007 Essential requirement for IRF7 in autoantibody production but not development of glomerulonephritis in murine lupus

T Mizoguchi and H Asada Department of Dermatology, Nara Medical University, Kashihara, Japan

Using a murine model of systemic lupus erythematosus (SLE) induced by 2,6,10,14-tetramethyl-2,6,10,14-hexamethylene disulfoxide (THD), we previously reported that interferon regulatory factor 7 (IRF7) deficient mice failed to produce autoantibodies such as anti-dsDNA, anti-dsDNA, anti-nRNP and Sm autoantibodies. TMDP induced apoptosis similarly in wild-type (WT) and IRF7 deficient mice, suggesting that the dysregulation of apoptosis was not involved in the pathogenesis of SLE-like symptoms in these mice. Total IgG and IgM levels did not differ significantly between WT and IRF7 deficient mice after TMDP injection suggesting a specific requirement for IRF7 in autoantibody production. However, the lack of these autoantibodies did not affect the development of glomerulonephritis indicating that autoantibody production and glomerulonephritis are controlled by separate mechanisms in this model. Real-time PCR on spleen cells and flow cytometric analysis on peritoneal cells showed that IRF7 deficient mice expressed substantially lower levels of IFN-stimulated genes as compared with WT mice. However, NF-kB target genes were regulated in both strains, as demonstrated by real-time PCR on spleen cells and ELISA assay on sera. Moreover, inhibition of NF-kB pathway by multiple injections of NF-kB inhibitor in TMDP-treated IRF7 deficient mice markedly attenuated the development of glomerulonephritis confirming the importance of NF-kB pathway in glomerulonephritis. These results suggest that type I IFN pathway was critical in autoantibody production but NF-kB activation was sufficient for development of glomerulonephritis thus demonstrating that autoantibody production and tissue pathologies involve overlapping but not identical transcription pathways and that these two events can at times take place independent of each other. We propose that a concurrent inhibition of these multiple pathways might be a novel strategy in treating human SLE.

009 B10 cells suppress contact dermatitis in an antigen specific manner

M Kamata1, K Candandó1, E Kountikov1, A Yoshizaki1, T Miyagaki1, J Lykkens2, J Fue1, S Sato1 and T Tedder1 1 The University of Tokyo, Tokyo, Japan, 2 Duke University Medical Center, Durham, NC, 3 Department of Dermatology, Tokyo Medical and Dental University, Tokyo, Japan, 4 University of Tokyo Graduate School of Medicine, Tokyo, Japan

B cells secrete antigen-specific antibodies during immune responses to neutralize pathogens and foreign antigens. Despite this well-characterized pro-inflammatory B cell function, a rare B cell subset (B10 cells) negatively regulates inflammation and autoimmunity by producing the inhibitory cytokine interleukin-10 (IL-10). It has been reported that B10 cells have suppressive effects on autoimmune diseases in mouse models. Nonetheless, therapeutic effect on contact dermatitis has not been reported. Furthermore, several signaling pathways are involved in B10 cell development, including the Toll-like receptor, CD40 and B cell antigen receptor (BCR) pathway. Here, we report the development of an IL-10 expressing B cell subset in the lymph nodes of B10 mice. We investigated whether B10 cells could suppress contact dermatitis using a contact hypersensitivity (CHS) mouse model, and whether antigen specificity was required for B10 cell function. B10 cells are so rare as to make experiments difficult. Thereby, we first established regulatory B cells expanding culture system using NIH-3T3-CD154/BLyS cells and B10 cell development, including the Toll-like receptor, CD40 and B cell antigen receptor (BCR) pathway. Furthermore, we report the development of a regulatory B cell subset in the lymph nodes and spleen of B10 mice. Adoptively transferred cultured B10 cells with osazolone-specific BCR could suppress oxazolone CHS inflammation, whereas cultured B10 cells without osazolone-specific BCR did not suppress oxazolone CHS inflammation. Our results suggest that B10 cells have suppressive effect on contact dermatitis. Furthermore, antigen specificity is required for B10 cells to suppress contact dermatitis.

010 FcgRIIb is important for clonal ignorance and prevents pemphigus phenotype in pathogenic anti-desmoglein 3 antibody knock-in mice

H Nomura1, Y Kase1, Y Yamagami1, W Wada1, S Koyama1, H Takahashi1 and M Amano1 1 Dept. Dermatol., Keio Univ, Tokyo, Japan, 2 Japan Blood Products Organization, Tokyo, Japan, 3 RIKEN Center for Integrative Medical Sciences, Tokyo, Japan, 4 Keio Univ, Tokyo, Japan and 5 Dept. Dermatol., Keio Univ./ RIKEN-IMS, Tokyo, Japan

