suppression such as side effects or infection. We have reported the results of our long-term research about transplant tolerance, mainly induced by blocking of T-cell costimulatory signals. In 2005, we reported a protocol that makes possible long-term (>2 years) tolerance in monkey allogenic kidney transplantation (JCI 115:1896-902, 2005). Currently, we are performing clinical trials of the tolerance induction in living-donor kidney transplants as collaboration with Tokyo Women's Medical University and Juntendo University. We plan to apply same protocol to liver transplant cases in the near future in Hokkaido.

With the recent establishment of pluripotent stem cells (ES or iPS cells), hopes for development of regenerative medicine are growing. Although it is expected that immunological rejection will not occur when using iPS cells, the need for immunosuppression will still continue to exist with a framework of stem cell banking. In the future, new kinds of research fused with the study of organ transplantation including immunological tolerance will be necessary to advance clinically-oriented regenerative medicine. For instance, one new strategy is to use pluripotent stem cells to manufacture cells to serve as the source of tolerance, and administer these cells to recipients along with ES/iPS cell-derived transplant tissues.

This lecture will address mechanisms of transplant tolerance, expertise accumulated thus far through our clinical practice, as well as future possibilities for immune-regulation in the age of regenerative medicine.

LL3-6 The many roles of apoptotic cell death in the immune system

Chairperson: Shin Yonehara

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Apoptosis is a genetically programmed process for killing unwanted or dangerous cells. It plays a critical role in the immune system, reflected by the observation that defects in this process can cause autoimmune disease or lymphoid malignancies. Vertebrates have two distinct but ultimately converging apoptotic pathways. One is initiated by death receptors, members of the TNF-R family with an intra-cellular 'death domain' and requires the cysteine protease, caspase-8, and its adaptor FADD. The other pathway is triggered by developmentally programmed cues, growth factor deprivation and cytotoxic stimuli and is regulated by the interplay of pro- and anti-apoptotic members of the Bcl-2 protein family. We will present data from our analysis of multiple gene-targeted and transgenic mice on the identification of the functions of Bcl-2 family members and death receptors in the control of apoptosis of cells of the immune system, with emphasis on their roles in autoimmune disease and lymphoma/leukaemia.

LL3-7 Succesful Immunotherapy of established lesions induced by high risk HPV

Chairperson: Toshimitsu Uede

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Therapeutic vaccination with a synthetic long peptide (SLP®) vaccine mediated the eradication of established human papilloma virus type16 (HPV16)-positive tumors in mice and controlled wart growth and latent virus infection in rabbits persistently infected with cottontail rabbit papilloma virus. Subsequent phase I/II studies with an HPV16 SLP® vaccine, consisting of 13long peptides covering the HPV16 E6 and E7 antigens, in patients with advanced HPV16-positive cervical cancer, revealed that this vaccine was safe and highly immunogenic. The purpose of the current study was to test the clinical efficacy of this HPV16 SLP® vaccine in HPV16-induced high grade vulvar intraepithelial neoplasia (VIN3), a premalignant epithelial disorder, spontaneous regression of which occurs in less than 2%of patients and in which recurrence after standard treatment is high.

In a phase 2 trial, 20 women with VIN3 were vaccinated three times sc in the limbs with a mix of the HPV16 E6 and E7 synthetic long peptides formulated in Montanide ISA-51. The endpoints were objective clinical responses, defined as reduction of at least 50% in lesion size (partial response) or complete regressions, and HPV16-specific T-cell responses.

The vaccine was safe. At 3 and 12 months after the last vaccination an objective response was observed in 12/20(60%) and 15/19(79%) patients respectively. Nine of them showed a complete and durable regression of the lesions at 12 months and at 24 months. The strength of the vaccine-induced HPV16-specific T-cell response was significantly higher in the group of patients with a complete regression of their lesions compared to non-responders.

WS-045a Regulatory T cells 1

Chairpersons: Sayuri Yamazaki, Steven Ziegler

WS/PP-045a-01 Aryl hydrocarbon receptor (Ahr) potentially linked to indoleamine 2,3-dioxygenase (IDO) in dendritic cells regulates naïve T cell differentiation and proliferation

