Meeting Report

Molecular Pathobiology of Dendritic Cells and Macrophages

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The 12th International Symposium on Molecular Cell Biology of Macrophages (MP2003) was held in Utsunomiya, Japan from 19 and 20 June 2003.

The organizer of this Symposium, the Japanese Society for Molecular Cell Biology of Macrophages was founded in 1991 in order to promote basic science as well as clinical research on macrophage biology. This meeting has been held annually and made a large contribution to the world-wide advance of this research field. MP2003 consisted of three major sessions with 8 invited speakers from overseas and 9 Japanese speakers, each providing new insights in molecular pathobiology of dendritic cells and macrophages.

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

1. S. Ikehara's group (Kansai, Japan) has previously found that allo bone marrow transplantation (BMT) can be used to treat autoimmune diseases, and that autoimmune diseases are stem cell disorders. They have very recently found that the number of $CD11c^{+}CD3^{-}B220^{-}$ cells increases in the various organs (including the peripheral blood) of autoimmune-prone mice in comparison with normal mice. In addition, they have found that the injection of $CD11c^{+}CD3^{-}B220^{-}$ cells from old B/WF_1 mice (with thrombocytopenia) to young B/WF_1 mice transiently induces thrombocytopenia. This suggests that DCs play a crucial role in the induction and acceleration of autoimmune disease. Thus, the abnormalities of autoimmune-prone mice originate in the hematopoietic stem cells. This being the case, BMT from normal donors should be carried out. They have also established new strategies for BMT: They injected the BMCs directly into the bone marrow cavity of recipients (intra-bone marrow [IBM]-BMT). This "IBM-BMT" was found to be effective in treating autoimmune diseases in chimeric-resistant and radiation-sensitive MRL/lpr mice. They are now carrying out this new BMT method in monkeys in preparation for human application. This method heralds a new era in BMT, gene therapy, organ transplantation, and regeneration therapy.

2. R. K. Burt's group (Chicago, ILL, USA) has attempted to selectively induce the differentiation of hematopoietic cells from embryonic stem cells (ESCs). They have investigated the possibility of murine ESCs being maintained *ex vivo* and to form marrow and blood in lethally irradiated mice. The mouse ESC line, R1($H\text{-}2^{b}$), was cultured with irradiated embryonic fibroblasts. To induce the differentiation of the cell line toward HSCs *in vitro*, the embryoid body (EB) formation method in methylcellulose cultures and culturing EB-derived cells in methylcellulose-based hematopoietic differentiation medium were used. After 3-4 weeks, the suspension of these cells was injected intra-bone marrow. Irradiated (TBI: 5.5Gy) BALB/c mice ($H\text{-}2^{d}$) were used as recipients of ESC-derived cells. Four to eight weeks after ESC-derived cell injection (intra-bone marrow injection), the reconstitution of lymphocytes (~15%), monocytes/granulocytes (~30%) was observed. Embryonic stem cells successfully give rise to blood cells *in vivo* and might be used as an alternate hematopoietic and macrophage donor source.

3. In developed countries, *Campylobacter jejuni* is a leading cause of acute gastroenteritis. A prospective case-control study showed evidence of recent *C. jejuni* infection in 26% of the patients with GBS as compared with 2% of the household controls. In 1989, N. Yuki's group

(Dokkyo, Japan) first encountered the GBS case after *C. jejuni* infection. To reveal the pathogenic role of anti-GM_1 antibodies, they inoculated rabbits with GM_1. The rabbits developed flaccid paresis of the limbs. The rabbits' IgGs strongly bound to GM_1. IgG was deposited on axons of the peripheral nerves. In the cauda equina, macrophages were present in the periaxonal space. Electron microscopy confirmed that these macrophages directly attack the axon. To establish the molecular mimicry theory, they sensitized rabbits with the GM_1-like LPS. Some rabbits developed flaccid paresis. Possible pathogenesis of acute motor axonal neuropathy (AMAN) subsequent to *C. jejuni* enteritis associated with anti-GM_1 IgG : (1) Infection by *C. jejuni* bearing the GM_1-like LPS induces anti-GM_1 IgG production in patients with certain immunogenetic backgrounds. (2) Anti-GM_1 IgG binds to the nodal axolemma, which fixes complement. (3) Anti-GM_1 IgG and activated complement enter into the periaxonal space of the internode, which guide macrophages to the space. (4) Anti-GM_1 IgG antibody-dependent, complement- and macrophage-mediated cytotoxicity induces primary axonal degeneration.

