**007**

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

T Mizuguchi 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-tetramethylpentadecane (TMPD), we previously reported that interferon regulatory factor 7 (IRF7) deficient mice failed to produce autoantibodies such as anti-dsDNA, sDNA, rNRP and Sm autoantibodies. TMPD 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 TMPD injection suggesting a specific requirement for IRF7 in autoantibody production. However, the lack of these autoantibodies did not affect the development of glomerulonephritis confirming the importance of IRF7 in 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 pathology involves 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.

**008**

VGLL3 is a key regulator of transcriptome changes in gender biased autoimmune conditions

Y Liang,1,2 X Xing,1 MA Beerer,1 MK Sarkar,2 JJ Voorhees,1 PW Harms,1 JM Kahlenberg2 and JT Gordon

University of Michigan, Ann Arbor, MI

Men and women differ in their regulation of immune responses. Many autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis, are more frequent in females. However, the role of gender in the pathogenesis of specific diseases is poorly understood. For example, in a murine lupus model, female mice have a more severe disease than their male counterparts. Mice deficient in IFNα/β exhibit a similar female bias in disease severity. In this study, we investigated the role of sex on disease manifestations and tissue pathology in the murine lupus model. Male mice showed higher disease severity and antinuclear antibodies than female mice. In addition, we found that the disease in female mice was associated with increased expression of VGLL3, a transcriptional regulator involved in neutrophil function. VGLL3 expression was increased in the spleen and liver of female mice, and VGLL3 knockdown mouse had reduced disease severity. These findings suggest that VGLL3 is a key regulator of transcriptome changes in gender biased autoimmune conditions.

**009**

B10 cells suppress contact dermatitis in an antigen specific manner

M Kamata,1 K Candando2, E Kountikov,1 T Miyagaki,1 J Lykken2, HS Fiske,1 S Satoh1 and T Tedder2

1 The University of Tokyo, Tokyo, Japan, 2 Duke University Medical Center, Durham, NC, 3 Department of Dermatology, University of Tokyo Graduate School of Medicine, 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 (IL)-10. It has been reported that B10 cells have suppressive activity in contact dermatitis but their involvement in this pathological process 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) pathways. Here, we report an unidentified specific BCR signaling pathway essential for B10 cell function. We investigated whether B10 cells could suppress contact dermatitis via a contact hypersensitivity (CHS) mouse model, and whether antigen specificity was required for B10 cell function. B10 cells are so rare that to make experiments difficult. Therefore, we first established regulatory B cells expanding culture system using anti-CD43 T3.1 anti-CD40L cells and exogenous IL-4 and IL-21, which allowed splenic B cells to expand about 25,000-fold. We confirmed that cultured B cells acquired the competence to produce IL-10. Adoptively transferred cultured B10 cells suppressed skin inflammation in a CHS model. Moreover, adoptively transferred cultured B10 cells with oxazolone-specific BCR could suppress oxazolone CHS inflammation, whereas cultured B10 cells without oxazolone-specific BCR could not suppress oxazolone CHS inflammation. Our results suggest that B10 cells have suppressive activity on contact dermatitis. Furthermore, antigen specificity is required for B10 cells to suppress contact dermatitis.

**010**

FcgR1 is important for clonal ignorance and prevents psoriasis phenotype in pathogenic anti-desmoglein 3 antibody knock-in mice

H Nomura,1 Y Kase,1 Y Yamagami,1 N Wada,1 S Koya,1 H Takahashi1 and M Amano1

1 Department of Dermatology, Nara Medical University, Kashihara, Japan, 2 Keio University School of Medicine, Tokyo, Japan, 3 Keio University, Tokyo, Japan and 4 Department of Dermatology, Keio University, Tokyo, Japan

FcgR1 is important for clonal ignorance and prevents psoriasis phenotype in pathogenic anti-desmoglein 3 antibody knock-in 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 Fujita,1 T Miyagaki,1 S Murimura,1 S Shihata,1 Y Asano2 and S Satoh1

1 Department of Dermatology, University of Tokyo Graduate School of Medicine, Tokyo, Japan, 2 University of Tokyo, Tokyo, Japan, 3 Department of Dermatology, Nara Medical University, Kashihara, Japan

Emerging evidence has demonstrated that Toll-like receptors (TLRs) 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 deficiency in unexposed psoriatic 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 inflammatory cells than that of TLR2-deficient mice. Among mRNA levels of Foxp3 and IL-10 in the lesional skin were significantly decreased in TLR2-deficient mice. Furthermore, flow cytometric analyses of the lymph node revealed that the frequency of CD4+CD25+Foxp3+ regulatory T cells (Treg) among CD4-positive cells was 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-treated mice ameliorated skin inflammation. 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 Kagami2, 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

We have previously generated pathogenic anti-DSS IgG, AK23, knock-in mice which can potentially undergo class switch recombination of Ig immunoglobulin. However, AK23 mice did not develop PV phenotype in sterile 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 AK23 mice. Bone marrow (BM) cells from AK23 mice were transferred into the C57BL/6 strain or C57BL/6 mice. Adoptively transferred autoreactive B cells were deleted by regulatory T cells (Tregs) and IL-10 production by Tregs and DCs, suppressing imiquimod-induced psoriasis-like skin inflammation. In the presence or absence of DSS, AK23 B cells developed in BM and spleen similarly between both recipients. AK23 B cells from both recipients similarly responded upon anti-DSS IgG stimulation. These results suggest that AK23 B cells were not deleted or anergic, instead stayed in “clonal ignorance”. To further understand whether FcgR1 contributes to this state, AK23 mice were introduced with FcgR1B-deficient allele. AK23 mice with FcgR1B/FcgR1B mice spontaneously developed PV phenotype, such as skin erosions, acantholysis, and IgG deposition on keratinocyte surfaces, with higher levels of anti-DSS IgG compared to AK23 mice. Their survival rate at age of 12 weeks was also significantly lower than that of AK23 mice (39% vs 81%, p = 0.005), together indicating breakdown of clonal ignorance in FcgR1B deficiency. Thus FcgR1B plays an important role in maintaining clonal ignorance and preventing the development of PV phenotype in AK23 mice.