have developed. These observations identify CCL2 as a crucial mediator of the initiation and progression of chronic colitis-associated colon carcinogenesis and suggest that targeting CCL2 may be useful in treating colon cancers, particularly those associated with chronic inflammation.

Gastric tumorigenesis caused by cooperation of inflammation and oncogenic activation

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Gastric cancer development is tightly associated with *Helicobacter* infection that induces expression of COX-2 and mPGES-1 in gastric mucosa. Subsequently, cyclooxygenase (COX)-2/prostaglandin E_2 (PGE_2) pathway is activated, which further leads to chronic inflammation. Although it has been established that COX-2/PGE_2 pathway plays a key role in gastrointestinal tumorigenesis, mechanism(s) underlying tumorigenesis has not been fully understood yet. To investigate the role of PGE_2 in gastric tumorigenesis, we constructed transgenic mice (*K19-C2mE* mice) expressing COX-2 and mPGES-1 in the stomach. *K19-C2mE* mice showed gastric metaplasia and hyperplasia associated with inflammatory responses, however, tumors were not developed.

On the other hand, accumulation of β-catenin is found in 30-50% of human gastric cancer, suggesting that Wnt signaling activation is one of the major causes for gastric carcinogenesis. Moreover, genetic alterations in BMP signaling result in gastrointestinal hamartoma development in Juvenile polyposis patients. We thus generated *K19-Wnt1* and *K19-Noggin* transgenic mice, in which Wnt is activated and BMP is suppressed, respectively, in gastric epithelial cells. However, neither *K19-Wnt1* nor *K19-Noggin* mice developed gastric tumors. Importantly, induction of PGE_2 pathway in both transgenic lines by crossing with *K19-C2mE* mice resulted in development of large gastric tumors. Notably, *K19-Wnt1/C2mE* compound mice developed gastric adenocarcinomas, whereas *K19-Noggin/C2mE* mice developed gastric hamartomas. These results suggest that tumor phenotypes are determined by type of oncogenic signaling, such as Wnt activation or BMP suppression, and that COX-2/PGE_2 pathway is essential for gastric tumor formation regardless of tumor phenotypes possibly through inflammatory responses.

Related references

1. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, and Taketo MM. Suppression of intestinal polyposis in *Apc*^{D716} knockout mice by inhibition of cyclooxygenase 2 (COX-2). ***Cell*** 87: 803-809, 1996.
2. Oshima H, Oshima M, Inaba K, and Taketo MM. Hyperplastic gastric tumors induced by activated macrophages in COX-2/mPGES-1 transgenic mice. ***EMBO J*** 23: 1669-1678, 2004.
3. Oshima H, Matsunaga A, Fujimura T, Tsukamoto T, Taketo MM, and Oshima M. Carcinogenesis in mouse stomach by simultaneous activation of the Wnt signaling and prostaglandin E_2 pathway. ***Gastroenterology*** 131: 1086-1095, 2006.
4. Oguma K, Oshima H, Aoki M, Uchio R, Naka K, Nakamura S, Hirao A, Saya H, Taketo MM, and Oshima M. Activated macrophages promote Wnt signaling through tumor necrosis factor-α in gastric tumor cell. ***EMBO J*** 27: 1671-1681, 2008
5. Guo X, Oshima H, Kitamura T, Taketo, MM, and Oshima M. Stromal fiboblasts activated by tumor cells promote angiogenesis in mouse gastric cancer. ***J Biol Chem***, 283: 19864-19871, 2008.
6. Oshima H, Itadani H, Kotani H, Taketo MM, and Oshima M. Induction of prostaglandin E_2 pathway promotes gastric hamartoma development with suppression of bone morphogenetic protein signaling. ***Cancer Res***, 69: 2729-2733, 2009.

CXC Chemokines in the Regulation of Angiogenesis Relevant to Cancer

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Chemokines are a superfamily of homologous 8-10 kDa heparin-binding proteins, originally described for their role in mediating leukocyte recruitment to sites of inflammation. A subgroup of chemokines, the CXC family, plays a critical role in angiogenesis, in both physiologic and pathologic contexts, including chronic inflammation, fibrosis, and malignancy. Structurally, the defining feature of the CXC chemokine family is four conserved cysteine amino acid residues near the amino-terminus of the protein, the first two of which are separated by a non-conserved amino acid, thus constituting the Cys-X-Cys or 'CXC' motif. This family is further subdivided on the basis of the presence or absence of another three amino acid sequence, glutamic acid-leucine-arginine (the 'ELR' motif), immediately proximal to the CXC sequence. Unique amongst the mediators of angiogenesis, this structural motif determines the function of specific ligands: the ELR containing CXC chemokines, originally discovered for their potent neutrophil chemoattractant properties, promote angiogenesis via interaction with CXCR2, while the non-ELR ligands, which attract mononuclear leukocytes, inhibit angiogenesis via their interaction with CXCR3.

Angiogenesis is an essential feature of development and progression of cancers, and represents an intriguing, if as yet elusive, therapeutic target. CXCR2/CXCR2 ligand-mediated angiogenesis has been shown to play a critical role in tumor growth of multiple solid cancers. In contrast, the biological axis of CXCR3/CXCR3 ligands both inhibits angiogenesis and mediates Th1-type cell mediated immunity via recruitment of CXCR3-expressing T cells has led to the concept of "immunoangiostasis" in cancer. Taken collectively, focusing on these findings support the notion that CXCR2/CXCR2 ligands and CXCR3/CXCR3 ligands can be considered both as a target and for consideration of therapeutic intervention, respectively, in treating cancer.

Poster Abstracts

P1. The contribution of inflammatory macrophages, and CD8$^+$ effector T cells to adipose tissue remodeling, inflammation and insulin resistance in obesity

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Recently inflammatory macrophage infiltrations into obese adipose tissue was reported, and chronic inflammation is increasingly regarded as a key process underlying metabolic diseases in obese subjects. Therefore, to assess the dynamic interplay between multiple cell types, an *in vivo* visualization technique was developed. *In vivo* imaging revealed that macrophage infiltrations are associated with adipocyte differentiation and adipocyte cell death in obesity. (2007 Diabetes). In addition, increased leukocyte-platelet-endothelial cell interactions were observed in the microcirculation of obese adipose, a hallmark of inflammation (2008 J Clin Invest). Upregulated expression of adhesion molecules on macrophages and endothelial cells contribute to local activation of inflammatory processes. We also found that large numbers of CD8$^+$ effector T cells infiltrated obese adipose tissue, whereas the numbers of CD4$^+$ helper were diminished (2009 Nat Med). The infiltration by CD8$^+$ T cells preceded the accumulation of inflammatory M1 macrophages, and CD8$^+$ T cells depletion reduced M1 macrophage infiltration and adipose tissue inflammation, and ameliorated systemic insulin resistance. Coculture and other *in vitro* experiments revealed the vicious interactions between CD8$^+$ T cells, macrophages and adipose tissue. Our findings suggest that obese adipose tissue activates CD8$^+$ T cells, which in turn promote the recruitment and activation of macrophages, thereby initiating and maintaining inflammatory cascades in obese adipose tissue. Infiltration of CD8$^+$ T cells and M1 macrophages is, thus, essential for the initiation and development of adipose inflammation. Our results also demonstrate the power of our imaging technique to analyze multi-cellular interactions *in vivo* and to evaluate new therapeutic interventions against it.

