18th International Symposium on Molecular Cell Biology of Macrophages 2010

Date: May 20 (Thu) to May 21 (Fri), 2010
Venue: Kumamoto Parea Hall, Kumamoto, Japan

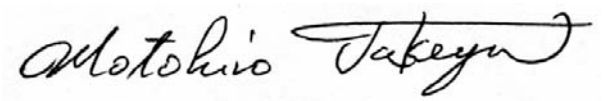
Welcome to Kumamoto

It is my great pleasure to invite you to join the 18th International Symposium on Molecular Cell Biology of Macrophages 2010. This Symposium is sponsored by The Japanese Society for Molecular Cell Biology of Macrophages which was founded in 1991 by Professor Kouji Matsushima with its aim to promote the basic science as well as the clinical research in the field of macrophage/dendritic cell research. The symposium has been held annually and made a large contribution to the world-wide advance of this research field.

This year's symposium will last two full days including lectures by 18 invited speakers from United States, The Netherlands, Italy, Korea and Japan, and poster presentations by society members. The main theme of the Symposium is "Macrophage Activation and Disease Process". Special emphasis will be given to the following four topics: "Regulation of Inflammation ", "Innate Immune Recognition", "Macrophage-related molecules and Diseases", and "Tumor Microenvironment". It is our intention to enable all participants from abroad and Japan to create international and interdisciplinary interactions as well as friendship.

Kumamoto City is located in the center of Kyushu Island and is the prefectural capital of Kumamoto Prefecture. It has a long history and is known a "City of Forests" since it is blessed with an abundance of nature. When seeing the sights of Kumamoto, the historical 400-year old Kumamoto Castle and Kumamoto's classic Japanese style garden known as Suizenji Park will surely fascinate you. It is my hope and belief that you will enjoy the symposium and have a wonderful stay in Kumamoto!

May 20, 2010



Motohiro Takeya
Conference Chairperson

Conference Chairperson:
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Yoshiro Kobayashi
(Toho University)

Kensuke Miyake
(The University of Tokyo)

Shigeo Koyasu
(Keio University)

Makoto Naito
(Niigata University)

Kenjiro Matsuno
(Dokkyo Medical University)

Naofumi Mukaida
(Kanazawa University)

General Information

Date: May 20 (Thu) to May 21 (Fri), 2010
Venue: Kumamoto Parea 9F, 10F　(テトリアくまもとビル 9 階・10 階)
Official Language: English
Home Page: http://secand.co.jp/macrophages/index.html

Get-Together Party (Fee JPY 2,000)
Date and Time: May 20 (Thu)　18:30-20:30
Place: Restaurant "SAI", City Hall 14F (See Access Map)
（カフェレストラン「彩」熊本市役所 14 階 地図参照）

Registration
Place: In front of Parea Hall (10F)
Opening Hour: May 20: 9:00 - 17:00, May 21: 9:00 - 16:40

Registration fee

Member (Before Apr 30)	JPY	5,000
Member (After May 1 or On site)	JPY	7,000
Non-member	JPY	10,000
Student	JPY	3,000

来年度の開催予告

第 19 回マクロファージ分子細胞生物学国際シンポジウム

（第 76 回日本インターフェロン・サイトカイン学会学術集会との合同開催）

日時：　２０１１年５月２５日（水）、２６日（木）、２７日（金）
会場：　全日空ゲートタワーホテル大阪　（大阪府泉佐野市）
〒598-8511　大阪府泉佐野市りんくう往来北 1 番地
TEL：072-460-1111　　Fax：072-460-1177
当番幹事（学術集会長）：　近畿大学医学部細菌学　　義江　修

Instructions for Speakers

Oral Session (Invited speakers)

An LCD projector will be provided. Please bring your Power Point file on a Windows readable USB memory stick or CD-ROM. Macintosh users or speakers using movie files should bring your own computers. In order to avoid technical problems, we ask you to kindly bring your Power Point Presentation to the PC center (10F) at least 30 min prior to the session.

Short oral presentation for Young Investigator Award

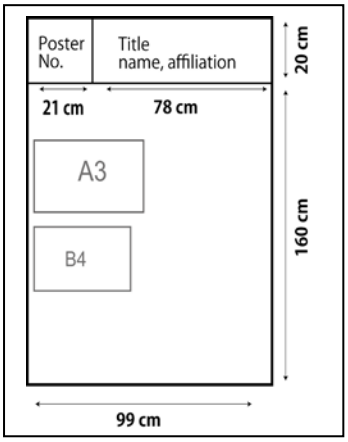
Young Investigator Award was established to encourage young investigators who have made significant contributions to this symposium. The committee will select two awardees from the first authors of the posters, who are graduated students or were awarded PhD degree within five years. The person eligible for this award will send the PDF (Power Point) file summarizing his/her paper in two pages to the Conference Chairperson by e-mail (cellpath@kumamoto-u.ac.jp) by May 14 and will present his/her paper with this slide in two minute in Session 3 in the evening of May 20. The awardees are kindly asked to make a short oral presentation of their accomplishment shown in their posters (10 min talk). 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 Room 1 (9F). (９階 第１会議室)

Poster Set Up	May 20 (Thu)	9:00-12:00
Poster Session	May 20 (Thu)	17:10-18:10
Poster Removal	May 21 (Fri)	16:40-17:00

The size of the poster board is 99-cm wide and 160-cm high. We will provide the poster numbers only. Please prepare the title (including names and affiliations of authors) of the poster by yourself. Mounting media will be available in the room.



Acknowledgments

This symposium is partly supprted by a Good Practice (GP) program “Advanced Medical Education Program” and a Kumamoto University Core Research Project B “Center for Frontier Research on Life Style and Stress Signal”.
The organizers sincerely appreciate the generous support and participation for 18th International Symposium on Molecular Cell Biology of Macrophages 2010 by the following foundations and groups.

第 18 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 組織的な大学院教育改革推進プログラム「臨床・基礎・社会医学一体型先端教育の実践」ならびに熊本大学拠点形成研究 B「ライフスタイルとストレスシグナルの先端研究拠点」の補助を受けています。

また、本シンポジウムの開催に対し、以下の財団および団体からご援助、ご寄付を戴いております。ここに厚く御礼申し上げます。

SPONSORS AND CONTRIBUTORS (2010 年 4 月 12 日現在)

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Kumamoto International Convention and Tourism Bureau
熊本大学細胞病理学分野同門会
Almuni Association of Department of Cell Pathology, Kumamoto University

Access Map


Kumamoto City
熊本博物館
家庭裁判所
教育センター
県伝統工芸館
県立美術館
熊本城公園
熊本城
国立病院機構熊本医療センター
合同庁舎
市役所花畑別館
坪井川
日本郵政グループ熊本ビル
通町筋
現代美術館
熊本市役所
市役所前
国際交流会館
崇城大学市民ホール［市民会館］
交通センターくまもと阪神
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藤崎八旛宮
熊本電鉄藤崎宮前
鶴屋
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中央郵便局
西辛島町
辛島公園地下駐車場
辛島町
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至上熊本駅
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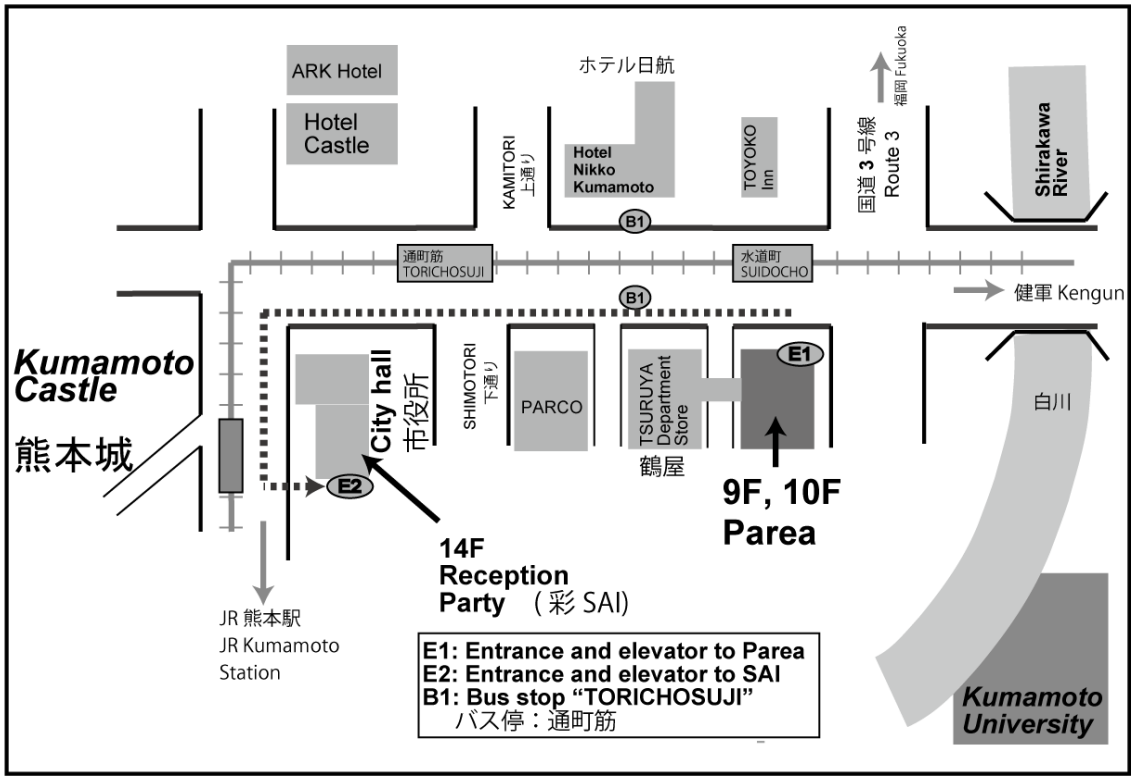
慶徳校前
河原町
至鹿児島
代継橋
銀座橋
新代継橋
熊大医学部付属病院


ARK Hotel
Hotel Castle
ホテル日航
Hotel Nikko Kumamoto
TOYOKO Inn
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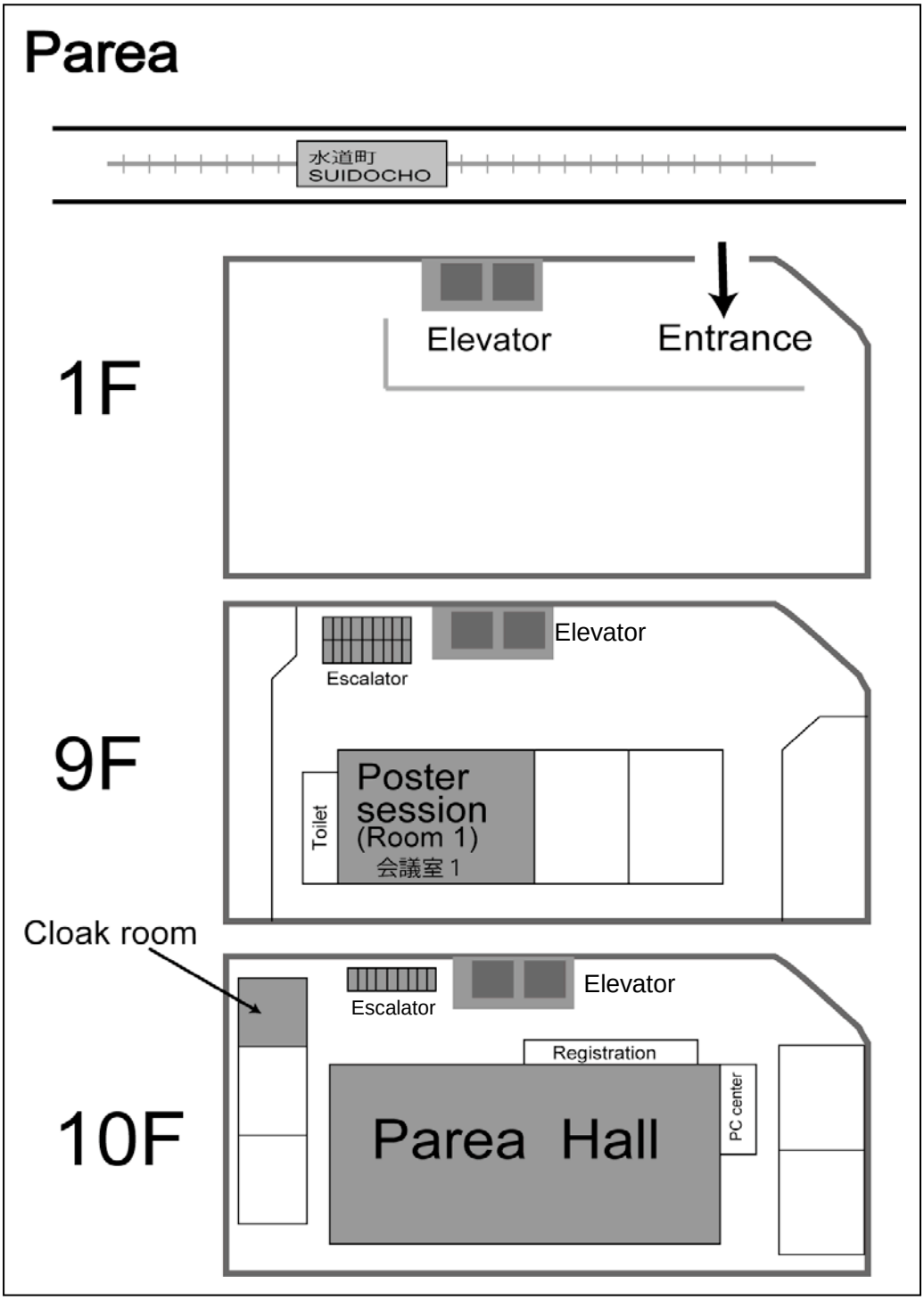
福岡 Fukuoka
Shirakawa River
B1
通町筋 TORICHOSUJI
水道町 SUIDOCHO
健軍 Kengun
Kumamoto Castle
熊本城
City hall 市役所
SHIMOTORI 下通り
PARCO
TSURUYA Department Store
鶴屋
E1
E2
9F, 10F Parea
14F Reception Party （彩 SAI）
白川
JR 熊本駅
JR Kumamoto Station
E1: Entrance and elevator to Parea
E2: Entrance and elevator to SAI
B1: Bus stop “TORICHOSUJI”
バス停：通町筋
Kumamoto University