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Ahr plays an important role in immune responses of T cells and macrophages. However, its function in dendritic cells (DCs) remains unknown. IDO, an enzyme catabolizing tryptophan into kynurenine, is induced in DCs by immune activation. IDO+ DCs are known to induce Treg cells and suppress T cell expansion. Recent studies show the possibility of kynurenine to activate Ahr in some types of cell causing immunosuppressive effects. Therefore, we hypothesize that there is a potential link between Ahr and IDO in DCs resulting in immunoregulation. We investigated Ahr and IDO expression in CpG-ODN-stimulated bone marrow-derived dendritic cells (BMDC) from wild-type (WT) and Ahr-deficient (Ahr−/−) mice. We found that CpG-ODN induces both Ahr and IDO expression in WT BMDC. But IDOmRNA was not induced by CpG-ODN in Ahr−/− BMDC. Blocking IDO by a specific inhibitor 1-methyltryptophan (1-MT) partly suppressed Ahr expression. In addition, surface activation markers were down-regulated and IL-10 production was abrogated in Ahr−/− compared to WT BMDC. Importantly, we demonstrated that stimulated Ahr−/− BMDC inhibited the differentiation of naïve T cells into regulatory T (Treg) cells and promoted the proliferation of naïve T cell. In conclusion, the deficiency of Ahr and IDO in DC may inhibit Treg cell development and the promote naïve T cell proliferation. Whether IDO-linked Ahr in DC participates in generation of Th17/Th1 cells leading to Treg versus Th17/Th1 imbalance-mediated inflammatory diseases is under investigation.

WS/PP-045a-02 Role of poly(ADP-ribose)polymerase-1 in Th17 and regulatory T cell differentiation

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Regulatory T cells (Tregs) maintain immunological self-tolerance and immune homeostasis. Th17 cells are involved in host defense against bacterial and fungal pathogens, and immune-mediated diseases. Recent findings showed that Poly(ADP)Ribose Polymerase-1 (PARP-1) plays an important role in inflammatory/immune responses. We wondered whether PARP-1 might contribute to the balance between regulatory and effector programs. Increased percentages and numbers of regulatory $CD4^+CD25^+/Foxp3^+$ T cells were found in thymus, spleen and lymph nodes of PARP-1KO mice as compared to WT controls. The increased Treg cell frequency at periphery resulted in impaired CD4 cell proliferation and IL-2 production, which could be restored by $CD25^+$ cell-depletion. Tregs from PARP-1KO were able to inhibit cell proliferation and cytokine production in freshly isolated $CD4^+CD25^-$, Th1- and Th2-polarized cells as efficiently as controls. Also their phenotype was similar, indicating that PARP-1 affects Treg cell differentiation rather than function. Purified naïve CD4 cells from PARP-1KO mice, stimulated *in vitro*, expressed Foxp3 mRNA at higher level and generated a higher number of $Foxp3^+$ cells (inducible Tregs, iTregs) than the WT counterpart. This finding was due to a higher rate of naïve CD4 cell to $Foxp3^+$ iTreg cell conversion rather than to higher resistance to apoptosis induction. Interestingly, PARP-1 deficiency did not affect RORγT mRNA expression, IL-17 production and differentiation of purified naïve CD4 cells to Th17 cells. PARP-1KO iTreg cells showed features similar to control cells. In conclusion, our findings demonstrate that PARP-1 affects Treg cell differentiation and open new perspectives on potential targets for modulating immune responses.

WS/PP-045a-03 TNF preferentially up-regulates expression of co-stimulatory TNFR superfamily members on CD4+FoxP3+ regulatory T cells

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TNF is a pleiotropic cytokine with intriguing biphasic pro-inflammatory and anti-inflammatory effects. Our previous study demonstrated that, in the presence of IL-2, TNF had the capacity to up-regulate FoxP3 expression and selectively activated and expanded CD4+FoxP3+ regulatory T cells (Tregs) by utilizing TNFR2 (J Immunol. 179:154). Furthermore, we found that TNFR2 expression identified the most suppressive subset of Tregs (J Immunol. 180:6467). In this study, we showed that TNF, in concert with IL-2 or some other common γ chain cytokines, preferentially up-regulated TNFR2 expression on Tregs. TNF also up-regulated the expression of T cell co-stimulatory TNF receptor superfamily (TNFRSF) member 4-1BB and OX-40 on Tregs, but not on FoxP3- effector T cells (Teffs). TNF-mediated up-regulation of 4-1BB and OX-40 was functional, since their agonist antibodies stimulated the proliferation of Tregs, while preserving and even enhancing the suppressive activity of Tregs. In addition in vivo treatment with LPS markedly increased the proportion of FoxP3+ cells in splenic CD4 cells, which was associated with up-regulation of expression of TNFR2, 4-1BB and OX40 on Tregs. Treatment with neutralizing anti-TNF Ab markedly blocked LPS-mediated increase in the proportion of Tregs present in splenic CD4 cells ($p<0.05$), suggesting that TNF attributed in part to the expansion of Tregs after LPS challenge. Our data indicate that TNF preferentially up-regulates members of co-stimulatory TNFRSF on Tregs, which may represent a positive feedback mechanism magnifying the stimulatory effect of TNF in the activation of Tregs.