P2. Regulation of T cell responses by a distinct subset of resident splenic macrophages

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The balance between immune activation and tolerance must be regulated to maintain immune homeostasis. It has been well demonstrated that dendritic cells are critical regulators to maintain such balance. Tissue macrophages constitutes the major cellular subsets of antigen presenting cells within body, however, it is poorly understood what kind of tissue resident macrophages (Mφs) is involved in the regulation of immune homeostasis in the peripheral lymphoid tissues. Here, we found that splenic Mφs could be divided into two subsets due to α9 integrin expression (F4/80^{int}Mac-1^{hi}α9^{-}and F4/80^{hi}Mac-1^{low}α9^{+}). F4/80^{int}Mac-1^{hi}α9^{-}Mφ spontaneously secreted IL-6 and IL-12p40 and induced robust proliferation and differentiation of naïve T cells. These characteristics were met with the features of conventional splenic Mφ (cMφ). Mφs expressing α9 integrin (α9Mφs) were mainly localized in the red pulp. Unlike cMφs, α9Mφs expressed TGF-β and inhibited T cell proliferation by a mechanism partially dependent on TGF-β. Furthermore, α9Mφs were capable of inducing the differentiation of naïve CD4^{+}T cells into Foxp3^{+} regulatory T cells via TGF-β. These results suggest that these distinct two Mφ subsets reciprocally control T cell immune responses in the spleen.

P3. The role of chemokine receptors, CCR2 and CX3CR1, in arthritis in IL-1 receptor antagonist-deficient mice

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Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the infiltration of T cells, B cells, macrophages, and neutrophils into the joint space. Chemokine receptors, CCR2 and CX3CR1, are expressed on macrophages and T cells infiltrating into the synovium of RA patients. However, it still remains unclear on the roles of CCR2- or CX3CR1-mediated signals in RA. Hence, we investigated the roles of CCR2- or CX3CR1-mediated signals in arthritis developed spontaneously in IL-1ra-deficient mice, by ablating CCR2 or CX3CR1 gene in IL-1ra-deficient mice. Neither single CCR2- nor CX3CR1-deficient mice developed any signs of arthritis until 1 year after birth. Consistent with the previous report, IL-1ra-deficient mice developed multiple arthritis until 12 weeks after birth. Ablation of CCR2 gene but not CX3CR1 gene exaggerated arthritis in IL-1ra-deficient mice, as evidenced by augmented arthritis clinical scores and histopathological scores. Moreover, IL-1ra-CCR2-double deficient mice exhibited lower bone mineral density on computer tomography and higher serum concentration of cartilage oligomeric matrix protein, than IL-1ra-deficient mice. Furthermore, tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts were increased in the joints and osteoclastogenesis was enhanced in IL-1ra-/-CCR2-/- mice compared with IL-1ra-deficient mice. These observations suggest that CCR2-mediated signals can modulate bone destruction in arthritis developed in IL-1ra-deficient mice.

P4. Pathogenic Roles of The CX3CL – CX3CR1 Intercations in Macrophage recruitment and function in Dextran Sodium Sulfate-Induced Acute Colitis in Mice

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Ulcerative colitis (UC) is an inflammatory bowel disease (IBD), characterized by inflammation in mucosa and submucosa and ulceration in colon. Dextran sodium sulfate (DSS) ingestion causes in rodents, massive granulocyte and macrophage infiltration, glandular architecture distortion, and ulceration in colon, mimicking pathological changes observed in the acute phase of UC patients. DSS ingestion enhanced the expression of a chemokine, CX3CL1/fractalkine and its receptor, CX3CR1 in colon, as well as inflammatory cell infiltration. Given the fact that CX3CR1 is expressed by a subset of macrophages, we examined the pathogenic roles of the CX3CL1-CX3CR1 interactions in DSS-induced acute colitis, by using mice deficient in CX3CR1 gene. When 2 % DSS solution was given orally to wild-type mice for 5 days, body weight loss and shortening of colon developed. Histopathological examination revealed a massive infiltration of granulocytes, macrophages, and lymphocytes, and destruction of glandular structure. On the contrary, when CX3CR1-deficient mice were given DSS solution in the same way, they did not exhibit body weight loss and shortening of colon. Moreover, infiltration of inflammatory cells and destruction of glandular architecture was attenuated in CX3CR1-deficient mice. Ameliorated inflammation in CX3CR1 KO mice corresponded to lower levels of expression of inducible NO synthetase (iNOS) by macrophages compared to WT mice in the course of colitis induction. Chimeric mice with CX3CR1 deficient bone marrow cells also exhibited milder inflammation, comparable to CX3CR1 KO mice. Furthermore transplantation of WT bone marrow to CX3CR1-deficient mice restored inflammatory cell infiltration, iNOS expression, and finally resulted in colitis with severity similar to that observed in WT mice.
These observations suggest the pathogenic roles of CX3CR1-mediated signals for recruitment and activation of inflammatory cells, particularly macrophages, in DSS-induced acute colitis.

P5. Critical roles of NK and CD8$^+$ T cells in central nervous system listeriosis

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Listeria monocytogenes (LM) causes a life-threatening infectious disease affecting the brain of humans and domestic animals. Unfortunately, no adequate murine models for central nervous system (CNS) listeriosis exist. Using intraparenchymal injection, we have established a new murine model for CNS listeriosis. Injection of a small volume of bacterial suspension limits the bacteria to the brain parenchyma with no leakage into the ventricular system. This new method enabled us to investigate the progression of and recovery from listerial brain infection, revealing roles for both innate and adaptive immune cells in CNS listeriosis. In the early phase of CNS listeriosis, NK cell-derived IFNγ is a critical cytokine in the limitation of bacterial growth by the host defense. During the later phase, CD8$^+$ but not CD4$^+$ T cells play a critical role and LM-specific CD8$^+$ T cells kill LM-infected microglia. Thus, innate and adaptive immune responses combine to successfully eliminate bacteria from the brain.

P6. Cigarette smoke condensate extracts induce proinflammatory cytokines in synovial cells and exacerbate collagen-induced arthritis in mice

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Cigarette smoking is a solid environmental risk factor for rheumatoid arthritis (RA) as revealed by epidemiological studies. However, there are no reports supporting the association between cigarette smoking and RA at scientific basis. RA is characterized by proliferation of synoviocytes that produce proinflammatory cytokines, which are implicated in the pathogenesis of RA. When human fibroblast-like synoviocytes line MH7A was treated with cigarette smoke condensate (CSC), either mainstream or sidestream, expression levels of IL-1α, IL-1β, IL-6 and IL-8 mRNA were up-regulated in both time- and dose–dependent manners. CSC also induced these cytokines at protein level and further augmented the effects of TNFα. When main stream CSC was administered into DBA/1J male mice at the time of immunization with collagen and Freund's complete adjuvant, CSC augmented the development of arthritis. These results support the epidemiological studies indicating a strong association between heavy cigarette smoking and pathogenesis of RA.