4. Plasmacytoid dendritic cells (PDC) are characterized by the ability to rapidly synthesize large amounts of type I IFN (IFN-α and IFN-ß) in response to viral infection. In contrast to other dendritic cell subsets, which express a broad profile of TLRs, the TLR profile in PDC is restricted to TLR7 and TLR9. So far, CpG DNA is the only defined microbial molecule recognized by PDC, while other microbial molecules such as LPS or poly I:C, due to the lack of the corresponding TLRs, do not stimulate PDC. G. Hartmann's group (Munich, Germany) propose a model in which the PDC functions as a switchboard for regulating Th1 versus Th2/Th0 responses: in the presence of appropriate microbial stimulation (such as CpG DNA), PDC trigger a Th1 response; in the absence of appropriate microbial stimulation, PDC promote an unbiased T helper cell response (Th0) or Th2. Novel techniques allow us to use PDC in combination with CpG ODN for immunotherapy of cancer. First animal studies provide evidence that large murine tumors can be cured by CpG ODN and dendritic cells. In conclusion, the CpG ODN represents a unique microbial stimulus for PDC. The PDC is a promising candidate for cell-based vaccine protocols currently under evaluation for the treatment of cancer.

Session 2: Role of Spleen in the Host Defense

1. K. Matsuno and his colleagues (Dokkyo, Japan) provided 5 questions concerning the spleen: (1) What are homing receptors for lymphocytes to enter the white pulp? (2) What is a role of marginal zone B cells? Relevance to B1 B cells? (3) What is a role of marginal zone macrophages and marginal metallophilic macrophages? (4) Why the draining blood enters the portal vein, then to the liver? (5) Is the spleen really not necessary for host defense? Temporary answers after discussion were (1) MadCAM1 on endothelial cells of the marginal sinus may be a candidate. Sialoadhesin on marginal metallophilic macrophages may also be involved. (2) These cells become antibody-forming cells to encapsulated bacteria. Marginal zone B cells are also involved in antigen presentation. (3) Still unclear. (4) Some effects of PDGF from platelets in the red pulp on hepatocyte growth were suggested. (5) Splenectomy delayed hepatic resistance to *lesihmaniasis* and hepatic granuloma formation. Patients with anatomic or functional asplenia are at particular risk of severe sepsis due to encapsulated bacteria and from malaria.

2. H. Tsurui (Juntendo, Japan) developed a hyper-multicolor (more than 7 color) fluorescence imaging method based on Fourier spectroscopy and singular value decomposition. When mice received CFSE-labeled apoptosis-induced cells, most of signals from CFSE were detected in the macrophages in red pulp ($F4/80^+$, $BM8^+$, $Mac3^-$, $MOMA2^-$), follicle and PALS

($F4/80^-$, $BM8^-$, $Mac3^+$, $MOMA2^+$). All the DCs that engulfed CFSE-labeled cells were $CD8^+CD11b^-$ cells. In contrast, in case of Zymosan, a fairly good portion of the particles were ingested by $CD11b^+$ DCs. DCs in the spleen showed very low phagocytotic activity for E. coli and all the DCs engulfed the bacteria were $CD11b^+$.

