

The 26th International Symposium of Sapporo Cancer Seminar: Innate immunity in cancer and infectious diseases

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The 26th International Symposium of the Sapporo Cancer Seminar was held at Hokkaido University, Sapporo, Japan, on July 21–23, 2006. More than 120 researchers from various countries participated in the meeting (Fig. 1). The weather was fine every day, and the participants enjoyed walks around the university campus and dining at Chara Restaurant and Kirin Beer Garden. The symposium consisted of seminars presented by leading researchers from Japan and overseas in the field of innate immunity. Oral presentations were given by 12 researchers and poster presentations were given by 21 researchers. The symposium was organized by Dr Tsukasa Seya (Hokkaido University, Japan), as the chairman, and committee members Drs Misako Matsumoto, Hirofumi Sawa, Tokiyoshi Ayabe, and Roberto Cattaneo. Ms Saoko Kume was particularly involved in the organization of this meeting.

Sapporo Cancer Seminar

The international symposiums of the Sapporo Cancer Seminar are funded by a foundation established almost 30 years ago by Dr Hiroshi Kobayashi, a Professor Emeritus at Hokkaido University. Dr Kobayashi had been inspired by the academically liberal atmosphere of the Gordon Research Conference held in New Hampshire, USA, and conceived the idea of the Sapporo Cancer Seminar to provide opportunities for medical researchers to share their knowledge in a relaxed atmosphere. The first Sapporo Cancer Seminar was held in 1981, and the Sapporo Cancer Seminar was established as a foundation in 1983 with the support of businesses, pharmaceutical organizations and the general public. Since 1987, the Sapporo Cancer Seminar has been a twice-yearly event, with seminars held in winter and summer. The winter seminar is scheduled to coincide with Sapporo's Snow Festival, an internationally famous event, and is focused on cancer-related clinical investigations, whereas the summer seminar is focused on basic aspects of cancer research.

Innate immunity in cancer and infectious diseases

The field of innate immunity has rapidly expanded since the discovery of the Toll-like receptor (TLR) and establishment of the concept that microbial pattern-recognition receptors trigger activation of the immune system. This concept was first proposed by Dr Charles Janeway Jr in 1989 in the Cold Spring Harbor Symposium. In 1997, his first report on human TLR was published in *Nature* and led to a breakthrough in this field. Many immunologists, virologists and microbiologists became involved in research on TLRs. During this period of expansion, it was found that the peptide vaccine for cancer immunotherapy was effective in only 2.6% of patients who were vaccinated. Results obtained by Rosenberg *et al.* (published in *Nature*

Medical in 2004) suggested that simultaneous stimulation of the innate system with peptide loading is a prerequisite for efficient elicitation of effector lymphocytes, such as cytotoxic T cells, antibody-producing B cells and natural killer (NK) cells. The molecular mechanisms by which these effectors are activated remained unknown, but the importance of TLRs on dendritic cells for driving T, B and NK cell activation has been widely accepted. Dr Akira's TLR knockout mice have facilitated the identification of TLR ligands and their signal pathways. Now we have a map of the molecular pathways of TLR signaling and cellular outcome in dendritic cells. We hope that the current knowledge on TLRs will be helpful to develop TLR agonists for clinical use as antitumor therapies for cancer and infectious diseases.

Topics at the 26th International Symposium

Twelve excellent oral presentations were given at the meeting.

Dr Bowie (Trinity College, Dublin) identified the function of the fifth Toll-IL-1R (TIR) homology domain-containing adapter, which is sterile alpha motifs and beta-catenin/armadillo repeats (SARM). SARM acts as a negative regulator of TIR-domain-containing adapter-inducing interferon (IFN)- β (TRIF)-mediated responses, including the pathway for induction of type I IFN. He has been studying the vaccinia virus TIR-containing protein (A52R), which acts as an antagonist of TLR signaling that induces nuclear factor (NF)- κ B activation. He reported that the additional vaccinia virus TIR-containing protein (A46R) was found to antagonize TRIF (also called TICAM-1) signaling that induces interferon regulatory factor (IRF)-3 and IFN- β promoter activation. He extended this work to the function-unknown TIR-containing protein SARM. SARM is now known to be an endogenous inhibitor of the TLR3/4-TRIF-dependent pathway.