P7. 5-hydroxytryptamine 4 receptor stimulus of myenteric neuron induces anti-inflammatory actions via alpha7nACh receptor of muscularis macrophages in postoperative ileus.

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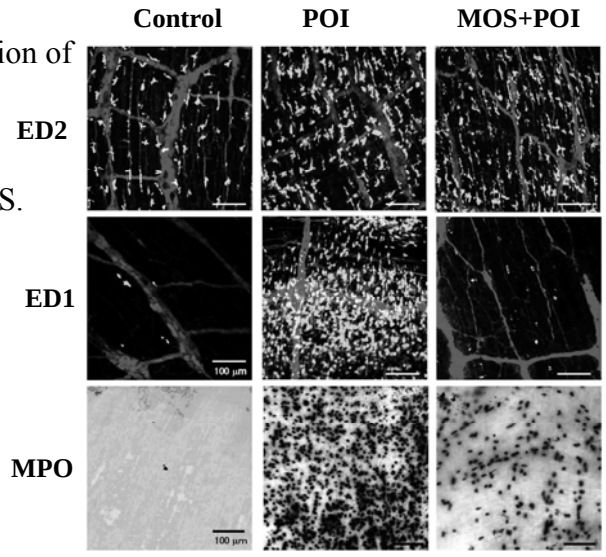
Postoperative ileus (POI) is often found in gastroenterological surgery. Previous evidence indicates that POI is mainly caused by inflammatory mechanisms in intestinal muscle layer. In the inflammatory actions, muscularis macrophages play a crucial role to induce gastrointestinal (GI) dysmotility via NO production. Mosapride citrate (MOS), that is a potent 5-HT_4 agonist, is actually used in clinical practice in Japan as a potent gastroprokinetic agent. Therefore, we expected that MOS may be useful to ameliorate GI dysmotility in POI. Intestinal manipulation was performed at rat ileum under anesthesia to make POI model rat. At 24 h after intestinal manipulation, the manipulated lower intestine was picked out. Subcutaneously administration of MOS (0.3-3 mg/kg) or CJ-033466 (another selective 5-HT_4 agonist: 1 mg/kg) significantly amerilrated GI dysmotility in POI as we had expected. In addition, infiltration of macrophages and leukocytes into the inflamed muscle layer was also inhibited by MOS and CJ-033466. 5-HT_4 antagonist, GR113808 (0.3-3 mg/kg i.p.) reduced the anti-inflamatory action by MOS (1 mg/kg), indicating that 5-HT_4 stimulus could have an anti-inflammatory action.

Non-selective ganglionic blocking agent, hexametonium (1 mg/kg i.p.) abolished the anti-inflammatory actions and recovery action of GI motility mediated by MOS. In addition, alpha7nACh receptor antagonist, methyllycaconitine citrate (MLA) also attenuated anti-inflammatory action of MOS. Taken together, it is suggested that 5-HT_4 stimulus may activate serotonergic neuron in the myenteric plexus, then released acethylcholine can activate alpha7nACh receptor of muscularis macrophages to ameliorate POI.



Inhibitory action of 5-HT_4 agonist on intestinal muscularis inflammation by POI.

P8. Staphylococcal superantigen-like protein 5 (SSL5) inhibits the activity of matrix metalloproteinase-9 from human neutrophils

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Staphylococcal superantigen-like proteins (SSLs) constitute a family of exoproteins exhibiting structural similarities with superantigens and enterotoxins but no superantigenic activity. It was previously reported that SSL5 bound to P-selectin glycoprotein ligand-1 (PSGL-1) and receptors for anaphylatoxins and chemokines, and interfered their functions. In this study, we present evidence that SSL5 specifically binds to matrix metalloproteinase (MMP)-9 and inhibits its enzymatic activity. When human neutrophil cell lysate was applied to SSL5-conjugated Sepharose, a major protein of approximately 100 kDa was recovered in the bound fraction. This protein was identified as the proform of MMP-9 (proMMP-9) by peptide mass fingerprinting analysis. Surface plasmon resonance analysis revealed that SSL5 bound to proMMP-9 with rather high affinity (K_d = 1.9 nM). SSL5 was found to effectively inhibit MMP-9-catalyzed hydrolysis of gelatin and a synthetic fluorogenic peptide in a noncompetitive manner (K_i = 0.097 nM), as assessed by zymography and the fluorescence quenching method. Finally, the neutrophil transmigration across Matrigel basement membranes in response to a formyl peptide and the leukocyte recruitment into the murine peritoneal cavity in response to thioglycolate were suppressed by the presence of SSL5. These findings suggest possible roles that SSL5 may play in immune evasion of staphylococci by inhibiting MMP and interfering with leukocyte trafficking.

P9. Stat3 mediates the cell-cell interaction of macrophages and tumor cells in human glioblastoma.

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Tumor-associated macrophages (TAM) actively participate in development of tumor microenvironment by inducing angiogenesis, immunosuppression, and tumor invasion. Promotion of angiogenesis by TAMs in tumor development has been well established. We have recently shown that the infiltration of TAMs polarized to M2 phenotype are correlated well with tumor proliferation and poor prognosis in human glioblastoma. In this study, we investigated the mechanisms of cell-cell interaction between TAMs and glioblastoma cells. Immunostaining studies of human glioblastoma specimens showed that signal transducer and activator of transcription-3 (Stat3) activation was detected not only in tumor cells but also in TAMs. Tumor cell supernatant (TCS) induced Stat3 activation in macrophages and differentiation toward M2 phenotype. Inhibition of Stat3 by RNA interference suppressed TCS-induced macrophage differentiation toward M2 phenotype. Based on these results, we assumed that the direct cell-cell interaction of TAMs and tumor cells are positively involved in tumor development through Stat3 signaling. Therefore, we investigated the direct cell-cell interaction of tumor cells and macrophages by means of co-culture system. Interestingly, Stat3 activation was detected not only in macrophages but also in tumor cells when these two kinds of cells were mixed and co-cultured for 2 days. Cyclin-D1 or Ki-67 expression in tumor cells was also increased by co-culture with macrophages. Activation of tumor cells was inhibited when Stat3 in macrophages was suppressed by RNA interference. Productions of oncostatin M, IL-10, and VEGF-A in macrophages were significantly increased by TCS. These increases were significantly suppressed by the inhibition of Stat3 by RNA interference. These findings indicate that cell-cell interaction via Stat3 plays an important role in development of tumor microenvironment.