3. P. Lane and his colleagues (Birmingham, UK) showed a presence of unique $CD4^+CD3^-$ stromal cells in the outer PALS and lymph follicle of spleen that interact with primed and memory T cells. These cells are distinguished from conventional DC by their lack of response to Flt3 ligand and their inability to process antigen. Unlike DC, the $CD4^+CD3^-$ cells have little CD80 or CD86 expression but do express high levels of the TNF ligands, OX40 ligand and CD30 ligand. Th2-primed cells express the receptors for these TNF ligands and preferentially survive when cocultured with these cells. Furthermore, the preferential survival of $OX40^+$ T cells and the support of memory T cell help for B cells are linked to their association with $CD4^+CD3^-$ cells in vivo.

4. J. Kearney's group (Birmingham, AL, USA) presented a specialized subpopulation of B cells, splenic marginal zone (MZ) B cells and B1 lymphocytes. MZ B and B1 cells participate jointly in the early immune response against T independent (TI) bacterial capsular antigens, by interacting with $CD11c^{low}$ immature dendritic cells, which capture and transport bacteria from blood to the spleen. $CD11c^{low}$ DCs provide critical survival signals, TAC1, to antigen specific MZ B cells and promote their differentiation into IgM secreting plasmablasts.

5. P. Kaye and his colleagues (London, UK) reported a persistent chronic infection model of murine visceral *leishmaniasis* generated in the spleen. They found loss of marginal zone macrophages and associated depression in T cell migration; loss of T zone stromal cells and T zone production of CCL21 and CCL19; and deficient T zone migration of DCs, resulting from TNFα-dependent, IL-10-mediated inhibition of CCR7 expression. The elevated number of splenic DCs with migratory phenotype was due to stroma from infected mice, but not uninfected mice. Infected stroma had the capacity to support DC development via cognate interaction in the absence of exogenous cytokines. The DCs that develop under these conditions had an immature $CD11c^{low}$ phenotype and uniformly express CD11b.

Session 3: Trafficking of macrophages and DCs in immune responses I

1. During T cell development, thymocytes migrate from the cortex to the medulla within the thymus and emigrate to the circulation. Y.Takahama's group (Tokushima and RIKEN, Japan) have devised a time-lapse visualization system to directly evaluate the migration of developing thymocytes. They firstly showed that chemokines CCL19 and CCL21 play a major role in the emigration of mature thymocytes into the circulation in newborn mice. Impressive movies using two-photon laser microscopy were presented next showing very dormant movement of immature T cells in the cortex. This was impaired when using $TCR^{-/-}$ thymocytes, suggesting the importance of TCR signal in controlling such motility. They concluded that TCR-mediated self-recognition in the cortex contributes to initiating the relocation of positively selected T cells out of the cortex to the medulla.

2. Atherosclerosis is developed by a chronic inflammatory process in which monocytes interact with dysfunctional endothelium of arteries. K. Ley and his colleagues (Virginia, VA, USA) have investigated the arrest of monocytes on endothelial cells under flow conditions. They firstly introduced that monocyte arrest is triggered by immobilized chemokines such as CCL5 on microvascular or aortic endothelium. Using stroboscopic epifluorescence video

microscopy, they next showed that circulating activated platelets and platelet-monocyte aggregates interact with atherosclerotic lesions. Activated platelets deliver CCL5 on endothelial surface, promote monocyte binding of vascular cell adhesion molecule (VCAM)-1, and increase monocyte adhesiveness to inflamed endothelium. Injection of P-selectin$^{-/-}$ platelets decreased monocyte arrest on the surface of atherosclerotic lesions in apolipoprotein-E-deficient mice. These results demonstrate that platelet P-selectin-mediated delivery of chemokines to monocyte and vessel wall is a crucial factor in accelerating monocyte recruitment to form atherosclerosis.

3. G. MacPherson's group (Oxford, UK) has been studying the migration of DCs in pseudo-afferent intestinal lymph in the rat system. (DCs in the intestinal lymph were obtained by cannulating the thoracic duct after mesenteric lymphadenectomy.) They showed constitutive migration of DCs from the intestine to mesenteric lymph nodes via afferent lymphatics. Steady-state OX41^{-} DCs can transport apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes, suggesting a role of this population in inducing self-tolerance. In contrast, inflammatory stimuli increase OX41^{+} DC exit from the gut into T cell areas of lymph nodes, suggesting this subset to be immunogenic type. In mouse transmissible spongiform encephalopathy (TSE) model, DCs can sample disease-associated forms of prion protein (PrPsc) from the gut lumen and carry them to mesenteric lymph nodes possibly leading to TSE replication. These studies prompted us to reconsider the mechanisms regulating homeostatic DC migration and its relationship to peripheral tolerance.