Kumamoto Parea
（熊本県民交流館パレア）



Program

May 20 (Thursday)

9:00- Registration

9:25-9:30 Opening Address (Motohiro Takeya)

Session 1: Regulation of Inflammation
Chairpersons: Osamu Yoshie (Kinki University)
Yoshiro Kobayashi (Toho University)

9:30-10:00 Evaluating the role of macrophage as a source of the chemokine MCP-1/CCL2 during the inflammatory responses.
Teizo Yoshimura (National Cancer Institute Frederick, USA)

10:00-10:40 Regulation of M-CSF receptor expression by mononuclear phagocytes in inflammation.
Pieter JM Leenen (Erasmus Medical Center, Netherland)

10:40-10:55 Coffee Break

10:55-11:25 Spred-2, a negative regulator of MAP kinase cascade, controls inflammatory responses.
Akihiro Matsukawa (Okayama University, Japan)

11:25-12:05 Role of macrophages and T lymphocytes in inflammatory lymphangiogenesis.
Gou Young Koh (Advanced Institute of Science and Technology, KAIST, Korea)

12:05-13:20 Lunch and Poster Viewing

Session2: Innate Immune Recognition
Chairpersons: Tatsuro Irimura (The University of Tokyo)
Norimitsu Kadowaki (Kyoto University)

13:20-13:50 Innate inflammation by Th2 cytokines derived from “Natural Helper” cell.
Shigeo Koyasu (Keio University School of Medicine, Japan)

13:50-14:15 Distribution and immunological implications of MGL2.
Kaori Denda-Nagai (Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan)

14:15-14:40 Hierarchical nucleic acid sensing system for innate immune responses.
Hideyuki Yanai (Graduate School of Medicine, The University of Tokyo, Japan)

14:40-15:20 Redox remodeling controls innate immunity.
Anna Rubartelli (Istituto Nazionale per la Ricerca sul Cancro, Italy)

15:20-15:35 Coffee Break

15:35-16:15 Self and non-self recognition through C-type lectin receptors.
Sho Yamasaki (Research Center for Infection Network, Kyushu University)

16:20-16:50 Session 3: Short poster presentation
Chairperson: Naomi Sakashita (Kumamoto University)

16:50-17:10 **Business Meeting**

17:10-18:10 **Poster Viewing and Discussion**

18:30-20:30 **Get-Together Party**

May 21, Friday

9:00- Registration

Session 4: Macrophage-related molecules and Diseases
Chairpersons: Kenjiro Matsuno (Dokkyo University)
Akihiro Matsukawa (Okayama University)

9:20- 9:50 Different activity and downstream signaling of IL-34 and M-CSF, which share the receptor Fms.
Shinya Suzu (Center for AIDS Research, Kumamoto University)

9:50-10:20 Galectin-9 beneficially modulates macrophage functions in inflammation and cancer.
Mitsuomi Hirashima (Faculty of Medicine, Kagawa University)

10:20-10:35 Coffee Break

10:35-11:15 The ACAT1 enzyme and its pathophysiological roles in atherosclerosis and Alzheimer disease.
Ta-Yuan Chang (Dartmouth Medical School, USA)

11:15-11:45 Roles of Angiopoietin-like protein2 in inflammation and its-related diseases.
Yuichi Oike (Graduate School of Medical Sciences, Kumamoto University)

11:45-12:15 Session 5: Young Investigator Award Presentation
Chairpersons: Kouji Matsushima (The University of Tokyo)
Motohiro Takeya (Kumamoto University)

12:15-13:30 Lunch and Poster Viewing

Session 6: Tumor Microenvironments
Chairpersons: Teizo Yoshimura (National Cancer Institute Frederick, USA)
Motohiro Takeya (Kumamoto University)

13:30-13:55 Significance of M2 macrophage infiltration in malignant tumors.
Yoshihiro Komohara (Kumamoto University)

13:55-14:20 Dynamics of CCR7+ tumor-infiltrating dendritic cells that regulate anti-tumor immune responses.
Satoshi Ueha (Graduate School of Medicine, The University of Tokyo)

14:20-15:00 S100 proteins and the tumor microenvironment.
Geetha Srikrishna (The Burnham Institute for Medical Research, USA)

15:00-15:15 Coffee Break

15:15-15:55 Macrophage polarization in tumour development.
Antonio Sica (Istituto Clinico Humanitas, Italy)

15:55-16:35 Macrophage Diversity Promotes Tumor Progression and Metastasis.
Jeffrey W Pollard (Albert Einstein College of Medicine, USA)

16:35- Closing Remarks Kouji Matsushima (The University of Tokyo)

Poster Session

(*Candidates for Young Investigator Award)

P1*

Suppression of TLR4-mediated inflammatory response by macrophage class A scavenger receptor (CD204).

Koji Ohnishi[1,2], Yoshihiro Komohara[1], Yukio Fujiwara[1], Kenichi Takemura[1], XiaoFeng Lei[1,3], Naomi Sakashita[1], and Motohiro Takeya[1]

[1]Department of Cell Pathology, Graduate School of Medical Sciences, Faculty of Life Sciences, Kumamoto University; [2]Department of Surgical Pathology, Kumamoto University Hospital, Kumamoto; [3]Department of Biochemistry, Showa University School of Medicine, Tokyo, Japan

P2

Apoptosis of macrophages by N-Arachidonyl Glycine is mediated by GPR18.

Rina Takenouchi, Kazuhiko Inoue, Atsuro Miyata

Department of Pharmacology, Graduate School of Medical and Dental Sciences, Kagoshima University

P3

EP2 and EP4 receptors on muscularis resident macrophages mediate LPS-induced intestinal dysmotility via iNOS upregulation through cAMP/ERK signals.

Masatoshi Hori[1], Tsuyoshi Tajima[1,2], Takahisa Murata[1], Kosuke Aritake[3], Yoshihiro Urade[3],

Toshiyuki Matsuoka[4], Syu Narumiya[4], and Hiroshi Ozaki[1]

[1]Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo, Japan, [2]Laboratory of Veterinary Pharmacology, Nippon Veterinary and Life Science University, Tokyo, Japan, [3]Department of Molecular Behavioral Biology, Osaka Bioscience Institute, Osaka, Japan, [4]Department of Pharmacology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

P4

Overexpression of Wiskott-Aldrich syndrome protein (WASP) N-terminal domain inhibits inflammatory responses in LPS-activated macrophages.

Chisato Sakuma[1, 2], Mitsuru Sato[1], Joe Chiba[2], Hiroshi Kitani[1]

[1]Transgenic Animal Research Center, National Institute of Agrobiological Science, [2]Department of Biological Science and Technology, Graduate school of Faculty of Industrial Science and Technology, Tokyo University of Science

P5

Silica and alum induce type 2 immunity via inflammasome-independent mechanisms.

Etsushi Kuroda[1], Ken J Ishii[2,3] and Yasuo Morimoto[4]
[1]Department of Immunology and Parasitology and [4]Department of Occupational Pneumology, University of Occupational and Environmental Health, Japan, [2]Department of Molecular Protozoology, Research Institute for Microbial Diseases, Osaka University, Japan, [3]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, Japan

P6

Mint3 enhances the activity of HIF-1 in macrophages by suppressing the activity of FIH-1.

Takeharu Sakamoto and Motoharu Seiki
Department of Cancer Cell Research, Institute of Medical Science, The University of Tokyo

P7*

Spermine involved methylation status on ITGAL promoter possibly regulates the expression of LFA-1.

Yoshihiko Kano, Kuniyasu Soda, and Fumio Konishi
Department of Surgery, Saitama Medical Center, Jichi Medical University

P8

Tim-3 mediates phagocytosis of apoptotic cells by inflammatory macrophage and CD8$^+$ dendritic cell subsets.

Kazuyoshi Takeda,[1] Masafumi Nakayama,[1,2] Hisaya Akiba,[1] Yuko Kojima,[3] Hideo Yagita,[1] Koetsu Ogasawara,[2] and Ko Okumura[1]
[1]Department of Immunology and [3]Division of Biomedical Imaging Research, Biomedical Research Center, Juntendo University School of Medicine, [3]Department of Immunobiology, Institute of Development, Aging and Cancer, Tohoku University

P9

M-CSF-dependent red pulp macrophages regulate CD4 T cell responses.

Daisuke Kurotaki[1,2], Kyeonghwa Bae[2], Toshimitsu Uede[1,2], and Junko Morimoto[2]
Division of [1]Matrix Medicine and [2]Molecular Immunology, Institute for Genetic Medicine, Hokkaido University

P10

Rheumatoid arthritis patient-derived synoviocytes are more sensitive to cigarette smoke condensate extracts-induced IL-1 beta expression through NF-kB activation than OA patient-derived synoviocytes, human lung fibroblasts and human lung epithelial cells.

K. Onozaki[1], S. Okamoto[1], M. Yokoyama[1], T. Arakawa[1], M. Adachi[1], K. Yamada[1], K. Akita[1], S. Itoh[1], T. Takii[1], Y. Waguri-Nagaya[2], T. Otsuka[2] and K. Hayakawa[3]
[1]Graduate School of Pharmaceutical Sciences, [2]Graduate School of Medical Sciences, Nagoya City University, [3]Kanazawa University

P11

Absence of IFN-γ accelerates thrombus resolution through enhanced MMP-9 and VEGF expression.

Mizuho Nosaka[1], Yuko Ishida[1], Akihiko Kimura[1], Yumi Kuninaka[1], Naofumi Mukaida[2], and Toshikazu Kondo[1]

[1]Department of Forensic Medicine, Wakayama Medical University, Wakayama, Japan, [2]Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, Kanazawa, Japan

P12

CCR7-independent trafficking of skin antigens to regional lymph nodes by cells in the dermis.

Miya Yoshino, Kazuki Okuyama, Akihiko Murata, Yuki Egawa, Atsuko Sawada, Shin-Ichi Hayashi

Division of Immunology, Department of Molecular and Cellular Biology, School of Life Science, Faculty of Medicine, Tottori University

P13

A proteasome inhibitor bortezomib suppresses immunostimulatory activity of human plasmacytoid dendritic cells by targeting intracellular trafficking of nucleic acid-sensing Toll-like receptors and endoplasmic reticulum homeostasis.