P10. Crucial contribution of thymic Sirpα+ conventional dendritic cells to central tolerance against blood-borne antigens

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Thymus is an important organ for development and education of T cells. In the thymus, T cell progenitor cells pass through two selection systems, positive and negative selections, to differentiate into the mature naïve T cells. Negative selection can decrease the risk of the development of autoimmune disorders, by inducing clonal deletion in potentially pathogenic autoreactive T progenitor cells. In central tolerance, thymic dendritic cells (DCs) are presumed to be a major sentinel to induce the apoptosis of autoreactive T progenitor cells. Several lines of evidence indicate the presence of heterogenous DC subsets in thymus, similarly observed on other lymphoid organs such as lymph nodes and spleen. However, the role of each DC subset in the central tolerance is still unclear, because unique microenvironment and a small number of DCs in thymus hinder the in vitro examination of the functions of each DC subset. Chemokine receptor expression by DCs prompted us to examine whether thymic DC subsets change in mice deficient in chemokine receptors including CCR1, CCR2, CCR5, and CX3CR1. We observed that a minor Sirpα$^+$ DC subset was selectively decreased in the thymus derived from CCR2-deficient mice but not other chemokine receptor gene-deficient mice. We further revealed that thymic Sirpα$^+$ cDCs migrated from bone marrow to thymus via peripheral blood and that they exhibited a unique intrathymic localization confined to perivascular and cortical areas, under the guidance of a CCR2 ligand, MCP-2. Moreover, Sirpα$^+$ cDCs had a greater capacity to uptake blood-borne antigens than Sirpα$^-$ cDCs, along with their unique intrathymic localization. Furthermore, OVA peptide induced clonal deletion of DO11.10 TCR-expressing CD4$^+$ CD8$^+$ thymocytes, when it was injected into peripheral blood of DO11.10 TCR transgenic mice, but the decrease was attenuated by CCR2 gene ablation. Concomitantly, CCR2 deficiency allowed releasing more autoreactive T cells against serum antigens into periphery. Thus, thymic Sirpα$^+$ cDCs may function as a specialized APC for the development of central tolerance to blood-borne antigens.

P11. *Glycyrrhiza inflata*-derived chalcones, Licochalcone A, Licochalcone B and Licochalcone D, inhibit phosphorylation of NF-κB p65 in LPS signaling pathway

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Licorice root, *Glycyrrhiza inflata,* has been used as a traditional medicine for the treatment of gastric ulcer and inflammation; however, the mechanism of its anti-inflammatory activity has not been clarified. Here, we investigated the effect of Licochalcone A, a major component of *G. inflata*, on the LPS signaling pathway. We found that Licochalcone A remarkably inhibited LPS-induced NO production, and TNFα expression and MCP-1 expression in RAW264.7 cells. Furthermore, when injected with Licochalcone A prior to injection of LPS, the serum level of TNFα and MCP-1 in C57BL/6 mice was clearly decreased, indicating that Licochalcone A has a potent anti-inflammatory effect both *in vitro* and *in vivo.* Strikingly, Licochalcone A significantly inhibited LPS-induced NF-κB transcriptional activity through inhibited activation of PKA, which is required for the phosphorylation of NF-κB p65 at serine 276 that is important site for NF-κB transcriptional activation. *G. inflata* contains not only Licochalcone A but also Licochalcone B, Licochalcone C, Licochalcone D, Echinatin and Isoliquiritigenin, harboring the common structure of chalcones. We also observed that Licochalcone B and Licochalcone D significantly inhibited LPS-induced phosphorylation at serine 276 and transcriptional activation of NF-κB, the same as Licochalcone A. Taken together, Licochalcone A, Licochalcone B and Licochalcone D might contribute to the potent anti-inflammatory effect of *G. inflata* through the mechanism of NF-κB inhibition.

P12. PROTECTIVE ROLE OF CX3CR1-MEDIATED SIGNAL PATHWAY IN *CLOSTRIDIUM DIFFICILE* TOXIN A-INDUCED ENTERITIS.

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We investigated the pathophysiological roles of CX3CR1 in *Clostridium difficile* toxin A-induced enteritis using CX3CR1-deficient (CX3CR1 KO) mice. Toxin A injection into the ileal loop of CX3CR1 KO mice induced the significant increase of fluid accumulation, as compared with WT mice treated with the same manner. Histopathologically, the severe destruction of intestinal epithelial architecture was observed with a concomitant of massive neutrophil infiltration in toxin A-treated ileum of CX3CR1 KO mice. Furthermore, toxin A treatment caused marked enhancement of the gene expression of proinflammatory cytokines in the ileum of CX3CR1 KO mice, compared with WT mice. However, the gene expression of cytoprotective molecule HO-1 was enhanced to a greater extent in WT mice than CX3CR1 KO mice. Double-color immunofluorescence analysis showed that HO-1 was mainly expressed on F4/80-possitive macrophages. Treatment with CX3CL1 in macrophages induced the gene expression of HO-1 via ERK activation. The suppression of HO-1 expression by treatment with HO-1 inhibitor exacerbated toxin A-induced enteritis in WT mice. Moreover, therapeutic treatment with CX3CL1 in WT mice before toxin A treatment inhibited the development of toxin A-induced enteritis. Thus, the absence of CX3CL1-CX3CR1 interaction exacerbated *C. difficile* toxin A-induced enteritis due to the impaired expression of HO-1. These observations indicated that CX3CL1-CX3CR1 interaction could play a protective role in *C. difficile* toxin A-induced enteritis.

P13. Presence of Acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1) on late endosome (LE) in cholesterol-rich macrophages: efficient cholesterol esterification on LE and possible therapeutic strategy for Niemann-Pick disease type C1

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Acyl coenzyme A:cholesterol acyltransferase 1 (ACAT1) is an endoplasmic reticulum (ER)-resident enzyme that catalyzes cholesterol esterification. We found that a significant portion of the total ACAT1 appears in small vesicles and *trans*-Golgi network associated membranes in cholesterol-rich macrophages (Mϕ). Here we investigated the possibility that a portion of the total ACAT1 may also be associated with the late endososomal membranes (LE) in cholesterol-rich Mϕ. Confocal laser microscopy revealed that no significant ACAT1 signal was colocalized with the signal for LAMP2, a marker protein for LE/lysosomes in intact Mϕ. However, approximately 20% of the total ACAT1 signals colocalized with the LAMP2 signal in cholesterol-rich Mϕ. In this situation, the ACAT1-positive organelles isolated by immunoadsorption using ACAT1 specific antibody contained LAMP2, demonstrating the association of ACAT1 and the LE. After incubating with ^{3}H-labeled cholesterol ([^{3}H]Chol)-aggregated LDL ([^{3}H]Chol-agLDL) for 30 min, significant esterification did not occur in intact Mϕ, but did occur in cholesterol-rich Mϕ. Cholesterol-rich Mϕ, not intact Mϕ, produced estrified [^{3}H]Chol ([^{3}H]CE) even after cells were simultaneously treated with [^{3}H]Chol-agLDL and the amphipathic amine U18666A, which blocks the Niemann-Pick type C (NPC) dependent cholesterol translocation from LE to ER. Mouse Mϕ with NPC-/- phenotype failed to esterify [^{3}H]Chol-agLDL, however, we further confirmed complete recovery of cholesterol re-esterification in choleterol-rich NPC-/- mouse Mϕ**.** Our results indicate that cholesterol-rich Mϕ produces ACAT1-positive LE and efficiently esterify modified LDL-derived cholesterol on LE in the manners independent of the NPC-dependent cholesterol translocation pathway. Because accumulation of free cholesterol on LE is critical pathogenesis of NPC disease, our findings possibly open therapeutic strategy for NPC disease.