Dr Seya (Hokkaido University, Sapporo) reported on the TLR pathway in dendritic cells leading to activation of NK cells by tumor biology method. Tumor cells tend to be diverged into major histocompatibility complex (MHC)-negative and -positive populations. CTL is a potent effector to kill MHC-positive cells. However, MHC-negative cells generally circumvent CTL-mediated killing. To eradicate MHC-negative cells by an immunological strategy, adjuvant agents that confer the directions on dendritic cells to activate NK cells must be established. Dr Seya showed that TLR signaling in dendritic cells determines the directions for activation of CTL or NK cells by selecting TLR adapter proteins.

Dr Kariko (University Pennsylvania, Philadelphia) gave an interesting presentation about nuclear modification in association with immunogenicity and translation-proof. Her results showed that unmodified RNA is immunogenic and responsible

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Fig. 1. Participants at the 26th International Symposium of the Sapporo Cancer Seminar "Innate Immunity in Cancer and Infectious Diseases", Hokkaido University, Sapporo, Japan, July 21–23, 2006.

for IFN- α induction. The aim of her research is to develop new vaccination modalities. An interesting finding is that pseudouridine-modified mRNA is more efficiently translatable than is natural (unmodified) mRNA.

Dr Fujita (Kyoto University, Kyoto) presented results of his epoch-making study on retinoic acid-inducible protein-I (RIG-I) and its family members, melanoma differentiation-associated gene-5 (MDA5) and RIG-I-like RNA helicase (LGP2). Dr Fujita also mentioned his recent work on the RIG-I-binding motif in the RNA duplex. The presence of an open 3'-stretch in the double-stranded RNA facilitated RIG-I binding to the RNA and initiated the RIG-I-mediated induction of type I IFN. He found a new RIG-I activator, AP20187, a small synthetic compound that assembles in RIG-I multimers.

Dr Cattaneo (Mayo Clinic, Rochester) attempted to develop virotherapy focusing on measles virus. The measles virus uses two receptors, CD46 and CD150, for entry into cells. Lymphoma and lymphatic malignancies often express these two receptors, enabling measles virus to target the tumor cells selectively. Dr Cattaneo produced measles targeting designated receptors. He found the measles virus without the host control evasion proteins replicate efficiently only in transformed cells with defective IFN response.

Dr Matsushima (University Tokyo, Tokyo) tried to clarify the mechanism by which memory T cells are formed after viral infection. His study revealed a novel function of dendritic cells, which explains the immunological missing link between innate immune activation and provoking acquired responses. The role of plasmacytoid dendritic cells in antigen presentation by myeloid dendritic cells appears to be crucial. He found by confocal technology that plasmacytoid dendritic cells physically interact with myeloid dendritic cells in draining lymph nodes when viral infection starts.

Dr Seth (South-western Medical Center, Dallas) added some new data to her recent finding on mitochondrial antiviral signaling (MAVS) recently published in *Cell*. MAVS is an adapter protein that links to RIG-I downstream of the RIG-I signaling pathway. RIG-I recognizes an RNA duplex and induces IFN- α / β . This pathway is blocked by viral factors in hepatitis C virus (HCV) infection. Her group identified the mechanism whereby HCV inhibited RIG-I-mediated IFN induction. NS3/4, a protease of HCV, efficiently cleaved MAVS into an inactive form, thereby facilitating escape of HCV from host immunity. MAVS and TICAM-1 (TRIF) are adapters lying downstream of virus pattern-recognition receptors, and both appear to be targets for HCV.

Dr Shimotohno (Kyoto University, Kyoto) presented two interesting topics. He has searched for a cellular factor that suppresses replication of the HCV genome and found that cyclosporine B, but not A, functionally enhances HCV RNA polymerase, resulting in up-regulation of HCV genome replication, a finding that was published in *Molecular Cell* recently. The second topic is related to a regulatory mechanism of the RIG-I pathway. Ubiquitin conjugation to RIG-I is suppressed by UbcH8, and the suppression is restored by co-expression of ISG-15. RIG-I may be a target of ubiquitination via RNF125 (Ringfinger-125), an E3 ligase. RNF125 functions with UbcH8, a ubiquitin E2 enzyme (conjugator), and this association is regulated by ISG-15. ISG-15 is induced by type I IFN and modifies many proteins, including RNF125. This schema may fit the fact that the intracellular RIG-I content is minimal in normal cells and is rapidly up-regulated in response to IFN production. When RIG-I is ubiquitinated, RIG-I-mediated IFN-induction is shut down.