Makiko Hirai,[1] Norimitsu Kadowaki,[2] Toshio Kitawaki,[2] Haruyuki Fujita,[2] Akifumi Takaori-Kondo,[2] Ryutaro Fukui,[3] Kensuke Miyake,[3] Takahiro Maeda,[4] Shimeru Kamihira,[5] Yoshiki Miyachi,[1] Takashi Uchiyama [2, 6]

[1]Department of Dermatology and [2]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [3]Division of Infectious Genetics, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [4]Department of Island and Community Medicine, and [5]Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [6]Kitano Hospital, The Tazuke Kofukai Medical Research Institute, Osaka, Japan

P14

Z39Ig is a novel cell surface marker of macrophages in murine large intestine.

Masashi Tanaka, Taku Nagai, Kazuhisa Hasui, Takami Matsuyama

Department of Immunology, Graduate School of Medical and Dental Sciences, Kagoshima University

P15*

High-mobility Group Box-1 Protein Promotes Granulomatous Nephritis in Adenine-induced nephropathy.

Yoko Oyama[1], Teruto Hashiguchi[1], Noboru Taniguchi[2], Salunya Tancharoen[3], Tomonori Uchimura[1], Kamal K. Biswas[1], Ko-ichi Kawahara[1], Takao Nitanda[4], Yoshihisa Umekita[5], Martin Lotz[2] and Ikuro Maruyama[1]

[1]Department of Laboratory and Vascular Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan, [2]Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California, [3]Pharmacology Department, Faculty of Dentistry, Mahidol University, Bangkok, Thailand. [4]Division of Surgical Pathology, Kagoshima University Hospital, Kagoshima, Japan. [5]Department of Tumor Pathology, Field of Oncology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan.

P16

Class A scavenger receptor promotes osteoclast differentiation via the enhanced expression of receptor activator of NF-κB (RANK).

Kenichi Takemura, Naomi Sakashita, Yukio Fujiwara, Yoshihiro Komohara, XiaoFeng Lei, Koji Ohnishi, Motohiro Takeya

Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University

P17

The defensive role of interferon-γ produced by myeloid cells in 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 I, National Institute of Infectious Diseases, Tokyo, Japan

P18

Targeting of folate receptor β–expressing macrophages in bleomycin induced pulmonary fibrosis.

Taku Nagai, Masashi Tanaka, Kazuhisa Hasui, Takami Matsuyama

Department of Immunology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

P19

What are effector cells in the liver transplant rejection ?

Hisashi Ueta, Xue-dong Xue, Bin Yu, Yasushi Sawanobori, Yusuke Kitazawa, Kenjiro Matsuno

Department of Anatomy, Dokkyo Medical University

P20*

Translocation of ACAT1 from ER to Late Endosomal Associated Membranes in Cholesterol-rich Human Macrophage.

XiaoFeng Lei[1,2], Naomi Sakashita[2], Yukio Fujiwara[2], Catherine CY Chang[3], Ta-Yuan Chang[3], Motohiro Takey[2], Akira Miyazaki[1]

[1] Department of Biochemistry, Showa University School of Medicine, Tokyo, Japan [2] Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan, [3] Department of Biochemistry, Dartmouth Medical School, Hanover, NH, USA

P21*

Macrophage and neutrophils have different response to hypoxia in expression of long pentraxin 3 (PTX3) in human atherosclerosis.

Alexander S. Savchenko[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, Japan; [2]Department of Cardiology, Juntendo University Nerima Hospital, Tokyo, Japan, [3]Perseus Proteomics Inc., Tokyo, Japan

P22

A symbiotic growth of nasal NK/T-cell lymphoma cells with CD204-positive macrophages would defense themselves from endogenous reactive oxygen spices-induced cell necrosis.

Kazuhisa Hasui[1], Xinshan Jia[2], Motohiro Takeya[3], Takuro Kanekura[1], Yoshifumi Kawano[1], Shuji Izumo[1], Yoshito Eizuru[1], Takami Matsuyama[1]

[1]Kagoshima University Graduate School of Medical and Dental Sciences,[2]China Medical University, and [3]Kumamoto University Graduate School of Medical Sciences

P23*

Involvement of M2 macrophages in the ascites of advanced epithelial ovarian cancer in tumor progression via Stat3 activation.

Kiyomi Takaishi[1, 2], Yoshihiro Komohara[1], Hironori Tashiro[2], Hidetaka Katabuchi[2], and Motohiro Takeya[1]

[1]Department of Cell Pathology and [2]Department of Gynecology, Graduate School of Medical Sciences, Kumamoto University

P24*

Infiltration of macrophages plays a key role in tumor angiogenesis and progression.

Kosuke Watari, Yuji Basaki, Michihiko Kuwano, Mayumi Ono

Department of Pharmaceutical Oncology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

Evaluating the role of macrophages as a source of the chemokine MCP-1/CCL2 during the inflammatory responses

Teizo Yoshimura
Laboratory of Molecular Immunoregulation, Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD 21702

MCP-1 is a chemokine regulating the recruitment of monocytes into sites of inflammation and cancer. MCP-1 can be produced by a variety of cell types, including macrophages, neutrophils, fibroblasts, endothelial cells and epithelial cells. Among them, macrophages have been shown to produce a high level of MCP-1 in vitro in response to proinflammatory stimuli, leading us to hypothesize that this cell type is a major source of MCP-1 produced in vivo during the inflammatory responses. To test the hypothesis, we constructed myeloid cell (neutrophil and macrophage)-specific conditional MCP-1-deficient mice using the Cre/loxP system and evaluated the role of these cells in MCP-1 production in a TG-induced peritonitis model. Contrary to the hypothesis, we did not detect a significant reduction in MCP-1 concentration in the peritoneal fluids of the conditional MCP-1-deficient mice. Furthermore, adoptive transfer of resident peritoneal cells of wild-type mice into the peritoneal cavities of systemic MCP-1-deficient mice did not alter MCP-1 concentration in the peritoneal fluids after TG injection. Similar results were obtained in a zymosan-induced peritonitis model. Taken together, our results indicated that non-myeloid cells, such as mesothelial cells, in the peritoneal cavity are the major source of MCP-1 in this model and that non-myeloid cells play a larger role in the development of inflammatory responses than we originally expected. We are currently using other disease models to further evaluate the role of macrophages as a source of MCP-1.

Regulation of M-CSF receptor expression by mononuclear phagocytes in inflammation

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Macrophages and dendritic cells (DC), together comprising the mononuclear phagocyte lineage, share precursor cells in the bone marrow (BM) that can be stimulated by different growth factors. M-CSF is a key factor for mononuclear phagocyte development, recognized by the M-CSF receptor (M-CSFR, CD115) encoded by the *c-fms* gene. In this presentation, I will elaborate two examples of M-CSFR regulation in mouse mononuclear phagocytes in inflammation. The first concerns the development of phenotypically distinct monocytes during infection with the facultative intracellular bacterium *Listeria monocytogenes*. Secondly, I will discuss the involvement of microRNAs regulating M-CSFR expression in inflammatory DC maturation.
Expression of the M-CSFR is thought to be a hallmark of mononuclear phagocytes, including early stages in the BM. However, we found that M-CSFR expression is strongly reduced at both mRNA and protein level in monocytes, identified as Ly-6Chi CD11b+ Ly-6G– cells, developing in BM during severe *Listeria* infection. This reduction in M-CSFR expression showed a direct correlation with the number of bacteria used for infection. Moreover, these infection-induced monocytes showed a flow cytometric scatter profile that appeared to be much more neutrophil-like compared to that of steady state monocytes.
Besides being effector cells in host defense, monocytes are also precursors of inflammatory DC. This development can be mimicked *in vitro* by GM-CSF stimulation of BM cells leading to generation of immature and mature DC that can be distinguished by immunophenotype. M-CSFR appears to be expressed at high levels by DC precursors and immature DC, but is strongly down-regulated in the final stage of DC maturation. In general, protein expression can be regulated at the transcriptional level, but also post-transcriptionally by microRNAs. To determine whether microRNAs, which are small RNAs that regulate mRNA stability and translation, are involved in M-CSFR down-regulation, we assessed microRNA profiles of GM-CSF-stimulated DC in different stages of development. Subsequently, we matched those profiles with

sequence data of the 3'UTR of M-CSFR mRNA, predicting putative regulatory microRNAs. Remarkably, all three predicted microRNAs, namely miR-155, -34a and -22, appeared to be up-regulated in mature DC compared to immature DC. Blockade of these microRNAs significantly inhibited down-regulation of M-CSFR during LPS-stimulated maturation of DC, thus showing their functional involvement with M-CSFR regulation. Moreover, other markers of DC maturation were blocked as well by such microRNA inhibition, suggesting that M-CSFR down-regulation is required for final DC maturation. This was confirmed by the finding that enforced over-expression of M-CSFR in developing DC significantly inhibited their final maturation. Therefore, these data indicate that microRNAs cause M-CSFR down-regulation in the final stage of inflammatory DC maturation and that this enables the development of mature, immune-stimulating DC.

Taken together, our findings indicate that expression of the M-CSFR is strongly down-regulated in monocytes that are newly generated during *Listeria* infection. M-CSFR expression is also down-regulated in the final maturation of inflammatory DC, a process that is mediated via microRNAs. Interestingly, M-CSFR down-regulation appears to be a prerequisite for final DC maturation.

Spred-2, a negative regulator of MAP kinase cascade, controls inflammatory responses

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Cytokines have been implicated in the progression of T cell-mediated liver injury, in which mitogen-activated protein (MAP) kinase cascades may play a role. In this study, we attempted to explore the role of Spred-2, a member of the Sprouty family proteins that negatively regulate the MAP kinase cascade, in a murine model of fulminant hepatitis induced by acetaminophen (APAP) and concanavalin A (ConA). In APAP-hepatitis model, liver injury in Spred-2KO mice were much severer than that in the WT mice, as evidenced by increased serum levels of ALT, exacerbated hepatic injured area and reduced mice survival relative to the WT mice. TUNEL-positive hepatocytes were augmented in the liver from Spred-2KO mice. Activities of caspase-3, -8, and -9 were significantly elevated in Spred-2KO liver. FACS analyses demonstrated that the numbers of hepatic NK cells and neutrophils in Spred-2KO liver were significantly higher than those in the WT control. In addition, elevated levels of cytokines and chemokines were found in Spred-2KO mice relative to the WT mice. In ConA-hepatitis model, the liver injury in Spred-2KO mice was exacerbated relative to the WT mice, an event that was accompanied by increased serum levels of ALT and augmented caspase-3, -8, and -9 activities. Spred-2KO mice demonstrated higher levels of circulating cytokines such as IFNg and IL-17 compared to the WT mice. The numbers of hepatic CD4+T cells in Spred-2KO liver were significantly higher than those in the WT control. Altogether, these results provide clear evidence that Spred-2 protects the liver from different types of hepatitis model. Spred-2 can be a therapeutic target in the treatment of liver injury.