P14. Draper-mediated phagocytosis of *Staphylococcus aureus* by *Drosophila* hemocytes

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Phagocytosis is central to cellular immunity against bacterial infections. Unlike in vertebrates, opsonophagocytosis is seemingly not a major mode of phagocytosis in *Drosophila*, and phagocytic cells presumably recognize components of the bacterial cell wall using specific reeceptors. Despite the identification of candidate receptors for phagocytosis, the cell wall components that act as their ligands remain to be found. We searched for *Staphylococcus aureus* genes required for phagocytosis by *Drosophila* hemocytes in a screening of mutant strains with defects in the structure of the cell wall. The genes identified included *ltaS* encoding an enzyme responsible for the synthesis of lipoteichoic acid. *ltaS*-dependent phagocytosis of *S. aureus* required the receptor Draper but not Eater or Nimrod C1, and Draper-lacking flies showed reduced resistance to a septic infection of *S. aureus* without a change in the humoral immune response. Finally, lipoteichoic acid bound to the extracellular region of Draper. We propose that lipoteichoic acid serves as a ligand for Draper in the phagocytosis of *S. aureus* by *Drosophila* hemocytes, and that the phagocytic elimination of invading bacteria is required for flies to sutvive the infection.

P15. Role of Pretaporter as a ligand for Draper in phagocytosis of apoptotic cells by *Drosophila* hemocytes

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We previously identified a *Drosophila* protein that binds to the phagocytosis receptor Draper, a homologue of *Caenorhabditis elegans* CED-1. Pretaporter is normally present in the endoplasmic reticulum and relocates to the cell surface upon the induction of apoptosis. Pretaporter is required for the Draper-mediated phagocytosis of apoptotic cells by *Drosophila* hemocytes. However, as to whether Pretaporter serves as a ligand for Draper was not certain. In the present study, we biochemically examined this issue.

After a series of in vitro experiments, we obtained the following results.
1. The amino-terminal one third of Pretaporter was sufficient for the binding to the extracellular region of Draper.
2. Pretaporter-coated latex beads were phagocytosed by hemocyte-derived culture cells in a Draper-mediated manner.
3. *Drosophila* culture cells artificially expressing Pretaporter at the cell surface became susceptible to Draper-mediated phagocytosis with no indication of apoptosis.
4. Draper in hemocyte-derived cultured cells was tyrosine phosphorylated upon the addition of Pretaporter.

These results collectively suggested that Pretaporter moves from the endoplasmic reticulum to the cell surface during apoptosis and acts as a ligand for Draper to induce the phagocytosis of apoptotic cells.

P16. Activation of Wnt signaling in gastric cancer cells by inflammatory macrophages

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Wnt/β-catenin signaling plays a key role in maintaining the intestinal stem cells and progenitor cells. It has been reported that Wnt activation and chronic inflammation are involved in intestinal tumor development. However, the correlation between inflammatory response and promotion of Wnt/β-catenin activity has not been fully investigated. We found that activated macrophages increase Wnt/β-catenin activity in gastric cancer cells through production of TNF-α that increases the level of unphosphorylated β-catenin. We constructed a TCF-reporter plasmid expressing green fluorescent protein (GFP) in response to activation of Wnt/β-catenin signaling. The vector was transfected to two gastric cancer cell lines, and Wnt activity was analyzed by flow cytometry. To investigate the role of macrophages in Wnt/β-catenin upregulation, the GFP expressing gastric cancer cells were cultured in conditioned medium (CM) from macrophages activated by LPS treatment. Surprisingly, high-GFP population was markedly increased in cells stimulated with the CM. We next investigated a relation between Wnt/β-catenin and TNF-α that is a key mediator of inflammatory response. Importantly, neutralizing antibody against TNF-α suppressed the effect by CM to enhance Wnt/β-catenin activity in gastric cancer cells. Furthermore, Wnt/β-catenin activity in gastric cancer cells was increased by TNF-α treatment in a dose-dependent manner. Consistently, the level of unphosphorylated β-catenin was increased significantly in gastric cancer cells treated with CM or TNF-α. These results indicate that TNF-α is one of the major factors derived from activated macrophages which stimulates Wnt/β-catenin pathway.

P17. *MafB* and *c-Maf* are required for clearance of apoptotic cells in macrophages

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MafB and c-Maf is the member of large Maf family transcription factors well known to be specifically expressed in monocyte-macrophage lineage of hematopoietic cells. Recently we and other groups reported the minor consequences of either MafB or c-Maf single deficiency in the fetal liver macrophages. To address the further function of MafB and c-Maf in macrophage, we performed microarray and RT-PCR analysis of *Mafb-/-* macrophage and *Mafb, c-Maf* compound double KO (*MafB-/-::c-Maf-/-*) fetal liver. Interestingly, we found that reduction of C1qa, a member of the first component of the classical complement pathway, was observed in both *Mafb-/-* and *MafB-/-::c-Maf-/-* macrophages compared with wild type. C1qa deficiency is known to result in defect of macrophages in clearance of apoptotic cells. Consistent with this evidence, *Mafb-/-* and *MafB, c-Maf* compound double hetero (*MafB+/-::c-Maf+/-*) macrophage failed to uptake of apoptosis induced Jurkat cells. Moreover, ectopic expression of MafB and c-Maf increased C1qa promoter–driven luciferase activity through the Maf recognition element in C1qa promoter. These results suggest that MafB and c-Maf was important for the clearance of apoptotic cells via regulation of C1qa.

P18. Lymphocyte recruitment to nasal-associated lymphoid tissues is regulated by two sulfotransferases

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The nasal-associated lymphoid tissue (NALT) is a mucosal immune tissue that functions as a first line of immunological defense against invading pathogens. In NALT, inhaled foreign antigens are efficiently trapped and concentrated to initiate adaptive immune responses. NALT contains various types of lymphoid cells that are required for the induction and regulation of mucosal immune responses to antigens delivered from the nasal cavity. However, molecular mechanisms underlying lymphocyte recruitment to NALT are still unknown. Immunohistochemical studies revealed that high endothelial venule (HEV) in NALT strongly expresses peripheral lymph node addressin (PNAd) bearing mucin-like domains that functions as scaffolding for sulfated *O*-glycans. In this study, we investigated the role of PNAd in lymphocyte recruitment to NALT using gene-targeting mice deficient in two sulfotransferases, GlcNAc6ST-1 and GlcNAc6ST-2, that are involved in PNAd biosynthesis (Kawashima *et al., Nat. Immunol.,* 6:1096-1104, 2005). NALT HEV in the double null (DKO) mice was devoid of immunoreactivity against MECA-79 monoclonal antibody which specifically recognizes PNAd, indicating that the two sulfotransferases are essential for PNAd biosynthesis in NALT HEV. Short-term homing assay indicated that lymphocyte recruitment to NALT was significantly decreased by approximately 80% in DKO mice. Production of IgE and number of sneezes in response to nasally administered ovalbumin were also substantially diminished in the DKO mice. These results demonstrate that the two sulfotransferases, GlcNAc6ST-1 and GlcNAc6ST-2, play an essential role in lymphocyte recruitment to NALT and nasal immune responses, suggesting a potential therapeutic approach to modulate allergic reactions by targeting sulfated glycan-mediated lymphocyte recruitment.