4. H.Yoneyama in Matsushima's laboratory (Tokyo, Japan) previously reported in vivo trafficking of murine circulating myeloid DC (mDC) precursors in a granulomatous liver disease model. In this meeting, He showed in vivo fate of murine plasmacytoid DC (pDC) precursors in herpes simplex virus infection model. Adoptive transfer experiments reveal that pDC precursors can directly transmigrate across high endothelial venule of inflamed LNs in a CXCL9 and E-selectin dependent manner. PDCs subsequently contact to antigen-presenting mDCs in T cell areas of draining lymph nodes. Finally, they showed recruitment of pDCs in the LNs is needed to anti-viral CTL induction by mDCs. This study unveils sophisticated orchestration of mDCs and pDCs in a host defense through distinct function and trafficking pathway.

Session 3: Trafficking of macrophages and DCs in immune responses II

1. T. Geijtenbeek and his collaborators (Amsterdam, Neth) have revealed the importance of carbohydrate recognition for DC functions. DC-SIGN, identified as a DC-specific C-type lectin, is a universal pathogen receptor that captures HIV-1, Ebola virus, cytomegalovirus, and mycobacteria, etc. Although DCs are vital in the defense against pathogens, it is now becoming evident that some pathogens subvert DC functions to escape immune surveillance. He presented recent efforts on DC-SIGN, especially its unique role as an inhibitory signal transducer for DC maturation. DC-SIGN captures and internalizes intact *Mycobacterium bovis* through the mycobacterial cell wall component mannose-capped lipoarabinomannan (ManLAM). ManLAM binding to DC-SIGN prevents mycobacteria- or LPS-induced DC maturation. Their results suggest that mycobacteria targets DC-SIGN both to infect DCs and to down-regulate DC-mediated immune responses, especially Toll-like receptor-mediated DC maturation.

2. Heparan sulfate proteoglycans (HSPGs), a major component of basement membranes, support the mechanical integrity and serve as reservoirs of growth factors and cytokines.

Therefore, degradation of HSPG should be a critical step in the regulation of many biological events including transendothelial migration. N. Higashi and his collaborators (Tokyo, Japan) studied regulatory mechanism of heparanases (heparan sulfate-specific endo-ß-glucuronidases) in macrophages, and indicated that cellular distribution of the enzyme potentially regulates the activity. Phorbol myristate acetate-treated U937 cells express the heparanase on the cell surface. The enzyme is accumulated into a restricted region that is thought to be lipid microdomains after adhesion, and redistributes along the gradient and toward the higher concentration of chemoattractants during migration. The results together with invasion inhibition test using anti-heparanase mAb suggest that the heparanase at the invasive edge is involved in monocyte transendothelial migration.

3. Comprehensive understanding of biological events will be possible by monitoring global RNA expression profile during the processes. T. Kodama's group (Tokyo, Japan) has established a comprehensive normal human tissue RNA expression database. By using this, they successfully describe macrophage differentiation as changes of RNA expression of a number of genes. Importance of time course was emphasized in the lecture. According to the comprehensive data, there are distinct sets of genes that classify early and late stages of macrophage differentiation. An example is the expression of adhesion molecules; ICAM-1 is expressed in the early stage, whereas VCAM-1 in the late stage. The difference is partly correlated with the involvement of GATA-3 transcription factor. Their database is a powerful tool to describe many cellular events such as cell differentiation and activation *in silico*.

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http://macro.dokkyomed.ac.jp/mp2003/ or http://www.prevent.m.u-tokyo.ac.jp/macrophage.html

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