Dr Nishijima (Doshisha, Kyoto) utilized biochemical strategies to identify the inflammasome. He focused on TLR4 ligands with high versus low toxicity. Monophosphoryl lipid A (MPL) works as a less toxic adjuvant than lipid A. MPL is capable of activating the TLR4-MyD88 pathway but notably incapable of activating caspase 1, in contrast to the known properties of lipid

A, which activates both MyD88 and caspase 1. As the inflammasome involves caspase 1, caspase 2, intracellular microbial sensors a cytoplasmic protein family with pyrin domain (PYD) (NALPs) and apoptosis-associated speck-like protein containing CARD (ASC), he analyzed molecular assembly occurring in RAW mouse macrophages secondary to MPL stimulation.

Dr Wagner (University of Munich, Germany) presented results of his recent study on TRAF3 and its association with the TLR pathway. He showed that TRAF3 is essential for TLR-mediated induction of type I IFN and interleukin (IL)-10. He also suggested that IRF-1 comes into play in IFN induction downstream of myeloid differentiation factor MyD88-IRAK1. MyD88 and IRF-1 colocalize in the cytoplasm, and nuclear translocation of IRF-1 occurs in response to stimulation with TLR9 ligand CpG DNA.

Dr Akira (Osaka University, Osaka) presented results of analyses in his knockout mice. He focused on the mechanism of type I IFN induction by viral infection. As two major adapters of TLR, MyD88 and TRIF, are not involved in type I IFN induction in mouse embryonic fibroblasts (MEF) prepared from knockout mice, he looked for the pathway responsible for virus-mediated IFN induction. He found that RIG-I is a main virus-mediated IFN inducer. This is true in myeloid dendritic cells. However, in plasmacytoid dendritic cells, a MyD88-containing molecular complex plays a key role in IFN induction. Data obtained from the knockout mice strongly suggest the presence of a cell type-specific IFN-inducing pathway for viral infection. He also identified the adapter for RIG-I, named IPS-1 (MAVS), and delineated the pathway initiating RIG-I/MDA5 to IFN induction. He suggested an additional IFN-inducing cytoplasmic pathway for recognition of the B-form of DNA. A variety of receptors appear to discriminate viral nucleotide patterns in a virus species-specific manner.

Dr Nunez (University of Michigan, Ann Arbor) focused on the NOD-like receptor (NLR) family. He first summarized the functions of NOD1 and NOD2, which recognize bacteria-specific peptides containing diaminopimelic acid and D-isoglutamic acid, respectively. His main talk was on NALP3 (cryopyrin) and ICE-protease activating factor (IPAF). He found that *Salmonella flagellin* mutants, particularly Δ FlhC, do not induce IL-1 beta secretion. Flagellin is a bacterial protein injected into host cells via type III secretion. Caspase 1 participates in activation (proteolytic cleavage of the propeptide of IL-1 β) of IL-1 β and extracellular liberation from the cytoplasm. His finding suggests that IPAF but not TLR5 intracellularly recognizes flagellin to activate caspase 1. More exactly, the IPAF-ASC-caspase 1 pathway is activated to induce the active form of IL-1 β . The same activation pathway of IL-1 β is shared by IL-18. Another topic was the requirement of cryopyrin for IL-1 β /IL-18 secretion induced by R837 and R848, two antiviral synthetic purine-like compounds in macrophages. IL-6 and TNF- α secretions are not affected by cryopyrin and these compounds. His subsequent report suggested that cryopyrin recognizes bacteria/virus-specific RNA and activates caspase 1. Results of *in vivo* knockout mouse studies supported his idea that the IPAF-ASC-caspase 1 pathway actually functions to activate IL-1 β and IL-18.

Social program: Barbecue dinner at Kirin Beer Garden

Lunches and dinners were served free according to the plan of the committee. The participants had lunch on July 22 and 23 at Chara Restaurant in the campus of Hokkaido University. Japanese-style food was served with drinks. In the evening of July 22, all participants enjoyed conversation over a barbecue dinner of lamb at Kirin Beer Garden. Japanese sake as well as several other drinks were provided and significantly enhanced the friendly atmosphere. Ms Kume and Ms Satoh were involved in the organization of these events. I appreciate their sincere efforts.

Conclusion

The 26th International Symposium was a great success. With the help of the members of the Organizing Committee and the Sapporo Cancer Seminar Foundation, we were able to organize an outstanding scientific program in the field of basic immunology and its clinical applications. We hope that the fruitful outcome of this meeting will facilitate research interchange among scientists and universities, which may result in generation of appropriate adjuvants for cancer or vaccine immunotherapy.

On behalf of the Organizing Committee, the author extends appreciation to all of the participants for their contribution to the international symposium.

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