Role of macrophages and T lymphocytes in inflammatory lymphangiogenesis

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In adult mammals, new lymphatic vessels are formed mainly through lymphangiogenesis, which is a sequence of processes that include sprouting, migration, proliferation, and tubule formation by pre-existing lymphatic endothelial cells (LEC). In several types of disease models, we found that inflammation is one of the strongest stimuli for lymphangiogenesis in adult. Among several immune cells, macrophages play a critical role in the inflammation-induced lymphangiogenesis through secretions of lymphangiogenic growth factors VEGF-C, -D and –A. The macrophage-mediated lymphangiogenesis contributes to pathogen clearance and inflammation resolution in local inflamed tissue and draining lymph node. We also found that Toll-like receptor-4 is abundantly expressed in LEC and it plays an essential role in LPS-induced inflammatory lymphangiogenesis by chemotactic recruitment of macrophages. In contrast, interferon-gamma secreted from T-lymphocyte is a responsible molecule for reduction of pre-existing lymphatic vessels and for attenuation of inflammation-induced lymphangiogenesis through reduction of Prox-1 expression and activity in LEC. Thus, macrophages and T-lymphocytes may finely regulate homeostasis of lymphatic vessels and lymphangiogenesis by secreting lymphangiogenic and lymphangiostatic factors during activation and resolution of inflammation

Innate inflammation by Th2 cytokines derived from “Natural Helper” cell

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Inflammatory responses are involved in various phases of biological processes. Those include tissue development, defense mechanisms against infection, and pathophysiological processes such as autoimmunity and tumorigenesis. In this symposium, the role of a newly identified lymphocyte population in innate inflammatory responses through the production of Th2 cytokines will be discussed. Innate immune responses are important in combating various microbes during the early phases of infection. Natural killer (NK) cells are innate lymphocytes that, unlike T and B lymphocytes, do not express antigen receptors but rapidly exhibit cytotoxic activities against virus infected cells and produce various cytokines. We recently discovered a new type of innate lymphocyte present in a novel lymphoid structure associated with adipose tissues in the peritoneal cavity. These cells do not express lineage (Lin: CD3, CD4, CD8α, TCRβ, TCRδ, CD5, CD19, B220, NK1.1, TER119, Gr-1, Mac-1, CD11c, FceRIα) markers but express c-Kit, Sca-1, IL-7R and IL-33R. Similar lymphoid clusters were found in both human and mouse mesentery and we term this tissue “FALC” for fat-associated lymphoid cluster. FALC $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$ cells are distinct from lymphoid progenitors and lymphoid tissue inducer (LTi) cells. These cells proliferate in response to IL-2 and produce large amounts of Th2 cytokines such as IL-5, IL-6 and IL-13. IL-5 and IL-6 regulate B cell antibody production and self-renewal of B1 cells. Indeed, FALC $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$ cells support the self-renewal of B1 cells and enhance IgA production. IL-5 and IL-13 mediate allergic inflammation and protection against helminth infection. Upon helminth infection and in response to IL-33, FALC $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$ cells produce large amounts of IL-13, which leads to goblet cell hyperplasia, a critical step for helminth expulsion. In mice devoid of FALC $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$ cells such goblet cell hyperplasia was not induced. Thus, FALC $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$ cells are Th2-type innate lymphocytes playing an important role in the early phase of helminth infection and we propose that these cells be called “natural helper cells”.

Distribution and immunological implications of MGL2

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It is estimated that more than half of all proteins produced by eukaryotes are glycosylated. Most, though not all, cell surface and secreted proteins are thought to be glycosylated. Therefore, the immune responses to self and foreign proteins are likely to be modulated through the functions of carbohydrate-recognition molecules (i.e., lectins) expressed on antigen presenting cells. Surface lectins on antigen presenting cells are potentially involved in the regulation of immune response as endocytic receptors, signaling receptors, or regulators of cellular localization and trafficking. The macrophage galactose-type C-type lectin (MGL/CD301) is a sole galactose-type C-type lectin expressed on cells in the immune system. Though mice have two MGL genes *Mgl1* and *Mgl2*, their distinct roles have not been previously explored. The aim of our recent studies is to elucidate the tissue distribution of these lectins and the identification of the cells expressing these lectins, and to establish their immunological roles under homeostatic and pathological conditions.
The cellular distribution of MGL1 and MGL2 was examined by immunohistochemistry and flow cytometry. MGL2-expressing cells were shown to be a portion, mostly conventional dendritic cells (cDCs), of MGL1-expressing cells, which were macrophages, cDCs, and plasmacytoid DCs. In the skin-to-LN immune system, MGL2 expression was shown to be limited to the MGL1-expressing dermal DCs (DDCs). Contributions of these cells in contact hypersensitivity were investigated. $MGL2^+$ DDCs 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 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 $MGL2^+$DDCs played a key role in the initiation of immune responses. To assess the roles of MGL2 in uptake and presentation of glycosylated antigens, the binding and internalization of α-GalNAc or β-*N*-acetylglucosamine (GlcNAc) conjugated to soluble polyacrylamide carriers labeled with FITC (GalNAc- or GlcNAc-polymers) were examined by flow cytometry using DCs generated from BM cells of *Mgl2* knockout (KO) and their wild-type (WT) littermate mice. The binding and internalization for GalNAc-polymers were correlated with cell surface expression of MGL2. Streptavidin (SAv)-primed $CD4^+$ T cells, but

not $CD8^+$ T cells, proliferated more efficiently when they were co-cultured with WT BM-DCs pretreated with a complex of biotinylated GalNAc-polymers and SAv (GalNAc-SAv) than with BM-DCs pretreated with GlcNAc-SAv or SAv alone. The enhancement of Ag presentation by GalNAc-polymers was not observed with *Mgl2* KO BM-DCs. These results indicated that MGL2 is involved in the uptake and presentation of mucin-like antigens with terminal GalNAc residues to $CD4^+$ cells.
We demonstrated that MGL2 was preferentially expressed on cDCs and involved in the efficient uptake and presentation of antigens with GalNAc residues. Targeting of MGL by the addition of GalNAc residues to antigens should contribute to the development of a novel vaccine.

The hierarchical nucleic acid sensing system for innate immune responses

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The activation of innate immune responses by nucleic acids is crucial to protective and pathological immunities and is mediated by the transmembrane Toll-like receptors (TLRs) and cytosolic receptors. In mammals, TLR3, TLR7 and TLR9 respectively recognize double-stranded RNA, single-stranded and short double-stranded RNAs, and hypomethylated DNA, while the RIG-I-like receptors (RLRs), namely retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene-5 (MDA5), are best known as RNA-sensing receptors in the cytosol. In addition, cytosolic DNA-sensing receptors which include DNA-dependent activator of IRFs (DAI), absent in melanoma 2 (AIM2), and others also trigger the innate and adaptive immune systems. Recently, it has been shown that RLRs also participate to the cytosolic DNA-sensing system. The hallmark of innate immune responses activated by these receptors is the induction of type-I IFNs, proinflammatory cytokines and chemokines, except that by AIM2 which is a critical component of the inflammasome that typically promotes the secretion of interleukin-1b (IL-1b). However, it remains unknown whether a single mechanism integrates these nucleic acid-sensing systems. Here we show that high-mobility group box (HMGB) proteins 1, 2 and 3 function as universal sentinels for nucleic acids. Firstly, HMGBs bind to all immunogenic nucleic acids tested. *Hmgb1*$^{-/-}$ and *Hmgb2*$^{-/-}$ cells are defective in type-I interferon and inflammatory cytokine induction by DNA or RNA targeted to activate the cytosolic nucleic acid-sensing receptors. Moreover, cells in which expression of all three HMGBs is suppressed exhibit a more profound defect accompanied by impaired activation of the transcription factors IRF3 and NF-kB. The absence of HMGBs also severely impairs activation of TLR3, 7 and 9 by their cognate nucleic acids, but not by non-nucleic acids. Thus, our results indicate a hierarchy in the nucleic acid-mediated activation of immune responses, wherein the selective activation of nucleic acid-sensing receptors is contingent on the more promiscuous sensing of nucleic acids by HMGBs. These findings may have implications for treatment of immunological disorders.

Redox remodeling controls innate immunity

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Inflammation is deeply entangled with redox modulation. Triggering of pathogen recognition receptors on inflammatory cells by pathogen-associated molecular pattern (PAMP) or damage-associated molecular pattern (DAMP) molecules induces ROS generation. As a consequence, activated cells arrange anti-oxidant responses aimed at restoring the redox homeostasis. Here I will discuss some recent data on the effects of the inflammation-related redox remodelling on Interleukin-1β (IL-1β□ secretion. We have observed that a biphasic redox event, including a prompt oxidative stress and a delayed antioxidant response, is required for the correct process of IL-1β□maturation and secretion inhealthy monocytes. We have also studied a large number of patients affected by Cryopyrin-associated periodic syndromes (CAPS), a group of autoinflammatory diseases where Nod-like receptor family, pyrin domain containing-3 protein (*NLRP3)* mutations lead to increased IL-1β secretion. Our data show that unstimulated CAPS monocytes are under oxidative stress, resulting in chronic upregulation of antioxidant systems. This redox imbalance causes a deranged response to PAMP stimulation, resulting in accelerated IL-1β secretion. The functional link between *NLRP3* mutations and altered redox response as well as their cooperation in accelerating IL-1β secretion in CAPS patients will be discussed.

Self and non-self recognition through C-type lectin receptors

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C-type lectin receptors comprise a family of proteins that share a common structural motif and are involved in various immune responses in myeloid cells. Macrophage-inducible C-type lectin (Mincle) is expressed mainly in macrophages and is induced after exposure to various stimuli and stresses. We found that Mincle selectively associated with the Fc receptor γ-chain (FcRγ) and activated macrophages to produce inflammatory cytokines and chemokines. Mincle-expressing cells were activated in the presence of dead cells, which was blocked by anti-Mincle mAb. SAP130, a component of small nuclear ribonucleoprotein (snRNP), was identified as a Mincle ligand that is released from dead cells. Intriguingly, Mincle also recognizes 'non-self' pathogenic fungus, *Malassezia*, and is required for inflammatory response to this fungus. In addition, we have recently found that Mincle recognizes pathogenic bacteria, *Mycobacterium tuberculosis* as well. An unique mycobacterial glycolipid, trehalose dimycolate (TDM), was identified as a Mincle ligand. Thus, Mincle may function as a multitask receptor that senses "danger" derived from damaged self (damage associated molecular patterns: DAMPs) and invading non-self (pathogen associated molecular patterns: PAMPs). The molecular basis and physiological advantages of these recognitions will be discussed.

Different activity and downstream signaling of IL-34 and M-CSF, which share the receptor Fms

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Macrophage colony-stimulating factor (M-CSF) regulates the production, survival and function of macrophages through Fms, the receptor tyrosine kinase. Recently, interleukin-34 (IL-34), which shares no sequence homology with M-CSF, was identified as an alternative Fms ligand. Here, we provide the first evidence that these ligands indeed resemble but are not necessarily identical in biological activity and signal activation. In culture systems tested, IL-34 and M-CSF showed an equivalent ability to support cell growth or survival. However, they were different in the ability to induce the production of chemokines such as MCP-1 and eotaxin-2 in primary macrophages, the morphological change in TF-1-fms cells and the migration of J774A.1 cells. Importantly, IL-34 induced a stronger but transient tyrosine-phosphorylation of Fms and downstream molecules, and rapidly down-regulated Fms. Even in the comparison of active domains, these ligands showed no sequence homology including the position of cysteines. Interestingly, an anti-Fms MAb blocked both IL-34-Fms and M-CSF-Fms binding, but another MAb blocked only M-CSF-Fms binding. Consistent with the finding, a Fms mutant (R146E-substitution) lost its binding to M-CSF but retained its binding to IL-34. These results suggested that IL-34 and M-CSF were different in their structure and Fms domains that they bound, which caused different bioactivities and signal activation kinetics/strength. Our findings indicate that macrophage phenotype and function are differentially regulated even at the level of the single receptor, Fms.

Galectin-9 beneficially modulates macrophage functions in inflammation and cancer

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Galectins are evolutionarily conserved β-galactoside-binding proteins with diverse roles in innate and adaptive immune responses. Among of them, we first identified Galectin-9 (Gal-9) as an eosinophil chemoattractant derived from activated CD4 T cells in 1998. However, our further studies revealed that Gal-9 plays a pivotal role on immune suppression, because Gal-9 induces apoptosis of terminally differentiated Th1 and Th17 cells through Gal-9/Tim-3 interactions. We further found that Gal-9 promotes innate immune response by Mϕs and DCs. Actually, Gal-9 induces maturation of human monocyte-derived DCs *in vitro*. In Meth-A-bearing mice, Gal-9 expands Tim-3+ DCs and CD8+ T cells, and enhances antitumor immunity through Gal-9/Tim-3 interactions. Moreover, in B16F10-bearing mice, Gal-9 expands unique Mϕs having plasmacytoid DC (pDC)-like phenotypes that activate NK cells. Thus, Gal-9 treatment results in the prolonged survival of tumor-bearing mice.

Gal-9 also plays a critical role in viral infection: HCV infection enhances Gal-9 expression by Kupffer cells, and Gal-9 expands CD11b+Gr-1+ F4/80 cells in HSV infection together with T cell depletion, suggesting the possible involvement of Gal-9 in the innate and adaptive immune response even in common viral infection.

In contrast, Gal-9 ameliorates IC-induced arthritis by regulating FcγR expression on Mϕs. Gal-9 also expands immunosuppressive Mϕs to ameliorate T-cell-mediated lung inflammation. More recent work further revealed that Gal-9-expanded $CD14^-$ pDC-like Mϕs attenuate acute lung injury by regulating Mϕ TLR expression.

It is thus suggested that Gal-9 has a thermostat-like function: Immunopotentiation in immunocompromised conditions and immunosuppression in hyperimmune condition, and is a candidate of novel therapeutic agents for various inflammation including malignant tumor by its homeostatic function in immune responses.