P19. Involvement of IFN-γ and IL-6 in severe invasive group A *streptococcus* infection

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Streptococcus pyogenes (group A *streptococcus*; GAS) is one of the most versatile bacteria among human pathogens. Non-invasive GAS infection can cause common diseases, such as pharyngitis and impetigo. Whereas, invasive GAS infection can lead to rapid progressive and life-threatening manifestations, including sepsis, necrotizing fasciitis and streptococcal toxic shock-like syndrome with high mortality rates ranging from 20% to 60%. Although not only bacterial factors but also host factors are likely to be involved in severe invasive GAS infection, it remains unknown what kinds of host factors contribute to the protection against invasive GAS infection or pathophysiology of the infection.

To clarify the molecular pathogenesis of disease, here we determined the levels of cytokines in plasma from human patients infected with severe invasive GAS and from mice infected with clinical isolates of either severe invasive or non-invasive GAS. Interestingly, a large amount of IFN-γ and IL-6, but not other proinflammatory cytokines, including IL-1α, IL-17 and TNF-α, was detected in plasma from fatal human patients, but not found in those from survival human patients. Similar results were also found in mice infected experimentally with invasive GAS isolates, but not mice infected with non-invasive GAS isolates. Furthermore, analysis using flow cytometry revealed that IFN-γ was produced by Gr-1^+ Mac-1^+ inflammatory monocytes in mice infected with invasive GAS isolates. These results suggest that monocyte-derived IFN-γ may be a host factor that participates in the pathogenesis of invasive GAS infection.

P20. MafB is indispensable for the development of atherosclerosis

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Macrophages play a crucial role in the development of atherosclerosis through the accumulation of oxidized LDL (oxLDL). However, little is known about transcriptional regulation network of macrophage in atherosclerosis development. The protooncogene MafB of the basic leucine zipper family is expressed in monocytes and macrophages. We identified that MafB null macrophages were strongly impaired the expression of apoptosis inhibitory factor, AIM (Apoptosis Inhibitor expressed by Macrophage). AIM is reported to be a critical factor that protects macrophages from the apoptotic effects of oxidized lipids and is regulated by transcription factor LXR (Liver X Receptor) /RXR (Retinoid X Receptor) hetero dimmer. LXR and RXR agonists induced the expression of MafB transcript and MafB-null macrophages were more sensitive to oxLDL-induced apoptosis than wild type. Ectopic expression of MafB increased AIM promoter-driven luciferase activity through the Maf recognition element. Moreover, atherosclerosis lesions in *LDLR*-/- mice transplanted with *mafB-/-* fetal liver cells are dramatically reduced when compared to wild type cells. These results strongly suggest that MafB is an indispensable for AIM gene regulation and macrophage survival in the development of atherosclerosis.

P21. Involvement of a core protein of *Drosophila* Perlecan in the phagocytosis of apoptotic cells

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Phagocytes selectively recognize target apoptotic cells using specific receptors that either directly or indirectly bind to phagocytosis markers, also called eat-me signals, present at the surface of apoptotic cells. Presumably, there are two distinct receptors for phagocytosis in *Drosophila*, and Draper is the one while the other remains to be identified.

To search the other receptor, we screened a library of monoclonal antibodies raised against hemocytes of *Drosophila* embryos for their effects on the phagocytosis of apoptotic cells. Among five antibody clones that were found to inhibit phagocytosis, we identified one monoclonal antibody that recognizes Trol, a core protein of *Drosophila* proteoglycan corresponding to mammalian Perlecan. RNA interference-mediated inhibition of Trol expression in phagocytes led to a decrease of the phagocytosis activity, and a simultaneous knockdown of Draper further lowered the activity. These results suggest that Trol is involved in the phagocytosis mediated by a receptor other than Draper. Trol is not a membrane protein but contains the sequence Arg-Gly-Asp, a motif for the binding to integrin. We thus examined a role for integrin in the phagocytosis of apoptotic cells. Inhibition of the expression of integrin in phaocytes reduced the phagocytosis activity, and the incubation of phagocytes with recombinant Trol increased the level of phosphorylated Focal Adhesion Kinase, a mediator of the integrin-initiated signal pathway. Taken together, we propose that Trol and integrin compose the second phagocytosis receptor responsible for the phagocytosis of apoptotic cells in *Drosophila*.

P22. Relevance of long pentraxin 3 expression and release in macrophages and neutrophils *in vivo* and *in vitro*

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Pentraxin 3 (PTX3) is the first identified long pentraxin, and it is rapidly produced and released by several cell types in response to lipopolysaccharide (LPS) and others proinflammatory signals. The aim of this study was to investigate the expression pattern of PTX3 in human macrophages and polymorphonuclear neutrophils (PMNs) in cell culture experiments, as well as in human atherosclerotic lesions.
Human six-day-cultured monocyte-derived macrophages, as well as freshly isolated PMNs cultured in the medium with or without LPS were used. We performed an immunofluorescence analysis using confocal laser microscopy to specifically detect PTX3 protein, as well as immunoblotting using a novel monoclonal antibody against human PTX3 (clone No. 1228). We also examined coronary arterial thrombi removed from patients with acute myocardial infarction to confirm the expression pattern of PTX3 *in vivo*.
The results of our study showed that human monocyte-derived macrophages expressed PTX3 in the cytoplasm, whereas PMNs showed PTX3 positively in granules which were reduced upon LPS stimulation. We also have shown that foamy macrophages and PMNs are major cellular sources of PTX3 in the process of atherosclerotic plaque activation following rupture and thrombosis. Of interest, extracellular expression of PTX3 was observed in some thrombi containing abundant PMNs, suggesting that PTX3 may be released together with neutrophil granules. Recently, Jaillon et al. have shown that released PTX3 can partially localize in neutrophil extracellular traps (NETs) formed by extruded DNA. Nevertheless, we did not find any evidence of PTX3 released by foamy macrophages.
In summary, we suggest that PTX3 protein is stored in macrophages and neutrophils, whereas PTX3 may be released upon stimulation mainly from neutrophils.

P23. Role of bone marrow-derived cells on repair of mucosal damage caused by either irradiation or anticancer drugs

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Mucosal damage is a common side effect of many cancer treatments especially intensive chemotherapy and radiotherapy. Although it decreases QOL of patients seriously and sometimes changes or disrupts the therapy, there is no effective treatment.

Irradiation or treatment with anticancer drugs often induces bone marrow suppression and cytopenia. Mucosal damage is partly due to the cytopenia, therefore we made a hypothesis that recovery of hematocyte may repair mucosal damage. In this study, mucosal damage model was induced by acetic acid and we revealed that exacerbation or repair of mucosal damage depends on timing of irradiation. Additionally, monocyte/macrophage-associated cytokines and chemokines were upregulated by irradiation at certain point. According to the results, monocytes/macrophages are suspected of contributing to repair mucosal damage.

Bone marrow suppression caused by treatment with 5-FU in mice delayed mucosal repair compared with control mice. Bone marrow transplantation recovered the mucosal damage significantly. Furthermore, we confirmed accumulation of fluorescence-labeled bone marrow cells which were injected i.v., in mucosal damage area but not in normal area.