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WS/PP-045a-04 The role of neuritin in regulating the immune response

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Regulatory T cells (Tregs) have been described in many autoimmune and infectious disease models and are a mechanism for the maintenance of self-tolerance and protection from autoimmune disease. Unfortunately Tregs also pose an obstacle for anti-cancer immunotherapies as they promote T cell inactivity (anergy) in response to tumor antigens. Therefore understanding the mechanisms of Treg-mediated immune suppression is critical for improving immunotherapies for both cancer and autoimmune disease. To explore the molecular mechanism by which Tregs exert their function, we used Affymetrix gene chip expression profiling to identify molecules unique to the Treg phenotype. We found a surface GPI-anchored molecule called Neuritin was specifically expressed by natural Tregs and anergized T cells, but not memory or activated T cells. While crucial for some neuronal processes, Neuritin's role in the immune system was completely unexplored. Here we report that Neuritin dramatically modulates immune cell function. Transgenic mice having T cells over-expressing the molecule develop less severe disease in the Experimental Autoimmune Encephalomyelitis (EAE) model of Multiple Sclerosis. Dissection of the cellular participants in the EAE response revealed that Neuritin blockade with monoclonal antibody impacts Treg homeostasis and myelin-reactive T cell proliferation potentially worsening disease severity. Findings from other *in vivo* and *in vitro* approaches resonate with and elaborate these results supporting the notion that Neuritin significantly contributes to Treg-mediated control of the immune response. Neuritin's clear immune regulatory action makes this newly identified Treg factor an intriguing potential target for manipulation in novel interventions for numerous diseases including cancer and autoimmunity.

WS/PP-045a-05 P-selectin glycoprotein ligand-1 (PSGL-1) controls suppressor activity by CD4+CD25+FOXP3+ regulatory T cells

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Mucin P-selectin glycoprotein ligand-1 (PSGL-1) is involved in leukocyte trafficking under physiological and pathological conditions. Whereas PSGL-1 role is emerging in T cell homeostasis, controversial results were obtained in animal models of autoimmune diseases. Our goal was to clarify the role of PSGL-1 in T cell responses during experimental autoimmune encephalomyelitis (EAE).

Results: EAE induction was more severe in the presence of PSGL-1 deficiency suggesting a role for PSGL-1 in regulatory mechanisms. We next studied the role of PSGL-1 in the suppressor activity exerted by CD4+CD25+FOXP3+Tregs by using two-photon laser microscopy (TPLM) and visualized the behavior of encephalitogenic CD4+ T cells in the presence or absence of Tregs in intact lymph nodes during early (day 1 post-immunization) and late (day 7 post-immunization) preclinical phase of EAE. Myelin-Oligodendrocyte Glycoprotein (MOG) but not ovalbumin-primed T cells showed a significant increase in their speed and a decrease of the arrest coefficient in the presence of WT Tregs in draining lymph nodes of immunized mice. Interestingly, PSGL-1−/− Tregs failed to modulate the motility behavior and proliferation of MOG35-55 CD4+ T cells during late but not early preclinical phase of EAE. Moreover, PSGL-1 deficient Tregs showed reduced migration to the central nervous system (CNS) and adhesive interactions in brain venules in the preclinical phase of disease. Finally, PSGL-1 deficient Tregs lost the capacity to suppress EAE.

In conclusion our data suggest that PSGL-1 has a key role in CD4+CD25+FOXP3+Treg migration in the CNS and suppression of late antigen-dependent T cell activation.

WS/PP-045a-06 SOCS1 is necessary for stable expression of Foxp3 and suppression of IFN-gamma production in nTregs

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We have shown that T cell specific-SOCS1-deficient (SOCS1-cKO) mice develop mild autoimmune phenotypes such as dermatitis and autoantibody production, although the number of CD4+Foxp3 regulatory T cells (Tregs) in SOCS1-cKO mice was higher than that in wild type (WT) mice in both central and periphery. To clarify the functional role of SOCS1 in Tregs, in vitro and in vivo suppression assay was performed. In vitro suppression activity of SOCS1-deficeint Tregs was similar to that of WT Tregs. Then naive CD4+T cells were transferred into Rag2-deficient mice together with nTregs (CD4+CD25+ or Foxp3-GFP+) cells. WT nTregs efficiently suppressed colitis induced by naive CD4+T cell transfer. However, SOCS1-deficeint nTregs could not prevent it. Then, to examine the fate of nTregs, Foxp3-GFP+ T cells were transferred into Rag2−/− mice. Six weeks after transfer, Foxp3 expression was retained in 36% of expanded SOCS1-cKO T cells in LN, in comparison with 56% of WT T cells (n=3). These data suggest that SOCS1-deficeit Tregs loses Foxp3 expression more rapidly than WT nTregs in vivo. SOCS1-deficeint nTregs produced higher levels of IFN-g during culture in vitro in the presence of IL-2 and IL-12. STAT1 was hyperactivated in SOCS1-deficeint nTregs without any stimulation. Finally, Foxp3 was more stable in IFNg−/− nTregs as well as IFNg−/−SOCS1−/− nTregs both in vivo and in vitro. These results suggest that SOCS1 plays important role in the stable expression of Foxp3 and suppression of IFN-g in Tregs by repressing excessive STAT1 signaling.