Acyl-coenzyme A:cholesterol acyltransferase 1 and its pathophysiological roles in macrophages and in neurons.

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Acyl-coenzyme A:cholesterol acyltransferase (ACAT) converts free cholesterol to cholesterol ester, and is one of the key enzymes in cellular cholesterol metabolism. The first ACAT gene was identified in 1993. This work led to the discovery of the ACAT enzyme family, which is comprised of ACAT1, ACAT2, and acyl-coenzyme A:diacylglycerol acyltransferase 1 (DGAT1). The ACAT enzymes are founding members of the membrane-bound O-acyltransferase enzyme family (MBOAT). MBOATs are multi-span membrane proteins that utilize long-chain or medium-chain fatty acyl-coenzyme A and a hydrophobic substance as their substrates. Additional MBOATs include ghrelin octanoyl-coenzyme A acyltransferase (GOAT), and lysophospholipid acyltransferases (LPATs). Both ACAT1 and ACAT2 are potential drug targets for treating dyslipidemia and atherosclerosis. More recent evidence suggests that ACAT1 is also a potential target for treating Alzheimer disease. This presentation will review the biochemical properties of ACAT1, its physiological functions in macrophages and in neurons, and will discuss the pros and cons of ACAT1 specific inhibitors for treating atherosclerosis and for Alzheimer disease.

Roles of Angiopoietin-like protein 2 in lifestyle-related diseases

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Recent major increases in obesity and related metabolic diseases (known as metabolic syndrome), due to sedentary lifestyle and overnutrition in developed and developing countries, are an exploding medical and social problem. These conditions are associated with increased risk of cardiovascular disease, the leading cause of death. Thus, it is necessary to understand the molecular basis underlying metabolic syndrome and cardiovascular disease for the development of effective preventive and therapeutic approaches against cardiovascular disease. Recently, we found that adipose tissue-derived angiopoietin-like protein 2 (Angptl2) activates an inflammatory cascade in endothelial cells and induces chemotaxis of monocytes/macrophages in obesity, resulting in initiation and propagation of inflammation within adipose tissues and obesity-related metabolic diseases (Cell Metabolism 2009). Furthermore, we found that synoviocyte-derived Angptl2 contributes to synovial chronic inflammation in rheumatoid arthritis (Am J Pathol 2010 in press). In this presentation, I would like to focus my talk on the roles of Angptl2 in lifestyle-related diseases, and discuss the possibility that Angptl2 could function as molecular targets for prevention and treatment of metabolic syndrome and locomotive organ disorders.

Significance of M2 macrophage infiltration in human malignant tumors

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Tumor-associated macrophages (TAMs) participate in development of the tumor microenvironment by inducing angiogenesis, immunosuppression, and tumor invasion. Although many studies of the TAM population in various cancer tissues have been reported, only a few dealt with the activation of macrophages (such as M1 and M2 phenotypes) in human cancerous tissues. Therefore we investigated the significance of M2 macrophages in human cancers by means of immunohistochemical analysis using CD163 as one of M2-associated molecules.

As the results, the patients with increased infiltration of CD163+ TAMs showed significant poor clinical prognosis in glioma, intrahepatic cholangiocarcinoma, kidney cancer, and T-cell lymphoma, whereas no significant differences were found in prostatic cancer, gastric cancer, esophageal cancer, or primary CNS lymphoma. The significant correlation of increased CD163+ TAMs and poor clinical prognosis was also reported by others in melanoma, pancreatic cancer, and leiomyosarcoma. Significant association of high serum soluble CD163 level and poor clinical prognosis in patients with melanoma was also reported. In our study, CD163+ TAMs were significantly associated to tumor cell proliferation in high grade gliomas. *In vitro* co-culture study using T98G glioma cell line suggested the cell-cell interaction between macrophages and tumor cells contributes tumor cell activation and proliferation. The cell-cell interaction was up-regulated by direct cell-cell contact, and activation of STAT3 is involved in this process.

In summary, CD163+ TAMs polarized to M2 phenotype are considered to contribute to poor clinical prognosis in various kinds of human malignant tumors. Suppression of M2 polarization or inhibition of cell-cell interaction between tumor cells and TAMs might provide a promising approach to enhancing anticancer therapy.

Dynamics of CCR7^{+} tumor-infiltrating dendritic cells that regulate anti-tumor immune responses

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Tumor-infiltrating dendritic cells (tDCs) play a pivotal role in anti-tumor immune responses through their ability to migrate into draining LN (dLN) and present tumor associated antigens to T cells. However, the dynamics and developmental pathway of tDCs remain poorly understood. Here, we demonstrate the turnover and chemokine dependency of highly immunogenic tDCs expressing CCR7 and MHC class II (CCR7^{+} tDCs), a putative precursor of migratory-DCs (mDCs) that mediates tumor-specific cytotoxic T cell responses in dLN. Parabiosis and *in vivo* BrdU labeling experiments revealed that CCR7^{+} tDCs are replaced by blood precursors within 5 to 10 days. The turnover kinetics was parallel to that of CCR7-dependent mDCs in dLN but was distinct from that of tumor-infiltrating Ly-6C^{hi} monocytic cells (tMOs). We also found that CCR7^{+} tDCs developed from *CCR2*$^{-/-}$ bone marrow but tMOs did not under competitive-condition using bone marrow chimeric mice reconstituted with mixture of *CCR2*$^{-/-}$ and wild-type bone marrow, suggesting that CCR7^{+} tDCs are largely independent of monocytic-cell lineage. These results identify CCR7^{+} tDCs as bona fide DCs in tumor with classical non-lymphoid DC life-cycle and may provide a clue for the development of therapeutic approaches to augment endogenous anti-tumor immune responses.

S100 proteins and the tumor microenvironment

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Myeloid-derived suppressor cells (MDSC) accumulate in many tumors, and they suppress T-cell mediated immune responses, while stimulating angiogenesis and tumor growth. They probably explain why cancer vaccines fail in patients. It is becoming increasingly clear that therapeutic strategies for cancers should consider effective inhibition of MDSC expansion, or their function or both. However, mechanisms triggering and sustaining MDSC expansion in tumors are not well understood. Calcium-binding hetero-dimeric proteins S100A8 and S100A9 are pro-inflammatory mediators that are also highly expressed in many tumors. We earlier showed that S100A8/A9 bind to a subpopulation of RAGE enriched for carboxylated glycans. More recently, we found S100A8/A9 positive MDSC in mouse models of colitis-associated colon cancer (CAC), and in mice bearing CT26 and MC38 colon tumors, 4T1 metastatic mammary tumors, and B-cell lymphomas. S100A8/A9 bind to tumor cells in a RAGE and carboxylated-glycan dependent manner, and activate NF-κB signaling and tumor cell proliferation. We also found that MDSC synthesize and secrete S100A8/A9, which further promote mobilization of MDSC, thus providing an autocrine feedback loop that sustains accumulation of MDSC in tumors. In support of the role of RAGE and carboxylated glycans in S100A8/A9 mediated effects, we found that mAbGB3.1, an anti-glycan antibody, blocks the onset of colitis and CAC, and that mice deficient for S100A9 and RAGE show reduced incidence of CAC tumors. The anti-glycan antibody also reduces MDSC levels in blood and secondary lymphoid organs in mice with metastatic 4T1 mammary tumors. Our studies suggest that S100A8/A9, RAGE and carboxylated glycans are critical for the accumulation of MDSC in tumor microenvironments, thus providing novel therapeutic targets.

Macrophage polarization in tumour development

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Clinical and experimental evidence have highlighted that a major leukocyte population mainly present in the hypoxic areas of tumours, the so called tumour-associated macrophages (TAM), are the principal component of the leukocyte infiltrate supporting tumour growth. Evidence is accumulating for a 'switch' in macrophage phenotypes during the course of tumour progression. Whereas the functions of classically activated, 'M1' macrophages during chronic inflammation appear to predispose a given tissue to tumour initiation, in established tumours macrophages exhibit mainly the alternatively activated, 'M2' phenotype and are engaged in immunosuppression and the promotion of tumour angiogenesis and metastasis. Here I will discuss regulatory mechanisms driving the functional phenotype of TAM during the course of tumour development, along with their implication for anti-cancer therapies aimed at prompting TAM to mount an effective antitumor response.

Macrophage Diversity Promotes Tumor Progression and Metastasis.

Jeffrey W. Pollard

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There is persuasive clinical and experimental evidence that macrophages promote cancer initiation and malignant progression. Macrophages enhance malignancy at the primary site by stimulating angiogenesis, inducing tumor cell migration, invasion and intravasation and by suppressing anti-tumor immunity. At metastatic sites macrophages promote tumor cell extravasation, survival and subsequent growth. Each of these activities is stimulated by a different population of macrophages whose unique signaling pathways might represent new therapeutic targets (Qian and Pollard, 2010).

Qian BZ, Pollard JW (2010) Macrophage diversity enhances tumor progression and metastasis. Cell 141:39-51.

P1

Suppression of TLR4-mediated inflammatory response by macrophage class A scavenger receptor (CD204)

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The class A scavenger receptor (SR-A; CD204), one of the principal receptors expressed on macrophages, has been found to regulate inflammatory response and attenuate septic endotoxemia. However, the detailed mechanism of this process has not yet been well characterized. To clarify the regulative mechanisms of lipopolysaccharide (LPS)-induced macrophage activation by SR-A, we evaluated the activation of Toll-like receptor 4 (TLR4)-mediated signaling molecules in SR-A-deficient ($SR\text{-}A^{-/-}$) macrophages. In a septic shock model, the blood levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and interferon (IFN)-β were significantly increased in $SR\text{-}A^{-/-}$ mice compared to wild-type mice, and elevated nuclear factor kappa B (NFκB) activation was detected in $SR\text{-}A^{-/-}$ macrophages. SR-A deletion increased the production of pro-inflammatory cytokines, and the phosphorylation of mitogen-activated protein kinase (MAPK) and NFκB *in vitro*. SR-A deletion also promoted the nuclear translocation of NFκB and IFN regulatory factor (IRF)-3. These data indicated that SR-A suppresses both TLR4-mediated myeloid differentiation factor 88-dependent and -independent pathways. In addition, a competitive binding assay with acetylated low-density lipoprotein, an SR-A-specific ligand, and anti-SR-A antibody induced significant activation of TLR4-mediated signaling molecules in wild-type macrophages but not in $SR\text{-}A^{-/-}$ macrophages. These results indicate that SR-A suppresses the macrophage activation by inhibiting the binding of LPS to TLR4 in a competitive manner and it plays a pivotal role in the regulation of the LPS-induced inflammatory response.

P2

Apoptosis of macrophages by N-Arachidonyl Glycine is mediated by GPR18

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N-arachidonyl glycine (NAGly) is one of lipoamino acids which are fatty acid-amino acid conjugates. NAGly was first identified from the bovine and rat brain. Following study found that NAGly is present in a variety of tissues including spinal cord, small intestine, kidney, skin and blood. NAGly has close relationships with the anandamide (N-arachidonyl ethanol amide; AEA) not only structurally but also in terms of biological actions including analgesia, calcium ion mobilization and anti-inflammatory effects. At the same time, NAGly has a weak affinity for anandamide transporter, cannabinoid receptor CB1and vanilloid receptor VR1. It was also indicated that NAGly regulated AEA in macrophage. In addition, NAGly has been identified as a ligand of G-protein coupled receptor (GPCR) GPR18 and GPR92. So NAGly is an anticipated substance as pharmacological targets, but it has not demonstrated whether GPR18 and GPR92 were involved in these NAGly-induced physiological effects. Here, we focused physiological effects of NAGly in macrophage and the relations of GPCRs to the effects.

In this study, mouse macrophage derived cell line RAW264.7 were used. Cell apoptosis was determined by Cell counting kit-8 (CCK-8) and Annexin V stain. NAGly and AEA suppressed RAW264.7 cell survival rate and induced apoptosis but arachidonyl acid didnt. Westernblotting analysis revealed that NAGly activated MAP kinases (ERK1/2, JNK, p38 MAPK) and caspase-3 pathway. In particular, pretreatment with p38 MAPK inhibitor prevented NAGly-induced apoptosis. Pretreatment Gi protein inhibitor also prevented the action of NAGly, but it didn’t affect the action of AEA. Both GPR18 and GPR92 are expressed in RAW264.7 and GPR18 is coupled with Gi protein. GPR18 knocked-down RAW264.7 failed to induce apoptosis by NAGly. Our findings indicate that NAGly induced apoptosis in macrophage mediated by p38 MAPK activation and caspase-3 pathway.