In conclusion, our results demonstrate bone marrow cells, particularly monocyte/macrophages, contribute to the regeneration of mucosal damage.

P24. Identification of CCR7^{+}MHC class II^{hi} dendritic cells in mouse tumor

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Dendritic cells (DCs) play a pivotal role in immune responses and have emerged as a promising therapeutic target for tumors. Endogenous T cell responses to tumors are regulated by the migration of tumor-infiltrating DCs (TIDCs) to tumor-draining lymph nodes (DLN), however, clinical and experimental studies suggest that TIDCs are in an immature or tolerogenic state, leading to the induction of T cell tolerance. Although mouse TIDCs have been identified as CD11c^{+} cells or CD11c^{+}CD11b^{+} cells, a fraction of monocyte-derived macrophages also expresses CD11c and CD11b, and even MHC class II. Therefore, the previous observation on tumor-infiltrating CD11c^{+}CD11b^{+} cells might not reflect the exact nature of TIDCs.

Here, we demonstrate that the expression of chemokine receptor CCR7, a key homing receptor of interstitial tissue DCs to DLN, can discriminates DCs (CCR7^{+}TIDCs) from other tumor-infiltrating CD11c^{+}CD11b^{+} myeloid cells in a mouse subcutaneous tumor model. CCR7^{+}TIDCs comprise 0.5-1.5% of transplanted 3LL tumor-infiltrating CD45^{+} cells with a phenotype similar to that of tissue-derived migrating DCs (mDCs) in DLN. Parabiosis and *in vivo* BrdU labeling experiments revealed that CCR7^{+}TIDCs are blood-derived cells with a similar turnover to mDCs in DLN, which is slower than that of Ly-6C^{hi} inflammatory monocytes/macrophages. Interestingly, CCR7^{+}TIDC were unaffected in CCR2$^{-/-}$ mice, although the tumor-infiltrating inflammatory macrophages were diminished.

These results suggest that CCR7^{+}TIDCs are tumor-associated DCs (precursors), whose cell lineage is distinct from that of Ly-6C^{hi} inflammatory monocytes/macrophages. Further analyses for the function of CCR7^{+}TIDCs are now under investigation.

P25. Essential role of macrophages in the *in vitro* production of IL-4 or nonspecific IgE or IgG antibody by lymphocytes of submandibular lymph node from mice sensitized intranasally once with cedar pollen

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In a sensitized patient or animal, the production of specific IgE antibodies (Abs) directed toward an allergen has a mutual relation with the immunologic response leading to allergic rhinitis. The nasal mucosa is the first site of contact with inhaled antigen (*e.g.*, cedar pollen); and an increase in the IL-4-dependent production of serum nonspecific IgE Ab after an intranasal (*i.n.)* injection of cedar pollen into mice is a prerequisite for that of allergen-specific IgE Ab in the serum. In the present study, we explored which lymphoid organ was responsible to an *i.n.* injected allergen into mice and how IL-4 or nonspecific IgE or IgG Ab against the allergen was produced in the lymphocytes. Time-dependent changes in the total cell numbers and in the *in vitro* IgE Ab production by bulk cells of the lymphoid organs indicated that submandibular lymph node was a main organ responsive to *i.n.* injected allergen. Of particular interest, the lymphocyte-rich population alone in the submandibular lymph node produced a small amount of IL-4, IgE or IgG Ab; whereas the production was restored by the addition of Mac-1^+ cells in the macrophage-rich fraction to the lymphocyte-rich fraction.

These results implied that macrophages in the submandibular lymph node might play an essential role in the production of IL-4 or nonspecific IgE or IgG Ab by lymphocytes during the initial stage of *i.n.* sensitization of cedar pollen.

P26. High Mobility Group Protein-1 (HMGB1) and Receptor for Advanced Glycation End-Products (RAGE) Expression and Function in Human Inflamed Dental Pulp Cells and Macrophages: Possible role in dental pulpitis during pregnancy

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Pulpitis is characterized as the immune response that is mainly triggered by the invasion of dental caries-related micro-organisms into dental pulp chamber. It is known that macrophages are most likely the predominant population in severe pulpitis. Pregnancy does indeed increase the risk of starting dental caries and does increase the extent of existing dental caries. *Prevotella intermedia* (*P.i.*) is a gram-negative bacteria anaerobic pathogen closely involved in pregnancy gingivitis and suspected of inducing tooth pulp inflammation by its important role in prostaglandin stimulation. Recently, high mobility group protein-1 (HMGB1) has been identified as a novel inflammatory cytokine. The receptor for advanced glycation end-products (RAGE) is a receptor for extracellular HMGB1, and its up-regulation provide an amplification system including inflammation for HMGB1 effects on endothelial cells. To date, little is known regarding the precise mechanism of bacterial action in the pathology of pulp inflammation during pregnancy. Here we examined whether pregnancy women with caries risk have elevated levels of HMGB1 and RAGE protein in their infected pulp tissues compared with those of normal patients investigated by immunohistochemistry, ELISA and immunoblot techniques. To evaluate the effect of *P.i.* lipopolysaccharides (LPS) and sex hormone estrogen on the level of HMGB1, RAW 264.7 macrophages were stimulated with estrogen alone or in combination with LPS. The translocalization of HMGB1 from the nucleus to the cytosol and release into the cell culture supernatant were determined by immunocytochemistry, western blotting and ELISA. Moreover, odontoblast (OD) cells obtained from pulp tissue explants were exposed to recombinant HMGB1 (rHMGB1) in a concentration that based on the HMGB1 levels detected in the inflamed dental pulp tissues. The level of chemokine monocyte chemoattractant protein-1 (MCP-1) release and RAGE expression following rHMGB1 stimulation in OD was measured by ELISA and FACS analysis, respectively. Activated level of mitogen activated protein kinases (MAPKs) was measured by western blotting with phosphorylation-specific antibodies. We found that all pulp tissues express low levels of RAGE and nuclear HMGB1 normally. Both RAGE and HMGB1 proteins were markedly up-regulated in the odontoblast- like cells which line the pulp cavity, and in the macrophages, pulpal fibroblasts, endothelial cells and neurons in the inflamed pulp tissues of the pregnant patients. On the other hand, HMGB1 and RAGE expression was found at moderate levels in the odontoblast- like cells, macrophage and endothelial cells in the inflamed pulp tissues obtained from normal patients. In the in vitro experiments, estrogen abundantly enhanced LPS-induced HMGB1 translocation adn release in RAW264.7 macrophages in a dose-dependent fashion. Incubation of OD cultures with rHMGB1 resulted in increased expression of RAGE protein and augmented MCP-1 release significantly ($p<0.05$) compared with the control. MAPKs mediates rHMGB1- induced MCP-1 release. Taken together, our study is the first to implicate that hormonal fluctuations may have a surprisingly strong influence on the pulpal inflammatory cells recruitment and subsequent triggering the dental caries progression mediated though HMGB1 and its receptor. This information will be critical to an understanding of how sex steroids modulate untreated caries progress to pulpitis.