Pemphigus vulgaris (PV) is an autoimmune blistering disease caused by anti-desmoglein (Dsg) IgG. We previously generated a pathogenic anti-Dsg IgG, 2A3, knock-in mice which can potentially undergo class switch recombination of KI immunoglobulin. However, 2A3.KI mice did not develop PV phenotype in steady state, suggesting tolerance works in the mice. Autoreactive B cells are regulated by tolerance mechanisms including, deletion, anergy, receptor editing, and clonal ignorance. The purpose of this study is to clarify which mechanism is important in 2A3.KI mice. Bone marrow (BM) cells from 2A3.KI mice were transferred into wild type or Dsg3−/− mice to analyze whether clonal ignorance of 2A3.KI knock-in B cell in the presence or absence of Dsg3. 2A3.KI B cell development in BM and spleen similarly between both recipients. 2A3.KI B cells from both recipients similarly responded upon anti-Dsg3 IgG stimulation. These results suggested that 2A3.KI B cells were not deleted or anergic, instead stayed in “clonal ignorance”. To further understand whether FcgRIIb contributes to this state, 2A3.KI mice were introduced with FcgRIIb-deficient allele. 2A3.KI−/−FcgRIIb−/− or 2A3.KI−/−FcgRlIl−/− mice spontaneously developed PV phenotype, such as skin erosions, acantholyosis, and IgG deposition on keratinocyte surfaces, with higher levels of anti-Dsg3 IgG compared to 2A3.KI mice. Their survival rate at age of 12 weeks was also significantly lower than that of 2A3.KI mice (39% vs 81%, p = 0.005), together indicating breakdown of clonal ignorance in FcgRllb deficient mice. Thus FcgRllb plays an important role in maintaining clonal ignorance and preventing the development of PV phenotype in 2A3.KI mice.

011 TLR2 deficiency exacerbates imiquimod-induced psoriasis-like skin inflammation through downregulation of regulatory T cells and impaired IL-10 production by regulatory T cells and dendritic cells

M Nakao1, M Sugaya1, H Fujita1, T Miyagaki1, S Morimura1, S Shihata1, Y Ansou1 and S Sato1 1 Department of Dermatology, University of Tokyo Graduate School of Medicine, Tokyo, Japan, 2 Department of Dermatology, Tokyo Medical and Dental University, Tokyo, Japan, 3 Department of Dermatology, University of Tokyo Graduate School of Medicine, Tokyo, Japan and 4 University of Tokyo Graduate School of Medicine, Tokyo, Japan

Emerging evidence has demonstrated that Toll-like receptors (TLR) are associated with autoimmune diseases. We here investigate the role of TLR2 in psoriasis using a mouse model of imiquimod-induced dermatitis. Although TLR2 signaling plays a critical role in the induction of proinflammatory cytokines by dendritic cells (DCs), macrophages and T cells, TLR2 deficient mice unexpectedly exacerbated psoriasis-like skin inflammation. Clinical scores for disease severity and ear thickness were increased in TLR2-deficient mice compared with wild-type mice. Consistently, the lesional skin of TLR2-deficient mice exhibited a larger number of CD4+ T cells compared with that of TLR2-deficient mice. A (mRNA) levels of Foxp-3 and IL-10 were decreased in TLR2 deficient mice. Notably, selective stimulation with Pam3CSK4 (TLR2/1 ligand), but not Pam2CSK4 (TLR2/6), enhanced the proliferation of Tregs, while IL-10 production from Tregs and DCs was increased with either Pam2CSK4 or Pam3CSK4. Finally, adoptive transfer of Tregs into imiquimod-induced psoriasis-like mice restored IL-10 production by Tregs. Taken together, our results suggest that TLR2 signaling directly upregulates the proliferation of Tregs and IL-10 production by Tregs and DCs, suppressing imiquimod-induced psoriasis-like skin inflammation. Enhancement of TLR2 signaling may be a new therapeutic strategy for psoriasis.

012 Activation of 4-1BB signal and co-blockade of PD-1 and TIGIT signaling synergistically enhance melanoma-specific CTL responses during the effector phase

T Inoue1, T Yaguchi1, T Kawamura1, Y Kawakami1 and S Shimada1 1 Department of Dermatology, University of Yamanashi, Yamanashi, Japan, 2 Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan, 3 Dermatology, University of Yamanashi, Yamanashi, Japan and 4 University of Yamanashi, Yamanashi, Japan

Activation of tumor-infiltrating lymphocytes (TILs) is important for the removal of melanoma. Notably, the selective activation of tumor-specific T cells is important to avoid the stimulation of autoimmune responses as a side effect. We identified certain T cell suppressive molecules that are selectively expressed by tumor-specific T cells in tumor environments (PD-1, TIGIT, and Lag3) and revealed that their co-blockade synergistically enhances anti-tumor T cell responses (SID, 2016). In this report, we focused on T cell-activation molecules that are selectively expressed by tumor-specific T cells in tumor environments. We found that the activation receptor 4-1BB was highly expressed by tumor-infiltrating CTLs compared to peripheral blood CTLs in vivo: moreover, there was a clear correlation between the expression levels of PD-1 and 4-1BB in tumor-infiltrating CTLs. We co-cultured melanoma-specific CTLs and melanoma transfectants, which overexpress ligands for PD-1, TIGIT, and 4-1BB (PD-L1, CD155, and 4-1BB) to examine the impact of 4-1BB ligation on tumor-specific CTLs during the effector phase. As previously reported, co-transfection of PD-L1 and CD155 synergistically suppresses CTL activation. In contrast, overexpression of 4-1BB strongly enhances anti-melanoma CTL responses. Also, we confirmed the synergistic effects of 4-1BB and TIGIT signaling and the role of FcgR in the antitumor immunity. We conclude that more effective and safer manipulation of tumor-reactive T cells is possible for improvement of melanoma treatment.