Most recently it has been reported that macrophage apoptosis caused by lipids was mediated by the increase of fatty acid-binding protein-4(aP2), regulator of macrophage ER stress. Alteration of aP2 expression by NAGly in RAW264.7 is currently under investigation.

P3

EP2 and EP4 receptors on muscularis resident macrophages mediate LPS-induced intestinal dysmotility via iNOS upregulation through cAMP/ERK signals

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Intestinal resident macrophages play an important role in gastrointestinal dysmotility by producing prostaglandins (PGs) and nitric oxide (NO) in inflammatory conditions such as colitis, peritonitis and postoperative ileus. The causal correlation between PGs and NO in gastrointestinal inflammation has not been elucidated. In this study, we examined the possible role of PGE_2 in the LPS-inducible iNOS gene expression in murine distal ileal tissue and macrophages. Treatment of ileal tissue with LPS increased the iNOS and COX-2 gene expression, which lead to intestinal dysmotility. However, LPS did not induce the expression of iNOS and COX-2 in tissue from M-CSF-deficient *op/op* mice, indicating that these genes expressed in intestinal resident macrophages. iNOS and COX-2 protein was also expressed in dextran-phagocytized macrophages in the muscle layer. CAY10404, a COX-2 inhibitor, diminished LPS-dependent iNOS gene upregulation in wild-type mouse ileal tissue and also in RAW264.7 macrophages, indicating that PGs upregulate iNOS gene expression. EP2 and EP4 agonists upregulated iNOS gene expression in ileal tissue and macrophages, Gene expression of iNOS mediated by LPS was decreased in the ileum isolated from EP2 or EP4 KO mice. In addition, LPS failed to decrease the motility of EP2 and EP4 KO mice ileum. EP2- or EP4-mediated iNOS expression was attenuated by KT-5720, a PKA inhibitor and PD98059, an ERK inhibitor. Forskolin or db-cAMP can mimic upregulation of iNOS gene expression in macrophages. In conclusion, COX-2-derived PGE_2 induces iNOS expression through cAMP/ERK pathways by activating EP2 and EP4 receptors in muscularis macrophages. NO produced in muscularis macrophages induce dysmotility during gastrointestinal inflammation.

P4

Overexpression of Wiskott-Aldrich syndrome protein (WASP) N-terminal domain inhibits inflammatory responses in LPS-activated macrophages

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Wiskott-Aldrich syndrome (WAS) is an X-linked immunodeficiency caused by mutations in the responsible gene encoding the WAS protein (WASP). WASP plays a critical role as an adaptor molecule in signal transduction accompanied by cytoskeletal rearrangement in T cell, but its roles in signal transduction in innate immune cells are poorly understood. Previously, we generated WASP transgenic (Tg) mice overexpressing the WASP N-terminal Ena/VASP homology 1 (EVH1) domain. T cells from WASP-Tg mice were impaired in IL-2 production induced by T cell receptor stimulation, but the cytoskeletal rearrangement in these cells were not affected.

In the present study, to investigate the role of the WASP-EVH1 domain in the activation of macrophages, we established bone marrow derived macrophage (BMDM) cell lines from C57BL/6 and WASP-Tg mice. Wild-type BMDMs produced significant levels of inflammatory cytokines, such as TNF-α, IL-6 and IL-12p40 in response to LPS. But WASP-Tg BMDMs were impaired in production of these cytokines. Also, LPS-induced phosphorylation of NF-κB was reduced in WASP-Tg BMDMs. Furthermore, WASP-Tg BMDMs produced less amount of nitric oxide in response to LPS and IFN-γ than wild-type BMDMs. However, phagocytic activities against latex beads were comparable between wild-type and WASP-Tg BMDMs.

These results indicate that the overexpression of WASP-EVH1 domain inhibits the LPS signaling cascade. WASP may have important roles through the EVH1 domain in the LPS-induced inflammatory responses in macrophages.

P5

Silica and alum induce type 2 immunity via inflammasome-independent mechanisms

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Silica and alum are known to induce Th2-associated IgE production. However, the basis for the induction of type 2 immunity has not been elucidated. Recently, several reports indicate that these particulates activate the inflammasome, which mediates caspase-1 activation and secretion of the pro-inflammatory cytokines, IL-1β and IL-18 in macrophages (Mϕs) and dendritic cells and that the inflammasome might be involved in the induction of type 2 immunity. In this study, we show that silica and alum induce not only IL-1β and IL-18 but also lipid mediators, prostaglandin (PG). Silica and alum stimulated LPS-primed Mϕs to produce both IL-1β and PGE_2, however these particulates did not induce other inflammatory cytokines and chemokines. Caspase-1 inhibitor markedly suppressed IL-1β production but had no effect on PGE_2 production in silica and alum-stimulated Mϕs. Moreover, comparable levels of PGE_2 were produced from inflammasome-deficient (NALP3-/-, ACS-/- and caspase-1-/-) Mϕs in response to silica and alum. These results indicate a dispensable role of inflammasome on silica- and alum-induced PGE_2 production from Mϕs. PGE_2 production was regulated by the action of COX-2 and mPGES-1 because particulates-induced PGE_2 production was completely vanished in COX-2 inhibitor-treated or mPGES-1-deficient Mϕs. Interestingly, mPGES-1 deficient mice displayed reduced serum levels of antigen-specific IgE after the immunization with alum or silica but serum levels of antigen-specific IgG1 and IgG2c were comparable between WT and mPGES-1 deficient mice.
These results indicate that silica and alum regulate PGE_2 production via inflammasome-independent manner and PGE_2 induced by particulates controls type 2 immune responses in vivo.

P6

Mint3 enhances the activity of HIF-1 in macrophages by suppressing the activity of FIH-1

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Hypoxia Inducible Factor-1 (HIF-1) is a key transcription factor regulating cellular responses to hypoxia and is composed of α and ß subunits. During normoxia, Factor Inhibiting HIF-1 (FIH-1) inhibits the activity of HIF-1 by preventing HIF-1α binding to p300/CBP via modification of the Asn^{803} residue. However, it is not known whether FIH-1 activity can be regulated in an oxygen-independent manner. In this study, we survey possible binding proteins to FIH-1 and identify Mint3/APBA3, which has been reported to bind Alzheimer's beta-amyloid precursor protein (APP). Purified Mint3 binds FIH-1 and inhibits the ability of FIH-1 to modify HIF-1α in vitro. In a reporter assay, the activity of HIF-1α is suppressed due to endogenous FIH-1 in HEK293 cells and expression of Mint3 antagonizes this suppression. Macrophages are known to depend on glycolysis for ATP production due to elevated HIF-1 activity. FIH-1 activity is suppressed in macrophages by Mint3 so as to maintain HIF-1 activity. FIH-1 forms a complex with Mint3 and these two factors co-localize within the perinuclear region. Knockdown of Mint3 expression in macrophages leads to redistribution of FIH-1 to the cytoplasm and decreases glycolysis and ATP production. Thus, Mint3 regulates the FIH-1-HIF-1 pathway, which controls ATP production in macrophages, and therefore represents a potential new therapeutic target to regulate macrophage-mediated inflammation.

P7

Spermine involved methylation status on ITGAL promoter possibly regulates the expression of LFA-1.

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Polyamines are the natural substances that are indispensable for cell growth and cellular function. It is often observed that the concentration of polyamine in blood increases in cancer patients. On the other hand, the immune functions are often suppressed in cancer patients. We have reported that spermine suppresses the cellular function indispensable for cancer immunity. Among then, spermine selectively suppresses the expression of lymphocyte function-associated antigen-1 (LFA-1), one of the crucial adhesion molecules. In this study, the effects of polyamine metabolism on the methylation status of LFA-1 gene (ITGAL) promoter were examined. Human T cell line Jurkat was cultured in medium supplemented either with 3 mM α-difluoromethylornithine (DFMO) alone or with DFMO and 500 μM spermine. Intracellular concentrations of polyamines were measured by high performance liquid chromatography (HPLC). The expression of LFA-1 and the methylation status of the ITGAL promoter were measured by flow cytometry and bisulfite sequencing, respectively. In addition, the activities of DNA methyltransferase (Dnmt) were examined. LFA-1 expression was higher in cells cultured with DFMO than in cells cultured without any supplementation. Further, LFA-1 expressions were lower in cells cultured with DFMO and spermine than in those cultured with DFMO alone. ITGAL promoter was highly methylated in cells cultured with DFMO and spermine as compared with those cultured with DFMO alone. The activity of Dnmt was higher in cells cultured with DFMO and spermine than those cultured with DFMO alone. Increased methylation status of the ITGAL promoter appears to be one of the mechanisms by which spermine suppresses LFA-1 expression.

P8

Tim-3 mediates phagocytosis of apoptotic cells by inflammatory macrophage and CD8$^+$ dendritic cell subsets.

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T cell immunoglobulin and mucin domain-containing molecule-3 (Tim-3) is a type I cell surface molecule regulating autoimmune diseases and tolerance induction. In this study, we demonstrate that Tim-3 binds phosphatidylserine and mediates phagocytosis of apoptotic cells through the FG loop in the IgV domain, and is then recruited into the phagosome. Tim-3 is expressed on mouse peripheral blood Mac1$^+$ cells and thioglycollate-elicited peritoneal Mac1$^+$ cells (thio-Mac). Phagocytosis of apoptotic cells by thio-Mac is inhibited by neutralizing anti-Tim-3 mAb *in vitro* and *in vivo*. Moreover, Tim-3 is highly expressed on splenic CD8$^+$ dendritic cells (DC) able to present exogenous antigens on their MHC class I molecules, a process termed as cross-presentation. Masking of Tim-3 on CD8$^+$ DC by the anti-Tim-3 mAb shows significantly impaired recognition of apoptotic cells, resulting in a remarkable reduction in the cross-presentation of dying cell-associated ovalbumin to OT-I cells. These data provide a novel role of Tim-3 in phagocytosis of apoptotic cells and cross-presentation.

P9

M-CSF-dependent red pulp macrophages regulate CD4 T cell responses

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The balance between immune activation and suppression must be regulated to maintain immune homeostasis; dendritic cells are known to be critical regulators in maintaining such a balance. Tissue macrophages (Mφ) constitute the major cellular subsets of antigen presenting cells (APCs) within the body; however, how and what types of resident Mφ are involved in the regulation of immune homeostasis in the peripheral lymphoid tissues is poorly understood. Splenic red pulp Mφ (RPMs) remove self-antigens such as blood-borne particulates and aged erythrocytes from the blood. Although T cell responses usually occur in the T cell zone of the white pulp, it has been well known that there are numerous small patches within red pulp parenchyma (comprising in total a volume comparable to that the white pulp in human) which contain mainly T cells, B cells, and RPMs(7-9), indicating the possibility of interaction between RPMs and T cells in the red pulp. Therefore, T cell responses occurring in the red pulp should be strictly regulated, as majority of Ags are derived from self. Here, we found that murine splenic F4/80^{hi}Mac-1^{low}Mφ residing in the red pulp showed different expression patterns of surface markers compared with F4/80^{+}Mac-1^{hi} monocytes/Mφ. Studies with purified cell populations demonstrated that F4/80^{hi}Mac-1^{low}Mφ regulated CD4^{+}T cell responses by producing suppressive cytokines such as TGF-β and IL-10. Moreover, F4/80^{hi}Mac-1^{low}Mφ induced the differentiation of naïve CD4^{+}T cells into Foxp3^{+}regulatory T cells. Additionally, the differentiation of F4/80^{hi}Mac-1^{low}Mφ was highly regulated by M-CSF as F4/80^{hi}Mac-1^{low}Mφ was disappeared in *op/op* mice. *In vitro*-generated bone marrow-derived Mφ induced by M-CSF (M-Mφ) showed the similar expression pattern of surface molecules compared with F4/80^{hi}Mac-1^{low}Mφ. Furthermore, M-Mφ suppressed CD4^{+} T cell proliferation and induced the generation of Foxp3^{+}Tregs both *in vitro* and *in vivo*. These data suggest that M-CSF dependent F4/80^{hi}Mac-1^{low}Mφ is a subpopulation of RPMs and regulate T cell immune responses by several distinct mechanisms and maintain peripheral immune homeostasis.