P27. Nuclear protein nucleophosmin released from macrophages acts as an alarmin

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In case of severe inflammation or tissue damage, macrophages are major source of alarmins or danger-associated molecular patterns (DAMPs). Alarmins are endogenous molecules released from activated cells and/or dying cells, which activate the immune system and cause severe damage to cells and tissue organs. Nucleophosmin (NPM) is a major nucleolar multifunctional protein involved in ribosome biogenesis, centrosome duplication, cell cycle progression, apoptosis, cell differentiation, and sensing cellular stress. In the present work, stimulation of cells with the alarmin-inducible molecule endotoxin, for 16 hours, resulted in NPM release into the culture supernatants of RAW264.7 cells, a murine macrophage cell line. Extracellular NPM was detected in the ascites of the cecal ligation and puncture (CLP) model. NPM was translocated into the cytoplasm from the nucleus in lipopolysaccharide (LPS) -stimulated RAW264.7 cells; furthermore, NPM was detected in the cytosols of infiltrated macrophages in the CLP model. Recombinant NPM (rNPM) induced release of proinflammatory cytokines, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1) from RAW264.7 cells and increased the expression level of intercellular adhesion molecule-1 (ICAM-1) in human umbilical vein endothelial cells (HUVECs). NPM induced the phosphorylation of mitogen-activated kinases (MAPKs) in RAW264.7 cells. Our data indicate that NPM release from macrophages may have potent biological activities that contribute to systemic inflammation. Further investigations of the role of NPM may lead to new therapies for patients with sepsis or other inflammatory diseases.

P28. Peroxisome proliferator-activated receptor-γ contributes to peritoneal fibrosis through regulation of fibrocytes

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(Background) Peritoneal dialysis (PD) is a beneficial treatment for patients with end-stage renal diseases. However, long-term PD treatment has been shown induce histopathologic changes in the peritoneum, especially peritoneal fibrosis, resulting in the loss of dialytic capacity as well as ileus. Recently, bone marrow-derived collagen-producing cells, fibrocytes, have proposed to be involved in the pathogenesis of various fibrotic diseases dependent on chemokine/chemokine receptor system. However, the involvement of fibrocytes in the pathogenesis of peritoneal fibrosis has not been investigated. Peroxisome proliferator-activated receptor-γ (PPAR γ), which is a member of nuclear receptor family, has been revealed to have wide range of effects on metabolism as well as the immune systems. Recent studies have also documented that PPARγ has anti-fibrotic effect by decreasing the production of collagen in various organs. Taken together, these findings prompted us to examine the involvement of fibrocytes dependent on PPAR γ in the pathogenesis of peritoneal fibrosis.
(Methods and Results) Peritoneal fibrosis was induced by the injection of 0.1% chlorhexidine gluconate (CG) into the abdominal cavity in mice. The injection of CG induced marked thickness of the submesothelial compact zone and the up-regulation of pro-collagen type I α_1 (COLIAI) and monocyte chemoattractant protein (MCP-1)/CCL2. In addition, a considerable number of fibrocytes dual positive for CD45 and type I collagen infiltrated in the peritoneum accompanied with the progression of peritoneal fibrosis. To evaluate the contribution of PPAR γ in this model, telmisartan (Tel), angiotensin II receptor blocker with PPAR γ agonistic function, as well as a specific inhibitor of PPARγ, GW9662 (GW) were used. The treatment with Tel reduced the extent of peritoneal fibrosis, peritoneal transcripts of MCP-1 and the number of infiltrated fibrocytes. On the other hand, these inhibitory effects of Tel were partially cancelled by the co-treatment with GW. Furthermore, the direct impact of PPAR γ on fibrocytes was examined in vitro. In cultured fibrocytes, the expression of COLIAI induced by transforming growth factor (TGF)-β_1 was suppressed by the pre-treatment with Tel. In contrast, the treatment with Tel and GW increased TGF- β_1-induced COLIAI expression compared with pre-treatment with Tel.
(Conclusion) These results suggest that PPAR γ contributes to the pathogenesis of peritoneal fibrosis through infiltration and activation of fibrocytes.

P29. TAXIScan technology, used for analyses of chemotaxis of leukocytes from lung disease patients and for mast cell degranulation

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TAXIScan technology is a method of chemotaxis measurement using an optically accessible chemotactic device that provides various parameters of cell migration during chemotaxis (JIM 282, 2-11, 2003; JIM 320, 155-163, 2007). For advanced analysis, TAXIScan-FL has been developed, equipped with a fluorescent microscope and used for fluorescence and higher magnified view analyses up to x100 objective lens.

Using TAXIScan devices, we have investigated chemotaxis of various leukocytes from patients with lung diseases. We found that one parameter plot of leukocyte movement exhibited normal distribution whereas the other parameter plot showed binominal distribution. Less than 100 cells were required to estimate population distribution. Using these plot analyses, we could distinguish normal volunteers and COPD patients, and each severity class of the patients. Normal volunteers and asthma patients, and each severity class of the patients were also found to be distinguishable.

To deliver real time information of degranulation at a single cell level, we studied degranulation of mast cells dispersed in TAXIScan device. Since the device can gently hold the cells in the channel between glass plate and silicon substrate and since a reproducible concentration gradient of soluble stimulus can be formed in the channel, it is ideal to trace the degranulation process of mast cells by time-lapse image analysis. This method provides a powerful means for real time analysis of concentration- and stimulus-dependent degranulation of mast cells and allows comparison of cell responses under different conditions.

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P30. MGL2^{+} dermal dendritic cells are sufficient to initiate contact hypersensitivity *in vivo*

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Dendritic cells (DCs) are the most potent antigen-presenting cells in the mammalian immune system. In the skin, epidermal Langerhans cells (LCs) and dermal dendritic cells (DDCs) survey for invasive pathogens and present antigens to T cells after migration to the cutaneous lymph nodes (LNs). So far, functional and phenotypic differences between these two DC subsets remain unclear due to lack of markers to identify DDCs.

In the present report, we demonstrated that macrophage galactose-type C-type lectin (MGL) 2 was exclusively expressed in the DDC subset in the skin-to-LN immune system. In the skin, MGL2 was expressed on the majority (about 88%) of MHCII^{+}CD11c^{+} cells in the dermis. In the cutaneous LN, MGL2 expression was restricted to B220^{-}CD8α^{lo}CD11b^{+}CD11c^{+}MHCII^{hi} tissue-derived DC. MGL2^{+}DDC migrated from the dermis into the draining LNs within 24 h after skin sensitization with FITC. Distinct from LCs, MGL2^{+}DDCs localized near the high endothelial venules in the outer T cell cortex. In FITC-induced contact hypersensitivity (CHS), adoptive transfer of FITC^{+}MGL2^{+}DDCs, but not FITC^{+}MGL2^{-}DCs into naive mice resulted in the induction of FITC-specific ear swelling, indicating that DDCs played a key role in initiation of immune responses in the skin.

These results demonstrated the availability of MGL2 as a novel marker for DDCs and suggested the contribution of MGL2^{+}DDCs for initiating CHS.

References: Kumamoto Y, Denda-Nagai K, Aida S, Higashi N, Irimura T. MGL2^{+} dermal dendritic cells are sufficient to initiate contact hypersensitivity *in vivo*. *PLoS ONE*, 4(5): e561, 2009.