WS/PP-045a-07 TRAF6 controls commitment to regulatory T-cell lineage and T-cell receptor signals in thymocytes

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Naturally occurring regulatory T cells (T_{reg}s) are crucial for self-tolerance. However, the molecular mechanisms underlying their development remain largely unknown. TNF receptor-associated factor 6 (TRAF6) transduces signals from various cell surface receptors such as Toll/IL-1 receptor family and TNF receptor super family members to induce activation of NF-κB and AP-1. TRAF6-deficient mice show severe autoimmune-like phenotype and thymic $Foxp3^+$ T_{reg}s are almost absent even though development of conventional T cells is not impaired, suggesting that TRAF6-signaling directs the thymic T_{reg} development. We previously found that TRAF6 is essential for the development of medullary thymic epithelial cells (mTECs), which were proposed to induce thymic T_{reg} development. In order to determine cell types responsible for the lack of T_{reg}s in TRAF6-deficient thymus, we grafted fetal thymus to nude mice and generated hematopoietic cell chimeras. Although TRAF6 was required for the mTEC development, the absence of TRAF6 in thymic stroma did not largely affect T_{reg} development. Instead, mixed fetal liver transfer experiments revealed a cell autonomous requirement for TRAF6 in thymic T_{reg} development. To elucidate the mechanism of thymocyte-intrinsic TRAF6-signaling, we addressed if signals from T cell antigen receptor (TCR) are dependent on TRAF6. Indeed, we found that TCR-induced NF-κB and JNK activations were severely impaired by the absence of TRAF6 in thymocytes. On the other hand, TCR-induced Akt and ERK activations were rather enhanced in TRAF6-deficient thymocytes. Overall, our results suggest that the TRAF6 determines the commitment to the T_{reg} cell lineage by modulating TCR-induced signals in thymocytes.

WS/PP-045a-08 The role of regulatory T cells in the sensitization phase of mouse contact hypersensitivity

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Regulatory T cells (Tregs) play essential roles for the regulation of immune response in a variety of pathophyisiological conditions. Contact hypersensitivity (CHS) is one of the most frequently used mouse models on cutaneous immune responses, which consists of sensitization and challenge phases. It has been reported that transfer of Tregs leads to impaired CHS responses, suggesting that Tregs play suppressive roles in CHS. However, it remains unclear whether endogenous Tregs play such suppressive roles. In this study, we investigated the role of endogenous Tregs in CHS using Foxp3hCD2/hCD52 mice. Foxp3hCD2/hCD52 mice express human CD2 and human CD52 chimeric protein on cell surface of Foxp3+ Tregs. Therefore, Tregs can be conditionally depleted with neutralizing anti-human CD52 antibody. Using these mice, we depleted Tregs during sensitization or challenge phase, and examined CHS response. Mice depleted with Tregs either sensitization or challenge phase showed significantly increased ear swelling responses compared with the control mice. The number of memory CD4+ T cells and CD8+ T cells and IFN-g production in the draining lymph nodes (LNs) were significantly increased in Tregs depleted mice. The expression level of CD86 and CD80 on CD11c+ MHC class II+ cells was higher in Tregs depleted mice. Furthermore, the expression of IL-12 mRNA in migrated DCs was higher in Treg depleted mice compared with the control mice. Immunohistochemical analysis revealed that Tregs were in immediate contact with DCs in LNs. These results suggest that endogenous Tregs play suppressive role in CHS by down-regulating co-stimulatory expression and cytokine production on DCs.

WS-045b Regulatory T cells 2

Chairpersons: Naganari Ohkura, Takayuki Yoshimoto

WS/PP-045b-09 Impact of IPEX mutations on regulatory T cell development, homeostasis and function

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The molecular mechanisms by which the transcription factor Foxp3 controls regulatory T cell (T_{reg}) development and function remain elusive. To address this question, we have been investigating how Foxp3 gene mutations identified