P10

Rheumatoid arthritis patient-derived synoviocytes are more sensitive to cigarette smoke condensate extracts-induced IL-1 beta expression through NF-κB activation than OA patient-derived synoviocytes, human lung fibroblasts and human lung epithelial cells

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Proinflammatory cytokines produced by synoviocytes are implicated in the pathogenesis of rheumatoid arthritis (RA). Although cigarette smoking is a solid environmental risk factor for RA, the mechanism of the association is largely unknown. We have previously reported that cigarette smoke condensates (CSC) induced induction of proinflammatory cytokines, IL-1α, IL-1β, IL-6 and IL-8, at both mRNA and protein levels from human RA patient-derived synoviocytes line MH7A. CSC also augmented collagen-induced arthritis in mice. In this study the mechanism of CSC-induced IL-1β production in MH7A cells and sensitivity to CSC in comparison with other cell types were investigated.

Main stream CSC induced IL-1β protein and □mRNA expression in RA patient-derived synoviocytes and MH7A cells, while the induction was not found or quite low in osteoarthritis (OA) patient-derived synoviocytes, human lung fibroblast line MRC5 and human lung epithelial cells A549. NF-κB was activated by CSC in MH7A and MRC5 but not in A549, while AP-1 and C/EBPβ were activated only in MH7A cells. Luciferase assay indicated that IL-1β □promoter was activated in MH7A but not in MRC5 and A549. NF-κB activation appeared to be critical for the transactivation of the gene. These results suggest that RA-patient-derived synoviocytes are more sensitive to CSC in induction of IL-1β mRNA through activation of NF-κB, AP-1 and C/EBPβ, and NF-κB appeared to be quite important.

P11

Absence of IFN-γ accelerates thrombus resolution through enhanced MMP-9 and VEGF expression

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We examined the pathophysiological roles of IFN-γ in the resolution of deep vein thrombosis by the use of IFN-γ-deficient (IFN-$\gamma^{-/-}$) mice. Upon the ligation of the inferior vena cava of WT mice (Balb/c), venous thrombi formed and grew progressively until 5 days, and remained similar sizes at 10 days. Concomitantly, intrathrombotic IFN-γ contents were elevated progressively as post-ligation intervals was extended. When IFN-$\gamma^{-/-}$ mice were treated in the same manner, thrombus size was similar to that in WT mice until 5 days after the IVC ligation, but it was apparently smaller at 10 and 14 days, compared with WT mice. Intrathrombotic collagen-positive areas and intrathrombotic hydroxyproline (a major component of collagen) contents were remarkably reduced later than 10 days after IVC ligation in IFN-$\gamma^{-/-}$ mice, compared with WT mice. However, there were no significant differences in the intrathrombotic leukocyte numbers (neutrophils and macrophages) between WT and IFN-$\gamma^{-/-}$ mice. On the contrary, MMP-9 but not MMP-2 mRNA expression was higher at the late phase in IFN-$\gamma^{-/-}$ mice, than WT mice. Concomitantly, MMP-9 enzyme activities were higher in IFN-$\gamma^{-/-}$ mice than WT mice. Moreover, laser doppler imaging demonstrated that that blood flow was significantly recovered in IFN-$\gamma^{-/-}$ mice, compared with WT mice. Actually, intrathrombotic vascular areas were increased in IFN-$\gamma^{-/-}$ mice, with enhanced VEGF gene expression, compared with WT mice. Furthermore, the administration of anti-IFN-γ mAb accelerated the thrombus resolution in WT mice. Collectively, IFN-γ can have detrimental roles in the thrombus resolution by suppressing MMP-9 and VEGF expression and can be a good molecular target for the treatment of deep vein thrombosis.

P12

CCR7-independent trafficking of skin antigens to regional lymph nodes by cells in the dermis

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Continuous trafficking of self-antigens (Ags) in the periphery to regional lymph nodes (LNs) is considered to be critical to establish and maintain peripheral tolerance. CCR7 is known as a key molecule to regulate the trafficking of dendritic cells (DCs) from the periphery to regional LNs. Langerhans cells (LCs) are epidermal DCs, and transport both foreign and self Ags to regional LNs regulated by CCR7. It is possible that CCR7 deficiency would result in the disruption of skin immune tolerance. However, *Ccr7(-/-)* mice do not show skin autoimmune diseases. This finding implies that there is an alternative mechanism to maintain peripheral tolerance of skin. Therefore, we investigated the trafficking of skin Ags to regional LNs in *Ccr7(-/-)* mice.
Using epidermis-pigmented *KRT14-Kitl-Tg* mice system, in which the trafficking of epidermal Ags can be traced by melanin granules, we found that the epidermal Ag-trafficking to regional LNs was strongly suppressed in *Ccr7(-/-)* mice as compared with *Ccr7(+/-)* mice. On the other hand, using dermis-pigmented *KRT14-HGF-Tg* mice system, the trafficking of dermal Ags to regional LNs was clearly detected in *Ccr7(-/-)* mice. The amount of trafficked dermal Ags in the LNs of *Ccr7(-/-)* mice was around 43% to that in the LNs of *Ccr7(+/-)* mice. Immunohistochemistry revealed that most of the dermal Ag-transporting cells in the LNs of *Ccr7(-/-)* mice were $CD207^-$ but $CD11c^+$. These results suggest that; 1) the continuous trafficking of epidermal Ags is highly regulated by CCR7-signaling, 2) CCR7-independent trafficking cells should be present in the dermis, and the cells are DC-lineage cells other than recently reported $CD207^+$ dermal DCs. The alternative trafficking of skin Ags may be implicated in skin immune tolerance of *Ccr7(-/-)* mice.

P13

A proteasome inhibitor bortezomib suppresses immunostimulatory activity of human plasmacytoid dendritic cells by targeting intracellular trafficking of nucleic acid-sensing Toll-like receptors and endoplasmic reticulum homeostasis

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Dendritic cells (DCs) play a pivotal role in the pathogenesis of inflammatory disorders. Thus, suppressing the activity of DCs is instrumental in treating such diseases. Here we show that a proteasome inhibitor bortezomib suppresses immunostimulatory functions of human plasmacytoid DCs (pDCs) by targeting two critical points, intracellular trafficking of nucleic acid-sensing Toll-like receptors (TLRs) and endoplasmic reticulum (ER) homeostasis. pDCs were most susceptible to the killing effect of bortezomib among immune cells in blood. This correlates with a decrease in the spliced form of a transcription factor XBP-1, which rescues cells from apoptosis by maintaining ER homeostasis. Bortezomib suppressed the production of IFN-α and IL-6 by pDCs activated with a TLR9-stimulating CpG DNA and a TLR7-stimulating influenza virus, independently of the induction of apoptosis. Bortezomib inhibited translocation of TLR9 from the ER to endolysosomes but not of an ER membrane protein Unc93B1 that delivers TLR9 to endolysosomes. Thus, bortezomib suppresses the activity of pDCs (i) by inhibiting intracellular trafficking of TLRs through disrupting the coordinated translocation of TLRs and Unc93B1, and thereafter (ii) by disturbing ER homeostasis. This study suggests that proteasome inhibitors may alleviate inflammatory disorders that involve pDCs such as lupus and psoriasis.

P14

Z39Ig is a novel cell surface marker of macrophages in murine large intestine

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Z39Ig (CRIg) is a receptor for complement fragments (C3b and iC3b). Previously, we reported that Z39Ig is a marker of resident tissue macrophages (Mϕ) in human tissues. In this study, to characterize the $Z39Ig^{+}$ cells, we generated monoclonal anti-mouse Z39Ig antibody and examined the distribution of the $Z39Ig^{+}$ cells in mouse tissues. FACS and histological analysis showed that Z39Ig protein was expressed on resident Mϕ in peritoneum, heart and liver. Interestingly, histological analysis showed that the $Z39Ig^{+}$ cells were distributed in the large intestine, while the $Z39Ig^{+}$ cells were not detected in the small intestine. To further examine the $Z39Ig^{+}$ cells in the large intestine, cell surface markers were analyzed by FACS in comparison with those on the peritoneal $Z39Ig^{+}$ Mϕ. The peritoneal $Z39Ig^{+}$ Mϕ were $F4/80^{high}CD11b^{high}CD11c^{-}$. On the other hand, the intestinal $Z39Ig^{+}$ cells were $F4/80^{low}$ but $CD11b^{-}$ and $CD11c^{-}$. The intestinal $Z39Ig^{+}$ cells were also shown to have the ability of uptake of dextran and microspheres, indicating that these cells were Mϕ irrespective of the lack of CD11b expression on the cell surface. Therefore, it was suggested that Z39Ig is a novel cell surface marker of resident Mϕ in the large intestine and that Z39Ig plays important roles in the complement-mediated phagocytosis in resident Mϕ in the large intestine.

P15

High-mobility Group Box-1 Protein Promotes Granulomatous Nephritis in Adenine-induced nephropathy

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Granulomatous nephritis can be triggered by diverse factors and results in kidney failure. However, despite accumulating data about granulomatous inflammation, pathogenetic mechanisms in nephritis remain unclear. The DNA-binding high-mobility group box-1 protein (HMGB1) initiates and propagates inflammation when released by activated macrophages, functions as an “alarm cytokine” signaling tissue damage. In this study, we demonstrated elevated HMGB1 expression in renal granulomas in rats with crystal-induced granulomatous nephritis caused by feeding an adenine-rich diet. HMGB1 levels were also raised in urine and serum, as well as monocyte chemoattractant protein-1 (MCP-1), a mediator of granulomatous inflammation. Injection of HMGB1 worsened renal function and upregulated MCP-1 in rats with crystal-induced granulomatous nephritis. HMGB1 also induced MCP-1 secretion through mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) pathways in rat renal tubular epithelial cells *in vitro*. *Hmgb1*$^{+/-}$ mice with crystal-induced nephritis displayed reduced MCP-1 expression in the kidneys and in urine and the number of macrophages in the kidneys was significantly decreased. We conclude that HMGB1 is a new mediator involved in crystal-induced nephritis that amplifies granulomatous inflammation in a cycle where MCP-1 attracts activated macrophages, resulting in excessive and sustained HMGB1 release. HMGB1 could be a novel target for inhibiting chronic granulomatous diseases.

P16

Class A scavenger receptor promotes osteoclast differentiation via the enhanced expression of receptor activator of NF-κB (RANK)

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Class A scavenger receptor (SR-A) is one of the principal functional molecules of macrophages, and its level of expression declines during osteoclast differentiation. To investigate the role of SR-A in osteoclastogenesis, we examined pathological changes in femoral bone and the expression levels of osteoclastogenesis-related molecules in SR-$A^{-/-}$ mice. An *in vitro* differentiation assay revealed that the differentiation of multinucleated osteoclasts from bone marrow-derived progenitor cells is impaired in SR-$A^{-/-}$ mice. The expression levels of RANK and RANK-related osteoclast differentiation molecules such as nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATc1) and microphthalmia-associated transcription factor (MITF) significantly decreased in SR-$A^{-/-}$ mice. Furthermore, acetylated low-density lipoprotein (AcLDL), an SR-A ligand, significantly increased the expression level of RANK and MITF during osteoclast differentiation. The femoral osseous density of SR-$A^{-/-}$ mice was higher than that of SR-$A^{+/+}$ mice, and the number of multinucleated osteoclasts was significantly decreased. These data indicate that SR-A promotes osteoclastogenesis via augmentation of the expression level of RANK and its related molecules.

P17

The defensive role of interferon-γ produced by myeloid cells in invasive group A *Streptococcus* infection

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Severe invasive group A *streptococcus* (GAS) infection can lead to rapid progressive and life-threatening manifestations, including sepsis and streptococcal toxic shock syndrome. Although many studies have suggested that GAS-derived superantigens extraordinarily activate T cells to release excessive cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-2, which are considered to be participated in pathogenesis of such disease, the precise mechanism remains unknown. In this study, to clarify what kinds of cytokines are involved in severe invasive GAS infection, we first examined the levels of cytokines in plasma from mice infected with clinical isolates from either severe invasive or non-invasive GAS infection. Interestingly, a large amount of interferon (IFN)-γ was detected in plasma from mice infected with severe invasive GAS isolates, but not in those from non-invasive GAS infected mice, whereas other cytokines, such as TNF-α, IL-1α, IL-2 and IL-17, were scarcely detected in plasma samples of mice infected with either invasive or noninvasive isolates. Furthermore, analysis using flow cytometry of splenocytes, and peripheral blood cells revealed that IFN-γ was produced by Gr-1^+ F4/80^+ Mac-1^+ myeloid cells, but not by either T cells, NK cells, NKT cells or DCs, in mice infected with severe invasive GAS isolates. The result suggests that IFN-γ production is not caused by streptococcal superantigens, because T cells were unable to produce IFN-γ. To elucidate the role of IFN-γ in pathogenesis of severe invasive GAS infection, we administrated anti-mouse IFN-γ neutralizing antibody (R4-6A2) into mice, followed by GAS infection. The mice given R4-6A2 were more susceptible to infection with invasive GAS isolates than the mock administered mice. Collectively, our results suggest that IFN-γ production is independent on streptococcal superantigens and IFN-γ produced by myeloid cells acts as one of host defensive factors in severe invasive GAS infection.

P18

Targeting of folate receptor β–expressing macrophages in bleomycin induced pulmonary fibrosis

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BACKGROUNDS: It has been suggested that alveolar and interstitial macrophages by producing proinflammatory and/or fibrogenic cytokines play a key role in the pathogenesis of idiopathic pulmonary fibrosis (IPF). We have shown that inflammatory macrophages express folate receptor β (FRβ) while tissue resident macrophages in normal tissues express no or low levels of FRβ. In the present study, we examined the distribution of FRβ-expressing macrophages in the lungs of the patients with usual idiopathic pulmonary fibrosis (UIP) and mice with bleomycin-induced pulmonary fibrosis (PF) and tested whether the depletion of FRβ-expressing macrophages could suppress bleomycin-induced PF.
METHODS&RESULTS: Immunostaining with anti-human or mouse FRβ mAb revealed that FRβ-expressing macrophages were present predominantly in fibrotic areas of the lungs of patients with UIP and mice with bleomycin-induced PF. Intranasal administration of a recombinant immunotoxin, consisting of immunoglobulin heavy and light chain Fv portions of an anti-mouse FRβ mAb and truncated *Pseudomonas exotoxin A*, significantly increased the survival and reduced the levels of total hydroxyproline and fibrosis in bleomycin-induced PF. In immunohistochemical analysis, decreased numbers of TNF-α-, CCL2- and CCL12-producing cells were observed in the immunotoxin-treated group.
CONCLUSIONS: These findings suggest a pathogenic role of FRβ-expressing macrophages in IPF. Thus, targeting FRβ-expressing macrophages will be promising for the treatment of IPF.

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What are effector cells in the liver transplant rejection?

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Background: We previously reported that both donor DC migration and intrahost $CD8^+$T cell generation were completely inhibited by a tolerance inducing protocol, donor-specific transfusion (DST), where a specific cytotoxic alloantibody were responsible for elimination of migrating passenger leukocytes in recipient circulation. Provided that CD8+T cells are suppressed in DST group, what's happening in the graft liver? We examined a difference in cellular kinetics within the grafts between DST(+) and rejection [DST(-)] groups.
Methods: In DST(-) group, orthotopic liver transplantation of donor ACI ($RT1.A^aB^a$) to recipient Lewis ($RT1.A^lB^l$) rats was performed. In DST(+) group, 1-ml donor heparinized peripheral blood was i.v. injected to recipient 7 days prior to transplantation. In Ab(+) group, sera of DST treated rats were i.p. injected to naïve recipient 1d before transplantation.
Results and Discussion: In early phase of DST(-) group, recipient $CD8^+$T cells massively infiltrated in the non-sinusoidal (NS) area from and over half of proliferating cells were $CD8^+$T cells on day 4. In DST(+) group although earlier recipient cell infiltration was noted, any significant $CD8^+$T cell proliferation was noted in the NS area. Furthermore, $CD8^+$T cell response in the sinusoidal area was completely blocked. In DST(-) group, after manifestation (7d~) of rejection, liver became enlarged and NS area was expanded due to massively infiltrating recipient cells. Surprisingly most of them in the sinusoidal and NS areas were not $CD8^+$T cells but macrophages ($CD4^+ED1^+$), which up-regulated the expression of MHC-II and CD163. In DST(+) group, although later infiltrating cells were also recipient macrophages, their infiltration was much less dominant and their phenotypes seemed to be different. In the Ab(+) group, despite of inhibition in intrahost/graft $CD8^+$T cell responses and a significant MST elongation, late stage recipient cell infiltration was similar to DST(-) group.
Conclusion: Liver allograft rejection was participated accomplished by 2 types of effector cells, i.e. $CD8^+$T cells in the early stage and activated macrophages in the late stage. DST pretreatment would have multiple effects, such as suppression of the CD8+T

cell, modification of macrophage functions, and Treg induction, etc. Now we are investigating the phenotype and function of macrophage subsets between DST(-) and DST(+) groups.

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Translocation of ACAT1 from ER to Late Endosomal Associated Membranes in Cholesterol-rich Human Macrophage

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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 linoleate ([^{3}H]CL)-aggregated LDL ([^{3}H]CL-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 [^{3}H]Cholesterol ester ([^{3}H]CE) even after cells were simultaneously treated with [^{3}H]CL-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]CL-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.

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Macrophage and neutrophils have different response to hypoxia in expression of long pentraxin 3 (PTX3) in human atherosclerosis

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Expression of pentraxin 3 (PTX3) is induced as local response to inflammatory signals by various types of cells. The aim of the study was to investigate the response of macrophages and polymorphonuclear neutrophils (PMNs) to hypoxia in production of PTX3 protein in human atherosclerosis. Hypoxia-inducible factor-1 (HIF-1) was estimated as marker of adaptive response to hypoxia/ischemia.
We examined 31 coronary arterial thrombi removed from patients with acute myocardial infarction and angina pectoris. Paraffin-embedded samples were applied for immunohistochemistry using monoclonal antibodies against human PTX3 and HIF-1α, as well as cell-specific antibodies.
Unexpectedly, infiltrated neutrophils in ruptured atherosclerotic plaque and neutrophils in coronary thrombus displayed nuclear pattern staining against HIF-1α antibody, suggesting that HIF-1α protein was translocated to the nucleus in response to hypoxia. Some PMNs also showed the depletion of PTX3 protein in cytoplasm, suggesting their release in hypoxic condition. Moreover, apoptotic cells, probably neutrophils, were detected at the same thrombus samples, supporting the idea that PTX3 protein is able to bind and regulate the apoptotic cells clearance in human atherosclerosis. Of interest, hypoxic foamy macrophages displayed the ability to express PTX3 protein in atherosclerotic plaque, but the expression level of HIF-1α was low in foamy macrophages. These findings suggested that the sensitivity of neutrophils to hypoxia was higher than that of macrophages.

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A symbiotic growth of nasal NK/T-cell lymphoma cells with CD204-positive macrophages would defense themselves from endogenous reactive oxygen spices-induced cell necrosis

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Nasal NK/T-cell lymphoma (NKTCL) shows Epstein-Barr virus (EBV) infection-related high activation of autophagy and peculiar necrosis, and a symbiotic growth with $CD204^+$ macrophages. Endogenous reactive oxygen spices (ROS) derived from mitochondria induce cell necrosis and oxidize often Guanine (G) to Fapy G suppressing DNA synthesis and to 8-OHdG making a point mutation in genes. This study investigated growth patterns of 114 nasopharyngeal lymphomas, including 77 cases of NKTCL and 33 cases of B-cell neoplasm (B-ML), by means of immunostaining for 8-OHdG with that for LC3, the marker of autophagy and autophagic cell death. At least two kinds of decreasing growth patterns of lymphoma cells were recognized. The decreasing growth of lymphoma cells associated activation of autophagy, 8-OHdG-positive cellular debris, nuclear and somewhat cytoplasmal immunostain of 8-OHdG, suggesting cell necrosis due to endogenous ROS and suppression of DNA synthesis due to Fapy G, and was noted in 17 (52%) cases of B-ML and 6 (8%) cases of NKTCL. The other kind of decreasing growth of lymphoma cells often associated peculiar necrosis, showed sudden increase of autophagic cell death labeled densely with LC3 and 8-OHdG-positive cellular debris in the peculiar necrotic areas, and was noted in 4 (12%) cases of B-ML and 61 (79%) cases of NKTCL. The cell necrosis due to endogenous ROS occurred individually and in the late stage of autophagic cell death because the engulfed and mitochondria being digested in autophagolysosomes would supply ROS producing strongly oxidative radicals. Comparing these 2 kinds of decreasing growth patterns of lymphoma cells, a symbiotic growth of NKTCL cells with $CD204^+$ macrophages would defense themselves from endogenous ROS-induced cell necrosis when EBV enhanced autophagy in the cellular areas, and in the peculiar necrotic areas with the destruction of the symbiotic growth the persistent EBV-related enhanced autophagy would induce autophagic cell death.

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Involvement of M2 macrophages in the ascites of advanced epithelial ovarian cancer in tumor progression via Stat3 activation

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Objectives: Macrophages (Mφ) play a central role in the immune response. Peritoneal Mφ are also actively involved in the regulation of inflammation, immune response, and tumor growth in peritoneal cavity. Mφ in the ascites of advanced epithelial ovarian cancer (EOC) are polarized to M2 immunosuppressive phenotype and involved in tumor progression by modulating the tumor microenvironment. However, the cell-to-cell interaction between peritoneal macrophages and ovarian cancer cells is still unclear. This study focused on the activation of signal transducer and activator of transcription 3 (Stat3) which is a critical signal transduction molecule at a point of convergence for numerous oncogenic signaling pathways as well as controlling the M2-polarization of Mφ.

Methods: We examined the population of Mφ in the ascitic fluid of non-EOC (uterine myoma, n=4) and EOC (stage I, n=5; stages III + IV, n=15) by immunohistochemistry. Expressions of cytokines and growth factors in the ascitic fluid were analyzed by ELISA and immunocytochemistry. In addition, the proliferation of an ovarian cancer cell line, SKOV3 was evaluated when the ascitic fluid and cytokines were added into its culture medium. The status of Stat3 in the co-culture of Mφ and SKOV3 was also evaluated.

Results: Most Mφ in the ascites of EOC were polarized to the M2 phenotype. EOC ascites stimulated proliferation of SKOV3 cells. It also induced the activation of Stat3 in THP-1 macrophages. In addition, the co-culture with M-CSF-primed M2 Mφ, but not with GM-CSF-primed immature Mφ induced a strong Stat3 activation in SKOV3 cells. The expression of cyclin-D1 in SKOV3 cells was up-regulated by the co-culture with Mφ. Up-regulation of cyclin-D1 in SKOV3 cells was significantly suppressed after blocking Stat3 by small interfering (si)RNA in Mφ, thus indicating that Stat3-mediated M2 polarization of Mφ in the ascites is important for tumor cell survival.

Conclusion: These results indicate that the cell-to-cell interactions between Mφ and EOC cells through Stat3 activation are positively involved in EOC progression.

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Infiltration of macrophages plays a key role in tumor angiogenesis and progression

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[INTRODUCTION]
Tumor-supporting macrophages are known as tumor-associated macrophages (TAMs) supporting the invasion, metastasis and angiogenesis by cancer cells through enhanced production of inflammatory cytokines, chemokines, proteases, prostanoids, growth factors and angiogenic factors. In this study, we asked whether macrophages could play any role in malignant progressions such as tumor growth, angiogenesis, and bone metastasis, and also whether such malignant progressions could be blocked by macrophage-targeting drugs.

[METHODS]
The *in vivo* assay for angiogenesis and lymphangiogenesis was performed by mouse corneal assay.
Angiogenesis and lymphangiogenesis were determined by immune-cytochemical and -histochemical analyses using specific antibodies (CD31, LYVE 1).

[RESULTS]
[1] Administration of macrophage-targeting bisphosphonate-liposomes inhibited IL-1β-induced angiogenesis and lymphangiogenesis in mouse corneas, and also reduced production of angiogenesis and lymphangiogenesis factors in mouse corneas.
[2] Administration of macrophage-targeting bisphosphonate-liposomes inhibited tumor angiogenesis, tumor growth, and infiltration of macrophages in tumor.

[DISCUSSION]
Our present study demonstrated that tumor growth and angiogenesis were highly susceptible to macrophage targeting drug, suggesting that infiltrating macrophages could be a potent target for development of therapeutical drugs for malignant cancer.